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THE EFFECT OF ULTRAVIOLET LIGHT ON THE TOXICITY OF NATURAL PRODUCTS TOWARD THE EGGS OF *DROSOPHILA MELANOGASTER*

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ABSTRACT.—The ovicidal activity of the natural product 2-phenyl-5-(1′-propynyl)thiophene (**1**) toward *Drosophila melanogaster* can be enhanced more than 200-fold by a short treatment with long wave length ultraviolet light (UVA). Coumarin, another natural product with ovicidal activity, did not respond to the photochemical treatment. The ovicidal activities of **1** and of 2-phenyl-5-ethynylthiophene, a synthetic intermediate, were very similar, both in the dark and in the presence of UVA.

We recently recognized that some natural products were capable of displaying phototoxicity toward the eggs of *Drosophila melanogaster* in the presence of long wavelength ultraviolet light (UVA) (1). It appeared desirable to find other examples for this new type of phototoxicity, and it was particularly interesting to observe the behavior of molecules which were already known to possess ovicidal activity (2), in order to determine whether this activity could be modified by irradiation with UVA.

Nakajima and Kawazu have reported that coumarin and 2-phenyl-5-(1′-propynyl)thiophene (**1**) possessed ovicidal activity in their Drosophila test, with LD_{50} values of 12.5 and 2 $\mu g/cm^2$, respectively (2, 3). A reinvestigation of the activity of **1** was particularly interesting because this molecule is closely related biosynthetically and chemically to phenylheptatriyne, which is known to be phototoxic to several organisms (4-6), and which we have also shown to possess photoovicidal activity (1).

EXPERIMENTAL

5-PHENYL-2-THIOPHENECARBOXALDEHYDE.—A stirred mixture of 2-phenylthiophene (8 g, 0.05 mol), *N*-methylformanilide (9.5 g), and $POCl_3$ (11.25 g) in 50 ml of dry toluene was heated at 95-100° for 5 h. After cooling, an excess of aqueous sodium acetate was added. The organic layer was separated, and the aqueous layer was extracted with benzene (3 x 50 ml). The organic extracts were combined, dried over $MgSO_4$, and concentrated to 20 ml. The solid obtained after addition of petroleum ether and cooling was flash chromatographed over silica gel (40 μm). Elution with benzene-hexane (20:80) gave a light yellow product which was recrystallized from ethanol to yield 8.8 g (93.6%) of the aldehyde as colorless needles, m.p. 92-93° [Lit. m.p. 92°, 80% yield (7)]; ir ($CDCl_3$) 1660 cm^{-1}; nmr ($CDCl_3$): 7.2-7.8 (m, 7 H) and 9.85 ppm (s, 1 H).

2-PHENYL-5-(2′,2′-DIBROMOETHENYL)THIOPHENE.—Triphenylphosphine (3.32 g, 20 mmol) and carbon tetrabromide (3.32 g, 10 mmol) were placed in a dry 100-ml round-bottomed flask equipped with a magnetic stirrer and a gas inlet. Anhydrous dichloromethane (20 ml) was added under nitrogen at 0°, the mixture was stirred for 10 min, and 1.504 g (8 mmol) of 5-phenyl-2-thiophenecarboxaldehyde was added (8). After stirring for 1 h at room temperature under nitrogen, the solvent was removed and the residue washed repeatedly with CH_2Cl_2. These organic extracts were combined, dried, concentrated, and flash chromatographed over 40 μm silica gel. Elution with hexane-$CHCl_3$ (9:1) yielded 2.56 g (87.9%) of the product, which melted at 109° after recrystallization from hexane: ms 342, with isotopic peaks at 344 and 346; nmr ($CDCl_3$): 6.77-7.63 ppm.

