National Library of Canada
Canadian Theses Service
Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Service des thèses canadiennes

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

NL-339 (r. 88/04)

Canada

Synthesis and Photochemistry of the Phototoxin α-Terthienyl and Related Compounds

by

Anita Marie Mac Eachern

A thesis
presented to the University of Ottawa
in fulfillment of the degree of
M.Sc.
in
Chemistry

© Anita Marie Mac Eachern, Ottawa, Canada, 1987.

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-40719-0

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

To my Family

and

To Claude

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. Peter Morand for his patience, encouragement and support throughout my studies. A special thanks to Dr. L. C. Leitch, whose contribution to this work was substantial. His humorous anecdotes about the eminent researchers he has known often brightened the lab. I would like to recognize the contributions of Dr. Thor Arnason. The biological data in this thesis were from tests performed in his laboratory and I would like to thank him for countless helpful discussions. Dr. Tito Scaiano supervised the photochemical aspects of this thesis and was an enthusiastic and excellent teacher. Also, for assistance during long nights on the laser, my gratitude is extended to Dr. Doug Weir and Chris Evans who were always willing to help. I gratefully acknowledge the technical assistance received from Raj Capoor for NMR spectra, H. Sequin for elemental analysis and Dr. Clem Kazakoff for mass spectra.

The journey through graduate studies has not been without its rough spots. I would like to thank my colleagues, especially Jacinta Drew and Jeffrey Atkinson for helping me maintain my sense of humor.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS i
TABLE OF CONTENTS ii
LIST OF TABLES vi
LIST OF FIGURES viii
ABSTRACT xi

CHAPTER 1. Introduction

1.1 General 1

1.2 Isolation and Structure Determination 2

1.3 Biosynthesis of Naturally Occurring Polyacetylenes and Thiophenes 4

1.3.1 Acetylene Biosynthesis 4
1.3.2 Biosynthesis of α-Terthienyl 6
1.3.3 Source of Sulfur in Thiophene Biosynthesis 9
1.3.4 Mechanism of Sulfur Addition 10

1.4 Biological Activity of α-Terthienyl and Phenylheptatriyne 11

1.4.1 Mode of Action for α-Terthienyl 12
1.4.2 Photogenotoxic Studies 16
1.4.3 Mode of Action of Phenylheptatriyne ... 16
1.4.4 Range of toxicity of α-Terthienyl and Phenylheptatriyne 18
1.4.5 Site of Action 21

1.5 The Function of Polyacetylenes and Thiophenes in Asteraceae 22

CHAPTER 2. Synthesis of PHT, α-Terthienyl and Related Compounds

2.1 Previous Syntheses of α-Terthienyl 24

2.2 The Nickel-Complex Catalysed Grignard-Wurtz Reaction 28

2.2.1 General 28
2.2.2 Catalytic Cycle 29
2.2.3 Metal Catalysts other than Nickel 33
2.2.4 Synthesis of α-Terthienyl 36
2.2.5 Solvent Effects 37
2.2.6 Side Product Formation 40

2.3 Analogues of α-Terthienyl 42

2.3.1 2,6-(Di-2-Thienyl)-Pyridine 44
2.3.2 Phenyl Analogues 45
2.3.3 Naphthalene Analogues 47

2.4 Derivatives of α-Terthienyl 48

2.4.1 Halogenation of α-Terthienyl 50
2.4.2 Methylation of α-Terthienyl 53
2.4.3 Nitro and Cyano Deivatives 55
2.4.4 Formyl and Amide Derivatives 57
2.4.5 Acrylic Acid and Alcohol Derivatives .. 59

2.5 ^{1}H NMR of α-Terthienyl Derivatives 61

2.6 Synthesis of Phenylheptatriyne and Acetylenic Compounds 62

2.6.1 Previous Syntheses of Phenylhepta-triyne 62
2.6.2 Synthesis of Phenylheptatriyne 65
2.6.3 Bromination of Terminal Acetylenes 67
2.6.4 Coupling Reactions with $Ni[dppp]Cl_2$ ... 69

CHAPTER 3. Experimental

3.1 General 73

3.1.1 General Procedure for the Grignard-Wurtz Coupling Reaction 74
3.1.2 General Procedure for Selection of Grignard Solvent 75

3.2 Preparation of 2,2':5',2"-Terthienyl in Various Ether Solvents 75

3.2.1 Large Scale Purification of α-Terthienyl 77
3.2.2 Preparation of Dichloro[1,3-Bis(diphenylphosphino)-propane]-Nickel(II), ($Ni[dppp]Cl_2$) 78

3.3 Preparation of Analogues of α-Terthienyl 78

3.3.1 2,6-(Di-2-Thienyl)-Pyridine 78
3.3.2 1,3-(Di-2-Thienyl)-Benzene 79
3.3.3 1,4-(Di-2-Thienyl)-Benzene 80
3.3.4 1-(2-Thienyl)-Naphthalene 80
3.3.5 2-(2-Thienyl)-Naphthalene 81

3.4 Synthesis of α-Terthienyl Derivatives 82

3.4.1 5-Methyl-2,2':5',2"-Terthienyl 82
3.4.2 5,5"-Dimethyl-2,2':5',2"-Terthienyl ... 83
3.4.3 5-Iodo-2,2':5',2"-Terthienyl 84
3.4.4 5,5"-Diiodo-2,2':5',2"-Terthienyl 85
3.4.5 5-Bromo and 5,5"-Dibromo-2,2':5',2"-Terthienyl 85
3.4.6 5-Cyano-2,2':5',2"-Terthienyl 86
3.4.7 5,5"-Dicyano-2,2':5',2"-Terthienyl 87
3.4.8 5-Nitro-2,2':5',2"-Terthienyl 87
3.4.9 5-Formyl-2,2':5',2"-Terthienyl 88
3.4.10 5-(2-Hydroxyethyl)-2,2':5',2"-Terthienyl 89
3.4.11 5-(2,2':5',2"-Terthienyl)-Amide 90
3.4.12 5-(2,2':5',2"-Terthienyl)-Acrylic Acid 90

3.5 Preparation of Phenylheptatriyne and Acetylenic Compounds 91

3.5.1 1-Bromo-2-Phenylacetylene 91
3.5.2 1,4-Dichloro-2-Butyne 92
3.5.3 Pentadiyne 93
3.5.4 1-Phenylhepta-1,3,5-triyne 94
3.5.5 Bromopropiolic Acid 95
3.5.6 1-Bromopenta-1,3-diyne 96
3.5.7 1-(2-Thienyl)-Penta-1,3-diyne 96
3.5.8 2-(2-Thienyl)-Phenylacetylene 97

CHAPTER 4. Photochemistry of α-Terthienyl and Related Compounds

4.1 Introduction 99

4.2 Results and Discussion 103

4.2.1 Transient Spectroscopy 103
4.2.2 Triplet Lifetimes 111
4.2.3 Oxygen Quenching Studies 114
4.2.4 Introduction: Singlet Oxygen Quantum Yields 116
4.2.5 Singlet Oxygen Quantum Yields 118

4.3 Structure-Function Relationships 126

4.3.1 Relationship Between Chemical Structure and Biological Activity 126
4.3.2 Contribution of Physical Properties to Biological Activity 131

4.4 Experimental 138

4.4.1 General 138
4.4.2 Quenching Studies 138
4.4.3 Singlet Oxygen Quantum Yields 138
4.4.4 The Laser System 139

GENERAL CONCLUSIONS 144

CLAIMS TO ORIGINAL RESEARCH 146a

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS WORK 147

REFERENCES 149

LIST OF TABLES

Page

Table 1. Target Organisms Treated with α-Terthienyl and Subjected to UV Irradiation.....19

Table 2. Target Organisms Treated with Phenylheptatriyne and Subjected to UV Irradiation.....20

Table 3. Capillary GC Retention Times of α-Terthienyl and other Thiophenic Compounds.....37a

Table 4. Yields of α-Terthienyl Obtained using Various Ether Solvents.....38a

Table 5. Capillary GC Analysis of α-Terthienyl Product Mixtures in Various Ether Solvents.....38b

Table 6. HPLC Analysis of Reaction Mixtures using Different Reaction Conditions to Methylate α-Terthienyl.....55

Table 7. ^{1}H NMR Data for Disubstituted Derivatives of α-Terthienyl.....61a

Table 8. ^{1}H NMR Data for Monosubstituted Derivatives of α-Terthienyl.....61b

Table 9. Triplet- Triplet Absorption Characteristics of α-T, Derivatives and Analogues.....109

Table 10. Triplet Lifetime Data for the Substrates Examined.....113

Table 11. Rate Constants Obtained for Oxygen Quenching...115

Table 12. Singlet Oxygen Generating Efficiencies in $CDCl_3$ at Room Temperature.....124

Table 13. The LC_{50} Values for a Series of Compounds Against Mosquito Larvae (*A. atropalpus*) After Treatment With Near-UV.....128

Table 14. Partition Coefficients for α-T and Related Compounds for an Octanol-Water System.....133

Table 15. Percent Mortality of α-T and Related Compounds Toward the Cornborer, With or Without UV Light Treatment.....134

LIST OF FIGURES

Page

Figure 1. Chromatogram of the reaction products obtained from the Grignard-Wurtz reaction in Ether/THF (1:7) after column chromatography to isolate α-terthienyl.....37b

Figure 2. Trace recorded at 460 nm following 337 nm excitation of a 0.38 mM solution of Mα-T in methanol at full laser power.....104

Figure 3. Triplet-triplet absorption spectra of ONP (A), Mα-T (B) and Brα-T (C) in methanol under a nitrogen atmosphere.....105

Figure 4. Triplet-triplet absorption spectra of CNα-T (A), TNP (B) and BTP (C) in methanol under a nitrogen atmosphere.....106

Figure 5. Triplet-triplet absorption spectrum of PYT in methanol under a nitrogen atmosphere.....107

Figure 6. Time dependent triplet-triplet spectra of DBrα-T in methanol under a nitrogen atmosphere.....107

Figure 7. Variation in triplet decay rate as a function of laser dose (as represented by the maximum observed triplet optical density, termed the Top OD) for DBrα-T (0.084 mM) in $CDCl_3$.....108

Figure 8. Variation in triplet decay rate as a function of laser dose (as represented by the maximum observed triplet optical density, termed the Top OD) in methanol under a nitrogen atmosphere for ONP (A) and TNP (B)...112

Figure 9. Bleaching observed at 400 nm for 1.64 mM α-T and 0.85 mM DPBF (trace A) and for the same concentration of DPBF in the presence of sufficient OHBP to match the optical absorption with sample A (trace B). The insert shows a benzophenone trace monitored at 525 nm (OD_{337}=1.16 for an optical path length of 0.03 cm).....122

Figure 10. Mutiple regression plots of the changes in transient optical density as a function of the relative laser dose for 11.8 mM ONP and 0.82 mM DPBF (A), the DPBF/OHBP standard (B) and the corresponding benzophenone actinometer(C).....123

Figure 11. Plot of LC_{50} values for a series of α-T derivatives and analogues, versus the log of their quantum yield of singlet oxygen generation.....135

Figure 12. Plot of LC_{50} values for a series of α-T derivatives and analogues, versus the log of their partition coefficient.....136

Figure 13. Diagram of the laser facility at the National Research Council of Canada.....140

ABSTRACT

Interest in light activated pesticides and herbicides is dramatically increasing and coming to the forefront as they offer new alternatives in environmental control of pests or weeds. These substances are generally made and used by the plant which exploits both the light energy of the sun and the presence of oxygen in the environment. One of these naturally occurring compounds isolated from the plant family Asteraceae, α-Terthienyl (α-T), has proven its potential as a potent mosquito larvicide, both in laboratory tests and field trials.

A facile large scale synthesis of α-T was developed with potential after modifications for use as a commercial process. The influence of chemical structure on biological activity of the parent compound was studied by preparing a series of derivatives and analogues with extended or altered chromophores. All of the compounds synthesized were toxic to mosquito larvae. Two of the derivatives , 5-methyl-α-T, also found in Asteraceae, and 5-cyano-α-T, were more potent larvicides than the parent compound. The methylated derivative has been prepared in order to label it with tritium for biosynthetic incorporation experiments.

The final part of this work is a photochemical study of the prepared derivatives and analogues as their mode of action is light mediated. Specifically, their singlet oxygen quantum yields were measured and correlated with mosquito larva mortality data. Assessment of structure-function relationships may allow us to predict and prepare a more efficient phototoxic larvicide.

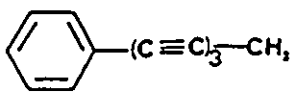
INTRODUCTION

1.1 GENERAL

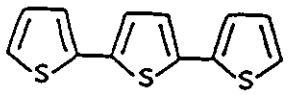
More than 700 naturally occurring polyacetylenes and biosynthetically related thiophene derivatives have now been isolated and characterized from the plant family Asteraceae[1]. Although polyacetylenes are especially prominent in Asteraceae, they can also be found in several other vascular plant families, e.g. in Apiaceae and Campanulaceae, in Basidiomycete fungi and in algae. Prior to 1950 less than 10 naturally occurring polyacetylenes were known[1]. Subsequent to this, rapid progress was made largely due to the work of three groups led by Sorensen, Bohlmann, and Jones. Sorensen, in his survey of Asteraceae, isolated many thiophenes and acetylenes including the widely distributed polyacetylene 1-phenylhepta-1,3,5-triyne (PHT, 1) which was first obtained from *Coreopsis grandiflora*[2]. Thiophene derivatives were also detected in the same extract during the purification of PHT[3].

