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REVIEWS

α-Terthienyl, Phototoxic Allelochemical

Review of Research on Its Mechanism of Action

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Research on the mechanism of biological action of the phototoxic allelochemical α-terthienyl is reviewed.

The plant-derived natural product α-terthienyl, first recognized as a phototoxin in the 1970s, has been a topic of considerable study in the last few years. Recent interest in the mechanisms of action of photoactivated toxins such as psoralen has stimulated several groups to study the means by which the structurally simple α-terthienyl exerts its effects on living organisms. The phenomenon of allelopathy has been much investigated as researchers have become more aware of the importance of natural products in ecological interactions, and their possible use in biocontrol. Thus attention has been drawn to this phototoxic compound produced in high concentrations by a number of species of the Compositae.

To our knowledge, no recent review encompassing this biochemical aspect of α-terthienyl has appeared. We now discuss the mechanisms of biological action of α-terthienyl.

The initial reports by Uhlenbroek and Bijloo (*1*, *2*) in the late 1950s described isolation of nematicidal principles α-terthienyl and BBT (5-(but-3-en-1-ynyl)-2,2′-bithienyl) from the marigold *Tagetes erecta*. This followed an earlier field observation by Slootweg (*3*) that *Tagetes* species were resistant to the root-lesion nematode *Pratylenchus*. A series of α-terthienyl analogs were also synthesized and tested for nematicidal activity (*4*). A bithienyl moiety was found to be a minimum structural requirement for biological activity. The marked enhancement of α-terthienyl toxicity in the presence of sunlight or uv light was not noticed by these workers. Following a lapse of a few years, a steady succession of articles has appeared concerning the toxicity of α-terthienyl and related compounds to a wide variety of organisms, and attempting to define the mechanism of this toxicity.

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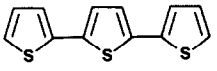
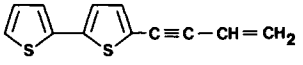


α- terthienyl



BBT

A more extensive investigation of the nematicidal aspects of α-terthienyl was made by F. Gommers and co-workers. The initial observation (reported in 1972) that α-terthienyl toxicity was enhanced by sunlight (*5, 6*) prompted a series of experiments. The increased potency was attributed to the effect of the uv component of sunlight. It was shown that the uv-absorption maximum of α-terthienyl coincided with the wavelength at which the light-induced enhancement of toxicity was the greatest. This observation led these authors to conclude that photoactivated α-terthienyl itself was the active species, rather than some other phototoxic compound whose level in the organism had been altered by the presence of α-terthienyl. Gommers proposed that an "activated mesomeric form" of α-terthienyl was responsible for its toxicity to isolated nematodes on exposure to uv light, but mentioned that in their hands the compound was minimally effective in soil, due to light blockage. He postulated that the nematicidal activity as seen in the field was not due to α-terthienyl "as such," but to "energy-rich related forms," presumably nontransient photoproducts rather than excited electronic states. Thus, Gommers felt at that time that two separate mechanisms of action were operable. Gommers also carried out an extensive series of bioassays of plant extracts from the members of the Compositae (*7*) against nematodes.

In 1975, the phototoxicity of α-terthienyl was rediscovered by Chan *et al.* (*8*), who were apparently unaware of the earlier published work of Gommers (*5*) and Gommers and Geerligs (*6*). The work of Chan *et al.* had been stimulated by a report by Daniels (*9*) that extracts of marigolds and other members of the Compositae exhibited phototoxic properties against the yeast *Candida albicans*. This observation had been made as part of a survey of phototoxic plant extracts, using uv-stimulated inhibition of the yeast's growth as a bioassay. Chan *et al.* repeated the extraction and identification of α-terthienyl and the related compound BBT from *T. erecta*. The presence of these compounds in *Tagetes* species was originally described in 1947 by Zechmeister and Sease (*10*), who did not report any biological activity attributable to these compounds. Towers discussed the enhancement of α-terthienyl toxicity to the yeast by uv light. Towers and co-workers (*11*) also conducted a survey of the Compositae for phototoxicity, using the *C. albicans* bioassay, but did not attribute any further instances of phototoxicity exhibited by plants of this family to the presence of α-terthienyl.