2-PHENYL-5-ETHYNYLTHIOPHENE.—A solution of the above dibromo compound (1.032 g, 3 mmol) in 30 ml of anhydrous ether was cooled to −78° under nitrogen, and 6.6 mmol of *n*-butyllithium was added dropwise. After stirring for 1 h at −78°, the mixture was allowed to return to room temperature and was stirred 1 h longer. The mixture was decomposed with ice-cold water, and was extracted with ether. The ether extract was dried over $MgSO_4$ and concentrated *in vacuo* to yield 376 mg of solid, which was recrystallized from *n*-pentane to afford 360 mg (63%) of light yellow product, m.p. 66-67°, [Lit. m.p. 65-67° (9)]; ir ($CHCl_3$): 3320 (C≡C-H), 2120 (C≡C), 1600 cm^{-1}; nmr ($CDCl_3$): 3.24 (s, 1 H, C≡C-H), 6.9-7.6 ppm (m, 7 H); ms: 184 (M^+), 152, 139, 115, 92, 77, 69.

2-PHENYL-5-(1′-PROPYNYL)THIOPHENE (**1**).—A solution of 2-phenyl-5-ethynylthiophene (92 mg, 0.5 mmol) in 10 ml of anhydrous tetrahydrofuran was cooled to −78° under nitrogen; 0.55 mmol of *n*-butyllithium was added dropwise, and the mixture was stirred for 45 min at −78°. Dimethyl sulfate (95 mg, 0.75 mmol) was dissolved in 5 ml of THF. After 1 ml of solution had been added dropwise, the mixture was allowed to warm up to the temperature of an ice-salt bath. The rest of the reagent was added dropwise at this temperature over 20 min. After stirring for 2 h at room temperature, the mixture was decomposed wih ice cold water and extracted with ether. The organic layer was dried over $MgSO_4$ and was concentrated *in vacuo* to yield 78 mg of residue, which gave 72 mg (72%) of **1** as light yellow crystals after recrystallization from *n*-pentane, m.p. 43°, [Lit. m.p. 43-44° (10)]; ms: *m/e* 198 (M^+); ir ($CHCl_3$): 2245, 805 cm^{-1}; uv (MeOH): λ max 310 (log ε 4.22); nmr ($CDCl_3$): 1.93 (s, 3 H, CH_3), 6.95 (br s, 2 H), 7.0-7.4 ppm (m, 5 H).

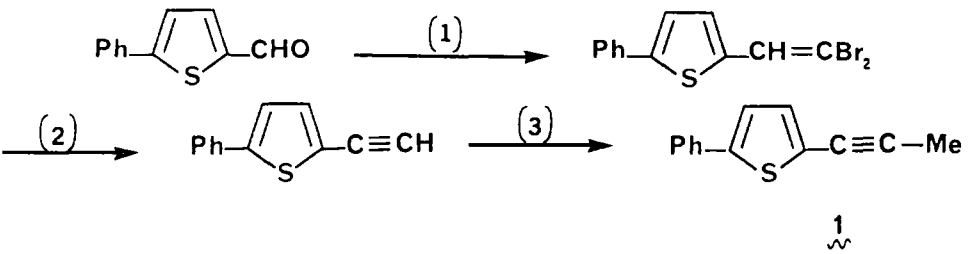
OVICIDAL ACTIVITY MEASUREMENTS.—Each Whatman No. 1 paper disc, diam. 1.5 cm, received 20 μl of an alcohol solution of sensitizer. The solvent was evaporated, and the disc was saturated with distilled water. Eggs less than 4 h old were transferred onto the discs in a darkroom dimly lit through amber Kodak OC Safelight filters. The eggs were incubated in total darkness, kept in a petri dish wrapped with a plastic film in order to prevent drying. The results provided the dark controls for the experiments in which the eggs were handled identically, except for a 45 min irradiation with long wave length ultraviolet light starting 1 h after the beginning of the incubation. Each paper disc had 10-40 eggs, and the petri dishes had pyrex glass covers during the irradiations under a bank of eight low pressure tubes, which had maximum emission at 350 nm (No RPR-3500A, Southern New England Co., Hamden, CT). These tubes were mounted horizontally 5 cm apart, 8.9 cm above the paper discs. Using a Yellow Springs radiometer Model 65A, the light intensity was found to be 13w/m^2.

