Occurrence of Homoserine Betaine and Valine Betaine in the Ovary of Shellfish *Callista brevisiphonata*

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From the ovary of shellfish *Callista brevisiphonata*, two quaternary amines were isolated by ion-exchange chromatography, followed by paper or thin layer chromatography. They were postulated to be α -trimethylammonio- γ -butyrolactone chloride and L-valine betaine on the basis of spectral analyses. Their structures were confirmed by comparison with synthesized specimens. A paper chromatographic survey of the initial extracts revealed that α -trimethylammonio- γ -butyrolactone chloride did not occur as such but was derived artificially from homoserine betaine by treatment in hydrochloric acid solution.

The ovary of the shellfish *Callista brevisiphonata* has been reported by ASANO and his coworkers to cause poisoning when ingested during the spawning season.¹⁻³⁾ The poisoning was assumed to be due to a high concentration of choline which presumably is derived from a choline ester in the organ. In an attempt to obtain more knowledge about the choline ester mentioned by them, an investigation on quaternary amines in ovary was undertaken. During the course of the study, a quaternary amine having a lactone ring (I) and a new betaine (II) were detected. They were isolated by ion-exchange chromatography, followed by paper or thin layer chromatography. Their structures were inferred from the mass spectra and confirmed by comparison with synthesized specimens to be α -trimethylammonio- γ -butyrolactone chloride and L-valine betaine, respectively. It was also found that I was not present as such in the extracts but was formed from homoserine betaine (III) by treatment with hydrochloric acid.

The present paper deals with the isolation and identification of the two betaines from the ovary of the shellfish.

Materials and Methods

Materials Specimens of *C. brevisiphonata* were collected at the coast of Morimachi, Hokkaido, in August 1973. Their ovaries were kept frozen at -20°C until used.

Extraction The minced ovary (600 g) was extracted three times with 1.8-liter portions of methanol under reflux for 30 min. The combined extracts were concentrated under reduced pressure, diluted with water, and extracted with diethyl ether in a separatory funnel. The extraction was repeated until the etherial layer became colorless. To the aqueous layer was added 4 volumes of ethanol, and the solution was allowed to stand

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overnight. The insoluble matters were filtered off and the filtrate was evaporated to dryness. A small portion of the residue obtained from the filtrate was used for paper chromatography with a solvent consisting of 1-butanol, acetic acid, and water (4: 1: 2, solvent A). The remaining of the residue was dissolved in water and the solution was transferred onto a column (4.5×45 cm) of Amberlite IR-120 (H⁺ form, 20–50 mesh). The column was washed successively with 2 liters of water and 3 liters of 4% ammonium hydroxide solution. The latter fraction was evaporated to dryness. After being redissolved in water, the residue was passed through a column (4.5×42 cm) of Amberlite IRA-400 (OH⁻ form, 20–50 mesh) and the column was further washed with 3 liters of water. The combined effluent was concentrated and put on top of a column (4.8×52 cm) of Dowex 50-X8 (H⁺ form, 200–400 mesh) and betaines were eluted with hydrochloric acid after the manner described by CHRISTIANSON, *et al.*⁴⁾ The eluate was collected in 17-ml portions and monitored for quaternary amines with ammonium reineckate solution. Thin layer chromatography on cellulose with solvent A was also carried out to check the separation of each component. The final stage of purification was carried out by either paper chromatography or by thin layer chromatography on Wako gel B-O with solvent A.

Synthesis of betaines DL-Homoserine and L-valine were treated respectively with methyl iodide in the presence of silver oxide after the manner of PATCHETT and WITKOP.⁵⁾ The crude products were treated with Amberlite IRA-400 (acetate form) and crystallized twice from ethanol. The lactone was prepared by heating homoserine betaine in 2 N hydrochloric acid for 2 hr. The product was crystallized from ethanol containing acetone.

Instruments Measurements of IR, ORD, and mass spectra were done on the following models: JASCO model IR-S, JASCO model ORD/UV-5, and JEOL model JMS-06.

Results

Presence of at least five compounds reactive to Dragendorff reagent was recognized by paper chromatography in the initial extracts; their R_f values being 0.27, 0.42, 0.47, 0.50 and 0.61. It may be pointed out that the chromatogram indicated presence of **III** (R_f 0.42) but not **I** which should appear at R_f 0.57.