Further investigations of the mechanism of action of α-terthienyl were reported in 1978 by Bakker and Gommers (*12*), in which they proposed that the photoinduced damage caused by α-terthienyl was due to the generation of singlet oxygen ($^1O_2$) in a photosensitization process analogous to that seen with dyes such as Rose Bengal or methylene blue. Using the nematode *Aphelenchus avenae*, an organism that tolerates anaerobic conditions, they demonstrated that oxygen was

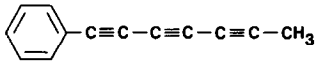
required for expression of toxicity against these multicellular eucaryotes (*13*). Proposing that inactivation of selected enzymes was a good model for α-terthienyl phototoxicity, they showed that there was a loss of activity brought about by α-terthienyl/uv light/oxygen seen in cholinesterase, glucose-6-phosphate dehydrogenase, and malate dehydrogenase isolated from the phytoparasitic nematode *Ditylenchus dipsaci*. This inactivation was blocked by singlet oxygen scavengers such as NaN_3, methionine, and histidine. The decrease in enzyme activity was not found to be inhibited by superoxide dismutase, mannitol, or catalase, which are reported to scavenge superoxide ion, hydroxyl radical, and peroxide, respectively. As an additional test for $^1O_2$ generation, the reaction of the olefin adamantylidene adamantane with uv-irradiated, oxygenated solutions of α-terthienyl was found to yield adamantanone by decomposition of the olefin's singlet oxygen adduct, the 1,2-dioxetane (*14*). The enhancement of the rate of enzyme inactivation in the presence of deuterium oxide relative to water further confirmed the production of singlet oxygen by the toxin system, due to the longer lifetime of singlet oxygen in D2O (*15*). Based on this evidence, Gommers attributed the toxicity of α-terthienyl entirely to the photosensitized production of singlet oxygen.

In a related study (*16*), Gommers *et al.* attempted to correlate the rate of photosensitized singlet oxygen production by a series of α-terthienyl analogs with the *in vivo* toxicities of these analogs to a nematode species and the *in vitro* inactivation of glucose-6-phosphate dehydrogenase. Using the photobleaching of *p*-nitrosodimethylaniline in the presence of imidazole as an assay for singlet oxygen, they examined the loss of enzyme activity and the *in vivo* toxicity of several dithienyl and 1,2-dithienyl ethene derivatives. They reported a significant correllation between the rate of singlet oxygen production and both the *in vivo* and the *in vitro* activities. Compounds with adjacent thiophene rings (the dithienyls) were more active in all assays than those with the two thiophene rings separated by a vinyl group (the 1,2-dithienyl ethenes). Despite the conjugation of the thiophene rings through the vinyl group, these compounds were poor singlet oxygen producers and relatively nontoxic both to the nematodes and to the enzyme.

At about the same time, a series of articles appeared in which Towers and coworkers examined the phototoxic properties of α-terthienyl using human erythroplasts (nonnucleated entities, inaccurately referred to as erythrocytes throughout Towers' work), *Escherichia coli,* and *Saccharomyces cerevisiae* as target systems (*17–21*). They also studied possible cytogenetic effects of α-terthienyl (*22*).

The phototoxicities of α-terthienyl and the polyacetylene phenylheptatriyne (PHT) were compared (*18*) with that of the known singlet oxygen producer methylene blue dye (MB) with respect to enzyme inactivation in erythroplasts. The activities of cytoplasmic enzymes such as lactate dehydrogenase (LDH), glyceraldehyde phosphate dehydrogenase (GAPDH), and glutamate-oxaloacetate transaminase (GOT), were assayed as a function of time under photosensitizing conditions versus the activity of acetylcholine esterase (AChE), an outer-membrane-bound enzyme. Prolonged irradiation of the erythroplasts in the presence of the toxins resulted in complete rupture of the cell membrane in all cases. Before hemolysis and exposure of the cellular contents to the solution, only the exposed

