POLYCYCLIC AROMATIC COMPOUNDS AS PHOTOTOXIC MOSQUITO LARVICIDES[1]

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ABSTRACT. The phototoxicity of 2-acetylnaphthalene, 1-acetylnaphthalene, 1-naphthalenecarboxaldehyde, 9-xanthenone, 9-thioxanthenone, 9-methylanthracene, anthracene, alpha-terthienyl, pyrene and fluoranthene was tested with larvae of *Aedes aegypti*, *Ae. taeniorhynchus* and *Culex quinquefasciatus*. The larvae were exposed to the chemicals in the presence of sunlight for intervals from 1 to 6 hr and the corresponding LC_{50} values calculated. The LC_{50} values determined when the 6-hr exposure was followed by 18 hr in the dark range from over 10 ppm to below 0.001 ppm. Naphthalene derivatives were the least active while alpha-terthienyl, anthracene, 9-methylanthracene, pyrene and fluoranthene were the most phototoxic.

INTRODUCTION

Phototoxic molecules display toxic properties in the presence of light, but not in its absence, and they have no increased toxicity when tested in the dark following an initial exposure to light. They belong to two main groups, depending upon whether or not oxygen participates in the phototoxic reactions. Molecules in the first group after reaching their excited states interact with oxygen, which is converted into more reactive forms (singlet oxygen or superoxide ion). Whereas molecules in the second group react directly with unsaturated components in cells, particularly DNA, with which they become covalently attached (Musajo and Rodighiero 1972). Consequently, they have found important applications in molecular biology and in medicine (Anderson and Voorhees 1980).

Recently, a number of organic molecules were shown to be highly toxic to insect larvae in the presence of light under laboratory conditions, but their activity in the presence of sunlight is poorly known. We were interested in evaluating the sunlight-dependent toxicity of several compounds which had been found to be phototoxic in the laboratory. They belonged to three groups of aromatic molecules, namely polycyclic aromatic hydrocarbons (compounds 1, 2, 3, 4, 5, 6 and 7), xanthene derivatives (8 and 9), and a thiophene derivative (10) (Fig. 1). These compounds provided an opportunity for analyzing trends between molecular structure and phototoxicity.

Polycyclic aromatic hydrocarbons are distributed throughout the atmosphere, soil and the food chain (Yang et al. 1978). The phototoxicity of polycyclic aromatic hydrocarbons to protozoa was reported by Mottram and Doniach (1938), Graef and Haller (1977) and Smith-Sonneborn (1983). Similar observations have been made with microorganisms (Mirson et al. 1971), mammalian cells (Van Gurp and Hankinson 1983), larvae of the fruit fly *Drosophila melanogaster* (Matolstsky and Fabian 1946, 1947), as well as with several aquatic organisms, such as immature frog *Rana pipiens* (Kagan et al. 1984), water flea (*Daphnia magna*), fish, and larvae of the mosquito *Aedes aegypti* (Linn.) (Kagan and Kagan 1986; Kagan et al. 1983, 1984, 1985). All these experiments, with one exception (Kagan et al. 1984) used artificial ultraviolet light rather than sunlight; thus, the phototoxicity measurement of selected polycyclic aromatic hydrocarbons under sunlight irradiation was of particular importance.

At the outset, we were especially interested in determining the phototoxicity of compounds 1 and 2 to mosquito larvae under sunlight, because we had previously found them to be phototoxic to the yeasts *Candida utilis* and *Saccharomycete cerevisiae*, to *Escherichia coli*, and to larvae of *Ae. aegypti* under UV light. In the case of compound 2, insecticidal activity in the mosquito *Culex quinquefasciatus* Say had been reported (Nagase 1942), and activity against yeast (Popov et al. 1953, Morris et al. 1979) and louse eggs (Eddy and Carson 1948) was also known, although the light-dependence of these effects was not investigated. These two molecules were the least phototoxic ones in our study, while hydrocarbons 4, 5, 6 and 7 proved to be much more phototoxic.

