powder per kg b.wt in a gelatin capsule surgically implanted in the abomasum or by abomasal injection of EL-979 in liquid preparation. Ruminal routes of administration by direct injection, drench, or gelatin capsule implantation were ineffective. Inactivity was due to conversion in the rumen by microflora of the nitro group at the four position to an amine compound i.e. 2-(1,1,2,2,tetrafluoroethyl)-6-(trifluoromethyl)-1H-benzimidazol-4-amine. This compound lacks anthelmintic activity.

The anthelmintic activity of EL-979 in sheep established nematode infections and treated with 1 and 2 mg/kg/day by abomasal infusion is presented in table 1. Inhibition of ova production was observed in both species at seven and 30 days of infusion, especially at the higher dose rate. Comparing total number of retained worms at necropsy, there was a 90% and 86% reduction in *H. contortus* mature and immature worm populations in sheep treated at the 1 mg/kg level. Approximately 91% of the *T. colubriformis* populations were reduced. Total worm reductions in sheep receiving the higher infusion rate were 98.5% and 93.5% for mature and immature *H. contortus* and 94% for *T. colubriformis* populations. Developing nematode data from sheep infused with 2 mg/kg/day indicate that the new infections were inhibited by the benzimidazoline treatment (table 2). The long term infusion resulted in a 94% reduction of *T. colubriformis* populations in the six treated sheep. Also, complete elimination of nematode ova in the feces and of both worm populations in the intestinal tract occurred. In vitro and in vivo data on the benzimidazoline compound indicate anthelmintic potential when introduced directly into the abomasum. The authors realize that the abomasal route of administration is impractical for control of helminths in the field. This method and the rumen infusion method provide basic dosage data needed to determine compound feasibility in control release rumen devices as described by Ludwig and Boisvenue[9].

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Occurrence of tetrodotoxin in the starfish *Astropecten latespinosus*[1]

J. Maruyama, T. Noguchi, J.K. Jeon, T. Harada[2] and K. Hashimoto[3]

Laboratory of Marine Biochemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo, Tokyo 113 (Japan),
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Summary. A toxin causing paralysis was detected in the starfish *Astropecten latespinosus*. The toxin was purified by a method consisting of charcoal treatment and chromatography on CM-Sephadex C-25 and Bio-Rex 70. The toxin was identified as tetrodotoxin by its behavior in thin-layer chromatography and electrophoresis and its ^{1}H-NMR spectrum.
Key words. Astropecten latespinosus; starfish; tetrodotoxin.

Tetrodotoxin (TTX) is one of the most potent neurotoxins. It was first isolated from the pufferfish[4], and then from several vertebrates (newts of the genus *Taricha,* a goby *Gobius criniger* and frogs of the genus *Atelopus*)[4] and an invertebrate (the blue-ringed octopus *Octopus maculosa*)[5]. Recently, we have detected TTX in some gastropod mollusks (a trumpet shell *Charonia sauliae*[6], the Japanese ivory shell *Babylonia japonica*[7] and a frog shell *Tutufa lissostoma*[8]). We have also revealed the presence of TTX in a starfish *Astropecten polyacanthus*[9], débris of which is often found in the trumpet shell. In the course of elucidating the mechanisms involved in the toxification of Japanese ivory shell, we detected paralysis-producing toxicity in another starfish, *A. latespinosus* (fig.). The present work was performed to demonstrate that this starfish also contains TTX.

Experimental procedure and results. A total of 1.5 kg of *A. latespinosus* specimens (average b.wt, 28.8 ± 6.7 g) were collected in June 1983 from Sakajiri Bay, Fukui Prefecture, Japan. They were repeatedly homogenized with 3 vol. of 1% acetic acid in methanol. The extracts were combined, evaporated in vacuo and defatted with dichloromethane. The aqueous layer (total toxicity: 6000 mouse units (MU)[10]) was concentrated to 500 ml, adjusted to pH 5.2 with 1 N NaOH, and treated batchwise with 300 g of activated charcoal (Wako Pure Chem.). The charcoal was washed with water. The toxin (5000 MU) was eluted with 1% acetic acid-20% ethanol and evaporated to dryness. The solid obtained was dissolved in a small amount of 0.1 M ammonium acetate (pH 6) and applied to a column (2.6 × 34 cm) of CM-Sephadex C-25 (NH_4^+ form, Pharmacia Fine Chem.). The column was developed by a linear gradient of 0.1 to 0.4 M ammonium acetate (pH 6). The toxic fractions were combined and lyophilized. The lyophilizate (4400 MU) was dissolved in a small amount of water and applied to a column (0.8 × 95 cm) of Bio-Rex 70 (H^+ form, Bio-Rad Lab.). The column was developed by a linear gradient of 0 to 0.1 M acetic acid. The toxic fractions were combined and freeze-dried to afford 0.8 mg white powder. The starfish toxin thus obtained exhibited a specific toxicity of 4600 MU/mg, the value which is almost comparable to that of an authentic TTX isolated from a pufferfish ovary by Goto's method[11].

