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Metal Binding and Resultant Loss of Phototoxicity of α-Terthienyl: Metal Detoxification Versus α-Terthienyl Inactivation

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The members of the plant family asteraceae are noted for their cosmopolitan distribution and their versatility is attributed to be largely due to their morphological adaptations. Interestingly members of this family are endowed with rich levels of secondary plant metabolites, many of which are photochemically active (Bakker et al. 1979; Kagan et al. 1989). The secondary plant metabolite ∝ -terthienyl derived form the plant family asteraceae is among the new class of light activated insecticide. The photobiocidal effects associated with ∝ -terthienyl in presence of sunlight and ultraviolet light (300-400 nm), has stimulated a great deal of interest in its toxic mechanism of action against a number of organisms including phytopathogenic fungi, nematodes and mosquito larvae. Trials under tropical conditions indicate a very high level of activity as a larvicide to mosquito. There is no cross resistance to this compound in malathion resistant mosquito larvae (Arnason et al. 1989).

Even though many researcher’s feel that the phototoxicity of secondary plant metabolites has arisen independently many times in evolution as a defense mechanism, the physiological impact of such biologically active compounds in the plant producing them also should be addressed (Arnason et al. 1987). Moreover, the accumulation of secondary plant metabolites in the roots of many asteraceae members as is the case with ∝ - terthienyi in marigold (Tagetes sp.) roots, hi hints towards their functional divergence. In this study, we demonstrate a potent heavy metal quenching activity of ∝ -terthienyl.

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MATERIALS AND METHODS

All of the four mosquito larval stages used for this study were reared in the laboratory and each stage was studied separately.

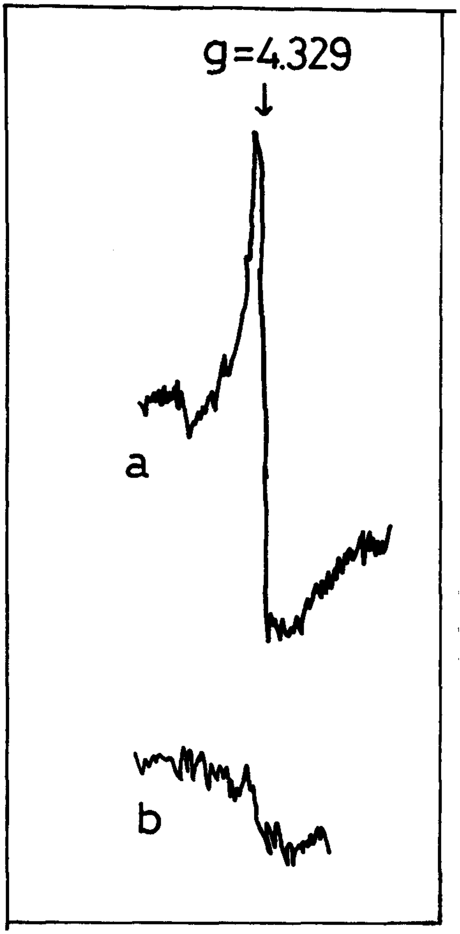
100 mM solution of cobalt chloride (Merck India Ltd.) was made in double distilled water and ∝ -terthienyl (a gift from Dr. J.T. Arnason, University of Ottawa, Canada) was prepared in ethyl alcohol 2 mg/ml. 100 ul of ∝ -terthienyl solution and 100 ul cobalt chloride solution were mixed in 1 ml of double distilled water.

The above-said reaction system was then subjected to UV visible spectroscopy using Shimadzu UV 160A recording double beam spectrophotometer using two matched quartz cells. Before starting the experiment the spectrophotometer was base line corrected. Similarly fluorescence studies were made using a Shimadzu RF540 spectrofluorophotometer. Finally the reaction system was subjected to electron paramagnetic studies using a Varian electron paramagnetic resonance (EPR) spectrometer. For this study, 100 ul aliquots was taken into a glass capillary tube (Clinicon Int., GmbH), and one end was flame sealed. The capillary was placed in EPR sample tube and was introduced into the cavity. The instrument settings were: Scan range- 10 x 1K; Field set- 1437G; Time constant- 1 minute; Scan time- 8 minutes; Modulation amplitude- 2 x 1G; Modulation frequency- 100KHz; Receiver gain- 4 x 10^3; Temperature- 27°C ± 5°C; Microwave power- 100mW; Microwave frequency- 9.01GHz.