AChE was affected to any great extent. All three compounds brought about a decrease in AChE activity, but the decrease attributable to α-terthienyl (64%) was about 2.5 times that caused by MB (24%): this was despite the facts that MB was present in higher concentration than α-terthienyl (50 μM vs 6 μM), the visible light used for MB activation (1.7 mW/cm^2) was more intense than the uv light that activated α-terthienyl (0.6 mW/cm^2), and the extinction coefficient of MB (92,000 at 665 nm (*23*)) is greater than that of α-terthienyl (24,000 at 350 nm (*16*)). We note that the discrepancy in photon fluxes in these two cases was even more pronounced than the energy difference due to the greater energy per photon in the uv region of the spectrum. It seems to us that if singlet oxygen production were the sole mechanism of α-terthienyl toxicity, one might expect equal toxicity with MB on a molar basis after normalization for photon fluxes, extinction coefficients, rates of intersystem crossing, and efficiencies of energy transfer to oxygen. None of these points were addressed. The question of α-terthienyl localization in the biological system was not discussed, although this might be partly responsible for the differential effects of α-terthienyl and MB. If α-terthienyl were confined to membranes, as is likely due to its lipophilic character, the *in situ* production of singlet oxygen could cause more pronounced damage to these membranes than singlet oxygen generated in the bulk aqueous phase by the water-soluble methylene blue. However, if α-terthienyl is restricted to membranes, it is more difficult to account for the different rates of inactivation of enzymes inside versus outside the erythroplasts, as one might expect equal rates of diffusion of the singlet oxygen to the outer and inner enzymes.



PHT

The small prehemolysis decrease in cytoplasmic enzyme activities caused by MB was not seen using α-terthienyl. The authors rationalized this disparity as either a noninvolvement of diffusible activated oxygen (singlet oxygen) in the α-terthienyl and PHT cases, or to a selective inactivation of α-terthienyl and PHT, but not MB, by an endogenous quencher. How MB might avoid such quenching was not addressed. It should be noted that no attempt was made to compare the efficiency of singlet oxygen production catalyzed by MB relative to α-terthienyl under these experimental conditions, which makes quantitative analysis of the results impossible.

A further study (*19*) of erythroplast damage by α-terthienyl and PHT using scanning electron microscopy revealed that both compounds caused massive membrane disruption in the presence of uv light and oxygen. It was not reported whether methylene blue brought about similar damage, or if the damage was reduced in the presence of singlet oxygen scavengers. The authors concluded that cell membrane damage "is likely to be largely responsible for the toxicity of these compounds to many organisms."

In a subsequent publication (*20*), the same group addressed the issue of an oxygen requirement in this system. Again using erythroplasts, the parameters of K^+ leakage, hemolysis, and AChE inactivation were evaluated relative to time of irradiation in the presence of oxygen, repeating the previous work on a more

quantitative basis. The experiment was then carried out under conditions presumed by the authors to be anaerobic. The results indicated that for α-terthienyl, hemolysis was almost entirely suppressed, suggesting an involvement of singlet oxygen in the membrane damage component of toxicity. AChE activity did decrease using α-terthienyl under nitrogen (24% after 1 h irradiation), but the rate of inactivation was substantially higher in the presence of oxygen (91% after 1 h irradiation), perhaps suggesting two operational mechanisms in this case. The authors also report an attempt to demonstrate qualitatively the generation of singlet oxygen in this system using trapping by cholesterol, and assay of the hydroperoxide conversion product. The cholesterol hydroperoxide was detected using MB, α-terthienyl, and PHT as activators, but no quantitative data were given. An additional result that casts doubt on the significane of the detection of singlet oxygen in this case is the discovery that PHT causes erythroplast hemolysis and AChE inactivation in the absence or presence of oxygen to about the same degree. However, the case for the involvement of oxygen in α-terthienyl toxicity appeared to be better. The authors concluded that singlet oxygen might be a significant contributor to α-terthienyl toxicity.

This conclusion was further supported by Arnason *et al.* (*21*) in an examination of the oxygen requirement in α-terthienyl phototoxicity to *E. coli* and *S. cerevisiae*. They showed that α-terthienyl bioactivity was enhanced about five orders of magnitude in the presence of oxygen, using cell survival as an assay. The singlet oxygen scavenger sodium azide increased cell survival by several orders of magnitude. Based on this evidence, Towers and co-workers concluded, as did Gommers and co-workers (*5, 6*), that singlet oxygen production is the main cause of α-terthienyl toxicity *in vivo*.

