Atrinine

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Atrinine, a New Betaine Isolated from the Adductor Muscle of Fan-mussel

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During the chromatographic analysis of quaternary ammonium bases in the adductor muscle of fan-mussel, *Atrina pectinata japonica*, the presence of an unknown betaine was recognized. It was isolated by ion-exchange column chromatography using Dowex 50, Amberlite IRA–400 and Amberlite IRC–50 and crystallized as its hydrochloride. From various analytical data, including the elementary composition, infrared absorption and nuclear magnetic resonance spectra, the hydrochloride was regarded as trimethyl-(2-carboxy-3-hydroxypropyl)-ammonium chloride. The new betaine was thus established to be an isomer of carnitine and named "atrinine" after the scientific name of the bivalve.

The present paper deals with these results.

Experimental and Results

Material and Preparation of Extract. The specimens of fan-mussel were caught off Yokosuka, Kanagawa Prefecture, late in March, 1969. Immediately after the animals were brought to our laboratory, the adductor muscle was removed and kept in a freezer until used.

One kilogram of the adductor muscle was homogenized and extracted with 1.5 l of ethanol. After centrifugation the residue was extracted twice with 2 l portions of 66% (v/v) ethanol in the same way. The supernatants were combined and left in a cold room at 4°C overnight. The resulting precipitates were filtered and the filtrate was condensed under reduced pressure to about 300 ml. The resulting turbid solution was treated thrice with 300 ml portions of diethyl ether to remove lipids and made up to 500 ml with water.

Isolation of Atrinine. One-third of the above extract (*ca.* 170 ml) was poured onto a column of Dowex 50 X-12 (H-form, 200–400 mesh, 9×30 cm) and developed with 1 N HCl at a flow rate of 90 ml per hour. Elution of quaternary ammonium bases from the column was monitored with ammonium reineckate reagent as previously reported[1]; a mixture of 0.5 ml of each eluate fraction and of the reagent was kept in an ice bath for 30 minutes to check precipitation. The first 8 l portion which gave no precipitate was discarded and 50 ml fractions were collected thereafter. Atrinine was eluted between Fr. 11 and Fr. 30 and was followed by carnitine. All fractions containing atrinine were

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pooled and evaporated to dryness *in vacuo*. The residue was taken up in a small amount of water. The remaining two-thirds of the extract was treated similarly and the combined aqueous solution was again subjected to chromatography with a longer column of Dowex 50 X-12 (H-form, 2×75 cm) as follows.

A half portion of the above solution (corresponding to 500 g of the adductor muscle) was introduced to the top of the column. After the column was rinsed with a small amount of water, 1 N HCl was allowed to flow through it at a rate of 60 ml per hour. The effluent was collected in 10 ml cuts and each fraction was examined with ammonium reineckate reagent; atrinine was found to be eluted between Fr. 85 and Fr. 100. The last few fractions were heavily contaminated with ninhydrin-positive substances and were discarded. The combined fractions were freed from excess HCl by repeated evaporations *in vacuo*. Water was added and the resulting aqueous solution was then passed through succesive connected columns of Amberlite IRA-400 (OH-form, 100 ml) and Amberlite IRC-50 (H-form, 40 ml). The percolate was acidified with 1 N HCl. After excess HCl was removed by repeated distillations *in vacuo*, the syrupy residue was taken up in the minimum amount of hot ethanol; diethyl ether was added until a permanent turbidity occurred. Colorless needles of atrinine hydrochloride soon appeared. By recrystallization from ethanol-diethyl ether in the same way, 165 mg of the pure substance was obtained. The second half of the run yielded 170 mg.

Properties and Structure of Atrinine Hydrochloride. The crystals are very hygroscopic and melt at 149–151°C. *Anal.* Found: C, 42.63; H, 8.15; N, 6.98; Cl, 18.06. Calcd. for $C_7H_{16}NO_3Cl$: C, 42.53; H, 8.16; N, 7.09; Cl, 17.93. R.D. in water (c=0.615), 16°C; positive Cotton-effect curve: $[\alpha]_{700}$ 0°, $[\alpha]_{589}$ 0°, $[\alpha]_{226}$ +7728° (peak), $[\alpha]_{195}$ −9192° (trough), $[\alpha]_{190}$−8134°. Atrinine gives an orange color with the Dragendorff reagent, but is negative to ninhydrin.

The infrared absorption spectrum of atrinine hydrochloride in nujol is shown in Fig. 1. Two peaks at 3400 cm^{-1} and 1726 cm^{-1} indicate the presence of hydroxyl and carboxyl groups, respectively.

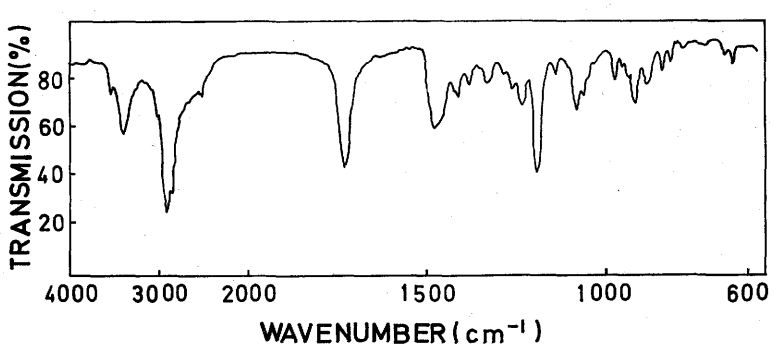

Fig. 1. Infrared absorption spectrum of atrinine hydrochloride in nujol.

