アワビ筋肉からの紅藻酸の単離

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Isolation of D-Rhodoic Acid from the Abalone Muscle*1

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In the strongly acidic fraction of the muscle extract of abalone *Haliotis discus hannai*, an unique compound which showed very weak ninhydrin reaction was detected by paper electrophoresis. It was isolated by ion exchange chromatography, and inferred to be D-rhodoic acid: *N*-(D-1-carboxyethyl)-taurine from the results of elemental analysis, NMR, ORD and IR spectrometries. This inference was confirmed by synthesis. It is very interesting to note that the structure of this compound is similar to that of octopine, alanopine and strombine, which are referred to as "opine".

In a previous paper,[1] it was demonstrated that several novel ninhydrin positive compounds were present in the acidic fraction of abalone muscle extracts. Some of these compounds were isolated and identified as chondrine,[1] L-pyrrolidine-2,5-dicarboxylic acid, D-α-iminopropioacetic acid (strombine),[2] and L-pyrrolidine-2,4-dicarboxylic acid.[3] In addition to these, we found an unique compound which showed very weak ninhydrin reaction in the strongly acidic fraction. This compound was inferred to be D-rhodoic acid. This inference was then confirmed by synthesis. The present paper deals with the isolation and identification of D-rhodoic acid from the foot and columella muscles of the abalone *Haliotis discus hannai*.

Experimental and Results

Materials

Specimens of abalone *Haliotis discus hannai* were collected in Okkirai Bay, Iwate Prefecture. One kilogram of the foot and columella muscles was used for the ethanolic extraction.

Isolation and Properties of D-Rhodoic Acid

Preparation of the ethanolic extract was carried out according to a previously published method.[4] The extract was applied on to a Dowex 50W-X8 (H^+ type, 4×22 cm) and washed thoroughly with deionized water. Column effluent was collected in 1 *l* fractions. Amino acids and other ninhydrin positive compounds were detected by the methods described previously.[2] The first 4 *l* (fractions 1–4) which contained large amounts of taurine was concentrated by the flash evaporator and then applied to a column of Dowex 1-X8 (acetate type, 4.6×22 cm). The column was washed thoroughly with deionized water to remove taurine, and then L-pyrrolidine-2,5-dicarboxylic acid and strombine were eluted with 6 *l* of 0.5 N acetic acid.

After this treatment, 6 *l* of 3 N acetic acid was passed through the column and the eluate was fractionated in to 500 m*l* portions. The fractions (numbers 5–8) showing a weak red spot with ninhydrin reagent at R_{CySO_3H} 0.84–0.86*3 (pH 3.7) and R_{Glu} (−)0.67–(−)0.69*4 (pH 1.9), were combined and concentrated under reduced pressure. The recovered compound was recrystallized from aqueous ethanol with a yield of 254 mg colorless crystal.

This compound was positive to sulfur test, but negative to phosphorus test after fusion with metallic sodium. The molecular formula of $C_5H_{11}NO_5S$ (MW 197) was determined by the elemental analysis. Anal. Found: C, 30.13; H, 5.71; N, 7.01%. Calcd. for $C_5H_{11}NO_5S$: C, 30.45; H, 5.62; N, 7.10%. The IR spectrum (Fig. 1) suggested the existence of both carboxyl group and sulfonic acid group in the molecule, judged from the large peaks at about 1700 and 1200 cm^{-1}, respectively. Further structural information was obtained from NMR spectrum, measured in trifluoroacetic acid (Fig. 2). This

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*3 Relative mobility to cysteic acid.[1]

*4 Relative mobility to glutamic acid.[1]

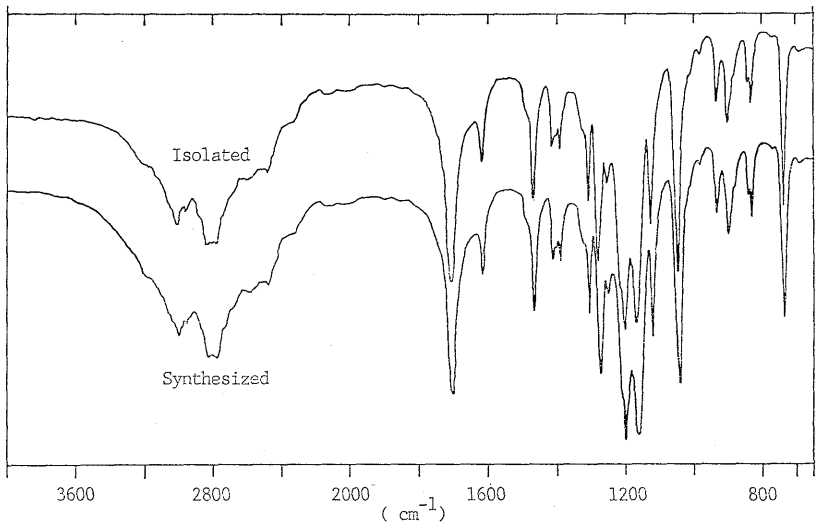


Fig. 1. IR spectra of isolated (from abalone and alga) and synthesized D-rhodoic acid.