Towers and co-workers (*22*) also examined possible cytogenetic effects of α-terthienyl relative to the known DNA crosslinking photoalkylator 8-methoxypsoralen (8-MOP) using sister chromatid exchange (SCE) frequency in Syrian hamster cell cultures as an indicator of direct DNA damage. In contrast to the high rate of SCE and marked chromosomal aberrations brought about by 8-MOP, α-terthienyl was found to cause no cytogenetic damage detectable in this assay. It was concluded that these data showed that α-terthienyl is not a DNA photoalkylator analogous to the psoralens.

Conflicting results have been presented by a third group working in this field. Kagan and co-workers (*24, 25*) reported a study using ^{14}C-radiolabeled α-terthienyl, in which they showed that on aerobic photolysis of α-terthienyl in the presence of purified calf thymus DNA, or *C. utilis* cultures, a fraction of the radioactivity became irreversibly bound to the polynucleotides.

In the earlier paper (*24*), Kagan had reported the use of the nonpathogenic yeast *C. utilis* as a replacement for the potentially pathogenic *C. albicans* used by Daniels (*9*) and later by Towers and co-workers (*18–21*). The substitution of *C. utilis* for *C. albicans* also resulted in a bioassay which was claimed to distinguish photodynamic compounds (singlet oxygen generators) like MB from nonphotodynamic phototoxins such as psoralen. MB gave a firm negative using *C. utilis*, whereas psoralen and α-terthienyl tested positive, allegedly supporting the hypothesis of nonphotodynamic mechanisms for both.

Kagan (*25*) experimentally ruled out the formation of persistent toxic photolysis products, and demonstrated the toxicity of α-terthienyl in the presence of uv light, but the absence of oxygen, against *E. coli* strain B, contradicting Towers' reports of an oxygen requirement. Oxygen was reported to *inhibit* the phototoxicity of α-terthienyl in this bacterial strain.

Examining the effects of the singlet oxygen scavenger sodium azide using *C. utilis* liquid cultures, they reported no reduction in aerobic α-terthienyl phototoxicity. Lower concentrations of NaN_3 were used in these experiments than in the work reported by Towers' group. The possibility that localization of α-terthienyl and subsequent singlet oxygen production in lipid-rich regions resulted in membrane damage before the water-soluble sodium azide had a chance to detoxify the singlet oxygen was not mentioned.

In a time-course study of the [^{14}C]-α-terthienyl/purified calf thymus DNA photoreaction, Kagan's group showed an exponential-like incorporation of the α-terthienyl into DNA, leveling off after about 2 h irradiation with about 25% of 0.05 μCi (2.6 μmol = 650 μg) α-terthienyl bound to 1 mg DNA. The amount of incoporation of radioactivity into the DNA also varied linearly with increasing α-terthienyl concentration from 0 to 160 μM. Sucrose gradient centrifugation of the calf thymus DNA/α-terthienyl photoproduct revealed only minimal changes in the sedimentation coefficients, suggesting that no crosslinking had occurred. This would seem to implicate α-terthienyl as a DNA monoalkylator in contrast to the psoralens, which can react either mono- or bifunctionally.

Using *C. utilis* liquid cultures, addition of 0.3 μCi (15.6 μmol = 3.9 mg) α-terthienyl to a culture containing 1.6 g dry wt of cells, followed by a 2 h irradiation, resulted in a 19% incorporation of radioactivity into the cells. However, only 0.26% of the cell-bound counts was associated with the DNA fraction isolated. Nevertheless, Kagan suggests that the interaction with polynucleotides is a significant component of the toxic effect.

