Toxicity Study on a Marine Snail, *Turbo argyrostoma*—II Identification of (3-methylthiopropyl)trimethylammonium chloride

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The previous work on a marine snail, *Turbo argyrostoma*, collected in Okinawa revealed the occurrence of two toxic amines containing sulfur. The main component, named toxin A, was newly shown to contain both trimethylammonium and methylthio moieties by its NMR spectrum. Further information obtained by mass spectral analysis of the thermal degradation products clearly indicated that toxin A is (3-methylthiopropyl) trimethylammonium chloride. The structure was also confirmed by synthesis.

In the previous report¹⁾ we have described isolation and some properties of two sulfur-containing toxic amines from a marine snail, *Turbo argyrostoma*, collected in Okinawa. The continued study disclosed that the main component, named toxin A, is a small molecule compound having a trimethylammonium function. Since both color reactions and IR spectrum indicated absence of other polar group in the molecule, it was postulated that thermal degradation of the quaternary ammonium group would yield volatile compounds suitable for a combined gas chromatography-mass spectrometry analysis. This hypothesis was proved to work satisfactorily and the results led us to assignment of toxin A as (3-methylthiopropyl)trimethylammonium chloride (I). The structure was further confirmed by comparison with a synthetic compound.

Experimental and Results

Material Isolation of toxin A used in the present study was described in the previous paper¹⁾. Although the sample thus obtained was not in a crystalline form, its homogeneity was confirmed by thin-layer and paper chromatography as reported previously.

NMR spectrum The NMR spectrum of toxin A was measured at 60 MHz as a 5% solution in D₂O by employing sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. In the spectrum two prominent peaks were observed at δ 3.2 (singlet, 9H) and δ 2.15 (singlet, 3H) which were assigned to trimethylammonium and methylthio moieties, respectively. Besides the above two peaks, presence of multiplets centered at about δ 2.3, 3.4, and 3.5 was indicated. Interpretation of these multiplets was not attempted owing to the poor resolution of the spectrum caused by the limited sample size. The relative intensities of the peaks in the spectrum suggested that the

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total number of the protons in the molecule might be less than 20.

Combined gas chromatography-mass spectrometry About 100 μg of toxin A dissolved in methanol was injected in a gas chromatographic instrument (JEOL, JGC-20K) of which injection port and column (1% OV-101, 1.5 m) were maintained at 80°C. After

Fig. 1. Gas chromatogram of toxin A.

Fig. 2. Mass spectra of thermal degradation products of toxin A.

elution of the solvent, the temperature of the injection port was raised to 250°C. Decomposition of the sample began at slightly above 200°C, and two peaks appeared in the chromatogram. After elution of the two peaks, the column temperature was raised gradually. A third peak of small size with flat shape appeared immediately. After that no peak appeared even when the column temperature reached 300°C. The chromatogram thus obtained is illustrated in Fig. 1. These peaks were analyzed by a mass spectrometer (JEOL, JMS-06) connected to the gas chromatographic instrument. Fig. 2 presents the mass spectra of two peaks (P-1 and P-2). The third peak (P-3) gave a spectrum

identical with that of P-2 and was judged to be the reminiscence of P-2. From the spectrum it is apparent that P-1 consists of CH_3Cl (m/e 50) and $(\text{CH}_3)_3\text{N}$ (m/e 59). In the spectrum of P-2, the peak of the highest mass number is at m/e 133. Presence of sulfur is easily recognizable through the existence of a peak at two mass units above this peak, due to the ^{34}S isotope. Similarly, ions at m/e 61 and 47 are likely to contain sulfur and may be assignable to $\text{CH}_3\text{S}^+\text{CH}_3$ and CH_3S^+ , respectively. A series of ions at m/e 58, 44, 42, and 20 may arise from the nitrogenous moiety $(\text{CH}_3)_2\text{NCH}_2$. Thus, the possible formula for the ion at m/e 133 can be written as $(\text{CH}_3)_2\text{N}(\text{CH}_2)_3\text{SCH}_3$. Existence of a peak at m/e 124 may seem inexplicable by this formula. However, a peak at two mass units above this with intensity of about the one-third strongly suggests the presence of chlorine and the possible formula may be $\text{Cl}(\text{CH}_2)_3\text{SCH}_3$. From these results, it is most probable that toxin A is (3-methylthiopropyl)trimethylammonium chloride and the thermal degradation proceeds as follows.

