Phytochemistry, Vol. 25, No. 7, pp. 1609–1611, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

PHOTOTOXICITY OF NATURALLY OCCURRING AND SYNTHETIC THIOPHENE AND ACETYLENE ANALOGUES TO MOSQUITO LARVAE

J. T. ARNASON, B. J. R. PHILOGÈNE, C. BERG, A. MACEACHERN, J. KAMINSKI, L. C. LEITCH, P. MORAND and J. LAM*

Ottawa Carleton Centre for Research and Graduate Studies in Biology and Chemistry, University of Ottawa, Ottawa, Canada K1N 6N5; *Department of Organic Chemistry, University of Aarhus, Aarhus 8000, Denmark

(*Received* 1 *October* 1985)

Key Word Index—Asteraceae; polyacetylenes; thiophenes; phototoxicity; mosquito larvae.

Abstract—Naturally occurring and synthetic analogues and derivatives of polyacetylenes and thiophenes of the Asteraceae were investigated for their phototoxic effects on mosquito larvae. Iodination, dimethylation in the 3′,4′-position or substitution of the middle ring of α-terthienyl reduced phototoxicity. Methylation at the 2′-position increased activity. In the acetylene series, retention of a triacetylene-furan chromophore was necessary for optimal activity.

INTRODUCTION

In a previous article [1] we demonstrated the phototoxic properties of naturally occurring acetylenes and thiophenes from members of the Asteraceae towards the larvae of the mosquito *Aedes aegypti*. Two of these compounds, α-terthienyl (**1**) and the furanoacetylene (**8**) were exceptionally phototoxic with LC_{50}s (lethal concentration for 50% mortality) of 19 and 79 ppb, respectively. The efficacy of α-terthienyl has recently been evaluated in field trials [2, 3] against the larvae of the mosquito *Aedes intrudens*. Complete control at one week was achieved in natural breeding pools with application rates as low as 10 g/ha. These rates compare favorably with currently used organophosphates such as temephos and pirimiphos methyl.

One report [4] of the phototoxicity of several analogues of **1** exists, but only qualitative (positive or negative) data is recorded. Considerable success has been achieved in the production of designed analogues of pyrethrin to improve efficacy and stability [5]. The present study was initiated to evaluate quantitatively the efficacy against *Aedes atropalpus* of synthetic analogues and derivatives of **1** and naturally occurring analogues of **8** as a first step towards understanding structure–function relationships of these compounds.

RESULTS AND DISCUSSION

Three series of analogues or derivatives of α-terthienyl (**1**) were investigated: a methyl substituted series (**6**, **7**), an iodo-substituted series (**2**, **3**) and a series in which the middle ring was substituted (**4**, **5**). Only the 2-methyl substituted derivative (**6**) increased phototoxicity (Table 1), while the 3′,4′-dimethyl derivative was an order of magnitude less toxic than **1**. The methyl terthienyl (**6**) is the only naturally occurring derivative, probably suggesting that evolutionary pressure by insects on plants has selected efficient structures for plant defence. In the iodo series, activity declines rapidly in the order of increasing iodination: **1** > **2** > **3**. The spectra of the above derivatives (**2**, **3**, **6** and **7**) are similar to α-terthienyl (**1**), suggesting that changes in phototoxicity are due to factors other than changes in light absorption. For example, the changes may be due to attachment to different sites in target cells or tissues which may depend on the partition coefficient and/or structure of the compound. In the third series, the substitution of the middle ring with a benzene ring (**4**) slightly reduces activity while pyridine substitution (**5**) reduces the phototoxic effect by an order of magnitude. In this series, the chromophore is altered and in the case of **4** there is no absorption in the photosensitizing region (300–400 nm). This latter compound is the only one in the series that shows no significant enhancement under irradiation as compared to dark conditions and its activity can be attributed to non-photosensitizing effects. This activity is much higher than the non-photosensitizing toxicity of α-terthienyl [LD_{50} = 0.74 ppm (**1**)] and may be the starting point for the design of a conventional non-phototoxic insecticide.

In series 2, **8** is the most active. Replacement of the triacetylene portion of the molecule with a thienylacetylene (**9**) reduces the activity by an order of magnitude. Similarly replacement of the furan of **8** with an epoxide in **10** produces an even greater reduction of activity. Compound **11**, with both a phenyl and a furan ring, showed the least activity. The results suggest that conservation of the furan and triacetylene moities is necessary for activity. The proportion of naturally occurring *cis–trans* isomers of **9** (1 : 1) and **8** (1 : 5) is altered on near-UV irradiation and this isomerization may play an important role in the phototoxic mechanism of these compounds.