In a related set of experiments, Kagan *et al.* (*26*) compared the compounds 1,4-diphenylbutadiyne and 2,5-diphenylthiophene with respect to their oxygen requirements for toxicity. Although the diyne proved to be phototoxic in the absence of oxygen, the related thiophene was not phototoxic at all. These workers then synthesized a larger series of diynes and their derivative thiophenes, and tested their oxygen-independent phototoxic properties (*27*). The pairs of compounds were α-terthienyl and 1,4-di-(2-thienyl)-1,3-butadiyne (DTBD), 2-phenyl-5-(2-thienyl)-thiophene (PTT), and 1-phenyl-4-(2-thienyl)-1,3-butadiyne (PTBD). Each pair of compounds represented a diyne unit or its thiophene derivative, flanked by phenyl or thiophene rings. All compounds tested were reported to show no requirement for oxygen for expression of phototoxicity against *E. coli* strain B and *S. cerevisiae*. However, the pair of compounds 1,4-diphenyl-1,3-butadiyne and 2,5-diphenylthiophene did not show comparable toxicity, indicating that the diynes and their thiophene derivatives did not operate by identical mechanisms in all cases. This type of nonphotodynamic phototoxicity was classified as type B phototoxicity by Kagan.

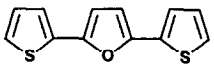
In subsequent papers Kagan shifted his attention to study of α-terthienyl as an insecticidal compound, and reported its activity against *Drosophila* eggs (*28, 29*)

and the larvae of both *Drosophila* and the mosquito *Aedes aegyptii* (*30*). He also demonstrated aerobic phototoxicity of α-terthienyl against late embryonic stages of the frog *Rana pipiens* (*31*), which may diminish the prospects for using α-terthienyl as an insecticide in aquatic environments due to its lack of selectivity. Apparently no further experiments have been carried out by this group concerning the mechanism of toxicity of α-terthienyl. However, statements appeared in one of the later publications (*31*) that "the mechanism by which the toxicity is produced is unknown," and "The requirement for the simultaneous presence of sunlight, the organisms and the sensitizer in the light-enhanced toxicity is in agreement with an oxygen-dependent process, as was proved to be the case in the phototoxicity toward micro-organisms and nematodes." Thus, it is unclear whether Kagan has modified his views on the matter and discounted his earlier work, or whether the above statement does not adequately reflect his current opinion. A report in the trade press (*32*) quotes Kagan as stating that the reaction of singlet oxygen produced via α-terthienyl photosensitization with cell components causes the damage to insect eggs. No additional substantiation of Kagan's earlier claims seems to have been obtained.

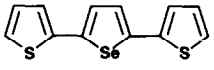
However, Kagan has reported (*33*) that the polyacetylene phenylheptatriyne (PHT) does require the presence of oxygen for expression of its phototoxicity toward *E. coli,* in direct contradiction of reports by Arnason's group (*34*) that polyacetylene phototoxicity is oxygen independent. It is difficult to reconcile this disparity, as in the case of the conflicting reports concerning an oxygen requirement of α-terthienyl toxicity. Kagan attributes the detection of anaerobic phototoxicity by PHT in Arnason's work to the use of impure, oxygen-contaminated nitrogen. However, Arnason seems quite confident in the lack of oxygen in their anaerobic experiments. In several cases he added glucose oxidase and glucose to the medium to scavenge any residual oxygen. Kagan does not suggest how oxygen may have escaped this treatment. No independent method for determination of trace levels of residual oxygen was used to prove the presence or absence of micromolar levels of oxygen in the medium.

Towers' group has also reported phototoxic effects of α-terthienyl against larvae of *A. aegyptii* (*35*), as well as against higher plants (*36*), fungi (*37*), and larvae of the Sphingid moth *Manduca sexta* (*38*) and of the Noctuiid moth *Euxoa messoria* (*39*). Two reviews of the use of α-terthienyl and other phototoxins for insect control have also appeared (*40, 41*).