The second group of molecules comprised compounds 8 and 9, two classical triplet sensitizers in organic photochemistry. A number of halogenated xanthene derivatives were known to be phototoxic to insect larvae, going back to Barbieri's original work (1928). Most notably, erythrosin B has found applications in the control of the house fly (Heitz 1982). However, little is known about the phototoxic properties of the simple xanthenes used in this work. There is one report of phototoxicity for 9 in Ehrlich

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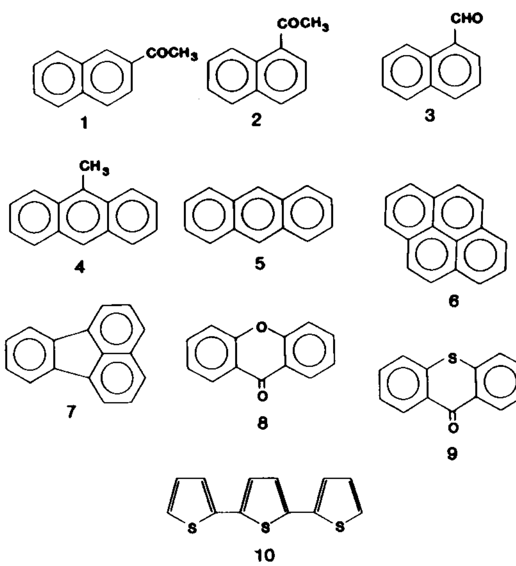

Fig. 1. Structure of polycyclic aromatic hydrocarbons: 1. 2-acetylnaphthalene, 2. 1-acetylnaphthalene, 3. 1-naphthalenecarboxaldehyde, 4. 9-methylanthracene, 5. anthracene, 6. pyrene, 7. fluoranthene, 8. xanthone, 9. 9-thioxanthone, 10. alpha-terthienyl.

ascites cells *in vitro* (Emmett et al. 1977), but nothing about compound 8, except for our earlier survey (Kagan et al. 1983), where we observed the phototoxicity of both 4 and 5 in yeasts, *E. coli*, *D. melanogaster* larvae, brine shrimp and mosquito larvae. Interestingly, compound 8 was reported to be useful for controlling mites in fruit orchards (Newcomer 1943, Newcomer et al. 1946). Although not considered at the time, the light-dependence of the acaricidal activity is strongly suggested by our results and by the discovery that a powerful acaricide activity is light-dependent (Relyea et al. 1983).

Finally, alpha-terthienyl (compound 10) was included for comparison. It has become one of the most thoroughly investigated phototoxic molecules, and it is known to exercise its effect through efficient generation of singlet oxygen (Reyftmann et al. 1985). It has shown light-dependent activity in a very large number of microorganisms, mammals, plants, insects and aquatic organisms (Kagan et al. 1986a, and references previously cited). Although Canadian workers have claimed that it was a desirable mosquito photolarvicide, suitable for use in field applications (Philogene et al. 1985, 1986), Kagan et al. (1984, 1986b, 1987a) have called attention to its lack of selectivity and to its very high activity against non-target organisms such as tadpoles, fish and *Daphnia* species. Its effect on *Ae. aegypti* in the laboratory has one remarkable feature, namely that it is phototoxic in pupae (Kagan et al. 1987b). Activity in the pupa has not been observed with any other phototoxic molecule. The mosquitoes tested so far with alpha-terthienyl were *Ae. aegypti*, *Ae. intrudens* Dyar and *Ae. atropalpus* (Coquillett).

We now report on our evaluation of the 10 phototoxic molecules as potential larvicides for the mosquitoes *Ae. aegypti*, *Ae. taeniorhynchus* Wiedemann and *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Chemicals. Alpha-terthienyl was synthesized in the laboratory (Beny et al. 1982). Fluoranthene was purchased from Aldrich (Milwaukee, WI), and all other compounds from J. T. Baker (Phillipsburg, NJ). They all gave a single peak when analyzed by high performance liquid chromatography.

Experimental animals. Larvae of *Ae. aegypti*, *Culex quinquefasciatus* and *Aedes taeniorhynchus* were reared at 26°C on a diet of brewer's yeast, lactalbumin, and lab chow (1:1:1), under a 16:8 hr light:dark cycle. Larvae were in the late 3rd or 4th instar when used in the experiments.

Larvicidal activity. Each compound (Fig. 1) was dissolved in 0.5 ml ethanol:dimethylsulfoxide (DMSO) (1:1) and appropriate five dilutions (10^{-3} to 10 ppm) were added to disposable plastic containers containing 500 ml water and 50 larvae. The containers were exposed to sunlight for intervals of 1–6 hr and the number of dead larvae at the end of each interval was recorded. After 6 hr exposure to sunlight, containers with survivors were kept in the dark (covered with black cloth) at 28°C for 18 hr, and dead larvae were again counted. Each experiment was repeated twice and the mean percent mortality was plotted against the five concentrations (ppm) of the compound and the LC_{50} read directly from the graph. Controls were treated in the same way except that only 0.5 ml of ethanol:DMSO (1:1) was added without any compound; each experiment was corrected for mortality (2–5%) that was not sensitizer-dependent. Another control was run simultaneously in which sensitizer in ethanol:DMSO was added but this experiment was run entirely in the dark at 28°C with mortality of 2 to 5% which was not light dependent.

