CHEMICAL AND PHARMACOLOGICAL STUDIES ON GLOBEFISH POISON

I. RF VALUES OF THE POISONOUS COMPONENT OF GLOBEFISH (SPHEROIDES RUBRIPES) LIVERS

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Since early days in this century, globefish poison has been studied by many workers chemically and pharmacologically. Tawara (1909[1]), 1911[2]) obtained a crude poison from an aqueous extract of the ovaries in globefish. Yokoho (1948[3]), 1950[4]) obtained this poisonous component in a crystalline form by chromatographic purification on alumina columns after removing impurities by usual chemical methods. Recently, Tsuda and Kawamura (1950–1960[5)-14)]) purified the crude poison through partition chromatography and absorption chromatography and further purification by means of chromatopile method and recrystaallization yielded tetrodotoxin in a crystalline form. They also studied the structure of the crystalline tetrodotoxin. Toxicology and pharmacology of the poison in the tissues and the organs of various species in globefish were well established by Yano (1937[17]), Nomiyama (1942[18]), and particulary Tani (1945[16]). However, the samples of globefish poison used in earlier investigations were perhaps of varying potency and purity. The availability of a crystalline globefish poison with a reproducible activity and a greater purity than any previous preparations other than possibly that of Yano (1950[4]), or Tsuda and Kawamura (1953[11]), led many workers to repeat some of the early pharmacological observations and undertake further studies Murtha, Stablie and Wills (1958[15]) have reported pharmacological effects of the crystalline globefish poison. Recently, in Japan, pharmacological or toxicological works of this crystalline have been conducted by Ogura (1959[19]), Nuki and Nagano (1959[20]). Most of these studies in the past were carried out with a poison of ovarian origin. Recently, poison of the liver and that of the ovary were pharmacologically compared by Nuki and Kawakami (1950[21]). In spite of these studies, properties of globefish poison of the liver has remained obscure. In the present investigation, paperchromatography of the constituents and the poisoonous fraction of globefish livers was first carried out using an aqueous extract of the liver, and such results were then

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compared with those obtained with ovaries and crystalline tetrodotoxin.

MATERIALS AND METHODS

The globefish (Spheroides rubripes) caught in the Inland Sea of Seto in January and February this year were used as the material. The weights of the livers ranged from 10 to 880 gm, and those of the ovaries, from 30 to 725 gm. The toxicity of the aqueous extracts and of fractions isolated chromatographycally were tested biologically in mice.

Extraction and toxicity of material: The extraction of poisonous component was carried out according to Tani's method, which was modified slightly in order to increase the yield. From 10 to 20 gm of livers and ovaries of Spheroides rubripes were taken in evaporting dishes containing 30–60 cc of 1% acetic acid solution and were heated at 80–90°C for 30 minutes on a steam bath. The boiled organ piece was homogenized with the remaining solution. These homogenates were heated again at 80–90°C for 30 minutes while adding a suitable quantity of water as needed and were filtered. The same operation was repeated three times with the residue. The filtrate was pooled and the upper oily layer was removed using a separatory funnel. The lower aqueous layer was evaporated to dryness and the resisidue was dissolved with a small quantity of warm warer. Water was added further to bring up the volume to the same numerical value as the weight of viscera used. Thus, the amount of toxin contained in 1 cc of this solution is equivalent to the amount in 1 gm of the viscera. A few cc of toluene was added to this solution, and the extract was preserved in a refrigerator for further studies.

Biological assay of samples: The method for estimating the toxic potency was that of Tani. Both sexes of cross-bred mice weighing 15 to 20 gm were used. The dose of the sample was 0.5 cc per animal and it was injected subcutaneously. The concentration which killed more than two mice in each group of three within one hour after injected was estimated by the mouse assay of serially diluted sample. The unitl of the toxicity was calculated by the following formula:

$$\text{Unit} = \frac{\text{The B. W. of Mouse (gram)}}{\text{The injected Dose (cc)}} \times \text{Dilution Factor}$$

The results of the assay individual toxicity are shown in Table 1, where the toxicity is graded as "deadly", "strong", "weak", and "none". The toxin contents of the 21 liver samples used for biological assays were further utilized in the following chromatographical and other studies.