Tower's group has also studied analogs of α-T (*42*) containing an oxygen or selenium atom instead of sulfur in the central ring. They related the rate of singlet oxygen production of the three compounds α-T,2,5-di-(2′-thienyl)-furan (DTF), and 2,5-di-(2′-thienyl)-selenophene (DTS), as measured by photooxidation of cholesterol, to the inactivation rate of yeast alcohol dehydrogenase (ADH) and the survival of *E. coli* on exposure to the compounds. The oxygen analog DTF, which was degraded by uv light under the experimental conditions, showed relatively little production of singlet oxygen (an order of magnitude less than the other two compounds), yet was almost half as effective in killing *E. coli*. DTF brought about little inactivation of ADH, and relatively little protection was provided by NaN_3. The selenium analog DTS was roughly comparable to α-T in singlet oxygen pro-



DTF



DTS

duction, ADH inactivation, and *E. coli* toxicity. The authors attributed the toxicity of the oxygen analog DTF to its photodegradation, but failed to carry out experiments to detect or isolate the putative toxic byproducts, which could have been phototoxins themselves. If, for instance, a DTF-singlet oxygen adduct, or its decomposition products such as the corresponding 1,4-diketone, were itself a phototoxin damaging to *E. coli,* this result could be rationalized. The scavening of singlet oxygen by DTF could account for the low levels detected in this system. No new data were presented to confirm or refute the alleged mechanism of action of α-T or DTS, which apparently acted similarly.

Arnason and Towers have also continued to work independently on α-terthienyl's mechanism of action. Using DNA repair-deficient mutants of *E. coli* strain B, Downum *et al.* (*43*) evaluated the width of growth inhibition zones of the different mutants on petri plates. The rationale was that if α-terthienyl attacked DNA *in vivo,* growth of the DNA repair-deficient mutants would be more strongly inhibited than that of the DNA repair-competent variety. Using 8-methoxypsoralen as a known DNA photoalkylator, and bleomycin as a control for light-independent DNA damage, they challenged the various bacterial clones with α-terthienyl. The psoralen control clearly showed a 3- to 4-fold increase in growth inhibition in the repair-deficient varieties, although the degree of growth inhibition in the repair-competent strain was low. Likewise, the growth inhibition brought about by bleomycin was greater by a factor of 1.5–2 in the repair-deficient strains. α-Terthienyl caused about the same amount of growth inhibition in all varieties, suggesting that attack on DNA is not a major factor in toxicity of this compound, or at least that the DNA is attacked in a manner not reversible by the bacterial DNA repair systems of the repair-competent strain.

In the same paper, the authors again studied the oxygen dependency of the toxicity against *E. coli* and *Pseudomonas aeruginosa* using an air-tight three-neck flask, rather than inverted beakers or glove boxes as had been done previously. They again reported an absolute requirement for the presence of oxygen, in direct contradiction to Kagan's results. The action spectrum of α-terthienyl was also determined, repeating the work of Gommer and co-workers (*5, 6*), in a more quantitative fashion. The action spectrum was again found to closely parallel the uv-absorption spectrum of α-terthienyl.

Gel electrophoretic separation of cell envelope membrane proteins from *E. coli* revealed that aerobic exposure to α-terthienyl brought about changes in the band pattern only after uv exposure, as expected. The irradiated cells were ruptured, and both cell membrane and soluble cytoplasmic proteins were recovered and analyzed by electrophoresis. Both protein fractions were reported to have suffered a general blurring of bands, and a dramatic increase in the amount of very-high-molecular-weight material, suggesting random protein crosslinking.

An interesting set of results were presented concerning temperature effects on *E. coli* survival. It was found that when the bacteria were preincubated with α-terthienyl (at 37°C), and then adjusted to various temperatures and irradiated, the cultures held at lower temperature (5°C) were better able to resist the toxic effects of the phototoxin than cultures irradiated at higher temperatures up to 42°C. However, when cultures were preincubated with α-terthienyl at 5 and at 37°C, the higher temperature culture was then cooled to 5°C, and both cultures were irradiated; no substantial differences in cell survival were observed. Thus. although the temperature during irradiation did influence α-terthienyl toxicity, preincubation temperatures had no effect. The authors suggested that the lack of effects of a higher preincubation temperature, during which time the α-terthienyl may have had more mobility to diffuse into the cell and partition into various compartments, suggested that entry of the α-terthienyl into the cell was not an important factor in toxicity. The dependence of toxicity on temperature during irradiation was rationalized as restriction of O_2 or $^1O_2$ diffusion at the lower temperatures. However, the authors conceded that this effect was not well understood. The overall conclusions reached were that membrane components such as proteins were important sites of attack for α-terthienyl-generated singlet oxygen, and that DNA was not involved in any significant way. It is not clear to us that the relatively minor temperature change (in terms of physical processes) from 37 to 5°C would be expected to significantly influence oxygen mobility. Perhaps the higher temperature increased the rate of important secondary reactions, such as thermal decomposition of lipid–singlet oxygen adducts.