EXPERIMENTAL

Insect rearing and toxicity tests. *Aedes atropalpus* larvae from a stock maintained in the laboratory were used [2]. Toxicity tests were performed as described in ref. [1].

Sources in compounds. Synthesis of analogues **2**–**8** will be described in detail elsewhere. (A) α-Terthienyl (**1**), 1,3-dithienylbenzene (**4**) and 2,6-dithienylpyridine (**5**), were prepared using a modified Grignard–Wurtz coupling reaction [6]. In each case, 2 equivalents of 2-thienylmagnesium bromide were added to the appropriate dibrominated compound. The reactions were carried

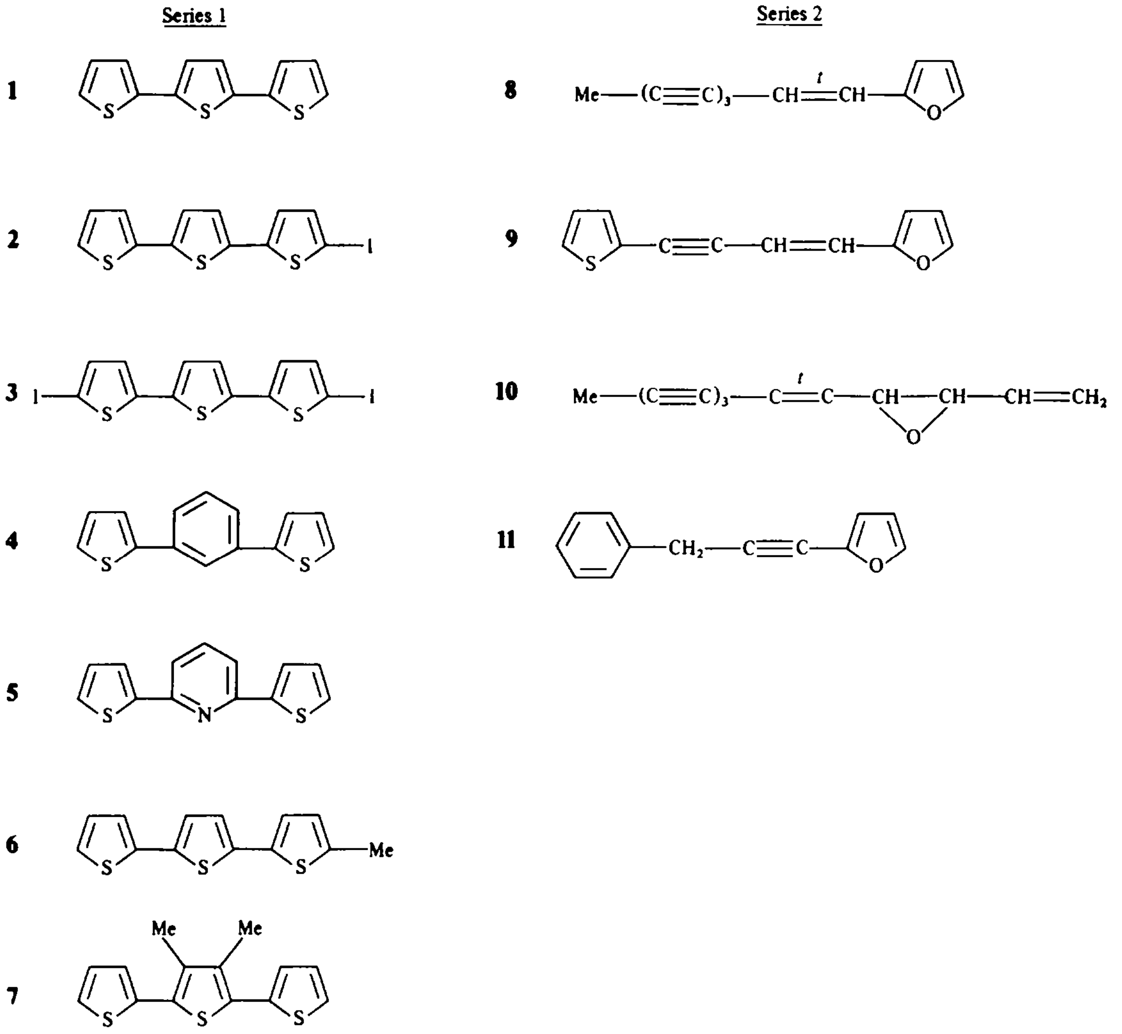

Fig. 1. Structure of synthetic analogues and derivatives of α-terthienyl, **1** (series 1) and naturally occurring analogues of the furano-acetylene **8** (series 2).