The viability of the eggs usually decreased by *ca.* 5-10% upon irradiation under the same conditions in the absence of added chemicals. No increased ovicidal activity was observed when the eggs were incubated in the dark over paper discs which had been previously irradiated after addition of the chemicals. All the experiments were repeated at least ten times with each compound at each concentration.

RESULTS AND DISCUSSION

THE SYNTHESIS OF 2-PHENYL-5-(1′-PROPYNYL)THIOPHENE.—In contrast to coumarin, which may be purchased commercially, **1** is not as readily available. It has been found in the plants *Coreopsis lanceolata* (3) and *C. grandiflora* (11), family Compositae, and was originally characterized by establishing the identity of the product of its catalytical hydrogenation reaction to that obtained by Friedel-Crafts acylation of 2-phenylthiophene with propionyl chloride, followed by Clemmensen reduction (11). Three syntheses have already been described. Atkinson *et al.* condensed the 5-iodo derivative of 2-phenylthiophene with the cuprous salt of 1-propyne (10), while Cymerman-Craig and Moyne (9) as well as Schulte and Bohn (12) methylated 2-phenyl-5-ethynylthiophene with dimethyl sulfate. In both cases, the intermediate had been prepared from 2-phenyl-5-acetylthiophene via the corresponding vinyl chloride or enol phosphate, from which the terminal acetylene was obtained after treatment with sodium amide.

We did not succeed in converting the propionyl side-chain of 2-phenyl-5-propionylthiophene into the desired acetylenic group, with either PCl_5 (13) or catecholophosphorus trichloride (14) followed by sodium amide, or by pyrolysis of the related selenodiazole (13).



SCHEME 1. The synthesis of 2-phenyl-5-ethynylthiophene.
[Reagents: 1, CBr_4, $(C_6H_5)_3P$; 2, *n*-BuLi; 3, *n*-BuLi, $(CH_3)_2SO_4$]

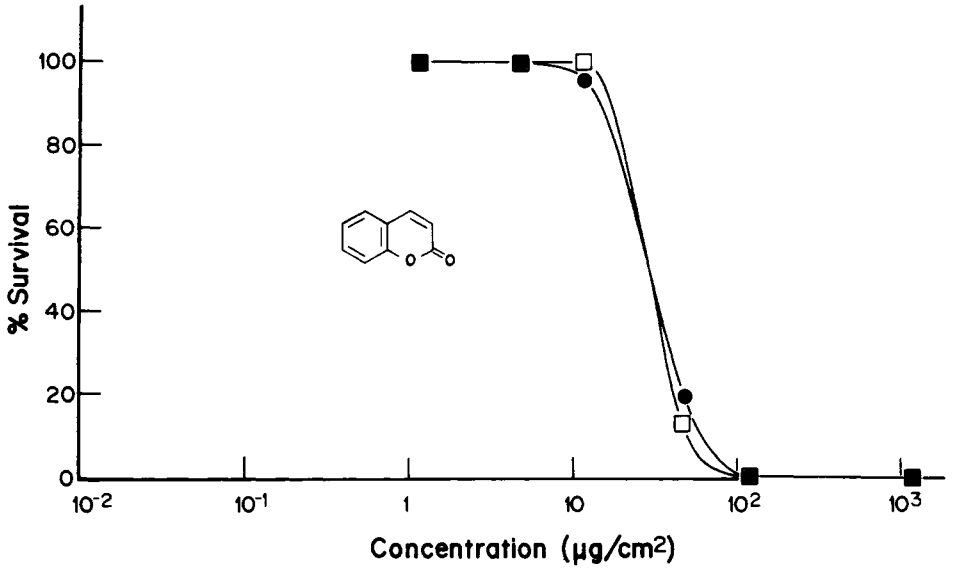


FIGURE 1. The ovicidal activity for coumarin. The survival of the eggs of *Drosophila melanogaster* is expressed as a function of the concentration of sensitizer on the paper discs, plotted on a logarithmic scale. (□ in the dark, ● in the light)

Our successful synthesis utilized the method of Corey and Fuchs (8), which we recently applied to the synthesis of several 1,3-butadiynes (15). Simple modifications of the published procedures led to the desired 2-phenyl-5-ethynylthiophene in 63% yield from 2-phenylthiophene (scheme 1). The lithium salt could be alkylated with dimethyl sulfate in better than 70% yield under mild conditions, and this synthetic material had physical properties matching those reported for the natural product.