Arnason *et al.* (*34*) did provide quantitative data relating the quantum yield of singlet oxygen production to the *in vivo* toxicity of six compounds, including α-terthienyl, PHT, BBT, and three other polyacetylenes. Their results indicate that α-terthienyl and BBT are good singlet oxygen producers relative to the polyacetylenes, and, once again, that oxygen is required for expression of α-terthienyl toxicity against both *E. coli* and *S. cerevisiae*. No quantitative toxicity data for BBT were provided. The polyacetylenes such as PHT, on the other hand, show anaerobic phototoxicity, a direct contradiction of the report of Kagan *et al.* (*33*). We note that an oxygen requirement for expression of phototoxicity does not prove singlet oxygen is the active species; for instance, a short-lived oxidation product of α-terthienyl might be responsible, as originally suggested by Gommers and co-workers (*5, 6*). Although psoralens can generate singlet oxygen (*33*), its effective mode of action does not involve singlet oxygen. This could clearly also be true of α-terthienyl.

The effect of α-terthienyl on photosynthesis in the alga *Chlorella*, and in spinach chloroplasts, was examined by Sinclair and Arnason (*44*). The results of this study suggest a more subtle mode of action than simple membrane destruction, at least in photosynthetic organisms. These authors showed an inhibitory effect on oxygen generation in the light, a smaller inhibition of oxygen uptake in the dark, and a stimulatory effect on Photosystem I (PS I) when uncoupled from Photosystem II. They suggest that the differential effects of α-terthienyl/uv light on oxygen evolution and consumption (in living algae) may reflect a differential availability of oxygen in the two organelles, the chloroplasts being more oxygen rich due to local oxygen generation. A second hypothesis was made that α-terthienyl may bind

selectively to the chloroplast thylakoid membranes, relative to mitochondrial membranes, and the differential effect was due to a targeting of the α-terthienyl, enhancing its concentration at certain loci.

The stimulatory effect on PS I, in contradistinction to its inhibition of the intact PS I/PS II, was difficult for the authors to explain. They proposed that α-terthienyl could act as an electron donor, or else influence the distribution of excitation energy between PS I and PS II. They ruled out the first possibility by demonstrating that α-terthienyl does not act as an electron donor for PS I. They then proposed that if α-terthienyl selectively entered the thylakoid membranes, it could alter energy transfer pathways. Again, this suggests a targeting component in α-terthienyl toxicity, rather than a general indiscriminate attack on membranes.

BBT, a structurally related compound to α-terthienyl, was tested by Downum *et al.* (*43*), using the *E. coli* DNA-repair-deficient mutants used earlier with α-terthienyl. Surprisingly, they reported a differential toxicity between the repair-deficient and the repair-competent *E. coli* strains using BBT, which was not observed with α-terthienyl. They proposed that BBT operates by a different mechanism than α-terthienyl, one more closely akin to the polyacetylenes, which may not require oxygen for toxicity (*20*), although this point is in dispute (*33*). However, BBT toxicity, unlike the toxicity exhibited by other polyacetylenes (according to Arnason), did require oxygen. Furthermore, it should be noted that BBT is not a polyacetylene; it contains only one acetylene moiety. If it did operate analogously to the polyacetylenes, other conjugated monoacetylenes such as phenylacetylene should exhibit this same property. No other compounds of this type were tested. The authors propose a "hybrid phototoxic mechanism of action," although the oxygen requirement by BBT may cast doubt on this analogy. It would perhaps be surprising if two such closely related compounds as α-terthienyl and BBT operated by wholly different mechanisms. It is interesting to recall Uhlenbroek and Bijloo's original structure–activity relationship study (*4*) in which they found that a bithienyl moiety was required for nematicidal activity.