In ion-exchange chromatography **I** was eluted from the Dowex 50 column with 2.5 N hydrochloric acid in fractions 110–118. Further purification by paper chromatography with solvent A yielded 7 mg of deliquescent residue. The presence of γ -lactone ring in the molecule is indicated by the absorption bands at 1780, 1230, and 1175 cm⁻¹ in the IR spectrum (Fig. 1). The mass spectrum (Fig. 2) strongly suggests that **I** decomposes upon heating into chloromethane (m/e 50) and dimethylaminobutyrolactone (m/e 129).

Fig. 1. IR spectra of isolated and synthesized α -trimethylammonio- γ -butyrolactone chloride (KBr).

Fig. 2. Mass spectrum of I (α -trimethylammonio- γ -butyrolactone chloride).

Fig. 3. IR spectra of isolated and synthesized homoserine betaine (KBr).

From these results it was postulated that **I** is α -trimethylammonio- γ -butyrolactone chloride, which had been derived from homoserine betaine by treatment in hydrochloric acid solution. Treatment of **I** with anion-exchange resin Amberlite IRA-400 (OH⁻ form) resulted in opening of the lactone ring. The IR spectrum of **III** (Fig. 3) thus obtained shows absorption band due to a carboxylate anion at 1620 cm⁻¹ and those due to a

hydroxyl function at around 3300, 1090, and 1060 cm^{-1} . In measurement of ORD, both **I** and **III** did not show any rotation, indicating that they had been racemized during the course of isolation.

II was eluted from the Dowex 50 column with 4 N hydrochloric acid in fractions 250–300, together with unidentifiable contaminants. The combined solution was evaporated and the hygroscopic residue was purified repeatedly by thin layer chromatography on Wako gel B–O: yield, 10.9 mg. In the IR spectrum (Fig. 4) presence of a carboxylate

Fig. 4. IR spectra of isolated and synthesized L-valine betaine (KBr).

Fig. 5. Mass spectrum of **II** (L-valine betaine).

anion is indicated by the absorption band at 1610 cm^{-1} . In the mass spectrum (Fig. 5) the molecular ion is seen at m/e 145 which fits formula $(\text{CH}_3)_2\text{CHCH}(\text{COOH})\text{N}(\text{CH}_3)_2$, a tertiary amine resulted from thermal decomposition of valine betaine. The fragment ions are also in support of this structure. The ORD curve of **II** showed a positive Cotton effect like the synthetic L-valine betaine: $[\phi]_{225}^{18} = +520$ ($c=0.305$, methanol).

Synthetic homoserine betaine was obtained in a yield of 50%. Plates, mp. 236–238°C. Anal. Found: C, 51.88; H, 9.21; N, 8.70%. Calcd. for $\text{C}_7\text{H}_{15}\text{NO}_3$: C, 52.15; H, 9.38; N, 8.69%. The lactone was obtained as deliquescent needles, mp. 220°C. The IR spectra of the synthesized specimens are shown in Figs. 1 and 3, respectively.

The synthesized specimen of L-valine betaine was obtained in a yield of 60%; mp. 207–209°C. *Anal.* Found: C, 59.70; H, 10.75; N, 8.81%. Calcd. for $C_8H_{17}NO_2$: C, 60.34; H, 10.76; N, 8.80%. The IR spectrum is shown in Fig. 4. The mass spectrum was identical with that of **II**. The ORD curve showed a positive Cotton effect with a peak at 225 nm: $[\phi]_{225}^{18} = +2918$, $[\phi]_{230}^{18} = +2714$, $[\phi]_{250}^{18} = +1082$, $[\phi]_{300}^{18} = +120$ ($c=0.45$, methanol).

Discussion

The data presented in this paper unequivocally indicate that homoserine betaine and L-valine betaine are present in the ovary of *C. brevishiphonata* and that the homoserine betaine is converted to the lactone during treatment in acidic solution.

Recently ABE and KANEDA⁶⁾ reported the presence of homoserine betaine in the hydrolyzates of a quaternary amine isolated from a green alga *Monostoma nitidum*. In our another investigation on a turbanshell *Turbo argyrostoma*, presence of homoserine betaine was also confirmed (unpublished data). Therefore, it is quite likely that this betaine is distributed widely among marine organisms. On the other hand, there is no available report on the occurrence of valine betaine in the nature.

During the course of the present study, occurrence of several other quaternary amines was recognized but the limited size of the sample did not allow us to proceed further. Their isolation and identification as well as examination of their pharmacological activities will be carried out in the near future.

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