Toxicity Study on a Marine Snail *Turbo argyrostoma*-IV
Occurrence of [3-(dimethylsulfonio) propyl]
trimethylammonium dichloride

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A new quaternary amine containing sulfur was isolated from a water soluble fraction of the viscera of *Turbo argyrostoma* by chromatography on columns of Amberlite IRA-400 and Dowex 50 followed by partition chromatography on a cellulose column. Mass spectral analysis indicated that the amine decomposes upon heating into CH_3Cl , $(\text{CH}_3)_3\text{N}$, $(\text{CH}_3)_2\text{S}$, $\text{CH}_3\text{S}(\text{CH}_2)_3\text{Cl}$, and $\text{CH}_3\text{S}(\text{CH}_2)_3\text{N}(\text{CH}_3)_2$, suggesting that it is [3-(dimethylsulfonio)propyl] trimethylammonium dichloride $(\text{CH}_3)_2\text{S}^+(\text{CH}_2)_3\text{N}^+(\text{CH}_3)_3 2\text{Cl}^-$. Treatment of the sample with potassium iodide yielded crystalline needles of diiodide $\text{C}_8\text{H}_{21}\text{I}_2\text{NS}$ (mp. 246°), which were identical with a synthesized specimen.

Previously, three pharmacologically active amines, 3-(dimethylthio) propyltrimethylammonium chloride, candicine chloride, and a quaternary amine named toxin B, were isolated from the viscera of *T. argyrostoma* collected in Okinawa.¹⁻³⁾ So far, all these amines were obtained from a 1-butanolic layer after shaking with the aqueous solution.

In the present study, quaternary amines retained in the aqueous solution were investigated. A preliminary survey by paper and thin layer chromatography revealed presence of another quaternary amine containing sulfur. This compound was first purified by paper and thin layer chromatography in a small scale to be tested by GC-MS analysis. The results strongly suggested that it is [3-(dimethylsulfonio)propyl]-trimethylammonium dichloride $(\text{CH}_3)_2\text{S}^+(\text{CH}_2)_3\text{N}^+(\text{CH}_3)_3 2\text{Cl}^-$ (I). Subsequently, it was isolated by chromatography on ion exchange columns followed by cellulose column chromatography. Finally I was converted to the diiodide (II) and the structure was confirmed by synthesis. This may be the first time that I was isolated from the natural source.

This paper deals with isolation and characterization of I.

Materials and Methods

Specimens Specimens of *T. argyrostoma* used in this study were the same as described in the previous paper.³⁾

Preparation of the water soluble fraction The methanolic extract obtained from

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1 kg of the viscera was concentrated, diluted with water, and extracted with diethyl ether and then with 1-butanol after the manner as described previously.¹⁾ The aqueous layer was concentrated to a syrup under reduced pressure and is described as the water soluble fraction in this paper.

Paper and thin layer chromatography Toyo Roshi No. 50 filter paper was used for paper chromatography, and cellulose powder MN 300 and Wako gel B-5 for thin layer chromatography with a solvent consisting of 1-butanol, acetic acid, and water (4: 1: 2). Detection of the spots was made by Dragendorff reagent.

GC-MS analysis A small amount of **I** for testing by mass spectral analysis was purified twice by paper chromatography and once by thin layer chromatography on Wako gel B-5 layer. The sample thus purified was heated in an injection port of a gas chromatographic instrument and the decomposition products were separated on a column (1% OV-101, 1.5 m) and analyzed by a mass spectrometer as described in the previous paper.²⁾

Isolation of **II** The water soluble fraction was passed through an anion exchange column (Amberlite IRA-400, OH⁻, 20-50 mesh, 3.5 × 60 cm) and the column was washed with 4 liters of water. The passing and washing were combined and percolated through a column of cation exchange resin (Dowex 50-X8, H⁺, 200-400 mesh, 3.5 × 70 cm). The column was washed with 3 liters of water and the basic substances were eluted with hydrochloric acid after the manner of CHRISTIANSON.⁴⁾ The eluate was tested by saturated ammonium reineckate solution for quaternary amines. Thin layer chromatography on cellulose layer was also carried out to locate **I** in the eluate. The fractions containing **I** were combined and evaporated to dryness. The residue was treated with acetic anhydride in sodium acetate solution in order to facilitate separation of **I** from the ninhydrin-positive contaminants. After heating in a boiling water bath for 30 min, the solution was evaporated to dryness and the residue was chromatographed on a cellulose column (3.2 × 40 cm) by using 1-butanol-acetic acid-water (4: 1: 2) as solvent. The fractions containing **I** were combined and evaporated to dryness. The residue was dissolved in a small volume of ethanol, and to this was added an equal volume of saturated ethanolic solution of potassium iodide. The mixture was left overnight in a refrigerator, and the resulting precipitates were crystallized twice from ethanol.

Synthesis of **II** 3-(Methylthio)propylamine was treated with methyl iodide and sodium methylate in methanol after the manner of SUYAMA and KANAO.⁵⁾ The product was crystallized once from water and twice from ethanol.

