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ISOLATION AND STRUCTURE DETERMINATION OF A NEW MARINE TOXIN, SURUGATOXIN FROM THE JAPANESE IVORY SHELL, BABYLONIA JAPONICA

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In September 1965, occurred intoxication from ingestion of a carnivorous gastropod, Babylonia Japonica captured in Suruga Bay located near Mount Fuji. The patients complained of visual defects, including amblyopia and mydriasis, with thirst, numbness of lips, speech disorders, constipation and dysuria[1].

Pulewka's method[2] which was developed for an assay of atropine activity on mydriasis of mice, was found to be useful for detection and separation of the toxin. The causative toxin was revealed only in the mid-gut gland of those gastropods collected in a very limited area of the bay[1,3,4]. When toxinless gastropods were kept in the limited area they gained the same toxicity in one month, while toxic gastropods entirely lost their toxicity when kept outside of the area.

In this communication the isolation and structure determination of the toxin, called surugatoxin by the authors in reference to Suruga Bay, will be described.

One kilogram of the mid-gut gland was thoroughly ground with a mixer and

extracted twice with 5ℓ of 1% of acetic acid each time. After centrifugation, the upper solution was mixed with ten volumes of acetone and the precipitates formed were again removed by centrifugation. The supernatant was concentrated to 100mℓ of brownish yellow syrup under reduced pressure at 30°C, after removal of ether-soluble fatty material.

The syrup was purified by gel filtration, bioassay of the toxin being followed by Pulewka's method.

Since it has already been found[3] that the toxin was not stable to mineral acids or alkalies but was fairly stable only in acetic acid, isolation columns other than 'Sephadex' and 'CM-Sephadex' were not suitable. The correct column selection permitted successful isolation of the toxin.

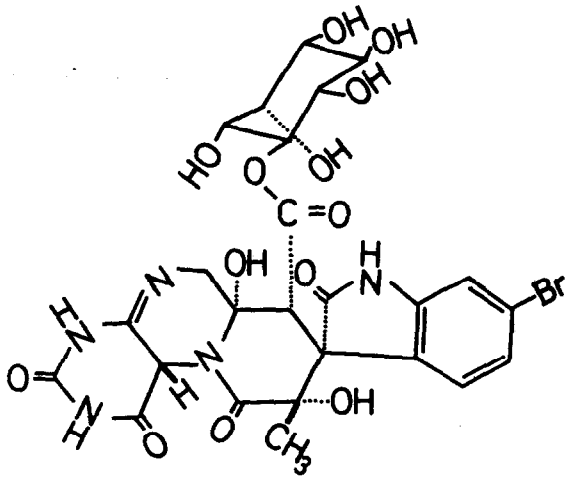
Gel filtration of the syrup through a 'Sephadex G-25' column was accomplished with water acidified with acetic acid at pH 3.9 as the elution solvent. The active eluted portion (4.3g, minimum dose to evoke mydriasis in mice --- M.M. 1μg/g) was further processed on a 'CM Sephadex C-25' column and eluted with the same solvent mentioned above, to give a light yellow powder (1.5g, M.M. 0.3μg/g). The powder was finally purified on a 'Sephadex G-15' column and a crystalline product (10mg) was obtained as colorless prisms from the most active fraction (0.5g).

The surugatoxin so isolated evoked mydriasis in mice at a minimum dose of 0.05μg/g body weight. Degradation of surugatoxin with 1N-hydrochloric acid or 2N-ammonium hydroxide at 100°C afforded a one molar ratio of myo-inositol.

Surugatoxin has the following properties;
mp>300°C, $C_{25}H_{26}N_5O_{13}Br$, UV$\lambda_{max}^{H_2O}$ 0.1NHCl 276(15,000), $\lambda_{max}^{0.1NNaOH}$ 279(19,000), mμ IR$\nu_{Cm^{-1}}^{KBV}$ 3200, 1740(sh), 1695 and 1640.

The formula unit $C_{25}H_{26}N_5O_{13}Br \cdot 7H_2O$ and the molecular structure including the absolute configration of surugatoxin as depicted in (I) were revealed by X-ray analysis of single crystals.

The crystals are orthorhombic, space group $P2_12_12_1$, with four formula units in a unit-cell of dimensions a=13.842(5), b=23.386(5), c=10.356(7) Å, and 1347 independent reflections were estimated visually on equi-inclination Weissenberg films taken along the c and a axes, with CuKα radiation.



(I)

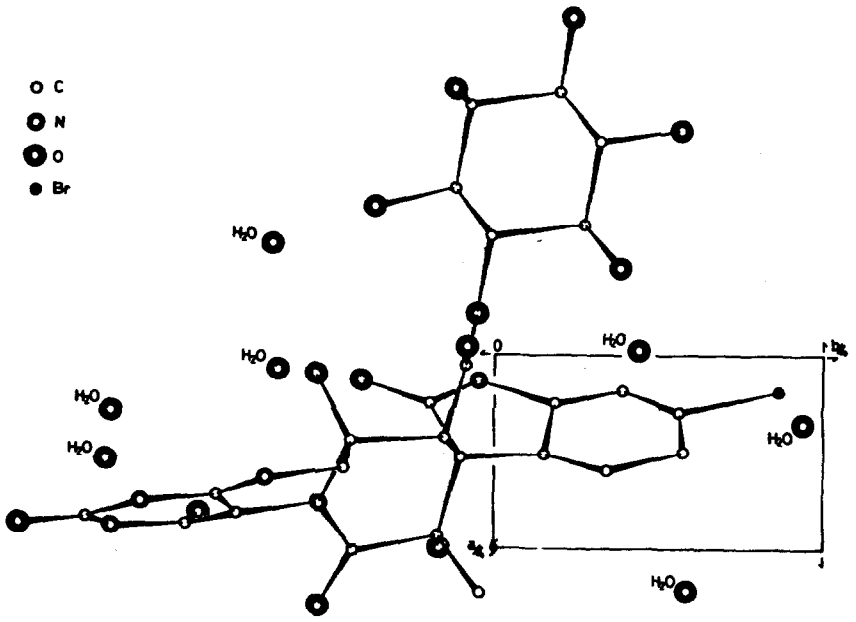


FIG. 1. THE MOLECULE OF SURUGATOXIN HEPTAHYDRATE VIEWED ALONG THE c AXIS.

Since this compound contains a bromine atom, the structure was solved by the heavy atom method and refined by block diagonal least-squares methods to an R-factor of 14.2%. The absolute configration was determined from the comparison of Bijvoet pairs caused by the anomalous dispersion effect of the bromine atom. The perspective view of the molecule along the c axis is shown in Fig.1, where all the atoms in the asymmetric unit of the crystal excluding hydrogen, are given. Further discussion related to the detailed structure will be published separately.

Surugatoxin is the first marine toxin so far discovered that contains indole and pteridine skeletons in the structure. It is some of interest that a marine toxin has a mydriatic action like atropine.

The reason for the occurrence of the toxic gastropod in the limited coast in Japan is not clarified yet. But the revealed structure of surugatoxin might be a powerful clue to clarify the poisoning mechanisms. Further studies on the problem and also on the physiological activity of surugatoxin are being carried on by the authors.

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