The following protocol was employed for testing toxicity with the complex formed against mosquito larvae: 25-30 mosquito larvae of all the four instars viz., 1st, 2nd, 3rd and 4th, were selected for the study. Larvae were cleaned with water taking care not to harm the larvae. They were transferred to petri dishes containing 15 ml of water. ∝ - terthienyl-cobalt complex was added to these petri dishes (1mg/10ml) and was then exposed to uv light (320-400 nm, model UVL 21 blackray lamp, Ultraviolet Pro, Inc., San Gabriel CA) for 1hr. Mortality (if any) of mosquito larvae was observed.

RESULTS AND DISCUSSION

Cobalt, a transition metal having a valance orbit configuration of $3s^2$, $3p^6$, $3d^7$, gives a prominent electron paramagnetic resonance at g=4.329 at a magnetic field 1487G (Fig. 1a). A study on the distortion of this spectrum has been used to investigate the reaction of Co^{++} with ∝ -terthienyl



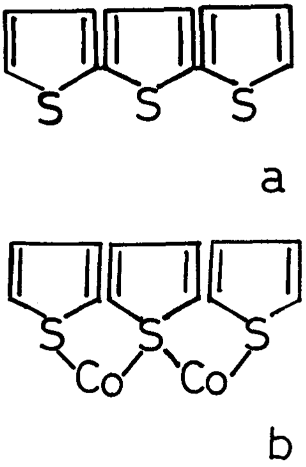


Figure 2. Structure of ∝ - terthienyl (a) and the proposed structure of ∝ -terthienyl - cobalt complex.

Figure 1. The EPR spectra of cobalt (a) and ∝ -terthienyl cobalt complex (b).

reflects the nature of complex formed (Fig. 1b). The most likely cause for the loss of EPR signal intensity when cobalt ion reacted with ∝ -terthienyl (Fig. 2a) is the formation of bridge species (Fig. 2b). In this event, Co^{++} spins can antiferromagnetically couple to produce a diamagnetic state or otherwise interact to change relaxation properties and reduce signal intensity.