Temperature in the containers throughout the experiments fluctuated from 28 to 32 °C. At these temperatures no mortality was observed in untreated larvae.

RESULTS

Acute toxicity. The dynamic response of the larvae of three species of mosquito to their treatment with sunlight in the presence of the sensitizers is shown in Figs. 2, 3 and 4. The following points should be made: 1) The larvae of *Ae. taeniorhynchus* are strikingly more resistant

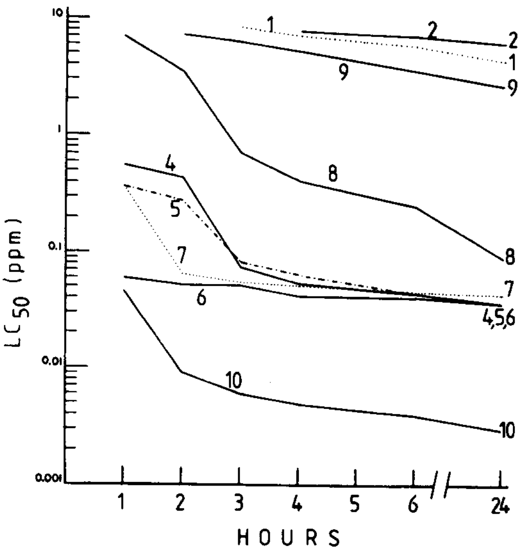

Fig. 2. The phototoxicity of polycyclic aromatic hydrocarbons towards larvae of *Culex quinquefasciatus*. The log of LC_{50} values in ppm of photosensitizers (1–10, see Fig. 1) were plotted against sunlight exposure time (1 to 6 hr) followed by 18 hr dark period (24 hr). LC_{50} values larger than 10 ppm were not plotted.

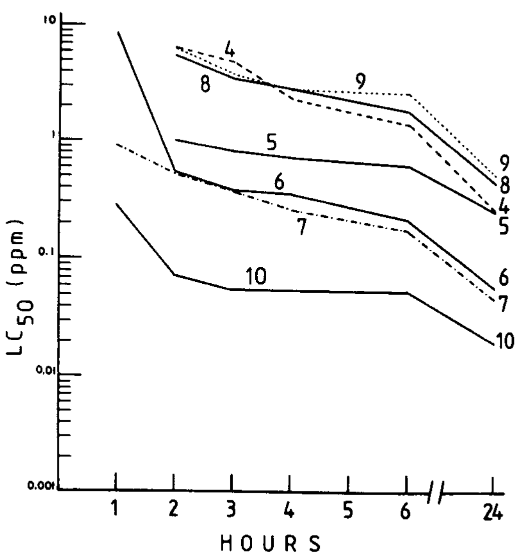

Fig. 3. The phototoxicity of polycyclic aromatic hydrocarbons towards larvae of *Aedes taeniorhynchus*. The LC_{50} values in ppm of photosensitizers (1–10, see Fig. 1) were plotted against sunlight exposure time (1–6 hr) followed by 18 hr dark period (24 hr) as in Fig. 2.

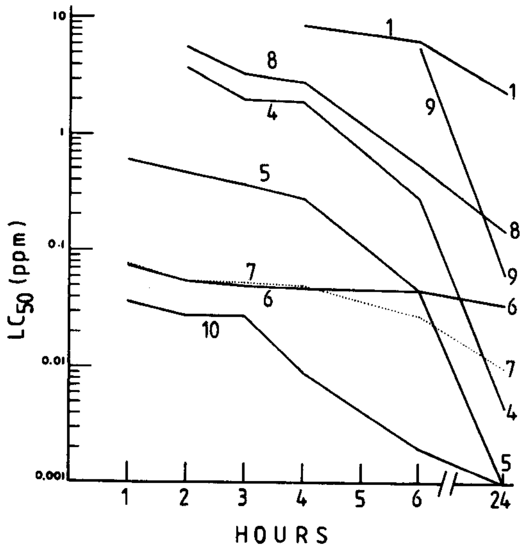

Fig. 4. The phototoxicity of polycyclic aromatic hydrocarbons towards larvae of *Aedes aegypti*. The LC_{50} values in ppm of photosensitizers (1–10, see Fig. 1) were plotted against sunlight exposure time (1–6 hr) followed by 18 hr dark period (24 hr) as in Fig. 2.