THE PHOTOOVICIDAL ACTIVITY.—The activity curve that we determined for coumarin was very similar to that published in the literature (2), with an LD_{50} value of 23 μg/cm^2, instead of 12.5 μg/cm^2 (figure 1). The activity curve was not affected when the eggs were irradiated for 45 min with UVA after the first hour of contact with the chemical. This lack of phototoxicity for coumarin is in accord with the other tests we performed on this compound using *Escherichia coli* B, the yeasts *Saccharomyces cerevisiae* and *Candida utilis*, and the larvae of the fruit fly *D. melanogaster* and those of the mosquito *Aedes aegypti* (17). All these tests gave negative results, in agreement with the known lack of phototoxicity for coumarin, a molecule which, in vitro, is known to undergo with great ease [2+2]-cycloaddition reactions via its pyrone double bond.

Our synthetic sample of 2-phenyl-5-(1′-propynyl)thiophene possessed ovicidal activity, and the activity curve was also quite similar to that published in the literature (3). The observed LD_{50} value (figure 2) was 2.9 μg/cm^2, compared with 2 μg/cm^2 reported by Nakajima and Kawazu (3). With this chemical, the effect of the photochemical treatment was dramatic, increasing the activity more than 225-fold, and lowering the LD_{50} value to 0.013 μg/cm^2 (figure 2). The high phototoxicity of this molecule toward the eggs of *D. melanogaster* was also found in tests with the larvae of this same organism, the larvae of the mosquito *A. aegypti*, and the microorganisms *E. coli* B, *S. cerevisiae*, and *C. utilis*.

The precursor to **1** in our synthesis, 2-phenyl-5-ethynylthiophene, also possessed ovicidal activity. With a LD_{50} value of 4.6 μg/cm^2, this compound is slightly less active than its propynyl analog **2**, but five times more active than coumarin (figure 3). In

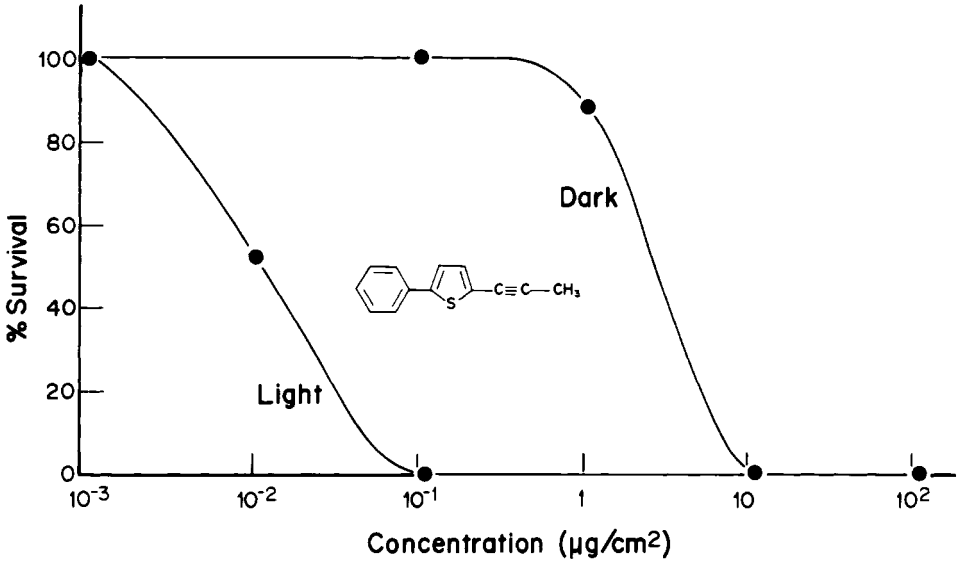


FIGURE 2. The ovicidal activity of 2-phenyl-5-(1′-propynyl)thiophene.