The first naturally occurring thiophene derivative isolated in a pure form from Asteraceae was α-terthienyl (α-T, 2)[4], from the African marigold, *Tagetes erecta*. The second, junipal (3) [5-(1-propynyl-2-formyl thiophene)], was isolated from *Daedelia juniperina* a Basidiomycete[5], and contained both a thiophene and an acetylenic moiety. Challenger and Holmes[6] first suggested that there was a biosynthetic connection between polyacetylenes and thiophenes in the same plant family. It is now known that thiophenes are produced by the cyclization of two adjacent acetylenic bonds[7], with the incorporation of a sulfur atom.

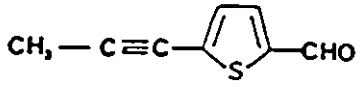
Recently, polyacetylenes and thiophene derivatives have received a great deal of attention because of their phototoxic activity[8]. This thesis describes the synthesis and biological activity of two highly active substances in this group of secondary metabolites, α-T and PHT. The photochemistry of α-T, derivatives and analogues was also examined.



1



2



3

1.2 ISOLATION AND STRUCTURE DETERMINATION

Naturally occurring acetylene and thiophene derivatives are usually isolated by ether/petroleum ether extraction[7] from the macerated roots, stems or leaves of certain plants. Repeated chromatography of the various fractions extracted, on silica gel or alumina, yields small quantities (often ug) of pure compounds. Because of the instability and sometimes explosive nature of polyacetylenes, great care must be taken in the isolation process to avoid degradation.

The structure elucidation of the isolated compound can usually be determined using spectroscopic techniques. In the earlier isolation work, the only technique available for such small quantities was ultraviolet (UV) spectroscopy.

It was, and still is, useful for detection of polyacetylenes as they have characteristic spectra[1,7] and high molar extinction coefficients. Infrared (IR) spectroscopy was not used as a primary method of identification but could be used to determine whether the alkynyl compound was mono-or disubstituted.[7]

Thiophenes with their broad UV maxima posed a major problem for early phytochemists. Sometimes a structure was suggested based on the UV spectrum and the suspected compound was then synthesized and compared to the isolated material[4,9]. This process was tedious, as synthetic methods were not as specific then, and reaction mixtures contained many side products requiring lengthy purification procedures. Identification of unknown substances was also facilitated by analyzing the products of chemical degradations, derivatizations and hydrogenations by mass spectrometry (MS) and gas chromatography (GC)[7].

Newer techniques such as GC-MS[10] and high performance liquid chromatography (HPLC)[11] are particularly suited for separation and identification of small quantities of material, and have been used successfully on thiophene and polyacetylene natural extracts[12].

The most powerful technique for complete structure elucidation of both thiophenes and polyacetylenes is ^{1}H NMR. Technological improvements are lowering the detection level so that on a 300 MHz spectrometer, a spectrum may be taken with 5-10 ug of sample. At such concentrations the solvent purity is a limiting factor. Other NMR techniques such as 2D NMR have also aided in structure determination of small quantities of natural products[13].

1.3 BIOSYNTHESIS OF NATURALLY OCCURRING POLYACETYLENES AND THIOPHENES

1.3.1 Acetylene Biosynthesis

The biosynthesis of acetylene compounds was first studied using radioisotope labeled precursors for incorporation experiments. Matricaria ester (4), a widespread polyacetylene isolated from Asteraceae and higher fungi (Agaricales), was labeled in a head to tail arrangement after feeding [1-^{14}C]-acetate to *Polyporus anthracophilus*[14].

$$CH_3\overset{*}{C}O_2^- \longrightarrow H_3C\text{-}\overset{*}{C}H\text{=}CH\text{-}\overset{*}{C}\equiv C\text{-}\overset{*}{C}\equiv C\text{-}\overset{*}{C}H\text{=}CH\text{-}\overset{*}{C}O_2CH_3$$

4

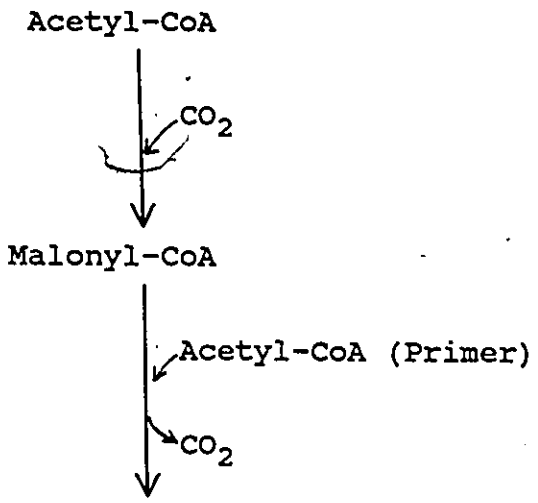
In a similar experiment, [2-^{14}C]-malonate was fed to *Tricholoma grammopoduin*. The C_{10} polyacetylene isolated (Scheme 1) contained a head to tail linkage of labeled carbons with the exception of the first two carbons, indicating that acetate was a probable precursor[15]. These results ultimately led to the hypothesis that acetylenes

$$CH_3COSCoA + 4\overset{*}{C}H_2(\overset{*}{C}O_2^-)COSCoA \longrightarrow H_3C\text{-}C\equiv\overset{*}{C}\text{-}C\equiv\overset{*}{C}\text{-}C\equiv\overset{*}{C}\text{-}CH\text{=}\overset{*}{C}HCH_2OH$$

Scheme 1.

were formed from fatty acids. In fatty acid biosynthesis[16,17] acetyl-CoA is carboxylated to yield malonyl-CoA (Scheme 2). The C_2 units of fatty acids are derived from malonyl-CoA by multistep condensations. For the condensation to begin, acetyl-CoA is required as a primer. Incorporations of both malonate and acetate

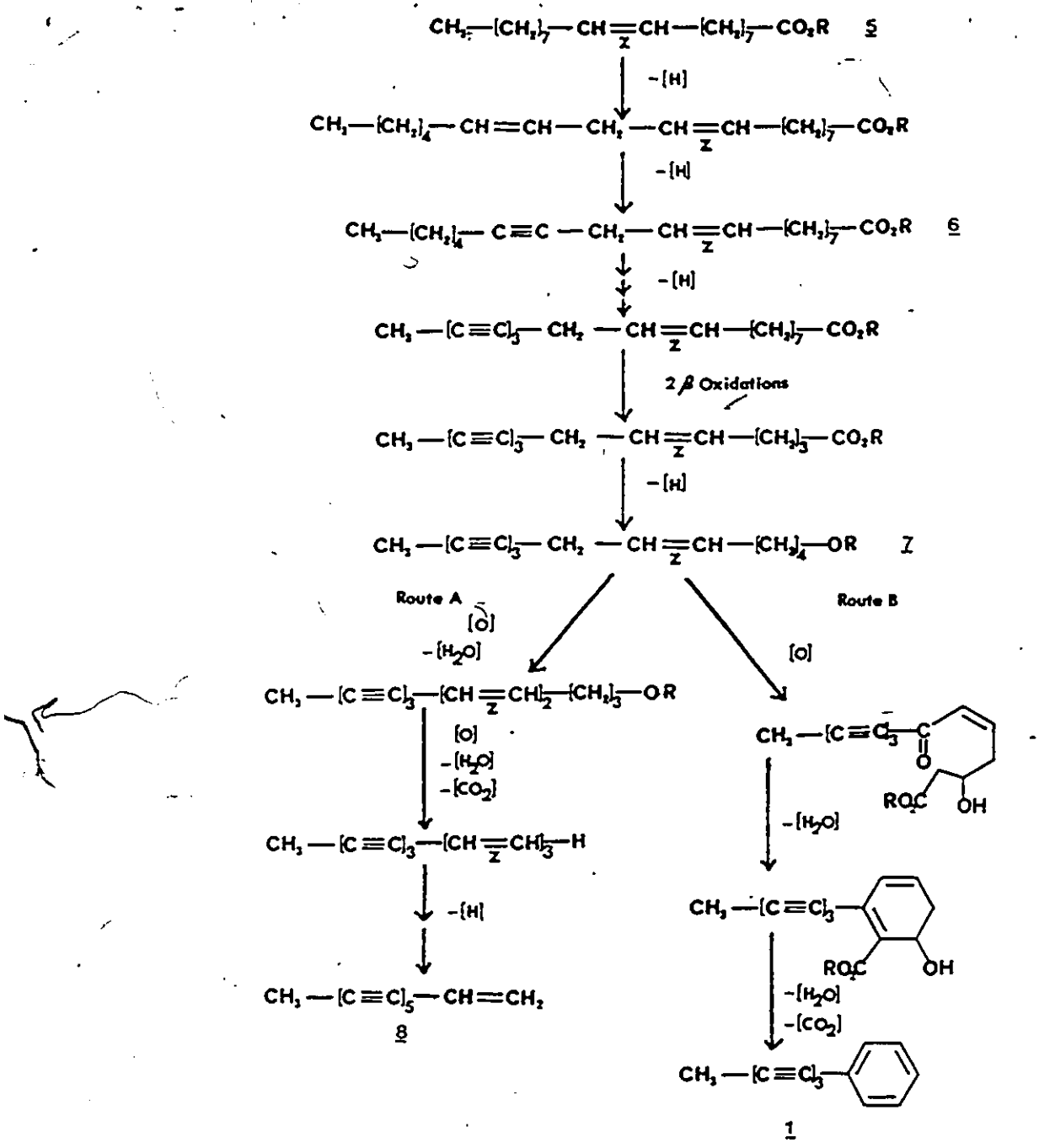
observed in polyacetylene biosynthesis is as expected for fatty acid derived molecules. In an incorporation study



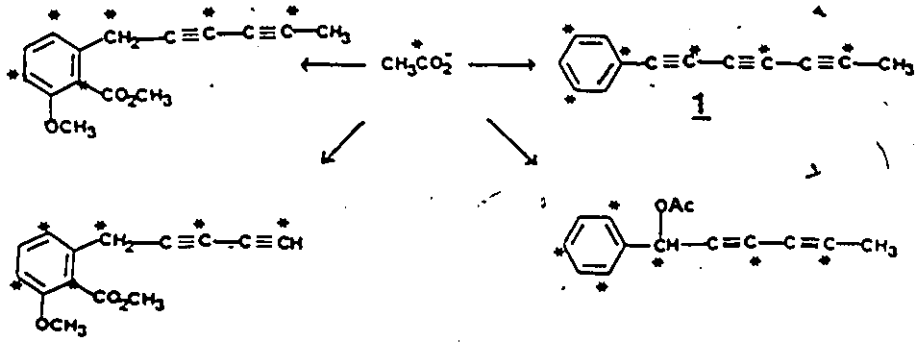
Scheme 2.

using oleic acid (5) as the labeled precursor[15], this hypothesis was confirmed. It was also determined that while oleic acid is a precursor, fatty acids with shortened chains[7] are not incorporated.

It is not really known at which stage the characteristic structural changes occur in the biosynthesis[7] of polyacetylenes. Scheme 3 shows how this pathway could proceed from oleic acid (5) using known mechanisms of fatty acid chain shortening (β oxidation) and hydrogenation systems. Key acetylenes such as crepenynic acid (6) may also branch off the pathway shown and lead to many other naturally occurring acetylenes. Route A ends with the important polyacetylene, tridecapentynene (8) and route B shows the biosynthesis of PHT (1). The experimental work reported in support of these pathways is described below.



Scheme 3.



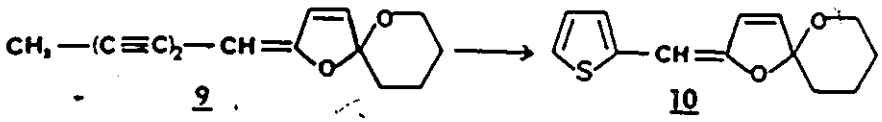
Scheme 4.

Tritium from doubly labeled [10,11-^{3}H]-oleic acid was incorporated into PHT[18], suggesting that aryl substituted acetylenes are derived from the same fatty acid precursor as other naturally occurring acetylenes. Feeding studies with [1-^{14}C]-acetate incorporated the labeled precursor into PHT and some closely related compounds (Scheme 4)[15]. With the methyl group of acetylenic precursor 7 tritium labeled and fed to *Coreopsis lanceolata*, PHT with a tritiated methyl group was isolated[19]. All of these labeling studies support the now generally accepted biosynthetic pathway to PHT. Definitive studies will require isolation and characterization of the key enzymes, none of which have yet been isolated, even in crude extracts.