Rf values of the poisonous component of livers: The Rf value of the poisonous component of livers was determined and compared with those of ovaries and crystalline tetrodotoxin. The materials consisted of two livers for each potency of "deadly", "strong" and "weak" each. Five ovaries were used for the "deadly"

potency, because no ovaries had "strong" or "weak" potency. For crystalline tetrodotoxin for control, RTE-2-3 supplied by Sankyo Co. Ltd., was used. This compound was dissolved in 30% acetic acid solution 100 γ and 200γ per ml. One-dimensional, ascending chromatography was carried ous using Toyo Roshi No. 50 (40 × 40 cm) in TN type two-dimensional apparatus (Natsume Works) at 20–22°C for 15 hours. About 1 cc of the test sample was applied to the starting line and chromatographed with a mixture of BuOH: AcOH: H_2O=4 : 1 : 3 or 90% phenol as the developing solvent. The filter paper sheet was subsequently cut in 20 equal portions and numbered 1 to 20 from the starting line to the solvent front. Each portion was reextracted with 1% acetic acid solution and assayed for locating the poisonous component

RESULTS

When the mixture of BuOH, AcOH and H_2O was used the developing-solvent, the toxicity was demonstrated only in No. 4, No. 5 and No. 6, the portions above No. 7 and No. 3 showing no poisonous activity. The results were the same whether the material was a liver or an ovary or tetrodotoxin, and regardless of the grade of potency. The toxic potency was found to be the strongest in No. 5 portion of each sample. In other words, the Rf value of the poisonous component was 0.15–0.30 (Fig. 1 and Table 1). When phenol was used as the solvent, the poison was found in No. 2 and No. 3, indicating that the Rf value of the poisonous component was 0–0.15 (Fig. 1 and Table 2).

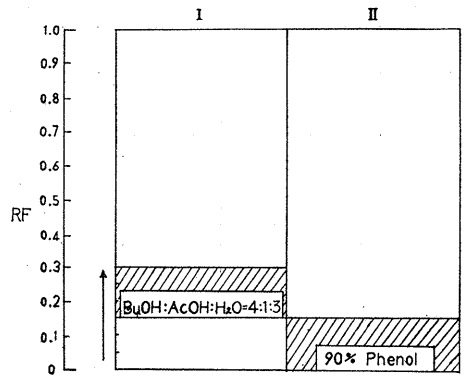

Fig. 1. One dimensional paper chromatogram of globefish poisonous component.

Note: Ascending system; Filter paper, Toyo roshi No. 50 (40 × 40 cm), at 20~22°c for 15 hours.

Table 1. Location of poisonous component of globefish livers and ovaries developed by butanol acetic acid solution

Sample No	Number of portions 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Toxicity
C 1	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
C 2	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
C 3	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
C 4	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
C 5	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
C 6	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
L 4	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Deadly P.
L 8	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Weak P.
L 11	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Strong P.
L 18	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Weak P.
L 19	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Strong P.
L 20	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Deadly P.
O 1	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Deadly P.
O 2	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	//
O 7	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	//
O 12	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	//
O 13	−	−	−	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	//

Notc: (1). Sample C; Crystalline tetrodotoxin (RTE-2-3) 100γ
L; Liver extract
O; Ovary extract
(2). Butanol acetic acid solution; BuOH : AcOH : H_2O=4 : 1 : 3

Table. 2 Location of poisonous compoent of globefish livers and ovaries developed by 90% phenol.

Sample No.	Numer of Portions 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Toxicity
C 1	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
C 2	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
C 3	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
C 4	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
C 5	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
C 6	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
L 4	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Deadly P.
L 8	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Weak P.
L 11	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Strong P.
L 18	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Weak P.
L 19	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Strong P.
L 20	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Deadly P.
O 1	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	//
O 2	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	//
O 7	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	//
O 12	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	//
O 13	+	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	//

Note: (1). Sample C: Crystalline tetrodotoxin (RTE−2−3) 100γ
L: Liver extract
O: Ovary extract

DISCUSSION

In 1952, Tsuda and Kawamura[10),11)] carried out paperchromatography with a crude poison extract of globefish ovaries using 90% phenol as the solvent. They determined the Rf value of the poison by mouse assays. The determined Rf value

aided them to purify further the poison of ovaries by column partition chromatography and they succeeded in further purification. Although they determined the Rf value of the ovary poison, that of the liver poison has never been studied. In this paper the Rf value of liver poison was determined and compared with those of ovary poison and crystalline tetrodotoxin, and it was founded that the Rf value for each poison was the same. It has been recognized in general that the pharmacological action of tetrodotoxin (commercial name for liver toxin). Recently, Nuki and Kawakami (1950[21]) reported that respiratory depressant action of the ovary poison was slightly stronger than that of the liver poison, whereas hypotensive action of the later was somewhat stronger than that of the former. In spite of these reports, the present study indicated that the liver poison is the same as or very closely related to the ovarypoison in chemical structure. Dr. Nuki[22] commented at the 19 Regional Meeting of Japanese Pharmacological Society in Kyoto that his recent studies showed no difference between the liver and ovary poison.

SUMMARY

1). Rf values of the poisonous conponents of globefish (Spheroides rubripes) livers and ovaries were determined chromatographically with the aid of mouse assay and they were compared with the Rf value of crystalline tetrodotoxin.
2). Irrespective of individual toxic potency and the organ, the Rf value was founded to be the same as that of crystalline tetrodotoxin.
3). These results strongly indicated that the liver poison is identical to the ovary poison, or that these poisons are very closely related in chemical structure.

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