Synthesis of (3-methylthiopropyl)trimethylammonium chloride In order to confirm the above structure, **I** was synthesized as follows. To 100 ml of ethanolic solution of γ -N,N-dimethylaminopropyl chloride hydrochloride (10 g) were added 3.5 g of sodium carbonate and 17 ml of 20% solution of methanethiol sodium salt, and the mixture was refluxed for 2 hr. After the solution was diluted with 300 ml of water, (3-methylthiopropyl)dimethylamine was extracted with four 50-ml portions of chloroform. The combined chloroform solution was dried over anhydrous sodium sulfate and refluxed with 10 ml of methyl iodide for 1 hr. The solvent and excess methyl iodide was removed by evaporation, and the residue was chromatographed on an acidic alumina column. By elution with chloroform-methanol (9:1), (3-methylthiopropyl)trimethylammonium iodide was obtained completely free from its corresponding sulfonium in a yield of 5.93 g. The methiodide thus obtained was converted to **I** by passing the aqueous solution through a column of Amberlite IRA-400 (OH^-) followed by acidification with hydrochloric acid. The residue was crystallized twice from methanol-acetone (9:1). Very hygroscopic needles were obtained: yield, 4.08 g. M.p. 141–142°C. *Anal.* Found: C, 45.57; H, 10.01; N, 7.62%. Calcd. for $\text{C}_7\text{H}_{18}\text{ClNS}$: C, 45.75; H, 9.87; N, 7.62%.

Comparison of toxin A with synthetic (3-methylthiopropyl)trimethylammonium chloride Mercuric chloride salt of **I** was prepared in a similar manner as described in the previous paper.¹⁾ After crystallization twice from acetone, the crystals melted at 122–123°C. The melting point is in a good agreement with that of the same salt derived from toxin A. Paper and thin-layer chromatographic analysis of both the synthetic compound and

toxin A was carried out by employing various adsorbents and solvent systems. The results, as presented in Table 1, indicate that they are indistinguishable from each other. The identity is also evidenced by their IR spectra, as shown in Fig. 3. Analysis of synthetic I by combined gas chromatography-mass spectrometry gave an identical result with that of toxin A.

Table 1. Comparison of *R_f* values of toxin A and synthetic (3-methylthiopropyl)trimethylammonium chloride by thin-layer and paper chromatography.

Sample Adsorbent	95%EtOH- 28%NH ₄ OH (95:5)		1-BuOH- AcOH-H ₂ O (4:1:2)		MeOH-Pyr- AcOH-H ₂ O (6:9:1:4)		1-BuOH-H ₂ O (saturated)	
	I	II	I	II	I	II	I	II
Aluminium oxide neutral (Merck)	0.70	0.70	0.78	0.78			0.41	0.40
Cellulose powder MN 300 (Macherey, Naged)	0.65	0.65	0.69	0.70			0.36	0.37
Wako gel B-5 (Wako Pure Chemical)			0.34	0.33	0.66	0.65		
Filter paper (Toyo Roshi, No. 50)	0.56	0.55	0.66	0.68				

Sample: I toxin A; II synthetic (3-methylthiopropyl)trimethylammonium chloride

Spraying reagent: Dragendorff reagent

Fig. 3. IR spectra of toxin A and synthetic (3-methylthiopropyl)trimethylammonium chloride.

Discussion

The data presented in this work established that toxin A isolated from the acetone-soluble fraction of *T. argyrostoma* is (3-methylthiopropyl)trimethylammonium chloride. There seems to be no previous report dealing with the isolation of it from the nature, and the present paper may be the first to report its occurrence. It has been well known that

some species of marine snail are able to produce quaternary ammonium compound having remarkable pharmacological activities.^{2,3)} However, this sulfur-containing amine of *T. argyrostoma* is likely to be obtained from their diet organism(s) rather than synthesized by the snail, as discussed in the previous paper. Identification of the true organism which elaborate **I** awaits further investigation.

Pharmacological activities of **I** and its related compounds were well investigated by LANDS and CAVALLITO⁴⁾ and by WEBB and MAUTNER.⁵⁾ They indicated that **I** is quite potent in exerting various cholinomimetic and other pharmacological activities. For instance, when compared on the basis of minimum lethal dose by *i.p.* injection to mice, toxicity of **I** (10 mg/kg)⁴⁾ exceeds that of tetramine (30 mg/kg)⁶⁾ that is known to induce symptoms in human when the salivary glands of marine snails, *Neptunea arthritica* and *N. intersculpta*, are ingested. Although the role of **I** in the poisoning caused by *T. argyrostoma*⁷⁾ remains questionable as discussed previously, occurrence of such a potent amine should be taken into consideration when this marine snail and other species with a similar feeding habit are taken as food.

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