1.3.2 Biosynthesis of α-Terthienyl

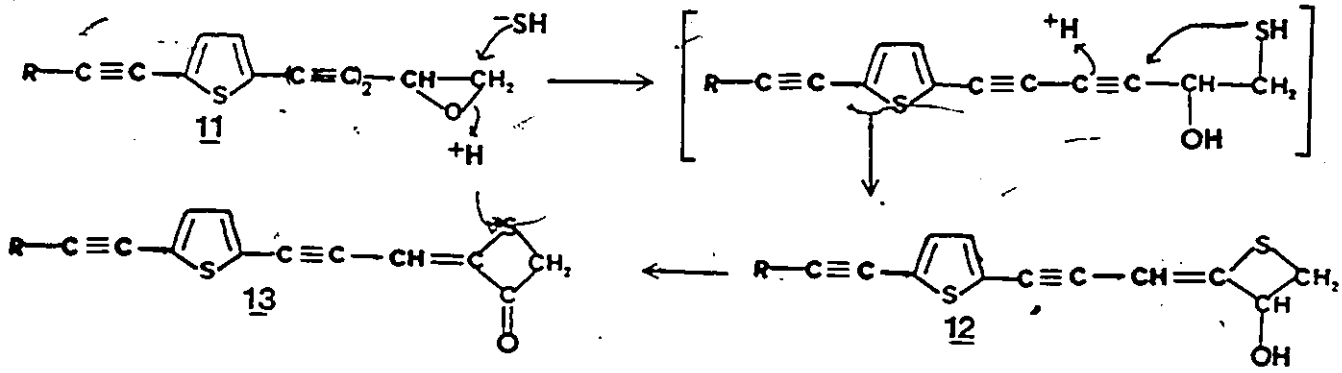
Through biosynthetic feeding studies with labeled precursors, it has been shown that most naturally occurring thiophenes have been formed via acetylenes[7]. An enormous diversity of functionalized thiophenes have been isolated, including such structures as a spiroenol ether (10) found in

Artemisia ludoviciana and the corresponding acetylene (9) from which it has been derived. Other unusual thiophenes



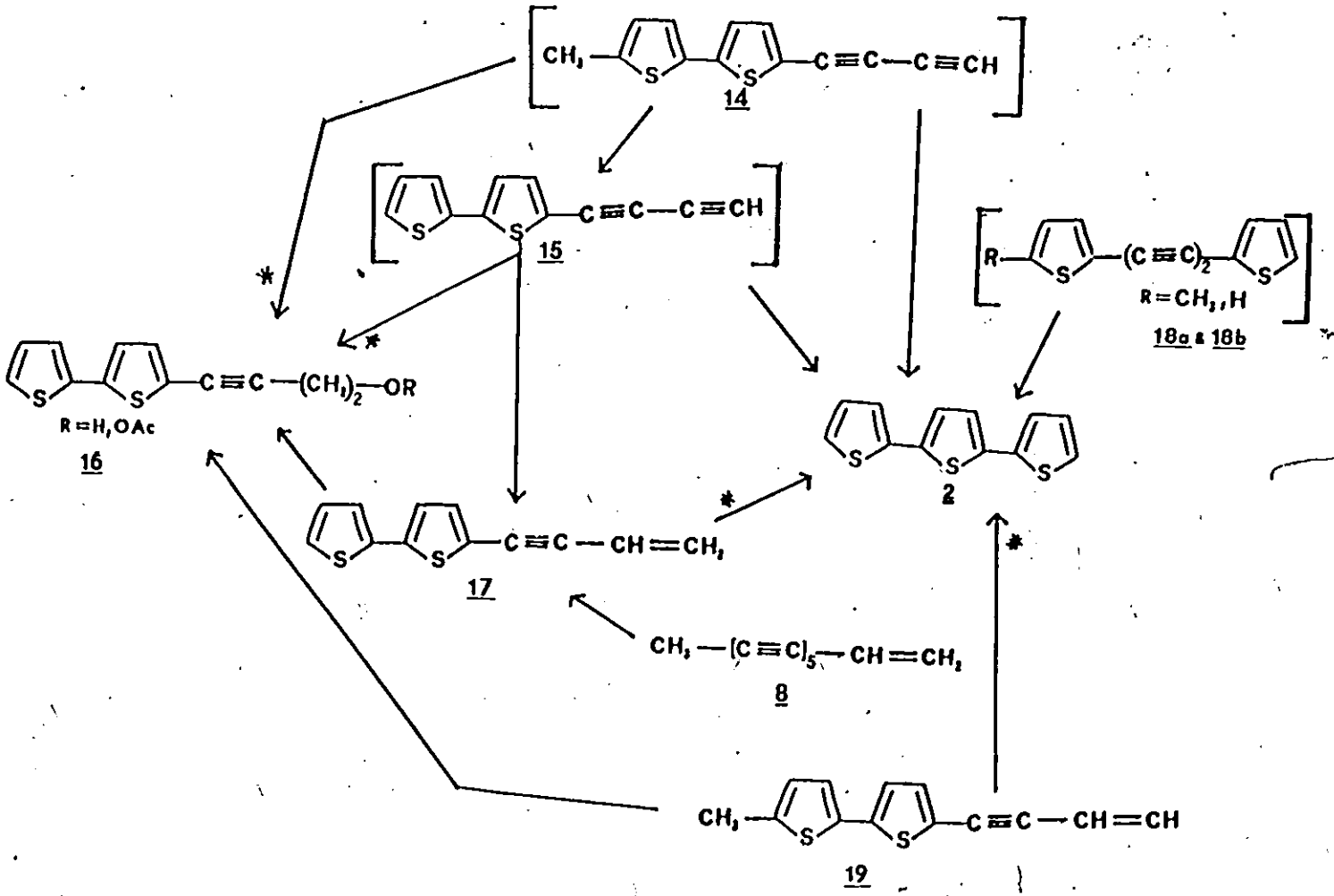
9 10

have been found in the *Berkheya*[20] and *Cullumia*[21] species (13,12) and are proposed to occur via an epoxide (11). α-T



has been shown by labeling studies[7] to originate from the long chain polyacetylene, tridecapentynene (8).

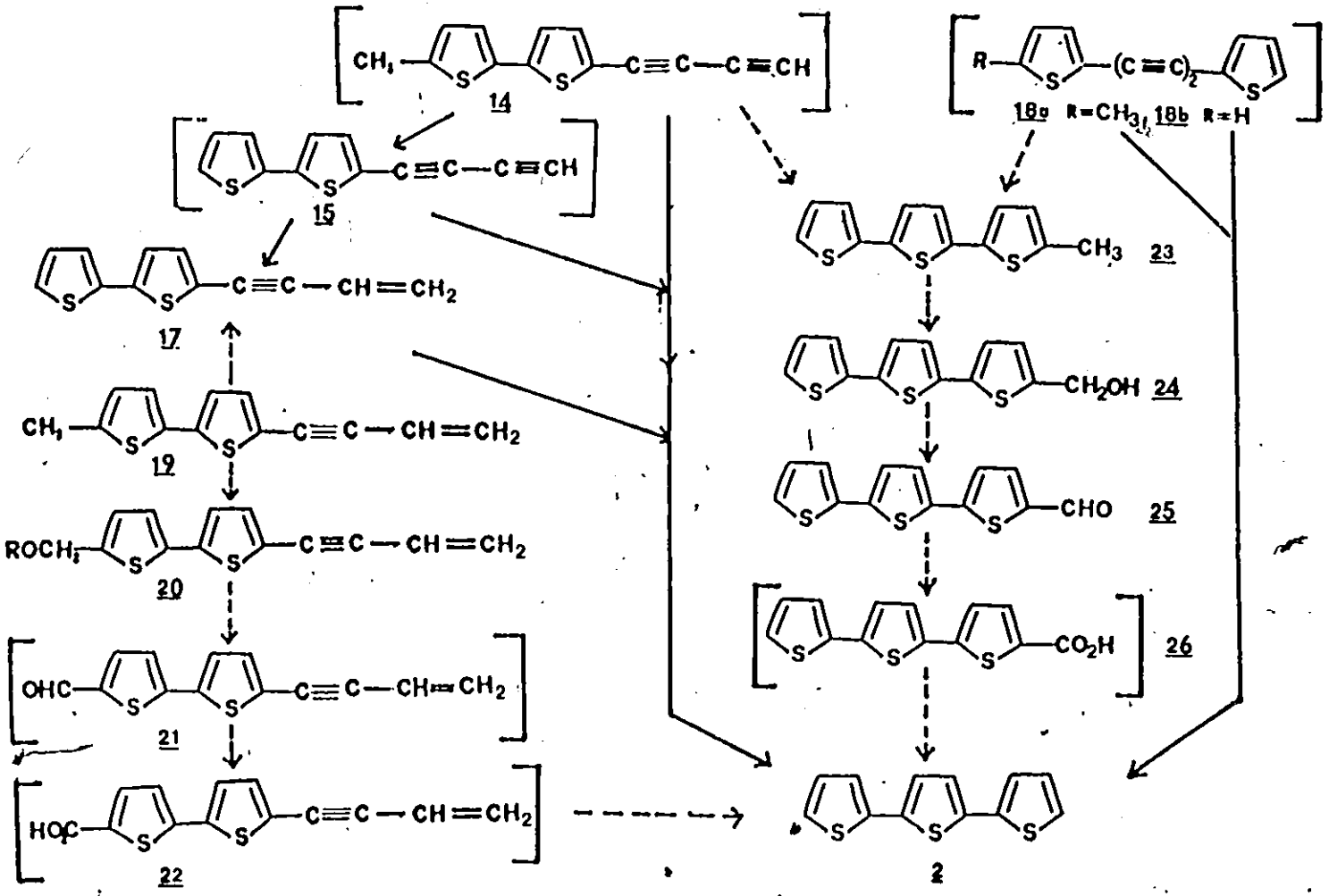
Feeding studies have been performed using the most widespread bithienyl compound (17) and suspected precursor to α-T. In two cases[7,22] it has been reported that incorporation into α-T does not occur. The feeding studies by Jente *et al.*[23] showed a low incorporation of labeled 17 and 19 into α-T. Scheme 5 illustrates the results of incorporation studies while Scheme 6 represents the possible biosynthetic routes to α-T based both on incorporation studies and isolation studies.



Scheme 5. Incorporation studies.

[ ] Indicates a compound not previously isolated from a plant source.

* Indicates a low incorporation (≤0.005%).



Scheme 6. Isolation and incorporation studies.

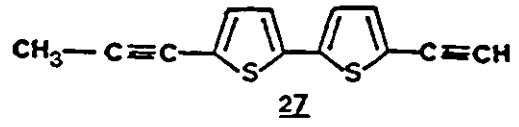
____ Incorporation study

-,- Hypothetical route

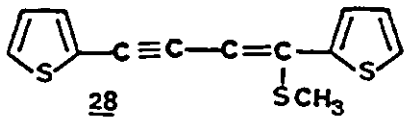
[ ] Indicates a compound not previously isolated from a plant source.

Further incorporation studies have shown that the bithienyl diynes 14 and 15 are the most likely precursors to α-T[22-24]. These postulated precursors have never been isolated from a plant source, although a closely related compound has been found (27). The terminal diyne group is so reactive that its presence *per se* in the plant environment would be very difficult to prove.

In feeding studies with 18a and 18b, incorporation into α-T was achieved[22] in high yields. As these derivatives cause no disruption in the plant metabolic path, it is probable that the order in which the rings are formed is not crucial in the formation of α-T. These compounds have not been found in a plant source although there is some support for their existence. For example the thioenol derivative (28) has been isolated from the *Berkheya*[20] species.



27



28

With labeled 15, plants are also able to synthesize 17 and the bithienylbutynol acetate (16)[22]. Later work[23] indicated that the plant, in this case *Tagetes patula*, had the ability to hydrogenate triple bonds and to form saturated carbon chains via the addition of water or acetic acid. It was also shown that the metabolic conversion of 14 to 15 was very rapid. There was no available information on the conversion of 19 to 17. The demethylation of the diynes is currently thought to be an

oxidative mechanism involving loss of CO_2. This may be an important step in the formation of α-T.

It is still not known at which stage the α-methyl group (Scheme 6) is removed in the biosynthesis of α-T, before or after the third thiophene ring is formed. The oxidative mechanism currently proposed suggesting 23 as a precursor to α-T is supported by other terthienyl derivatives that have been isolated[26]. The alcohol (24), aldehyde (25) and the methyl ester of the alcohol (24) have been isolated from *Eclipta erecta*.

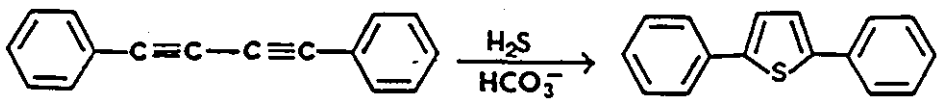
Definitive enzymatic studies of thiophene metabolism are just beginning with the first report by Sutfield and Towers[27] of a plant enzyme mediated polyacetylenic conversion. They have also studied the hydrolysis of 3,4-diacetylbutynyl-bithiophene in the same plant and were able to show that the compound is sequentially hydrolyzed[28] by two specific enzymes.

1.3.3 Source of Sulfur in Thiophene Biosynthesis

The exact source of sulfur in cyclizing conjugated triple bonds to thiophene rings has not been definitively identified for Asteraceae. In the laboratory, the addition of sulfur has been accomplished by adding H_2S to a diyne under slightly alkaline conditions[29] (Scheme 7), or by refluxing the diyne with $Na_2S \cdot 9H_2O$ in dioxane or tetrahydofuran (THF)[30].

The search for the biological equivalent of H_2S initiated labeling studies with sulfur containing amino acids, and salts. With [^{35}S]-cysteine in feeding studies with the plant *Chrysathemum vulgare*, an alkynal thiophene

was isolated[31] containing 14% of the activity incorporated into the lipid soluble compounds.



Scheme 7.