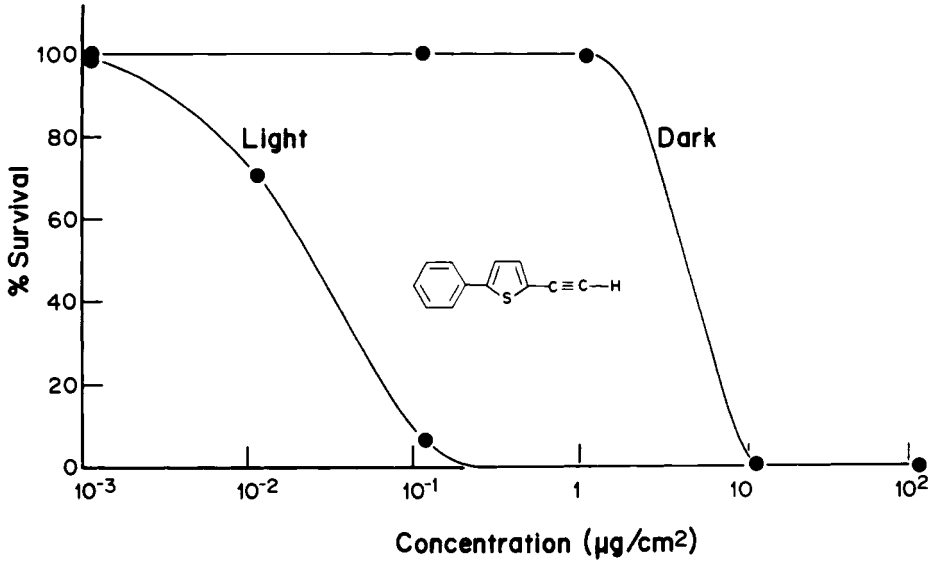


FIGURE 3. The ovicidal activity of 2-phenyl-5-ethynylthiophene.

contrast to the latter, its ovicidal activity was enhanced almost 200 times by the treatment with UVA, giving a LD_{50} value of 0.025 $\mu g/cm^2$ under the conditions used with the other sensitizers. The phototoxic properties of this acetylenic compound were also displayed in tests performed with the larvae of *D. melanogaster* and *A. aegypti*, and with the microorganisms *E. coli* B, *S. cerevisiae*, and *C. utilis*.

The mechanisms of either the ovicidal or the photoovicidal activities are totally unknown at this time. In the latter case, an oxygen dependent mechanism would be likely in view of the known ability of several thiophene and acetylenic compounds to activate oxygen, probably to its electronically excited singlet form (5, 18-20). However, this is made less certain by the fact that 8-methoxypsoralen also displayed photoovicidal activity (1), and this molecule is well-known for its ability to form covalent bonds with

DNA directly (21), even though it can also act as singlet oxygen sensitizer (22 and references cited therein).

In conclusion, this report identified two strong photoovicidal compounds, and proved that not all the ovicidal compounds necessarily have their activity enhanced by a treatment with UVA. Coumarin is a close chemical relative of 8-methoxypsoralen, which was found earlier to possess photoovicidal activity (1), but it is itself inactive. On the other hand, the two acetylenic compounds described in this work differ by one methyl group, but this structural change is not reflected in large differences in either the ovicidal or the photoovicidal activities. Clearly, much interesting work will have to be done in order to establish structure-activity relationships.

Finally, the very high ovicidal activity of the naturally occurring 2-phenyl-5-(1′-propynyl)thiophene in the presence of light suggests that the *Coreopsis* species in which it has been found may have a high degree of natural protection against herbivorous insects. A study of the interaction between insects and these plants *in vivo* should be rewarding.

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