Results

The paper chromatogram of the water soluble fraction showed the presence of several substances positive to Dragendorff reagent and indicated that **I** at R_f 0.16 was

the dominant.

The results of GC-MS analysis of **I** were found to be very similar to those previously obtained on $\text{CH}_3\text{S}(\text{CH}_2)_3\text{N}^+(\text{CH}_3)_3 \text{Cl}^-$ (**II**).²⁾ The thermal degradation products of **I** were separated into two peaks by gas chromatography. The mass spectral analysis revealed that the first peak contained HCl (m/e 36), CH_3Cl (m/e 50), $(\text{CH}_3)_3\text{N}$ (m/e 59, 58, 44), and $(\text{CH}_3)_2\text{S}$ (m/e 62, 47), and the second peak $\text{CH}_3\text{S}(\text{CH}_2)_3\text{Cl}$ (m/e 124) and $\text{CH}_3\text{S}(\text{CH}_2)_3\text{N}(\text{CH}_3)_2$ (m/e 133, 118, 105, 84, 70, 58, 47, 44). It may be pointed out that dimethyl sulfide was present in the degradation products of **I** but not in those of **II**. Therefore, it was postulated that **I** is a methyl sulfonium derivative of **II**.

The yield of **I** obtained by partition chromatography on the cellulose column was 74 mg. After subsequent treatment with potassium iodide and recrystallization, 23 mg of the diiodide was obtained as needles, mp. 246°C . *Anal.* Found: C, 23.00; H, 5.07; N, 3.26%. Calcd. for $\text{C}_8\text{H}_{21}\text{I}_2\text{NS}$: C, 23.01; H, 5.07; N, 3.36%.

Synthetic **II** was obtained in a yield of 70%. Mp. 246.5°C (lit.⁵⁾ mp. 246°C). *Anal.* Found: C, 22.93; H, 5.10; N, 3.40%.

Both the isolated and synthesized specimens of **II** agreed well with each other in melting point, IR spectra (Fig. 1), and mass spectra. Their identity was also confirmed by thin layer chromatography with 1-butanol-acetic acid-water (4: 1: 2), the R_f value being 0.41 on cellulose layer and 0.19 on Wako gel B-5.

Fig. 1. IR spectra of isolated and synthesized [3-(dimethylsulfonio)propyl] trimethylammonium diiodide (KBr).

Discussion

Any report on the occurrence of **I** in nature seems to be not available, although there are several reports on syntheses and pharmacological properties of **I** or **II**.⁵⁻⁷⁾ Since **I** is the methyl sulfonium of **II**, **I** is considered to be either a metabolite or a precursor of **II**. Another compound related to **I**, 3-(methylthio)propylamine, is known to occur in many species of marine algae^{8,9)} and may be taken up by the gastropods when they eat

the algae. At present, it is still uncertain whether **I** and **II** are present in algae or whether they are accumulated by the gastropods through diets.

Pharmacologically, **I** is far less potent than **II** and other quaternary amines found in the gastropod. The relative pharmacological activity of **II** on the basis of muscle paralyzing action in cat is reported to be about one fourteenth of that of **II** and the LD₅₀ of **II** determined by *i.v.* injection to mice is 140 mg/kg.⁷⁾ It is therefore unlikely that **I** was responsible as such for the poisoning caused by ingestion of the gastropod.¹⁰⁾ Nevertheless, its occurrence should be taken into consideration since it is contained in the viscera in a relatively high concentration and can be converted to **II** by heating or possibly by enzymatic action. Survey on distribution of **I** in other species of gastropods and marine algae will be carried out in the near future.

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References

- 1) T. YASUMOTO and M. ENDO: This Bull., **39**, 1055–1016 (1973).
- 2) T. YASUMOTO and M. ENDO: *ibid.*, **40**, 217–221 (1974).
- 3) T. YASUMOTO and M. ENDO: *ibid.*, **40**, 841–845 (1974).
- 4) D. D. CHRISTIANSON, J. S. WALL, R. J. DIMLER, and F. R. SENTI: *Anal. Chem.*, **32**, 874–878 (1960).
- 5) T. SUYAMA and S. KANAO: *Yakugaku Zasshi*, **84**, 1012–1014 (1964).
- 6) F. K. KIRCHNER, A. E. SORIA, and C. J. CAVALLITO: *J. Am. Chem. Soc.*, **77**, 4599–4601 (1955).
- 7) A. M. LANDS and C. J. CAVALLITO: *J. Pharmacol.*, **110**, 369–384 (1955).
- 8) M. SEINER and T. HARTMANN: *Planta*, **79**, 113–121 (1968).
- 9) T. HARTMANN: *Naturwissenschaften*, **55**, 391 (1968).
- 10) Y. HASHIMOTO, S. KONOSU, M. SHIBOTA, and K. WATANABE: This Bull., **36**, 1163–1171 (1970).