Other radioactive sulfur containing precursors were fed to *T. erecta* and the incorporation into α-T was measured. Incorporation into α-T occurred when fed sodium [^{35}S]-sulfate or DL[^{35}S]-methionine but not sodium hydrogen sulfide[32]. Carbon was incorporated with DL[2-^{14}C]-methionine (0.01% incorporation) and sodium [1-^{14}C]-acetate. The sodium acetate incorporation was very low (0.004%). No level of incorporation into α-T was observed with DL[2-^{14}C]-glutamic acid or [2,3-^{14}C]-succinic acid. From these results, it is very difficult to identify the source of sulfur in thiophene biosynthesis, although the lack of incorporation from the labeled sulfide suggests that H_2S is not involved. Further study with more convincing incorporation levels is required before the true source of sulfur *in vivo* can be established. For our purposes [S] will signify the biological source of sulfur.

1.3.4 Mechanism of Sulfur Addition

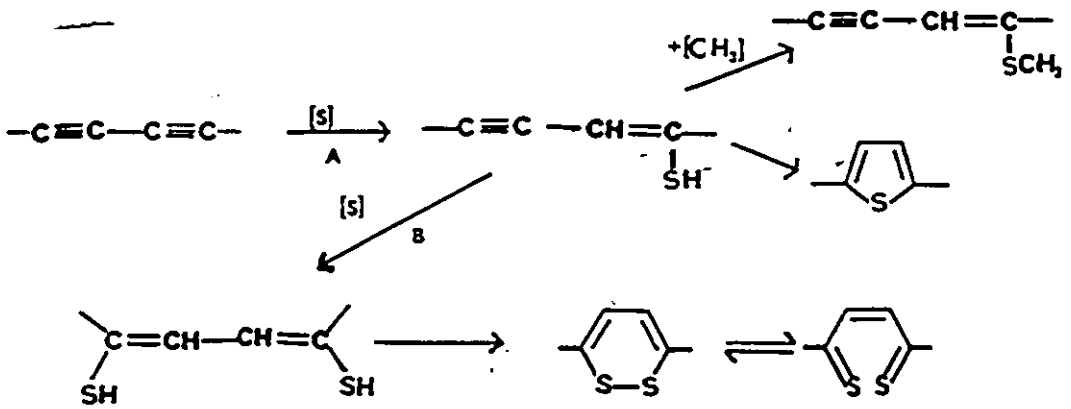
The mechanism of sulfur addition is thought to be a two-step process[7,33], due to the existence of many thioenol ethers. Tridecapentynene (8) and one of its

thioenol derivatives (29) have been isolated from *E. erecta*[26]. Single addition of [S] (Scheme 8A) may result in

$$CH_3[C{\equiv}C]_2\text{-}C(SCH_3){=}CH\text{-}[C{\equiv}C]_2\text{-}CH{=}CH_2$$

29

further methylation to the thio ether or cyclization to a thiophene ring. Double addition of [S] via route B leads to disulfide derivatives. A number of these red colored disulfides have been isolated from *Eriophyllum caespitosum* and yield thiophenes after heating [7,33].



Scheme 8.

1.4 BIOLOGICAL ACTIVITY OF α-TERTHIENYL AND PHENYLHEPTATRIYNE

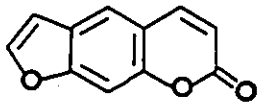
The isolation and identification of various phytochemicals including PHT[2] and α-T[4] were reported many years before their nematicidal properties were discovered[34,35]. A reinvestigation of previously and newly

isolated phytochemicals began, especially for thiophenes and polyacetylenes from Asteraceae, when it was shown that the biological toxicity of α-T was enhanced in sunlight[36,37]. Chan *et al.*[38] reported α-T as phototoxic to the yeast *Candida albicans* as were PHT and other polyacetylenes when subjected to UV irradiation. These unusual phototoxic properties prompted studies into their precise mode of action and further biological testing to evaluate the scope of their toxicity and commercial potential.

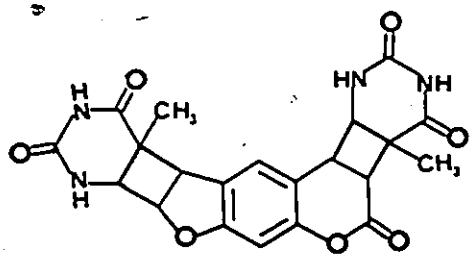
1.4.1 Mode of Action for α-Terthienyl

The photosensitizations involving oxygen such as occur with α-T are called photodynamic sensitizations. They are distinguished from nonphotodynamic sensitizations which do not involve oxygen. An example of this latter type are photosensitizations by the furanocoumarins(30), natural products which react with DNA when in their excited state. They form photoadducts with free pyrimidines, cross-linking the strands of DNA[39] (31), and interfering with crucial functions within the cell such as transcription.

In photodynamic sensitizations, type I or type II processes are possible. Anthraquinone dyes and flavins operate via a Type I process, producing superoxide radicals ($O_2^{-\cdot}$) through a series of electron processes after absorption of a photon. In Type II photosensitizations, singlet oxygen ($^1O_2$) is produced by energy transfer from the excited photosensitizer to molecular oxygen. For both types of photodynamic sensitizations, the activated oxygen species, whether superoxide radical or singlet oxygen, causes extensive biological damage.



30



31

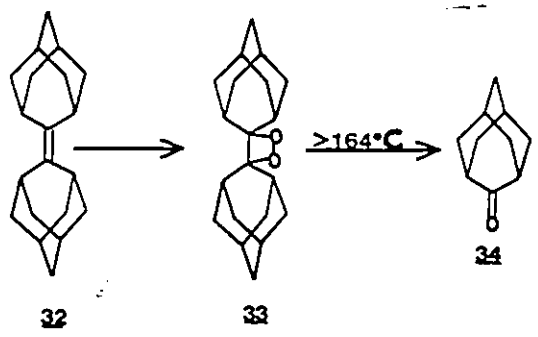
The first mechanistic study on α-T reported[40] the effect of α-T on glucose-6-P-dehydrogenase (G6-P), cholinesterase and malonate dehydrogenase, all isolated from homogenates of the plant nematode *Ditylenchus dipsaci*. It was found that the activities of the enzymes decreased dramatically when α-T was applied and subjected to UV irradiation. Dithionite and mercaptoethanol, both scavengers of oxygen, created an anaerobic environment which protected G6-P from damage by α-T. Only a slight decrease in activity was observed under a nitrogen atmosphere. These experiments demonstrated the dependence of α-T toxicity on the presence of oxygen *in vitro*.

In order to determine which activated oxygen species was being produced by α-T, Bakker and Gommers[40], performed a number of experiments. The lifetime of singlet oxygen is 10 times longer[41] in D_2O than in water, which means that biological damage is more extensive in the former. An enzyme sample (G6-P) when treated in D_2O with α-T and subjected to UV light for 25 min, retained 45% less activity than when subjected to the same treatment but in H_2O.

The investigators also tested other quenchers and inhibitors of singlet oxygen on the enzyme system. The

singlet oxygen scavengers methionine, histidine and sodium azide protected the enzyme from damage by α-T. Catalase, an enzyme which scavenges peroxide did not have any effect on the enzyme, nor did the addition of H_2O_2. Superoxide dismutase (SOD) also did not affect the enzyme, eliminating a Type I mechanism. Addition of EDTA to check for metal-oxygen complexation had no effect. The hydroxyl radical was eliminated as the active oxygen species since the hydroxyl radical scavengers mannitol and benzoate did not affect photosensitization of the enzyme.

These experiments led to the conclusion that singlet oxygen was the active oxygen species *in vitro*. Direct evidence was obtained for the presence of singlet oxygen by isolating a chemical product formed via singlet oxygen. After UV irradiation (325-400 nm) of an air saturated solution, containing the olefin adamantylidene adamantane(32) and a-T for 7.0 h, the dioxetane (33) formed and no trace of 32 could be detected (Scheme 9.). GC analysis indicated the formation of the ketone 34 from the dioxetane 33. When the experiment was repeated without α-T, 33 and 34 were not formed.



Scheme 9.

In the first *in vivo* experiments on the mechanism of photosensitization, Kagan *et al.* reported[42] on the non-photodynamic properties of α-T. The tests showed a-T was toxic to *Escherichia coli*, strain B, only in the absence of oxygen. They also found no protective effect to the yeast *Candida utilus* when adding sodium azide to irradiated α-T. They also reported that radioactive α-T intercalated with calf thymus DNA and with the DNA of *C. utilus*. This paper, with a conflicting nonphotodynamic mechanism, was later retracted by the authors[43] as were several other publications[44-46] stating that the toxicity of α-T was oxygen independent. The authors explain that there was "unexpected difficulty in obtaining a truly anaerobic environment".

Arnason *et al.*[47] also studied the mechanism of α-T *in vivo* on the microorganism *E. coli*. They reported that under anaerobic conditions, α-T displayed no photo-sensitizing activity. Protection from α-T was provided to the yeast *S. cerevisiae* by adding the singlet oxygen scavenger sodium azide to the cells. The authors explained[47] that the concentration utilized by Kagan in the previous study was too low to effectively quench $^1O_2$[48]. *E. coli* was slightly protected from α-T in the presence of UV by treatment with bovine SOD, so the production of superoxide radical could not be ruled out. They concluded that α-T acts as a photodynamic Type II sensitizer *in vivo*.

Chemical evidence of singlet oxygen production by α-T and PHT was obtained[49] using cholesterol. When the latter is oxidized by singlet oxygen it forms 3β-hydroxy-5α-cholest-6-ene 5-hydroperoxide. Radical oxidations yield the 7β-hydroperoxy compound as the main product[50]. Both α-T and

PHT irradiated in a solution with cholesterol formed the 5α-hydroperoxy derivative, with α-T yielding the larger amount.

1.4.2 Photogenotoxic Studies

The possibility of a photogenotoxic mechanism by α-T has been the subject of some conflicting studies. The results by Kagan[42], showing incorporation of radioactive α-T into calf thymus DNA, suggested that α-T was a monoalkylator and that this was an important part of its toxic effect. This *in vitro* study may not accurately portray the biological activity of α-T in a living organism. A subsequent *in vivo* study by MacRae *et al.*[51] found no evidence to show that *E. coli* DNA was damaged by α-T upon irradiation with UV light. Another study[52], using DNA repair-deficient *E. coli* mutants that are vulnerable to compounds which damage DNA, showed the same sensitivity to α-T upon irradiation as wild type *E. coli*. This strongly indicated that the damage to the microorganisms was not due to α-T/DNA intercalation. The mechanism for PHT also does not involve cross-linking of DNA[53].

1.4.3 Mode of Action of Phenylheptatriyne

While the mechanism of α-T has clearly been shown to be a photodynamic Type II process, the mechanism of action of PHT has been much harder to elucidate. The hemolysis of red blood cells[54] by PHT was enhanced in the presence of UV light but was oxygen independent. Photodegradation was a consideration when proposing a mechanism since more than 50% of PHT was destroyed after 30 min irradiation. An in depth study into the mode of action of PHT using *E. coli* and *S. cerevisiae* as the test organisms[55] showed that, unlike α-T, PHT was slightly less

effective in an oxygen environment. It was also confirmed that the mechanism was nonphotodynamic by testing the effects of sodium azide, D_2O and SOD. In each case, there was very little effect on *E. coli* survival curves. Slightly greater toxicity was observed when tests were carried out in D_2O instead of H_2O. The results indicated that PHT did not react like a classical Type II photosensitizer. A radical mechanism was proposed as this type of mechanism had previously been suggested for acetylenes[56,57] and a radical species can cause biological damage or polymerize.

A later more detailed study on several polyacetylenes by the same authors[58] suggested that for polyacetylenes both photodynamic and nonphotodynamic mechanisms compete for excitation. The *in vitro* generation of singlet oxygen was measured using the singlet oxygen scavenger, 2,5-dimethylfuran. It was found that α-T and PHT were good singlet oxygen generators and that long chain polyacetylenes were also generating some singlet oxygen although not as much as would be expected based on their toxicity to yeast and *E. coli*. This supported their suggestion of a nonphotodynamic oxygen independent mechanism for aliphatic acetylenes. PHT, a ring stabilized polyacetylene has an intermediate mechanism operating with competition between a radical based pathway and one involving singlet oxygen.

A conflicting interpretation was proposed for the mode of action of PHT by Kagan *et al.*[59]. In a study using *E coli*, the toxicity of PHT was found to be oxygen dependent and operating via a Type II mechanism analogous to that of α-T. This point is still not completely resolved and at

present, the author maintains that it is the correct mechanism for PHT[43].

1.4.4 Range of Toxicity of α-Terthienyl and Phenylheptatriyne

The toxicity of α-T and PHT toward microorganisms and nematodes (see Tables 1 and 2), both in the presence and absence of UV, eventually led to the discovery that these secondary metabolites were potent larvicides. Arnason *et al.*[66] examined a broad selection of polyacetylene compounds from Asteraceae, including α-T and PHT, for mosquito larvicidal activity when subjected to near UV. α-T and PHT were two of the compounds which induced 50% or greater mortality to fourth-instar mosquito larvae when irradiated. α-T was the most potent larvicide with an LC_{50} = 0.019 ppm (lethal concentration for half of the insects tested in parts per million), a lower LC_{50} than for the commercial insecticide DDT.