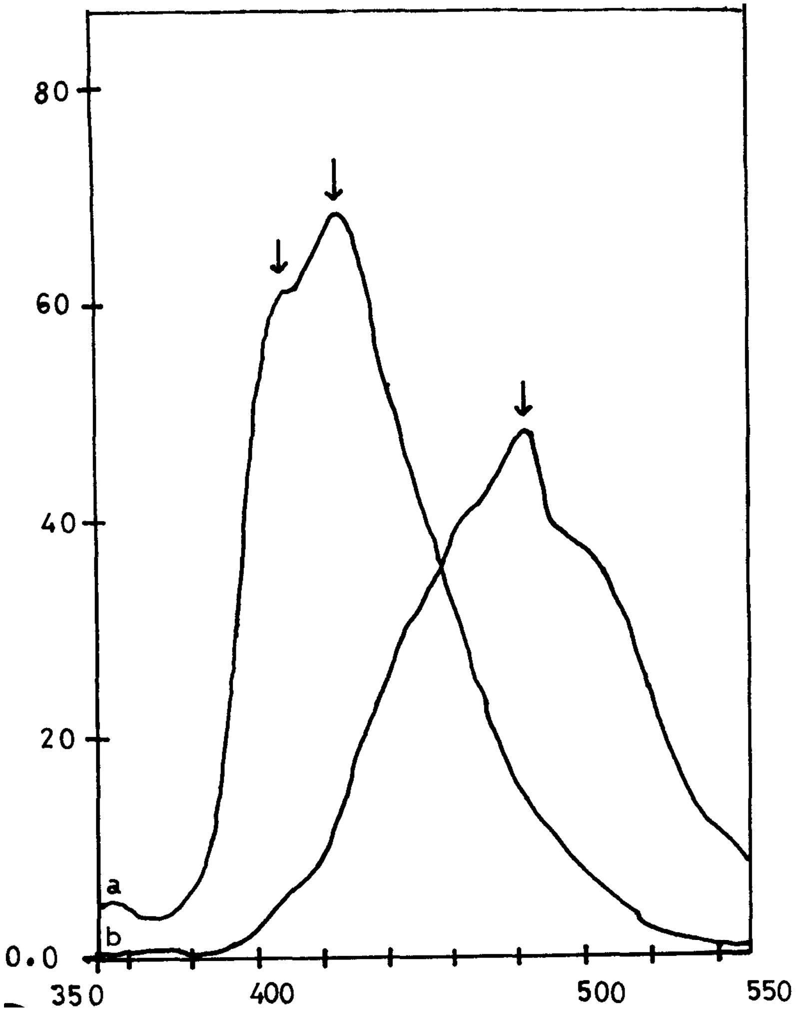


Figure 3. Absorption spectra of ∝ -terthienyl (a) and of ∝ - terthienyl-cobalt complex (b), arrows indicate the peak.

The characteristic absorption spectra of ∝ - terthienyl in ethyl alcohol (Fig 3a) gives a peak at 348 nm, but the addition of cobalt shifts this peak to 360nm (Fig 3b). Similar results are shown by fluorescence studies. ∝ - terthienyl gives a fluorescence peak at 406 and 423 nm and its complexing with Co^{++}shifts these peak to 486 nm (peaks shown by solid arrows) (Fig. 4a and b respectively).

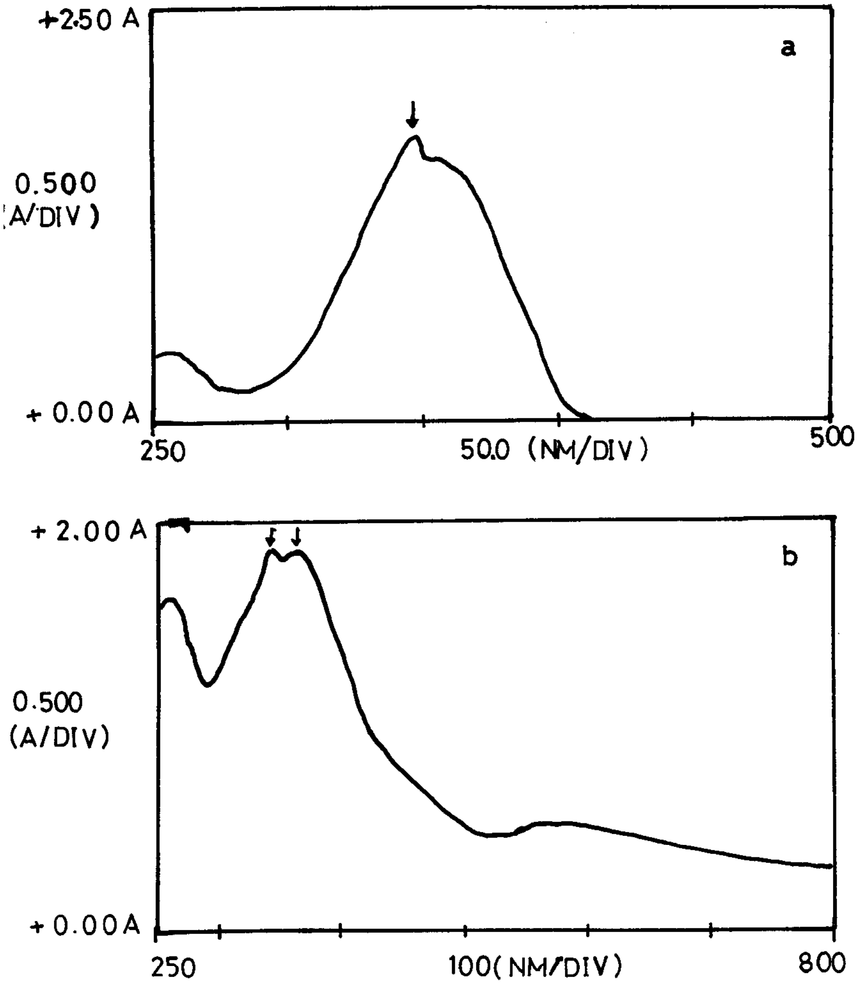


Figure 4. Fluorescence spectra of ∝ - terthienyl (a) and ∝ - terthienyl-cobalt complex (b), arrows indicate the peaks).

