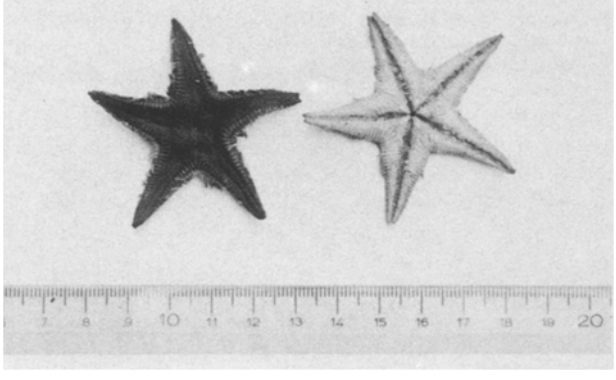
for 10 min[12]. In thin-layer chromatography, the starfish toxin showed a single spot whose Rf value (0.62) was in agreement with that of the authentic TTX. The toxin also gave a single spot in electrophoresis, the mobility coinciding well with that of the authentic TTX.

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

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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.

We now show an unexpected relationship between the duration of the initial incubation period and the phototoxicity.

Experimental. A stock solution of **1** in abs. ethanol (0.5 g/l = 0.0029 M) was utilized, and successive 10-fold dilutions afforded the solutions required in the experiments. To each Whatman paper No. 1 disc, 1.5 cm in diameter, was added 0.020 ml of desired sensitizer solution, and the solvent was allowed to evaporate with very gentle heating. The eggs of *D. melanogaster* were collected between 3 and 4 h after a standard food medium in a shallow plastic container had been exposed to adults, and 20–50 eggs were gently planted onto each treated paper disc which had been saturated with distilled water. The discs were then kept in a covered plastic disposable petri dish which also contained 1 set of controls, with eggs which had not been treated with any chemical. After the desired time of initial contact, the eggs were irradiated under a pyrex cover exactly as previously described[2,3]. Hatching was scored between 24 and 48 h after the irradiations. Dark controls were maintained in all the experiments, which comprized between 3 and 5 replicates. The survival values shown in the figures were all corrected for the mortality in the controls, which was usually less than 10%.

Results. When the irradiation experiments in the presence of **1** were performed with eggs of *D. melanogaster* exactly as described in our earlier papers[2,3], the survival curves resembled those observed with the other active compounds, both in the dark and in the UVA-treated specimens (fig. 1, curves A and B). Each point reported on these curves represents the average survival of 100–150 eggs. In the dark, the observed value of LD_{50} was 3.8 μg/cm^2, in excellent agreement with the value of 5 μg/cm^2 reported by Kawazu et al.[4]. The mortality increased by slightly more than a factor of 2 when the eggs were irradiated with UVA for 35 min, starting 30 min after the eggs had been deposited over the impregnated paper discs (LD_{50} = 1.7 μg/cm^2, fig. 1, curve B). In a control experiment, the impregnated paper discs were first irradiated with UVA. The eggs were then planted on these discs, and kept in the dark. No increased activity was observed compared to the ovicidal effect determined when no UVA had been used at all.The duration of the UVA exposure seemed to have no effect on the activity, all other conditions being identical. These experiments were conducted with irradiation times of 35, 45, 55, 65, and 75 min, and the results were all as expressed in figure 1 (curve B).

We now report that the photoinduced enhancement of the ovicidal activity can be appreciably increased by properly selecting the start of the irradiation period, following the initial contact with the chemical. Figure 2 illustrates how the eggs' survival in the presence of a fixed concentration of **1** responded to increasing contact time prior to the irradiations. At each concentration level shown, there is a minimum, corresponding to a contact time of 4–5 h prior to the UVA treatment, and curve C on figure 1 summarizes the experiments performed under conditions of maximum phototoxicity. The observed LD_{50} is now 0.13 μg/cm^3, corresponding to a 13-fold increase in phototoxicity compared to the initial experiments.

The shape of the survival curve on figure 2 is noteworthy. It demonstrates that, contrary to expectations, a longer exposure of the eggs to the chemical in the dark prior to irradiation (more than 6 h) is not necessarily more lethal than a shorter one (between 3 and 6 h). It is well-known that the chorion of *Drosophila* eggs cannot be penetrated by most chemicals, but we have not yet established whether this is the case with the sensitizer used in this study, or with any of those previously investigated by us. In any event, the results illustrated on figure 2 demonstrate the complexity of the photobiology of the ovicidal activity of **1**, and invite further research toward understanding the biological events responsible for the main phases of photoresponse uncovered in this work.

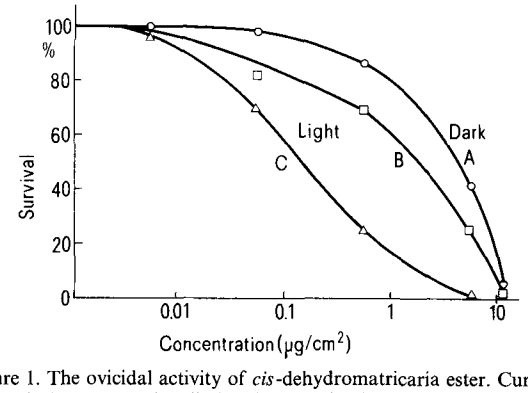

Figure 1. The ovicidal activity of *cis*-dehydromatricaria ester. Curve A, in the dark; curve B, irradiation for 35 min, following 30 min of contact; curve C, irradiation for 35 min at the activity maximum determined in figure 2 (5 h). The concentration scale is logarithmic.

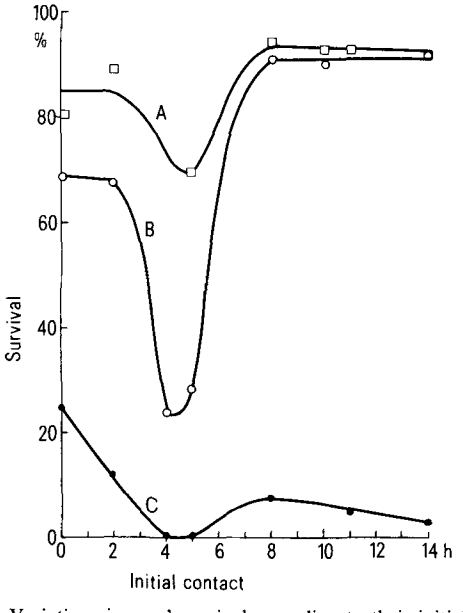

Figure 2. Variations in eggs' survival according to their initial contact time with **1** (curve A, 0.057 μg/cm^2; curve B, 0.57 μg/cm^2; curve C, 5.7 μg/cm^2).

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