Table 1. Acute 24 hr phototoxicity of analogues to *Aedes atropalpus* larvae

Compound	LC_{50} (ppm)	LC_{90} (ppm)	r
1	0.0275	0.0422	1.00
2	30.7	9330	0.989
3	> 1	> 1	—
4	0.060	0.320	0.820
5	0.261	0.680	1.00
6	0.0153	0.0269	0.932
7	0.164	0.209	0.998
8	0.100	0.300	0.952
9	0.858	1.57	0.992
10	1.47	8.91	0.999
11	3.71	6.30	1.00

Larvae in 100 ml of water were treated with 4 hr near-UV irradiation from a bank of four blacklight blue tubes (4 w/m^2) immediately following addition of test compound, then held in the dark for a further 20 hr. No mortality was seen in controls.

out in anhydrous Et_2O under N_2 with the catalyst dichloro(1,3-bis[diphenylphosphinopropane]) nickel. After refluxing for 2 hr, the reactions were stirred for 24 hr and then worked up with 3 N HCl. The Et_2O layer was separated and evaporated under red. pres. yielding the coupled product. (B) The iodination of α-T was done using HgO and I_2 in C_6H_6, although $CHCl_3$ and CCl_4 could also be used as solvents. The reaction yielded a mixture of mono (**2**) and diiodinated (**3**) products which were subsequently separated chromatographically. (C) 2-Methyl terthienyl (**6**) was prepared by lithiating α-T in anhydrous Et_2O at room temp. using BuLi and then quenching with MeI. A mixture of monomethylated and dimethylated α-T was obtained. (D) 3′4′-Dimethyl-2,2′,5′,2″-terthienyl (**7**) was synthesized using a modified Wittig reaction [7] involving the addition of 2-thienyl dithiocarboxymethyl ester to ethylidene-triphenylphosphorane to give a thiocarbonyl-stabilized ylide. The dimethyl derivative was obtained after pyrolysis of the ylide. The products of the above reactions were purified by CC or HPLC. Complete experimental details and structural assignment for new compounds will be published separately. (E) Compounds **9**–**11** were extracted by one of us (Lam) from plant sources. Compound **8** was isolated from roots of *Chrysanthemum leucanthemum* L., **11** (carlina oxide) from *Carlina acaulis* (caulescens) L., **10** (pontica epoxide) from *Artemisia pontica* L. and **9** from *Santolina chamaecyparissus* L. [8–11].

Acknowledgement—This work was supported by DND (Canada) and NSERC.

REFERENCES

1. Arnason, T., Swain, T., Wat, C. K., Graham, E. A., Partington, S., Towers, G. H. N. and Lam, J. (1981) *Biochem. Syst. Ecol.* **9**, 63.
2. Philogène, B. J. R., Arnason, J. T., Berg, C. W., Duval, F., Champagne, D., Taylor, R. C., Leitch, L. C. and Morand, P. (1985) *J. Econ. Entomol* **78**, 121.
3. Philogène, B. J. R., Arnason, J. T., Berg, C. W., Duval, F. and Morand, P., *J. Chem. Ecol.* (in press).
4. Kagan, J., Beny, J. P., Chan, G., Dhawan, S., Jaworski, J. A., Kagan, E. D., Kassner, P. D., Murphy, M. and Rogers, J. A. (1983) *Insect. Sci. Applic.* **4**, 377.
5. Casida, J. E. (1983) in *Natural Products for Innovative Pest Management* (Whitehead, D. L. and Bowers, W. S., eds) pp. 109–125.
6. Kumada, M., Tamao, K. and Sumitani, K. (1978) *Org. Synth.* **58**, 127.
7. Bestmann, H. J. and Schaper, X., Jr. (1979) *Tetrahedron Letters* 243.
8. Bohlmann, F., Kap-Herr, W. v., Fanghänel, L. and Arndt, C. (1965) *Chem. Ber.* **98**, 1411.
9. Semmler, F. W. (1889) *Chemiker-Zeitung* **13**, 1158.
10. Bohlmann, F., Arndt, C. and Bornowski, H. (1960) *Chem. Ber.* **93**, 1937.
11. Bohlmann, F. and Arndt, C. (1966) *Chem. Ber.* **99**, 135.