Recently, another study of the effects of α-terthienyl on erythroplasts has appeared (*45*). Examining the effects of singlet oxygen and radical scavengers on α-terthienyl hemolysis and lipid peroxidation, Towers and co-workers reported that whereas the singlet oxygen scavenger sodium azide partially inhibited hemolysis, the radical scavenger BHT (2,6-di-*tert.*-butyl-4-methylphenol) had no effect. Analysis of cell membrane lipids after α-terthienyl/uv light treatment showed that lipid peroxidation had taken place. This peroxidation was found to be inhibited by BHT but not by sodium azide. This suggested that simple lipid peroxidation is not a significant factor in erythroplast hemolysis. Membrane protein crosslinking was observed, but tritiated α-terthienyl was not incorporated into the protein fraction. Since erythroplasts lack DNA, along with many other normal cellular components, no evaluation of 3H-α-terthienyl reaction with DNA or any of the cellular organelles could be made. The rate of hemolysis was not enhanced in D_2O using α-terthienyl, but was enhanced using known photodynamic compounds methylene blue and protoporphyrin. Furthermore, the inhibition of hemolysis by sodium azide was marginal, increasing the half-time for hemolysis only from 27 min in the control to 34 min using 10 mM NaN_3. It is quite possible that localization of α-terthienyl in lipophilic regions, and *in situ* generation and consumption of singlet

oxygen in those areas inaccessible to sodium azide and D_2O, could account for these results. However, Towers and co-workers seem to overlook this explanation, or at least do not invoke it.

Again, no quantification of the rates of singlet oxygen production by α-terthienyl compared to methylene blue or protoporphyrin was provided. The concentrations of methylene blue and protoporphyrin used to produce hemolysis were not given, but it seems likely they were substantially higher than that of α-terthienyl, as in the previous work done by several members of the same group (*18*).

The question remains: if α-terthienyl and methylene blue both operate by a photodynamic production of singlet oxygen, why is α-terthienyl so much more potent on a molar basis? Clearly, there must at least be selective binding of α-terthienyl at sites where its toxic properties are more strongly expressed.

Downum *et al.* (*46*) do discuss the possibility of specific binding of α-terthienyl, similar to that mentioned earlier by Sinclair and Arnason (*44*), which could cause localized higher concentrations of singlet oxygen. However, the small degree of protection (about a 25% difference) provided by NaN_3 in this study is in strong contrast to earlier reports (*21*) of the substantial protection afforded yeast cells by 23 mM NaN_3, allowing survival rates several orders of magnitude higher in the NaN_3-protected cultures than in unprotected cultures. These workers (*43*) do continue to hold the position that singlet oxygen and its effect on membrane protein crosslinking are the major factors in erythroplast hemolysis and, they seem to argue, in the toxic properties of α-terthienyl exhibited in intact organisms. The suitability of erythroplasts as models for nucleated organisms seems questionable, when the other major proposed mechanism of action involves selective interactions with polynucleotides. Thus, the actual operative mechanism of α-terthienyl phototoxicity still seems to be in question.

Several major points remain to be resolved. The conflicting reports of the requirement for oxygen in α-terthienyl phototoxicity need to be reconciled. A quantitative study of α-terthienyl as a singlet oxygen producer relative to other singlet oxygen producers, and comparisons of their *in vivo* toxicities, should be carried out. The question of α-terthienyl targeting, or selective binding prior to irradiation, should be examined. Use of nucleated cells rather than erythroplasts lacking nuclei would be more useful in determining relative contributions of attack on nucleic acids or cellular organelles versus membrane components. Autoradiographic studies of the distribution of tritiated α-terthienyl in whole cells prior to, and following, irradiation could be useful in ascertaining possible localization of the toxin in membranes, etc. Model studies of the interaction of α-terthienyl with unsaturated lipids could provide information about possible lipid oxidation reactions.

Although the exact mechanism of biological action of α-terthienyl is as yet undetermined, the available evidence seems to indicate that α-terthienyl represents a unique class of phototoxic agents, rather than simply another example of a singlet oxygen generator. Once the details of the toxic interaction with living systems are worked out, α-terthienyl and its analogs may prove to be valuable biochemical probes, as with the psoralens, which have found much use in the study of polynucleotides.

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