Short Paper

Identification of Iodomethyltrimethylammonium as a Minor Toxin of the Green Turban Shell*

In the previous study¹⁾ we reported occurrence of saxitoxin in the viscera of the green turban shell *Turbo marmorata* collected at Okinawa. During the course of the study it was noticed that this snail contained minor toxins besides saxitoxin. Our subsequent effort enabled us to isolate and identify one of the minor toxins as described below.

Two kilograms of the viscera of the green turban shell collected at Ishigaki Island, Okinawa Prefecture, was extracted thrice with boiling methanol and the extracts were partitioned first between diethyl ether and water and then between 1-butanol and water. The aqueous fraction retained saxitoxin (1125 MU) while the butanol fraction afforded a mixture of the minor toxin and saxitoxin (1125 MU). The minor toxin in the butanol fraction was purified by adsorption to Amberlite CG-50 (H⁺ form) column followed by elution with 0.1 N hydrochloric acid. Succeedingly the toxin was adsorbed to an acidic alumina column from chloroform solution and then, after washing with chloroform and chloroform-methanol (9:1) solutions, was eluted from the column with chloroform-methanol (7:3) solution. It was further purified on Sephadex G-10 column equilibrated with 0.05 N acetic acid. Elution volume of the toxin indicated that it was a small molecule compound. The final stage of purification was done by paper chromatography (ppc) on Toyo Filter Paper No. 50 with the solvent consisting of 1-butanol, acetic acid, and water (4:1:2 v/v). The toxic band as tested by mice assay was located at Rf 0.6. The toxin was positive to the Dragendorff reagent indicating that it contained a tertiary or quaternary nitrogen. The presence of iodine in the molecule was soon recognized by the mass spectral analysis. The ions observed in the spectrum were assigned as follows: m/e 50 CH₃Cl, 58 (CH₃)₂NCH₂, 127 I, 128 HI, 142 CH₃I, 185 (CH₃)₂NCH₂I, and 254 I₂. The assignment of the ions containing iodine was supported by the absence of significant p+1 peaks. From the spectrum it was possible to postulate two compounds, tetramine iodide and iodomethyltrimethylammonium chloride. However, the possibility of the toxin being the former was soon denied after comparison with authentic specimen by thin layer chromatography (tlc). The toxin was easily

separable from the authentic specimen of tetramine iodide. Presence of the ion at m/e 50 (CH₃Cl) also indicated that the counterpart ion of the amine was chlorine rather than iodine. On the other hand, the isolated specimen was indistinguishable by ppc and tlc from synthetic iodomethyltrimethylammonium chloride, which was prepared by the reaction of methylene iodide with trimethylamine²⁾ followed by conversion into chloride with ion exchange resin. The comparison was carried out on filter paper (Toyo Filter Paper), cellulose layer (Macherey, Nagel), silica gel layer (Wako gel B-5) and aluminum oxide layer (Merck) with the same solvent used for preparative ppc (Rf 0.53, 0.71, 0.08, and 0.73 in order). The mass spectrum of the synthetic specimen was also identical with that of the isolated toxin. On the basis of these evidences it was concluded that the isolated toxin was iodomethyltrimethylammonium chloride, (CH₃)₃N⁺CH₂I Cl⁻.

The pharmacological activity of this compound is already known by the test with synthetic specimen.²⁾ However, it seems that this is the first time to isolate present quaternary ammonium from the natural source. Its occurrence in the green turban shell may be interesting in view of the fact that its hydrogen substituted form, tetramine, is a well known product of the salivary gland of marine snails such as *Neptunea arthritica* and *N. inter-sculpta*. Despite the resemblance in chemical structure the present toxin differs from tetramine in possibly being of exogenous origin. The toxin content in the snail was quite variable depending upon the place and time of the catch.

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