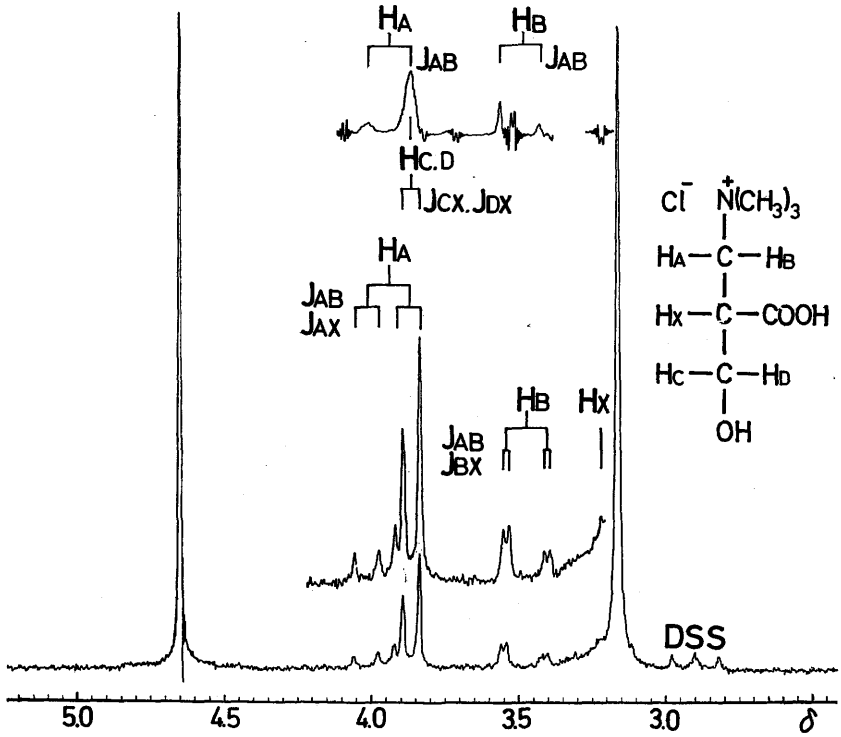

Fig. 2. Nuclear magnetic resonance spectra of atrinine hydrochloride. Spectrometer, Varian HA–100; internal standard, DSS; solvent, D_2O; sweep time, 1,000 sec; sweep width, 500 cps. The spectrum obtained in the decoupling test irradiating at H_X is given in the upper part.

Its proton resonance spectrum was measured on the Varian HA–100 NMR spectrometer at 100 MHz using deuterium oxide as solvent and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal reference (Fig. 2). Three peaks at δ 2.82, 2.90 and 2.98 are due to the reference DSS and a sharp peak at δ 4.65 to HDO. A strong resonance line at δ 3.16 representing 9 protons is assigned to the N-trimethyl group corresponding to the chemical shift of the N-trimethyl protons of authentic carnitine hydrochloride (δ 3.23). Five peaks at δ 3.84–4.06 (3 protons) are attributable to H_A, H_C and H_D as shown in Fig. 2; H_A is split into a quartet by coupling with H_B and H_X, and magnetically equivalent H_C and H_D are split into a doublet by coupling with H_X. Their coupling constants, J_{AB}, J_{AX} and J_{CX} (J_{DX}), are 14, 8 and 6 cps, respectively. Under the influence of H_A and H_X, H_B gives a quartet centered at δ 3.48, the coupling constant, J_{BX}, being 2 cps. The signal due to H_X (δ 3.22) partially overlaps with the peak of the N-trimethyl group.

For confirming these assignments, a spin-spin decoupling test irradiating at H_X was carried out. Both H_A and H_B are decoupled to give a doublet and H_C (H_D) a singlet (Fig. 2).

From these findings the structure of atrinine hydrochloride was considered to be

trimethyl-(2-carboxy-3-hydroxypropyl)-ammonium chloride, $[(CH_3)_3N^+ \cdot CH_2 \cdot CH(COOH) \cdot CH_2OH]Cl^-$.

Derivatives of Atrinine. The chloraurate and chloroplatinate were prepared from atrinine hydrochloride by the usual method. Chloraurate: orange yellow needles, m.p. 128–131°C. *Anal.* Calcd. for $C_7H_{16}NO_3 \cdot AuCl_4$: N, 2.80. Found: N, 2.75. Chloroplatinate: yellow elongated needles, m.p. 177–179°C. *Anal.* Calcd. for $C_{14}H_{32}N_2O_6 \cdot PtCl_6$: N, 3.83. Found: N, 3.75.

Discussion

Atrinine may be reasonably represented by formula (I) as a betaine. This formula had been once advanced by ENGELAND[2] in 1921 as a possible formula for carnitine. It does not seem, however, that a substance having this structure has been detected in biological sources or synthesized so far. To confirm the structure, its synthesis is now being attempted. Since atrinine has an asymmetric carbon atom, its configuration should also be elucidated.

$$(CH_3)_3N^+ \cdot CH_2 \cdot \underset{\displaystyle COO^-}{\underset{|}{CH}} \cdot CH_2OH$$

(I)

Atrinine was isolated in fairly good yield from the adductor muscle of fan-mussel. Its distribution in the other anatomical parts of the bivalve as well as in the other species of aquatic animals is an interesting subject. Furthermore, its physiological activity may be worth studying in view of the fact that carnitine, an isomer of atrinine, is known to be the essential nutrient for the larvae of the mealworm, *Tenebrio molitor*[3].

Summary

A new betaine, named atrinine was isolated from the adductor muscle of fan-mussel, *Atrina pectinata japonica*, and crystallized as its hydrochloride. From various analytical data, including the elementary composition, color reactions, proton resonance and infrared absorption spectra, atrinine hydrochloride was postulated to be trimethyl-(2-carboxy-3-hydroxypropyl)-ammonium chloride. The chloraurate and chloroplatinate of atrinine were also prepared from the hydrochloride.

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