As a potent phototoxin against mosquito larvae, α-T was the best candidate for further evaluation. A large scale synthesis allowed field trials and laboratory tests to be conducted against mosquito larvae (*Aedes intrudens*) and blackfly larvae (*Simulium verecundum*)[81]. α-T was slightly less toxic to blackfly larvae with an LC_{50} = 0.028 ppm, but able to control third and fourth-instar mosquito larvae with an application of only 0.1 kg/ha to the natural pools. In these experiments the half-life of α-T was determined as 4 h in the outside pools and a gradual reduction in mortality was obtained when larvae were added to the pools on subsequent days after treatment.

Table 1. Target Organisms Treated with α-Terthienyl and Subjected to UV Irradiation.

Target Organism	References
Microorganisms	38,42,44,47,52,60-62
Fungi	38,47,63
Nematodes	37,64
Insects	59,65-71
Plants	72
Human Skin	38
Red Blood Cell	73,74
Algae	75

This same group examined nontarget effects of α-T on a variety of organisms[82] under field or laboratory conditions. Many of the test organisms survived α-T treatment better than the target mosquito and only the closely related midge larvae and daphnia were killed at the concentrations necessary to control *A. intrudens*. This study, showing acceptable nontarget effects, is encouraging for further development of the most extensively studied naturally occurring Type II phototoxin.

As well as larvicidal activity, α-T and PHT have antifeedant activity which can be extremely potent to some species of phytophagous insects. PHT was a strong feeding deterrent to late instar larvae of the dark-sided cutworm *Euxoa messoria*[83], a herbivore whose diet does not contain members of the Asteraceae. PHT interfered with their diet

Table 2. Target Organisms Treated with Phenylheptatriyne and Subjected to UV Irradiation.

Target Organism	References
Microorganisms	47,53,59,76
Nematodes	37,65
Trematodes	77
Fungi	76
Insects	65,66
Algae	75
Plants	72
Bacteriophages	78
Animal Viruses	79
Red Blood Cells	74,80

at concentrations of 10-300 ppm and only 1/3 of the control feeding was observed at the lowest concentration. The PC_{50} (protective concentration) of PHT was determined as 119 ug/g[70]. α-T was a more potent antifeedant with a PC_{50} = 6.2 ug/g for the same insect. Neonates of the tobacco hornworm (*Manduca sexta*), which usually feed on nonphototoxic Solanaceae, were strongly deterred by both α-T and PHT. The cornborer (*Ostrinia nubilalis*) has a host range which includes Asteraceae and was able to avoid photosensitization by behavioral adaptations such as spinning silk and boring into diet cubes during testing. In their natural environment they bore into the stem of the

plant. Slight inhibition of feeding was found for both α-T and PHT with the thiophenes more inhibitory than the polyacetylenes and greater inhibition with increasing number of thiophene rings in the compound.

PHT and α-T have been shown to be phototoxic to a broad range of test organisms, plants and insects. Tables 1 and 2 summarize these studies using α-T and PHT respectively, as photosensitizers. The target organism is indicated as well as the literature reference.

1.4.5 Site of Action

The site of action of α-T and PHT is thought to be the cell membrane. Membrane components such as unsaturated fatty acids and cholesterol are known to react with singlet oxygen *in vitro*[84] and initially form hydroperoxides. Yamamoto *et al.*[73] studied the inactivation of enzymes in erythrocytes by α-T and PHT. After irradiation and before hemolysis of the red blood cell acetylcholinesterase, an enzyme located on the outer membrane was strongly inactivated. The cytoplasmic enzymes were inactivated only after hemolysis. It was concluded that the membrane was the primary target and that membrane proteins can be damaged by PHT and α-T. Damage to the red blood cell membrane before complete hemolysis during a similar experiment has been observed[74] by electron scanning microscopy. Confirmation of protein damage was found by Downum *et al.*[62] on their studies with *E. Coli* where, after α-T treatment, the membrane proteins were crosslinked and formed high molecular weight aggregates. The partition coefficients of α-T[85] and PHT[58,85] are very high indicating strongly lipophilic molecules which would certainly be soluble in membranes.

A new test designed to elucidate both mutagenicity and mode of action of a phototoxin[86] was performed on α-T. A series of four *E. coli* mutants with specific genes rendering them sensitive to far or near UV irradiation were treated with α-T. The inactivation kinetics for α-T for the four strains were all the same with the *E. coli* sensitivity to near or far UV having no effect on mortality. α-T was also not mutagenic to the *E. coli*. This result was consistent for a photosensitizer with a mode of action involving destruction of the membranes.

1.5 THE FUNCTION OF POLYACETYLENES AND THIOPHENES IN ASTERACEAE

Plants do not have the same protection against predators or pathogens utilized by most other animals or insects, i.e. the ability to avoid a threat or stress by physically changing location. They are sedentary and so it has been suggested that they have developed a chemical method of discouraging predation[87]. The current hypothesis concerning the function of secondary metabolites such as the polyacetylenes and thiophenes suggest they have evolved in order to provide a protective advantage against insect herbivores and other pathogens[88].

Studies[89] on the concentration of secondary metabolites in plants experiencing environmental stress have reinforced this position. Treatment of two week old *T. erecta* seedlings by a moderately virulent fungus, *Fusarium oxysporum* var *callistephi* race 2 (FOVC race 2) elicited a phytoalexin response as indicated by the accumulation of thiophene derivatives in the the roots and stems. The thiophenes identified were α-T, 5-(4-hydroxy-1-butenyl)-2,2'-bithienyl (Scheme 5., 16) and 5-(but-3-en-1-

ynyl)-2,2'-bithienyl (Scheme 5., 17). Treatment of 4 day old seedlings with the highly pathogenic fungi, *F. oxysporum* f.sp. *radicis lycoperisici* (FORL) resulted in a decrease of thiophenes isolated. This recent work supports the hypothesis that thiophenes and polyacetylenes function as part of a sophisticated plant defense system.

The taxonomic importance of these compounds is also currently being explored[7,11]. Genera of the Pectidinae contain a biochemical difference in their ability to synthesize acetylenic thiophenes and this provides a subclassification into two groups, Pectis, which does not contain these secondary metabolites and the remaining genera which have the ability to synthesize them[11]. Further studies on the distribution of these phototoxins in plants, plant- insect interactions, and their biological activity against pathogens, will enhance understanding of the ecological and environmental importance of these compounds[90,91].

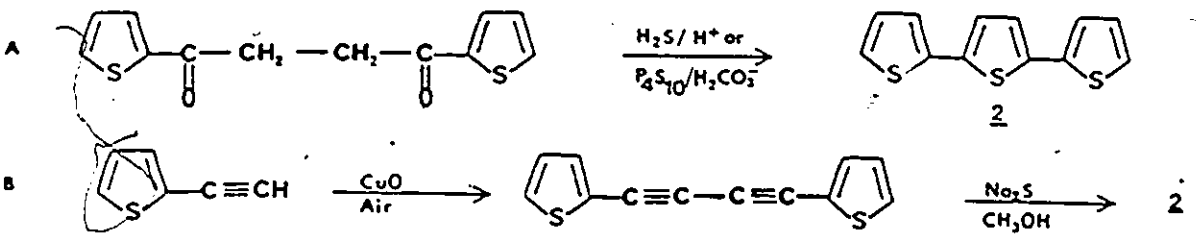
SYNTHESIS OF PHT, α-TERTHIENYL AND RELATED COMPOUNDS

2.1 PREVIOUS SYNTHESES OF α-TERTHIENYL

The structure of α-T essentially limits the number of synthetic approaches to this molecule. Several main routes have been developed since the first synthesis was reported in 1937[92]. The earliest syntheses employed the most obvious approach, coupling thiophene moieties to produce the trimer. Zechmeister and Sease[9] used the Ullmann reaction, heating 2-iodothiophene to a temperature over 200 °C with copper bronze as catalyst. This reaction gave a variety of products, from 2,2'-bithienyl (10% yield) to α-quinquethienyl (0.04% yield), with only a 3% yield of α-T. The separation of this mixture required tedious, repeated chromatography. The low yield of α-T by this method is largely due to the lack of control in the number of coupled thiophene monomers. The same lack of control is evident in an α-T synthesis patented[93] in 1959 for which no yield is given; large quantities of sulfur were added to saturated or unsaturated hydrocarbons (C_{12}-C_{20}) at high temperatures (200-600 °C) for 15 h.

Another approach for preparing α-T is to make an intermediate with a thiophene ring on either end and then to generate the middle ring by cyclization. The 1,4-di-(2-thienyl) diketone (Scheme 10A) has been cyclized successfully to yield α-T when treated with acid and H_2S[94,95] or tetraphosphorus decasulfide and sodium hydrogen carbonate[96]. In the latter case, Lawessons's reagent ($P_2S_4[p\text{-}C_6H_5\text{-}OCH_3]_2$) was equally effective. Treating the diketone with acetic anhydride and hydrochloric acid gave 2,5-di-(2-thienyl)-furan in 87% yield[94].

Another intermediate, 1,3-di-(2-thienyl)-butadiyne, has been cyclized to α-T[30], and has been postulated as a precursor in the biosynthetic pathway to the target molecule. The ethynyl thiophene was synthesized by



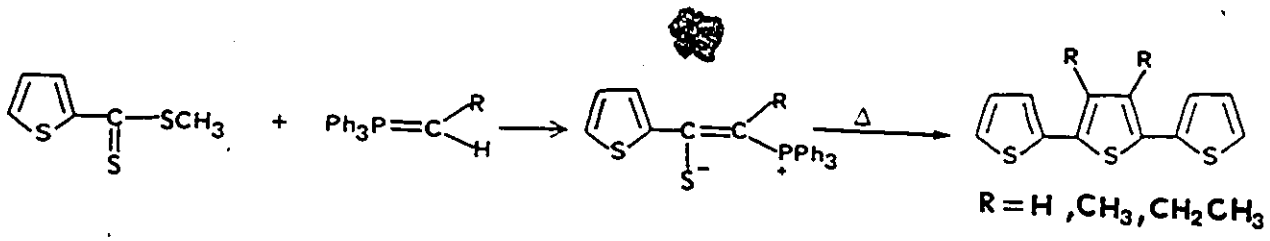
Scheme 10.

performing a Wittig reaction on 2-formylthiophene with CBr_4, followed by treatment of the product with butyllithium. The ethynyl thiophene was coupled by the Glaser method and cyclized to give α-T in 66% yield (Scheme 10B). ^{14}C-(2',5") labelled α-T[97] was synthesized in 12% yield by starting with labelled aldehyde and following the same synthetic scheme.

Bestmann and Schaper[98] presented a different approach to the synthesis of α-T using a modified Wittig reaction in which a stabilized ylide was prepared from the dithio methyl ester of thiophene and a methyl phosphorane (Scheme 11). Pyrolysis of the ylide gave α-T in 45% yield. An advantage of this procedure is the possibility of introducing substituents in the middle ring of α-T.

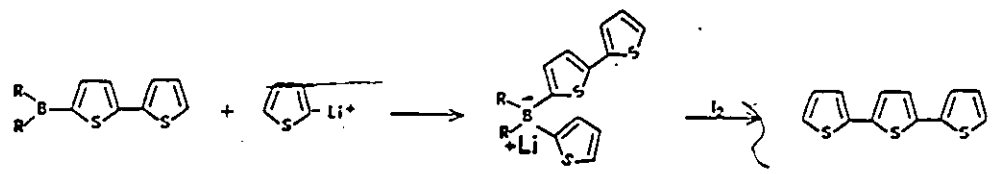
Unlike the earlier coupling reactions that gave α-T in low yield with many side products, new coupling

reactions have been developed to prepare α-T. The improvement in methodology was due largely to the diversity and better understanding of organometallics as mediators and catalysts.



Scheme 11.

A stepwise approach of adding thiophene units is now possible using organoborane reagents[99]. In one preparation (Scheme 12) B-methoxy-9-borabicyclo [3.3.1] nonane (B-methoxy-9BBN) was treated with lithiobithienyl, neutralized with boron trifluoride and treated again with lithiothiophene. Iodine oxidation of the complex formed gave α-T in 37% yield. The scope of this one-pot coupling reaction has not been determined for substituted thiophenes but the reaction is particularly convenient for

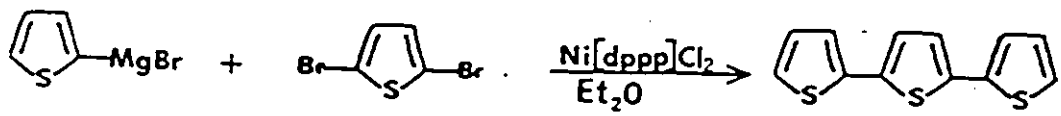


Scheme 12.

polythiophene oligomers which can be synthesized in high yields. This method allows for the sequential addition of thiophene rings and is not plagued by formation of significant amounts of side products. Lithium salts of terminal acetylenes can also be successively introduced onto B-methoxy-9BBN and coupled to produce the diacetylene[30]. This method is especially useful for unsymmetrical coupling and is an alternative to the Cadiot-Chodkiewicz procedure[100].

A versatile one-pot coupling reaction was developed by Kumada and Tamao *et al*[101-103] in which halogenated compounds are treated with Grignard reagents in the presence of a nickel-phosphine catalyst. The method has wide application and can be used to couple alkyl, alkenyl, aryl, and heteroaryl Grignard reagents with aryl, heteroaryl, and alkenyl halides.