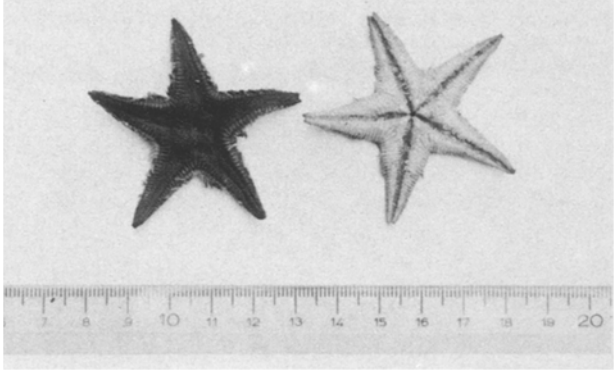
Attempts were made to identify the starfish toxin by thin-layer chromatography and electrophoresis. The former was carried out on Whatman LHP-K high-performance plates, with a solvent system of pyridine-ethylacetate-acetic acid-water (15:5:3:4). Electrophoresis was conducted on Chemetron cellulose acetate membranes under a constant current of 0.8 mA/cm for 30 min, using 0.08 M Tris-HCl buffer (pH 8.7). The starfish toxin was detected as a pink spot with the Weber reagent, or as a yellow fluorescent spot under UV light (365 nm) after spraying the plate with 10% KOH and heating at 110 °C

for 10 min[12]. In thin-layer chromatography, the starfish toxin showed a single spot whose Rf value (0.62) was in agreement with that of the authentic TTX. The toxin also gave a single spot in electrophoresis, the mobility coinciding well with that of the authentic TTX.

A portion of the toxin was dissolved in D_2O containing 1% CD_3CO_2D, and measured for ^{1}H-NMR spectrum at 100 MHz by a JEOL JNM FX-100 spectrometer. The spectrum was characterized by δ 2.08 s (CH_3CO_2D), δ 2.20 s (acetone, internal standard), δ 2.35 d (J = 9.5 Hz, C_{4a}-H), δ 4.02 m, δ 4.28 m, δ 4.78 br s (HDO) and δ 5.47 d (J = 9.5 Hz, C_4-H) ppm. The pair of doublets (J = 9.5 Hz) at δ 2.35 and δ 5.47 ppm was confirmed to couple with each other by means of double irradiation. The spectrum of the starfish toxin was indistinguishable from that of the authentic TTX[6, 11, 13].

Discussion. It was demonstrated by the present studies that the *A. latespinosus* specimens collected from Sakajiri Bay contained TTX. This, along with our previous finding with *A. polyacanthus*[9], suggests that TTX has a fairly wide distribution in the starfish, especially in the genus *Astropecten*. In connection with this, we have observed that the extract from another starfish *A. scoparius* also induces paralysis in mice on i.p. injection.

The origin of TTX in any of TTX-containing organisms still remains unknown, except for some carnivorous gastropod mollusks such as trumpet shell and frog shell in which TTX comes from the starfish *A. polyacanthus* on which the mollusks feed[6, 8]. It is not clear, however, whether in Sakajiri Bay the Japanese ivory shell feeds on the starfish *A. latespinosus* routinely, since the former is rather smaller than the latter when they reach adult size. It seems more reasonable to suppose that some foods common to both organisms contain an elaborator(s) of TTX or its precursor.



Astropecten latespinosus.

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The ovicidal activity of *cis*-dehydromatricaria ester: time-dependance of its enhancement by UV light

J. Kagan, C.P. Kolyvas and J. Lam[1]

Department of Chemistry, University of Illinois at Chicago, Box 4348, Chicago (Illinois 60680, USA), and Department of Chemistry, Aarhus University, DK-8000 Aarhus C (Denmark), 21 December 1983

Summary. Freshly laid eggs of *Drosophila melanogaster* were treated with the polyacetylenic compound *cis*-dehydromatricaria ester. The toxicity of the chemical was enhanced by treatments with long-wavelength ultraviolet light, and the maximum effect was observed when the UV irradiation was performed 4–5 h after the initial contact of the eggs with the chemical.
Key words. Drosophila melanogaster; ovicidal activity; dehydromatricaria ester; toxicity, enhancement by UV; polyacetylenic compounds.

Recent publications[2, 3] demonstrated that the activity of several ovicidal compounds could be significantly enhanced by a treatment with long-wavelength ultraviolet light (UVA). With the eggs of *Drosophila melanogaster,* this effect was selective, and there was one example of an active molecule which did not respond significantly to the UVA treatment, and of one which was inactive under normal conditions, but which acquired ovicidal activity when a UVA treatment was applied. In order to gain a clearer understanding of the phenomenon, it was desirable to investigate the effect of the timing and duration of the irradiation treatments on the photoovicidal activity. *Cis*-dehydromatricaria ester (**1**), a polyacetylenic molecule widely distributed among plants of the family Compositae, was selected for these studies.

$$CH_3-(C\equiv C)_3-CH=CH-COOCH_3$$
1

The ovicidal activity of this molecule had been previously reported[4], but the effect of UVA on this activity was unknown.