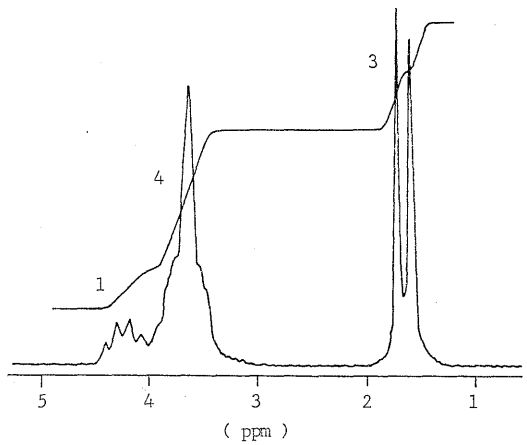


Fig. 2. NMR spectrum of D-rhodoic acid isolated from abalone.

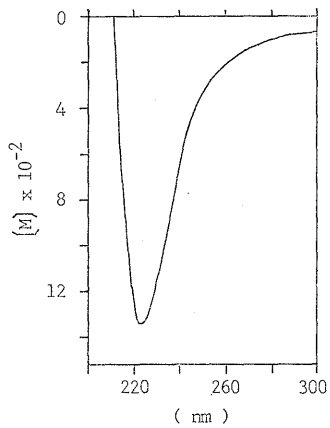
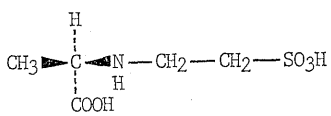


Fig. 3. ORD spectrum of D-rhodoic acid isolated from abalone.

spectrum revealed a doublet at 1.63 ppm (J=7.04 Hz, equivalent to three protons), a A_2B_2 spinning type singlet at 3.63 ppm (four protons) and a quartet at 4.23 ppm (J=7.04 Hz, one proton). From the chemical shifts and integration ratios of these signals, the signals at 1.63 and 4.23 ppm were assigned as methyl and methine groups, respectively, and the signal at 3.63 ppm was assigned as two methylene groups consisting a $-CH_2-CH_2-$ group. It was also concluded that the methyl and methine groups formed a CH_3-CH-group owing to the consistency of their coupling constants (J=7.04 Hz), and from the result of spin decoupling test. This compound showed a negative Cotton effect curve with a peak at 222 nm in ORD spectrum (Fig. 3). The molecular rotatory power at each wavelength was a followed: $[M]_{222}=-1385$, $[M]_{230}=-1159$, $[M]_{250}=-331$, $[M]_{270}=-142$ and $[M]_{300}=-68$ (c=0.167, H_2O at 22°C).

From the above data, the structure of this compound was inferred to be D-rhodoic acid: *N*-(D-1-carboxyethyl)-taurine.



Synthesis of D-Rhodoic Acid

D-Rhodoic acid was synthesized from taurine (0.8 g) and L-α-bromopropionic acid (1.0 g), which was derived from L-alanine, by the method of Abderhalden and Haase.[5] After 3 weeks at

room temperature, the reaction mixture was washed with ether to remove remainder of bromopropionic acid. The newly synthesized D-rhodoic acid was then separated as described above. The colorless crystals were obtained: yield 315 mg. Anal. Found: C, 30.26; H, 5.69; N, 7.08%. $[M]_{222}=-1316$, $[M]_{230}=-1110$, $[M]_{250}=-328$, $[M]_{270}=-157$, $[M]_{300}=-62$ (c=0.242, H_2O at 21°C). IR spectrum is shown in Fig. 1.

Isolation of D-Rhodoic Acid from Red Alga

Five kilograms of red alga *Rhodoglossum japonicum* were collected in Okkirai Bay, at May. The preparation of extracts and isolation of D-rhodoic acid were carried out by almost the same manner as mentioned above. The colorless crystals were obtained from aqueous ethanol: yield 1.4 g. The IR spectrum of this compound was shown in Fig. 1. Molecular rotatory power was as following: $[M]_{222}=-1347$, $[M]_{230}=-1177$, $[M]_{250}=-355$, $[M]_{270}=-146$, $[M]_{300}=-70$ (c=0.320, H_2O at 19°C).

Comparison of Natural and Synthesized D-Rhodoic Acid

The IR spectra of the two natural samples isolated from abalone and red alga, and synthesized one, were virtually identical (Fig. 1). They also showed the same optical configuration judged from their optical rotatory powers.

This compound showed a peak almost the same position of cysteic acid in the liquid chromatography.[2] But the color intensity with ninhydrin reagent was substantially weaker, about one hundredth, than that of cysteic acid.

Discussion

The occurrence of D-rhodoic acid: *N*-(D-1-carboxyethyl)-taurine is thus established in this paper as one of the predominant nitrogenous components in the muscle of abalone *Haliotis discus hannai*. This compound has already been isolated from some red algae by KURIYAMA in 1961.[6] But this case is the first finding of D-rhodoic acid in animal tissues, so far as the authors aware.

Concerning the metabolism of D-rhodoic acid in marine algae, it has been postulated that D-rhodoic acid might be derived from chondrin by oxidative ring opening reaction.[6] However, the optical configuration of two compounds differ from each other, D form for D-rhodoic acid and L form for chondrin. Thus, there must be a Walden inversion within that conversional reaction. The details of this problem still remain to be resolved.

Rhodoic acid is a member of the family of compounds often referred to as "opines" in the comparative physiology literature. These compounds are glycolytic end products, and are typically produced during periods of elevated energy demands in muscles.[7-9] Since strombine is present in only trace amounts and octopine and alanopine are absent in abalone muscle, it is likely that rhodoic acid formation assumes the same physiological role as does "opine" formation in other molluscs. Studies of the origin of D-rhodoic acid in the abalone muscle will be reported in detail elsewhere.

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