In a recent report detailing the scope of the Grignard-Wurtz reaction[104] for heteroaryl halides with various Grignard reagents, α-T was prepared (Scheme 13) in 80% yield (millimolar scale, GC analysis). This nickel catalyzed coupling procedure appeared to be the most promising synthetic scheme to adapt for preparing large scale quantities of α-T.



Scheme 13.

2.2 THE NICKEL-COMPLEX CATALYSED GRIGNARD-WURTZ REACTION

2.2.1 General

Cross-coupling reactions using organometallic reagents and organic halides are straightforward, efficient methods of forming carbon-carbon bonds. Grignard reagents initially were limited in terms of synthetic utility as coupling agents due to their inability to undergo direct substitution reactions with organic halides. Other problems included low chemoselectivity, competitive eliminations and halogen-metal exchange reactions leading to homo-coupled side products. The use of nickel(II) complexes to catalyze the reactions between Grignard reagents and organic halides, was independently discovered by Kumada and Tamao *et al.*[105] and Corriu *et al.*[106] and has eliminated many of these problems. A special feature of this reaction is that alkyl Grignard reagents containing β-hydrogen atoms do not undergo elimination but give selective cross-coupling products in high yield. This selective coupling reaction has been a facile and convenient method for preparing various substituted indans and tetralins[107], lignan skeletons[108] and shikimate or acetate derived products[109].

The Grignard reagent in the presence of $Ni[dppp]Cl_2$ (dichloro[1,3-bis{diphenylphosphino}-propane]-nickel(II)) has also been treated with thiols or sulfides to selectively introduce aryl and alkyl groups into purine derivatives in good yield[110]. Five membered ring heterocycles such as furan, thiophene and selenophene when treated with phenyl or methyl magnesium bromide in the presence of a nickel(II) catalyst, undergo alkylative ring opening yielding in most cases the Z,Z-buta-1,3-diene

stereospecifically[111]. The Z olefin configuration of thiophene was also retained for fused thiophene systems[112].

Although the scope of the nickel-complex catalyzed Grignard reaction is broad in terms of selection of organic halide and Grignard reagent, the major disadvantage of the reaction is that the substituents of either part are restricted to those which do not react with Grignard reagents. Groups with active hydrogen may be present in their salt form but others like C=O, NO_2, COOR and C≡N react with the Grignard reagent. For these reasons anhydrous conditions must be used and all carbonyls or reactive substituents must be masked. In contrast to the reactions between alkyl Grignard reagents and various aryl or alkenyl halides, the reactions between alkyl halides and Grignard reagents often result in complex product mixtures[103].

2.2.2 Catalytic Cycle

The proposed mechanisms for the Grignard-Wurtz nickel-complex catalyzed reaction are divided into those which involve Ni(II) and those which invoke other nickel species. A route proposed[113] involving Ni(0) however, is not consistent with rigorous experimental results obtained by varying phosphine ligands and the substrates. The addition of halogenated compounds to Ni(0) is known[114] although the process is much slower than that observed for the cross-coupling reaction. Also, according to Kochi[115], the following reaction

$$L_2Ni(II)(Ar)CH_3 \longrightarrow ArCH_3 + L_2Ni(0) \qquad Ar = \text{Aryl}$$

is too slow to be a catalytic process. Kochi *et al.* have studied the mechanism of nickel catalysis in cross-coupling

reactions and suggest a mechanism involving Ni(I), Ni(II) and Ni(III) species[116,117] (Scheme 14 - the phosphine ligands have been omitted). The proposed mechanism is initiated by electron transfer to the aryl halide producing the active Ni(III) species (Rxn A). The Ni(III) species undergoes aryl transfer with 35 giving the diaryl Ni(III)X intermediate (Rxn B). The latter species reductively eliminates Ni(I)X to yield the coupled product (Rxn C). The Ni(I) species can then produce the active Ni(III) species by oxidative addition with the aryl halide (Rxn D). One problem with this pathway identified by these workers, is that it involves aryl radicals when experimental results have precluded radicals as intermediates.

A $\mathbf{Ar}Ni(II)X + ArX \longrightarrow \mathbf{Ar}Ni(III)X^+ + Ar\bullet + X^-$

B $ArNi(III)X^+ + \mathbf{Ar}Ni(II)X \longrightarrow \mathbf{Ar}ArNi(III)X + Ni(II)X_2$

35

C $\mathbf{Ar}ArNi(III)X \longrightarrow \mathbf{Ar}\text{-}Ar + Ni(I)X$

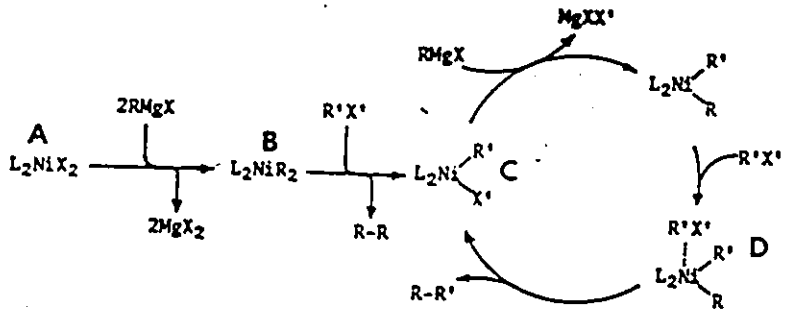
D $Ni(I)X + ArX \longrightarrow ArNi(III)X^+$

Scheme 14.

Two proposed catalytic cycles which do not require oxidation or reduction of the metal differ in terms of the complex which is formed with the catalyst. Scheme 15 is the catalytic cycle[103] designed by Kumada *et al.*

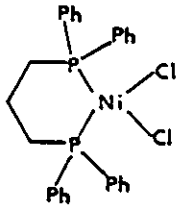
The catalyst (A) forms the intermediate diorganonickel complex (B) which is then converted by the organic halide to C. Further reaction with the Grignard reagent forms a nickel complex with both of the substrates to be coupled (D). Release of the cross-coupled product is

facilitated by the attack of organic halide via a penta-coordinated intermediate. The release of the product and addition of R'X' allows the catalytic cycle to continue.



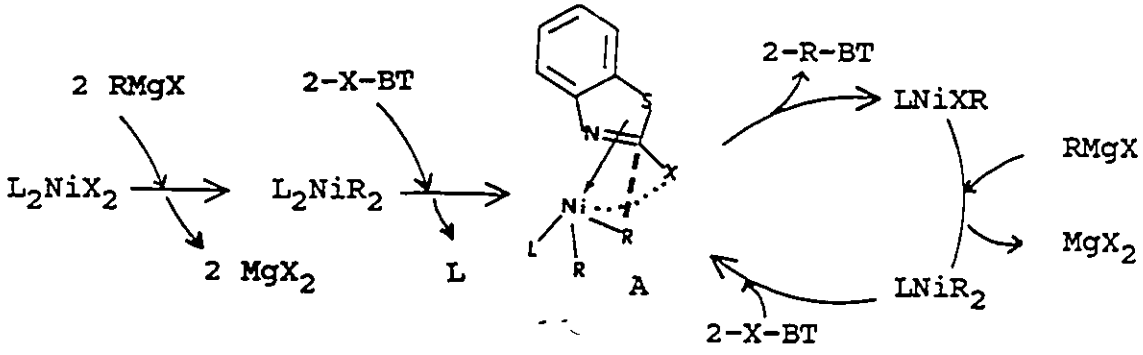
Scheme 15.

Kumada *et al.* investigated the role of the phosphine ligands in their reactions of aryl and alkenyl halides with Grignard reagents[103,104]. The nature of the ligand greatly influenced catalytic activity. This implies that the catalytically active species contained at least one ligand on the nickel. Using a standard reaction, it was found that bidentate ligands were more active than unidentate ligands and of the former, Ni[dppp]Cl_2 (36) was the most efficient. A three carbon methylene bridge was the optimum length in terms of activity. With Ni[dppp]Cl_2, the order of reactivity of halobenzenes was I >Cl >Br >F while with the ethyl diphosphine catalyst the order was as expected Br >Cl >F. The catalyst Ni[dppp]Cl_2 when treated with butylmagnesium bromide to form the catalytically active species, after being left stirring for 16 h maintained its stability and reacted with chlorobenzene to give the usual yield (98%) of butylbenzene.



36

Investigation of cross-coupling Grignard reagents with 2-halogenobenzothiazoles (2X-BT) using nickel(II) catalysts resulted in another catalytic cycle,[118] (Scheme 16) which derives a catalytically active complex (A) from the reaction of L_2NiR_2 with organic halide, and the replacement of one of the phosphine ligands. After the release of the cross-coupled product the catalytic species is regenerated by reaction with the Grignard reagent first and then the organic halide. The major differences between this catalytic route and the previous one are the loss of the phosphine ligand and the inclusion of the organic halide as a ligand instead of as an attacker promoting the coupling.



Scheme 16.

In the reactions with halogenobenzothiazoles the phosphine ligands were also shown to have an influence on activity. Contrary to Kûmada's work[103], bidentate ligands were inferior to unidentate ligands. This is consistent with the proposed mechanism. Ligand loss would be more difficult with the ring stabilized bidentate phosphine ligands than for a unidentate one. The mechanism is also consistent with the halogen reactivity order of F >Cl >I. Fluorine with its small volume and high metal affinity would coordinate better with the nickel. The activity of the unidentate ligands increased with increasing basicity, with $Ni(PPh_3)_2Cl_2$ being the most active catalyst.

The synthesis of the catalyst $NiBr_2$(2Cl-BT)$[P(CH_3)_3]$ when treated with CH_3MgI resulted in an almost quantitative yield of product ($2CH_3$-BT) via the postulated catalytically active complex A, $Ni(CH_3)_2$(2Cl-BT)$[P(CH_3)_3]$ in this case. This phosphine-organic halide nickel complex was more active than any of the previous nickel-diphosphine catalysts and provides compelling evidence for this catalytic cycle at least for the methylated derivative.

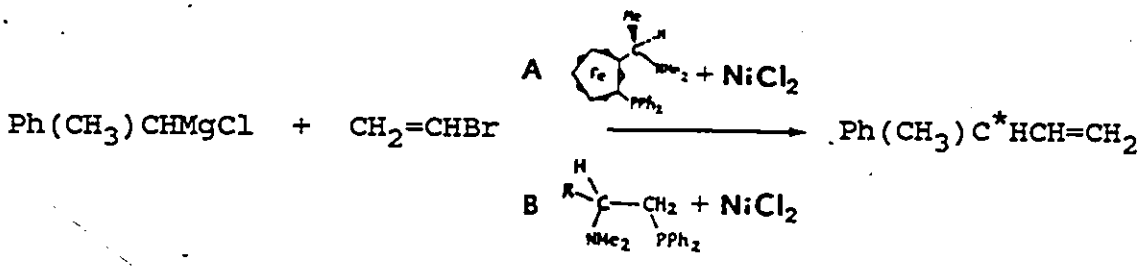
The latter two catalytic cycles proposed for the nickel catalyzed Grignard-Wurtz reaction may be a reflection of the specific nature of the substrates rather than a general mechanism which is appropriate regardless of the substate. This would explain the differences in the reactivity order of the halogens and the preferred type of phosphine ligand.

2.2.3 Metal Catalysts other than Nickel

A number of catalysts other than nickel have been used in the cross-coupling of Grignard reagents with organic

halides. A nickel(II) complex of 1,1'-bis(diphenylphosphino)ferrocene proved to be an efficient catalyst in cross-coupling reactions between tertiary alkyl Grignard reagents and β-bromostyrene[119]. E-β-tert-butylstyrene was the exclusive product without any of the isomeric isobutylstyrene which usually forms when palladium or nickel catalysts are used.

Chiral (aminoalkylferrocenyl) phosphines[120] are effective ligands for the asymmetric Grignard-organic halide cross-coupling reaction. The aminoalkyl side chain was responsible for high asymmetric induction of up to 63% ee in the optically active hydrocarbon (Scheme 17A). More recently, Ni(II) catalysts with chiral aminoalkyl phosphine ligands have resulted in asymmetric cross-coupling for the same reaction (Scheme 17B) in 94% ee[121]. The highest asymmetric induction was achieved with the bulky tert-butyl group.



Scheme 17.

The catalyst tris(dibenzoylmethido)iron(III) when treated with aryl Grignard reagents and alkenyl halides in dimethoxyethane (DME), give good yields of the coupled product but is not as stereospecific with E-alkenyl halides

as other nickel and palladium catalytic complexes[122]. The scope of iron catalysts in Grignard-Wurtz coupling reactions has not been fully realized but, like the nickel catalysts cost is an advantage and they are reasonably insensitive to light, moisture and air.

The cross-coupling of alkyl groups with Grignard reagents has been accomplished using silver(I) salts,[123,124] although yields are low and mixtures of products are common. Vinylic Grignard reagents can be alkylated by primary alkyl iodides and tosylates in the presence of a catalytic amount of Cu salts (CuI) in high yields[125].

Copper(I) catalyses cross-coupling between Grignard reagents and primary alkyl bromides[126] in THF solutions at 0 °C or lower. The homo dimers formed under these conditions are small. The structure of the halide, rather than the Grignard reagent is important, with secondary and tertiary alkyl halides being inert and giving poor yields of coupled products.

Palladium catalysts have been used in place of nickel catalysts with favorable results. Product yields have been generally higher particularly for aryl-alkenyl and alkenyl-alkenyl coupling reactions[122]. The catalyst $Pd(PPh_3)_4$ is also the best catalyst when other organometallic reagents such as lithium[127], boron[128], and aluminum[129] are used. A major disadvantage of palladium catalysts is their expense and sensitivity to air and moisture.