than those of the other two species, while the larvae of *Ae. aegypti* are the most sensitive. 2) With the most active sensitizers, a very significant portion of the acute toxicity is registered within the first hour of irradiation. This effect is particularly pronounced in the case of pyrene (6) with *Cx. quinquefasciatus* and with *Ae. aegypti*, and in the case of fluoranthene (7) with *Ae. aegypti*. 3) In general, the relative phototoxicity of the sensitizers tested is the same in the three mosquito species, with 10 being clearly the most active, followed by 7, 6 and 5. The activity of 4 is practically identical to that of 5, 6 and 7 in *Cx. quinquefasciatus*, but lower in the other two species.

24-hour survival. Table 1 and figures 2 to 4 report the 24-hour survival data for all the larvae which had been exposed to sunlight for 6 hr, as described above. Comparison of LC_{50} values at the end of the irradiations and at the end of the 24 hr periods disclosed a significant difference in response between *Cx. quinquefasciatus* and *Ae. taeniorhynchus* on one hand, and *Ae. aegypti* on the other. There was very little difference between the LC_{50} values at the end of the 6- and 24-hr periods in the first two species, while in *Ae. aegypti*, the LC_{50} values changed considerably in the cases of 4, 5, 9 and perhaps 10.

Since this survey arbitrarily limited the sensitizer concentrations to the range 0.001 to 10 ppm, the LC_{50} values shown at the low end of the range represent an upper limit for the toxicity levels.

Table 1. The effect of prolonged exposure to photosensitizers on larvae of *Culex quinquefasciatus*, *Aedes taeniorhynchus* and *Aedes aegypti*.[1]

	Species		
Photosensitizers	*Culex quinquefasciatus*	*Aedes taeniorhynchus*	*Aedes aegypti*
1. 2-acetylnaphthalene	4.64[2]	7.8	2.37
2. 1-acetylnaphthalene	6.46	>10	10
3. 1-naphthalenecarboxaldehyde	>10	>10	>10
4. 9-methylanthracene	0.037	0.27	0.0064
5. Anthracene	0.037	0.26	<0.001
6. Pyrene	0.037	0.06	0.035
7. Fluoranthene	0.045	0.048	0.01
8. Xanthone	0.092	0.46	0.15
9. 9-thioxanthone	2.82	0.54	0.0064
10. Alpha-tertienyl	0.003	0.02	<0.001

[1] Mosquito larvae in disposable containers were exposed to sunlight for 6 hr and then kept in the dark for 18 hr in the presence of photosensitizers.
[2] LC_{50} (ppm) values are average of two determinations. N.D. not determined.

DISCUSSION

The results of this study permit the following conclusions: 1) The naphthalene derivatives 1, 2 and 3, with only two aromatic rings, are distinctly less active than the other compounds tested, which possess at least three aromatic rings. 2) Alpha-terthienyl is, overall, the most active photolarvicide. 3) Results obtained with sunlight irradiation can be quite different from laboratory tests with UV light. This is particularly striking in the case of anthracene (5) in *Ae. aegypti*, which has an activity similar to that of 10 here, but is much less active in laboratory tests (Kagan et al. 1985, 1987a). 4) Although further work concerning the toxic effects of the most active compounds in non-target organisms under competitive conditions would be desirable, it is clear that light-dependent mosquito larvicides which do not contain halogens, carbamate or phosphorous derivatives present an alternative to the use of traditional pesticides. It has already been observed that strains of *Ae. aegypti* which were resistant to DDT or malathion were equally sensitive to phototreatments in the laboratory (Kagan et al., unpublished data). Thus, photolarvicides have potential for controlling mosquitoes which had become resistant to standard pesticides. However, it is not known whether mosquitoes will develop resistance to photoinsecticides and, if they do, whether the resistance will be specific to the one particular compound with which they have been treated or will include all photoinsecticides. Further work will be directed towards obtaining accurate determinations of the LC_{50} values resulting from longer sunlight exposures with the most active compounds.

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