Further Purification of the Ivory Shell Toxin*

In the previous paper1), it was described that a toxin in the Japanese ivory shell Babylonia japonica was purified to the extent to evoke mydriasis in mice at a dose as low as 0.02 μg/g body weight and postulated to be a bromo-compound of small molecule with carbonyl, hydroxyl, and unknown nitrogen functions. This paper deals with the observation that the final preparation in the previous work was further separable into two active components closely resembling each other.

Seventy-eight milligram of the preparation, which was obtained by silica gel column chromatography and showed a toxicity of 0.03 μg/g, was dissolved in 15 ml of water and transferred onto a column (2.6 x 70 cm) of Sephadex G-25 (fine). The column was percolated with water at a flow rate of 20 ml/hr. The eluate was collected in a 10-ml portion and examined on both toxicity and optical density at 280 mμ. As shown in Fig. 1, two toxic components, designated A and B, were eluted in order. Fractions containing each component were combined, concentrated under reduced pressure, and lyophilized. The both toxic principles were obtained as faintly yellow powder. Their minimum dose to evoke mydriasis in mice and yield of solid per g of the raw mid-gut gland were 0.017 μg/g and 0.051 mg/g for Component A and 0.018 μg/g and 0.057 mg/g for Component B, respectively.

On thin-layer plate of Silica gel G, the both components gave a few degradation products, as reported previously. Component A showed 5 or 4 and Component B 4 or 3 spots respectively, when developed with n-butanol-acetic acid-water (4:1:1.5) or 70% acetone. In each case, the toxicity was detected only in the main spot of the lowest Rf value which was slightly higher in Component B than in Component A.

In IR, UV, and elemental analyses, the two components were similar to each other and also to the final preparation in the previous work. Anal. Found: C, 40.59; H, 5.06; N, 8.85; Br, 11.87% for Component A and C, 40.34; H, 4.86; N, 9.74; Br, 12.29% for Component B. Bromine was estimated by potentiometric titration with silver nitrate solution and confirmed as such by the test with sodium hypochlorite after sodium fusion. Components A and B did not melt below 300ºC and gradually became dark at about 150ºC. The ivory shell toxin was thus found to consist of two closely related components.

The preparation before separation into Components A and B and showing the toxicities from 0.019 to 0.027 μg/g was subjected to the following reactions: hydrolysis with 6 N hydrochloric acid, degradation with hot water, oxidation with potassium permanganate in dilute sodium hydroxide, photolysis in 1% ammonia, hydrogenation with Adams’ platinic oxide catalyst, reduction by tin-hydrochloric acid, methylation with diazomethane, acetylation with pyridine-acetic anhydride, and trimethylsilylation. All of these reactions, however, gave a series of the very labile products, none of which served as the clue to chemical structure of the toxin.

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Reference

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