2.2.4 Synthesis of α-Terthienyl

As indicated earlier, it was decided to adapt the Grignard-Wurtz coupling reaction[104] using Ni[dppp]Cl_2 as catalyst, for a scale-up one-pot preparation of α-T. This was necessary in order to begin field trials with this compound. The starting materials were readily available from Aldrich and used without further purification. Yields of the order of 75%, were obtained even for 50g (2,5-dibromothiophene) scale runs[81].

It was not feasible to attempt to purify such large amounts of crude product by column chromatography, particularly when a purity of about 98% was needed for field testing. Crystallization from ethanol or methanol improved product purity, but not sufficiently. Soxhlet extraction of a crude α-T/silica gel mixture (1:1) with hexane as solvent proved to be an excellent purification method. Continuous extraction of this mixture with hexane provided crystalline α-T on concentration of the solvent and cooling. The bright yellow solid isolated using this procedure was >99% pure based on capillary GC analysis. This purification method is only limited by the size of the extraction apparatus and thimble and could probably be applied to a pilot plant or industrial scale preparation of α-T.

Although the synthesis and method of purification of α-T were both satisfactory, one obstacle remained for making this a more feasible commericial process. This obstacle was the use of diethyl ether as solvent for the reaction.

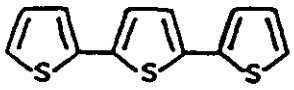
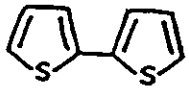
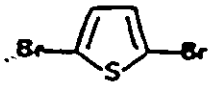
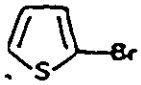
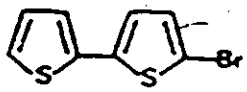
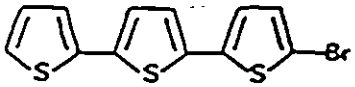
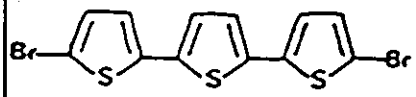
2.2.5 Solvent Effects

It was considered important to evaluate the effects of various solvents on the Grignard-Wurtz reaction and to find a more acceptable solvent than diethyl ether. Alternative solvents were evaluated in terms of yield and side product formation using the Grignard-Wurtz method to make α-T. The ideal solvent would form the Grignard readily, preferably without heat, and give a high yield of cross-coupled product.

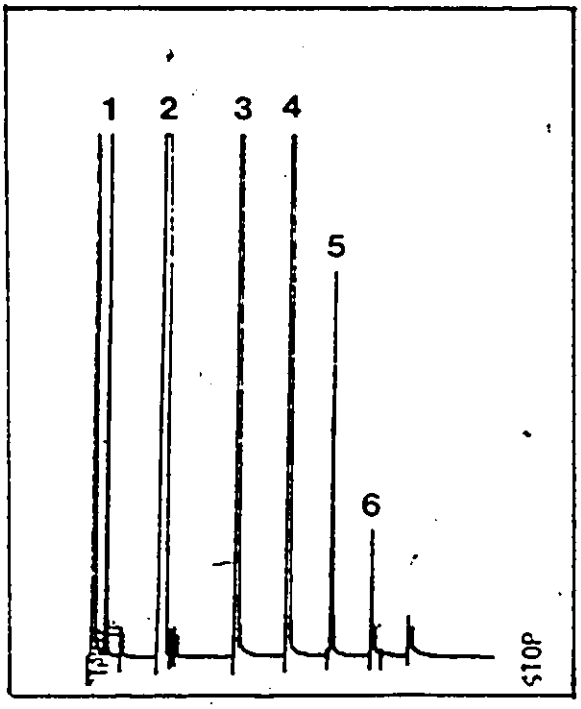
Reaction products were identified by comparing GC retention times to the retention times of known standards. Table 3 shows the retention times of the standards at various initial temperatures. The chromatogram (Figure 1) of reaction products from the Grignard-Wurtz reaction in ether/THF (1:7), after column chromatography to isolate α-T, is an example of this separation. The shorter 10 metre capillary column eliminated the problems associated with longer conventional columns in which degradation of the products was common.

The choice of solvent is an important factor in the formation of the Grignard reagent. In solution, the Grignard reagent is part of the Schlenk equilibrium[130]. The position of the equilibrium depends largely on solvent although concentration and temperature[131,132] can also influence it. For diethyl ether, the equilibrium lies to the left, coordinated with two molecules of ether (37). NMR studies of aryl Grignard reagents have shown that there is a decrease of ArMgX in tetrahydrofuran (THF) compared to ether with some aryl groups. Both of these solvents are widely used for the preparation of Grignard reagents.

Table 3. Capillary GC Retention Times* of α-Terthienyl and Other Thiophenic Compounds.

Standards	Retention Times (min)	
	Initial Temperature 100°C	70°C
	16.75	19.83
	6.13	10.49
	2.08	5.67
	–	1.44
	–	14.21
	–	16.96
	19.04	–
	21.71	–

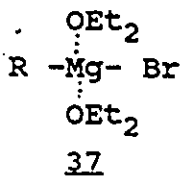
* GC analysis was carried out on a Varian 6000 VISTA GC model with an SEA 30, 10 m capillary column and a HEWLETT-PACKARD integrator. The injection temperature was 200 °C. The initial temperature was maintained for 5 min and then increased at a rate of 10 °C/ min to a temperature of 250 °C.



Peak	Retention Time(min)	Area%
1	1.23	3.4
2	5.36	37.0
3	10.47	29.7
4	14.07	27.4
5	16.94	1.5
6	19.75	0.9

Figure 1. Chromatogram of the Reaction Products Obtained from the Grignard-Wurtz Reaction in Ether/THF (1:7) after Column Chromatography to Isolate α-Terthienyl. The GC conditions are described in Table 3. for the initial temperature of 70 °C with the retention times to identify the compounds.

$$2RMgX \rightleftharpoons R_2Mg + MgX_2$$



37

For both the preparation of the Grignard reagent and the cross-coupling reaction, Kumada[103] has found diethyl ether to be a superior solvent to THF. The reaction proceeded faster with better yields and less reduction product. With THF, the Grignard reaction occurs without difficulty but the coupling reaction does not proceed as well as for diethyl ether. THF, a more basic solvent could bind with the nickel catalyst inhibiting the approach of the organic halide[103] or Grignard reagent, preventing the formation of the active organo-nickel complex.

For the preparation of α-T THF was also found to be an inferior solvent (Table 4) compared to diethyl ether (Table 5). α-T was synthesized in only 34% yield with a similar amount of unreacted starting material and a large amount of homo-coupled Grignard product. In an effort to improve the yield of α-T, the Grignard reagent of 2-bromothiophene was prepared in THF and then added to the organic halide and nickel catalyst in a diethyl ether/THF mixture (1:7). This solvent mixture did not improve the yield of the cross-coupling reaction and slightly less α-T was obtained compared to THF alone as solvent.

n-Propyl ether gave a yield similar to diethyl ether and contained only bithienyl as a side product. This reaction was not optimized so it is not unreasonable to assume that the yield could be improved and the amount of

Table 4. Yields of α-Terthienyl Obtained using Various Ether Solvents.

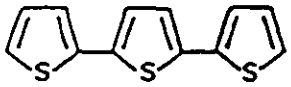
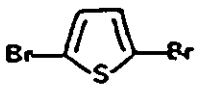
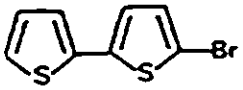
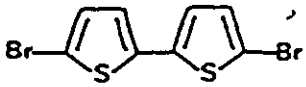
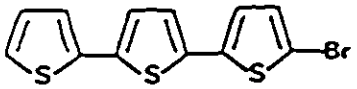
Solvent	Yield** of α-Terthienyl* (%)	Isolated Yield of α-Terthienyl* (%)
Diethyl ether	95.3	73.2
n-Propyl ether	91.5	70.2
n-Butyl ether	68.5	55.0
Diisopropyl ether	no reaction	no reaction
Dimethoxyethane	***	16.0
Tetrahydrofuran	67.0	47.7
Tetrahydrofuran and Diethyl ether	***	45.0
1,4-Dioxane	no reaction	no reaction

* taking account of unreacted 2,5-Dibromothiophene

** Yield determined by GC Analysis

***Gas Chromatogram not taken of the crude reaction mixture

Table 5. Capillary GC Analysis* of α-Terthienyl Product Mixtures in Various Ether Solvents.

Product	Solvent			
	Diethyl ether	n-Propyl ether	n-Butyl ether	THF
	% 77.9	% 70.6	% 42.1	% 34.0
	17.4	20.9	25.5	33.1
	2.6	8.5	10.2	23.6
	–	–	–	9.3
	2.1	–	–	–
	–	–	21.2	–

* The GC conditions are described in Table 3 for the initial temperature of 70 °C.

bithienyl formed decreased by altering the reaction conditions. The only obvious disadvantage when considering large scale reactions with this solvent is the expense of n-propyl ether. An attempt to do the reaction on a larger scale (0.1 m of halide) with a diisopropyl/n-propyl ether mixture (1:1.4) gave a poor yield of α-T.

The next higher homologue, n-butyl ether, gave only a modest yield (43.1%) of α-T. The n-butyl ether contained just over 25% of unreacted starting material and gave rise to larger amounts of the homo-coupled product bithienyl (10%) and a side product, 5-bromo-α-T, in 21% yield. As n-butyl ether is an economical solvent, experiments on over ten times the scale of the standard reaction were carried out to try to improve the yield of α-T and decrease side product formation. The initial large scale (0.05 m of organic halide) attempts were unpromising with low yields and the isolation of charred material due to the abrupt rise in temperature during the addition of the Grignard reagent. External cooling to maintain the reaction temperature at 40 °C reduced the charring and gave yields of 25-32%. Higher yields for the smaller scale experiments may be due to the higher molar dilution,[132] 0.1 M of halide as compared to 1.15 M in the large scale experiments. Larger volumes of n-butyl ether would not facilitate work-up which involves removal of solvent by distillation under reduced pressure. n-Butyl ether is therefore not the best choice of solvent for large scale work in preparing α-T.

Other solvents investigated included diisopropyl ether and 1,4-dioxane which were unsuccessful as solvents in the formation of the Grignard reagent even with the addition of ethyl iodide and the application of heat. Forcing

conditions were necessary in order to form 2-thienyl magnesium bromide with DME as solvent and the yield of α-T was poor (16% isolated yield).

2.2.6 Side Product Formation

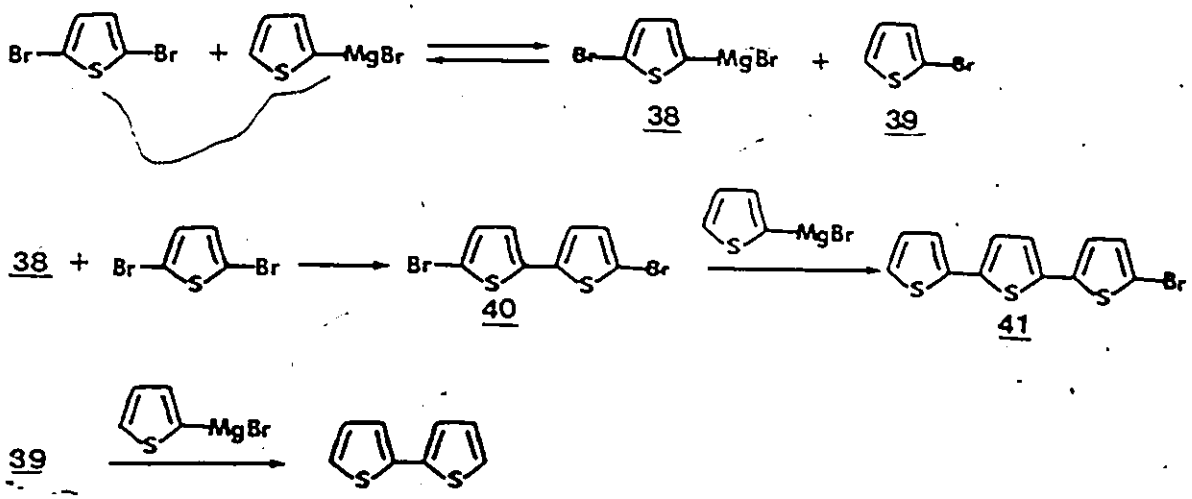
Bithienyl is a common side product formed in all of the ether solvents at various molar dilutions. The preparation of 2-thienyl magnesium bromide from 2-bromothiophene is quantitative, precluding the formation of 2,2'-bithienyl by a coupling reaction between the Grignard reagent and unreacted starting material. TLC immediately after preparing the 2-thienyl Grignard reagent indicated the absence of starting material and 2,2'-bithienyl. However, bithienyl was detected by capillary GC and TLC when the Grignard reagent was left overnight. The bithienyl is probably formed from the Grignard reagent via a disproportionation reaction with a free-radical type mechanism[132]. Small amounts of bithienyl may also be formed via either of the proposed catalytic cycles for a nickel (II) species. The double addition of the Grignard reagent to the nickel chloride catalyst and the subsequent displacement by organic halide, liberates the homo coupled Grignard product (Scheme 15 and 16).