An attempt to study the toxicity effect of this complex formed against mosquito larvae, gave some very interesting results. It was observed that this complex is totally non-toxic to mosquito larvae, no mortality was observed in any of the four larval stages (results not presented), as against 100% mortality shown by ∝ - terthienyl against mosquito larvae.

It is well known that thiophenes are several fold concentrated as waste products produced during the different biochemical reactions undergoing in the plant body but, later were assigned an insecticidal role. Several reports are known for their toxicity such as inactivation of a number of enzymes (Wat et al. 1981; Kagan et al. 1984; Nivsarkar et al. 1991 a, b), damage to DNA (Kagan et al. 1979), toxicity to microorganisms (Philogene et al. 1985), superoxide anion radical generation and toxicity (Nivsarkar et al. 1992) etc. But, the results shown above present a very different aspect in ∝ -terthienyl chemistry, i.e., besides having such toxic roles, ∝ -terthienyl possess a unique complexing property with cobalt.

Thus, the light induced phototoxicity could not be the proper explanation for the localization of ∝ -terthienyl in the underground parts of the plants especially roots. But, the heavy metal binding, the plausible heavy metal detoxification and its inactivation could be classed as a evolutionary prospect. This could have arisen as an adaptive biochemical evolution which makes the members of asteraceae cosmopolitan.

REFERENCES

Arnason JT, Philogene BJR, Morand P, Scaiano JC, Werstiuk N, Lam J (1987) Thiophenes and acetylenes : phototoxic agents to herbivorous and blood feeding insects In: Heitz JR, Downum R (eds) Light Activated Pesticides. Amer Chem Soc Symp Ser 339: 257-264.

Arnason JT, Philogene BJR, Morand P, Imire K, Iyengar S, Duval F, Souey-Breau C, Scaiano JC, Werstiuk NH, Hasspiler B, Downe AER Naturally occurring and synthetic thiophenes as photoactivated insecticides (1989) In: Arnason JT, Philogene BJR, Morand P (eds) Insecticides of plant origin. Ame Chem Soc Symp Ser, Washington DC 387: 164-172.

Bakker J, Gommers FJ, Nieuwenhuis I, Wynberg H (1979) Photoactivation of the nematicidal compound a-terthienyl from roots of marigold (Tagetes species): A possible singlet oxygen role. J Biol Chem 254: 1841-1844.

Kagan J, Gabriel R, Reed SA (1979) ∝ - terthienyl a non- photodynamic compound. Photochem Photobiol 31:465-469.

Kagan J, Hasan M, Grynspan F (1984) The inactivation of acetylcholinesterase by ∝ - terthienyl and UV light: Studies in vitro and in the larvae of mosquito Aedes aegypti. Biochem Biophys Acta 802:442-447.

Kagan J, Bazin M, Santus R (1989) Photosensitization with ∝ - terthienyl : The formation of superoxide anion in aqueous medium. J Photochem Photobiol B 3:165-174.

Nivsarkar M, Kumar GP, Laloraya M, Laloraya MM (1991a) Superoxide in the anal gills of larvae of Aedes aegypti : Its inhibition by ∝ - terthienyl. Arch Insect Biochem Physiol 16:249-255.

Nivsarkar M, Kumar GP, Laloraya M, Laloraya MM (1991b) Superoxide dismutase inactivation by ∝ - terthienyl : A novel observation in thiophene photochemistry. Pest Biochem Physiol 41:53-59.

Nivsarkar M, Kumar GP, Laloraya M, Laloraya MM (1992) Generation of superoxide anion radical by ∝ - terthienyl in the anal gills of mosquito larvae of Aedes aegypti: A new aspect in ∝ - terthienyl phototoxicity. Arch Insect biochem Physiol 19:261- 270.

Philogene BJR, Arnason JT, Berg CW, Duval F, Champagne D, Taylor RG, Leitch LC, Morand P (1985) Synthesis and evalution of the naturally occurring phototoxin, ∝ - terthienyl as a control agent for the larvae of Aedes intrudens, A. atropalpus, and Simulium verecundum. J Econ Entomol 78:121-126.

Wat CK, Prasad S, Graham E, Partington S, Arnason JT, Towers GHN, Lam J (1981) Photosensitization of invertebrates by polyacetylenes isolated from species of Asteraceae. Biochem Sys Ecol 9:59-62.