Solvents in which the yield of α-T was low (THF, THF/Ether, DME) gave rise to an intermediate product, 5-bromo-2,2'-bithienyl, which accounted for 20% of the reaction mixture when the reaction was carried out in the latter two solvents. In order to produce α-T, this intermediate product must go through the catalytic pathway once more, reacting with another equivalent of 2-thienyl magnesium bromide. The difficulty in forming the catalytic complex with 5-bromobithienyl may be due to a more favorable

solvent-catalyst complex which could prevent the approach of the bromobithienyl and subsequent coupling. The nature of the solvent cage is not known but could involve a tight nickel complex between the nickel and the oxygen of the ether.

GC analysis of the chromatographed products from the reactions using THF/ether or DME as solvent indicated small amounts of 5,5'-dibromobithienyl (40). This product could be formed by a free radical type process from two molecules of 2,5-dibromothiophene or from a halogen-magnesium exchange product, 38. The exchange product could react with 2,5-dibromothiophene using the Ni(II) catalyst (Scheme 18) to give 40. It is known that halogen-magnesium exchanges are enhanced in the presence of transition metals, and that the rate of exchange depends greatly on the solvating power of the ether solvent[133,134]. We observed the same trend as Zakharkin *et al.*[134] for halogen magnesium exchange, i.e. DME >THF/diethyl ether >diethyl ether. In the case of diethyl ether no exchange product was observed. This route could also produce the bithienyl compound, a common product in all of the reactions.

Another side product, 5-bromo-α-T(41), was observed in 21% yield under the standard reaction conditions with n-butyl ether. This product may form from a butyl ether enhanced halogen magnesium exchange mechanism. The production of 40 (Scheme 18) and its subsequent reaction with an equivalent of 2-thienyl magnesium bromide could produce 5-bromo-α-T. It is interesting to note that 40 was not detected in the reaction mixture.



Scheme 18.

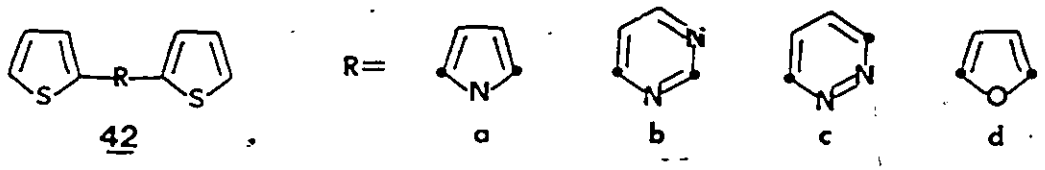
Our results show that maximum yield and minimum side product formation can be obtained in the order n=2 >n=3 >>n=4 for ethers of the type $H(CH_2)_nO(CH_2)_nH$ for the nickel(II) catalyzed Grignard-Wurtz reaction. Propyl ether was found to be a suitable alternative solvent to diethyl ether, giving a good yield of α-T, and being less of a safety hazard. The cyclic ether THF was the next best solvent for this reaction. The worst results were obtained with the branched alkyl ether and the diethers DME and 1,4-dioxane. Although the number of solvents tested was not exhaustive, our results agree with previous generalizations based on more complete and larger solvent studies[132].

2.3 ANALOGUES OF α-TERTHIENYL

The biological activity of α-T stimulated our interest in preparing analogues that may have similar biological activity. Some of the characteristics necessary

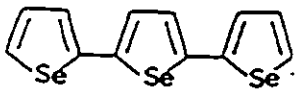
in order to consider a compound an analogue of α-T are three unfused rings (usually) with one ring at least containing a heteroatom, and all rings being aromatic.

Synthesis of various terthienyl structural isomers[95,135] and analogues of the general structure 42 have been synthesized where the center ring is an oxygen,[94,136] selenium[137] or nitrogen[96,138] containing heterocycle. Many of these analogues have unknown biological activity. A complete study of the synthesis and biological activity of α-T analogues containing different combinations of thiophene and furan rings[136] substituted at C-2, has shown that α-T and 43b are phototoxic in *in vitro* tests against the fungus *Pythium irregulare*. The analogues with furan between two thiophenes (42d) and thiophene substituted in the α position by two furan rings (43a) are toxic *in vitro* toward *Botrytis cinerea* in the dark. 1,4-Benzene analogs substituted by functionalized and unfunctionalized thiophene or benzene rings have been prepared but not biologically tested[139].



42

43a X=O Y=S Z=O
b X=S Y=S Z=O



44

Biological tests on analogues with selenophene or furan as the centre ring have been performed and compared to

the parent compound α-T[137]. The oxygen and selenium analogues were phototoxic to a variety of bacteria and fungi with the selenium analogue the more toxic of the two. The selenium analogue was just slightly less toxic than α-T and also appeared to operate via a mechanism involving singlet oxygen.

The Kumada procedure[103,104] was a very convenient route for synthesizing analogues of α-T for biological testing. In each case the Grignard reagent used was 2-thienyl magnesium bromide in diethyl ether solvent with the selected halide and Ni[dppp]Cl_2 as catalyst.

2.3.1 2,6-(Di-2-Thienyl)-Pyridine

Selection of the organic halide was very important and could dramatically alter the yield of the Grignard-Wurtz coupling reaction. Tamao *et al.*,[104] in their synthesis of 2,6-(di-2-thienyl)-pyridine, used 2,6-dichloropyridine in diethyl ether and refluxed with two equivalents of the thienyl Grignard reagent for 21 h obtaining a yield of only 11%. In our preparation using 2,6-dibromopyridine, a vigorous reaction occurred on addition of the Grignard reagent and the yield of cross-coupled product was 86%.

Initial halogen reactivity studies by Tamao *et al.*[103] based on the yields obtained for a standard reaction, using the Ni[dppp]Cl_2 catalyst and an alkyl Grignard reagent with different aryl halides, showed an unusual aryl halogen order (PhI >PhCl > PhBr >PhF). This result was not confirmed by these same workers in further competitive reactivity experiments which support a regular reactivity order. Our results support this as well with

bromides being more active and giving higher yields than the corresponding chlorides.

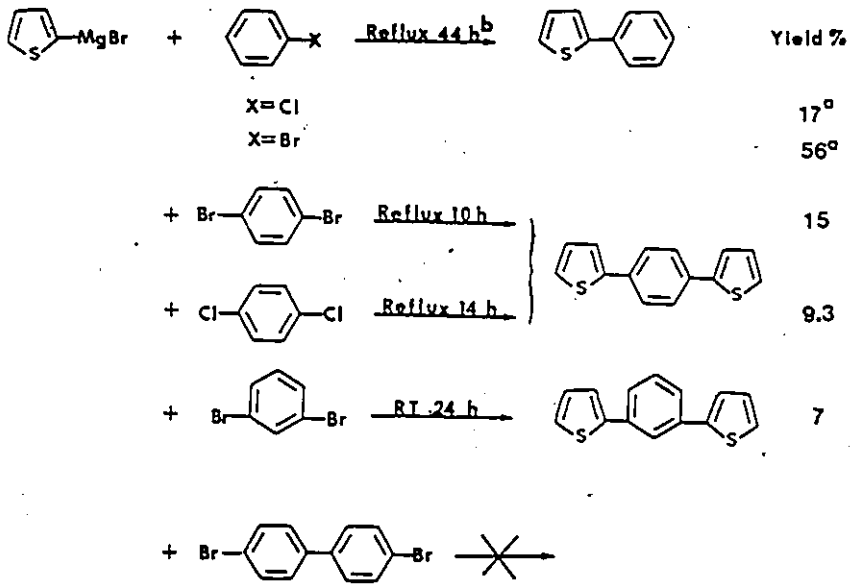
Similar results were obtained when 2,5-dichlorothiophene was used in some large scale experiments instead of the brominated thiophene in the preparation of α-T. The best result was a crude yield of 29% with α-T isolated as an orange -yellow powder. The reddish impurity was not identified but was of high molecular mass (m/z >382) with a mp 140-160 °C, possibly a thiophenic polymer.

2.3.2 Phenyl Analogues

The coupling between 2-thienyl magnesium bromide and aryl dihalides was not a complete success (Scheme 19). The yield of coupled product after stirring 1,4-dichlorobenzene with two equivalents of the Grignard reagent at RT was poor (3%) with the major product being 1-thienyl-4-chlorobenzene (38% yield). Repeating this experiment but refluxing for 14 h and stirring 48 h at RT only slightly improved the yield (9.3%). Using the more active 1,4-dibromobenzene and refluxing for 10 h further improved the yield to 15%. Low yields (7.6%) of coupled product were also obtained when treating 1,3-dibromobenzene with the 2-thienyl Grignard reagent. The observed unreactivity of 4,4'-dibromobiphenyl was first attributed to its low solubility in ether, but the coupling reaction would not proceed even after adding more diethyl ether or by further refluxing.

Tamao *et al.* also reported problems[104] with the Ni(II) catalyzed coupling reactions between thienyl Grignard reagents and aryl halides (Scheme 19), especially for chlorides. They propose that the low yields may be

attributed to an interaction between the sulfur atom of the thienyl Grignard reagent and the catalytically active species. This interaction could prevent effective coordination of the less reactive chloride[104]. This



Scheme 19. (a) From reference 104
(b) All reactions were in diethyl ether with $Ni[dppp]Cl_2$.

explanation receives some support from the observation that the diphenyldihalide which did not react with the 2-thienyl Grignard reagent, reacted in good yield with trimethylsilylmethylmagnesium chloride or cyclohexylmagnesium bromide[103] using the same nickel-phosphine catalyst.

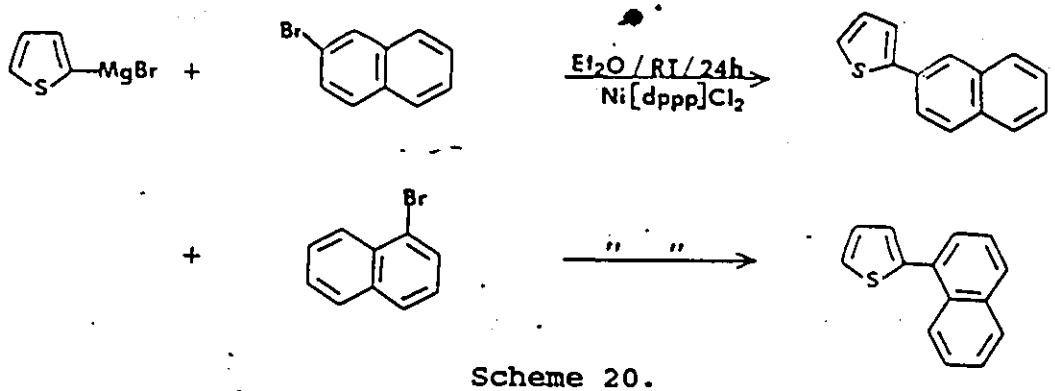
Similar difficulties have occurred when using vinylic Grignard reagents[103]. The yields can be improved if a vinyl halide is used to couple with the Grignard reagent. The vinylic Grignard reagent may compete with the halide for coordination onto the organonickel intermediate. Strong interaction between the halide and the catalyst favors the

catalytic process. The lower yields when reacting thienyl magnesium bromide with aryl dihalides reflect the stronger interaction of the Grignard reagent with the catalyst. It has been shown both in previous work[104] and our own, that aromatic halides with a heteroatom usually react well with the thienyl Grignard reagent. The presence of the heteroatom may improve the coordination between the halide and the catalyst, eliminating the problems associated with less reactive halides. The yield drops slightly if the heterocycle is a dihalide and more noticeably if the heterocycle is a dichloride.

Para-terphenyl has been prepared using the same reaction but a different nickel catalyst[106]. Instead of phenyl phosphine ligands, the catalyst has two acetoacetone ligands attached to the nickel. With this catalyst, 1,4-dibromobenzene was coupled at both positions to phenyl magnesium bromide in diethyl ether solvent producing para-terphenyl in greater than 80% yield. It would be interesting to repeat this experiment with the thienyl Grignard reagent, to see if the coordination between the catalyst and the reagents would be different. If the Grignard bound less strongly to the $Ni[acac]_2$ catalyst, the yields of our aryl dihalide couplings may improve.

2.3.3 Naphthalene Analogues

In order to prepare an analogue distinctly different from those described above, and still maintain a highly conjugated system like the one found in α-T, thienyl naphthalene analogues were prepared. These conjugated systems with the last two rings fused were of interest to us for both biological testing and for examination of the photochemical properties.



Scheme 20.

The coupling reaction (Scheme 20) to make the 1-(2-thienyl)-naphthalene product was exothermic with an isolated yield of 71%. This reaction was done at RT but Tamao *et al.*[104] reported a similar yield of 76% (GC analysis) by refluxing for 20 h. The 2-(2-thienyl)-naphthalene analogue was formed in 73% yield based on recovered starting material (Scheme 20). The 2-bromo-naphthalene reacted much less vigorously with the Grignard reagent. The greater reactivity of the α-bromo starting material parallels unsubstituted naphthalene where the α position is the preferred reaction site of free radicals, nucleophiles or electrophiles[130].

2.4 DERIVATIVES OF α-TERTHIENYL

Although many derivatives of α-T have been synthesized, only in rare instances have they been systematically prepared to test their effectiveness against a target organism[85,140-142]. The following derivatives were prepared in order to study how electron donating or withdrawing groups altered biological toxicity. The derivatives also contained substituents of various polarities and substituents to extend the α-T chromophore.

Derivatization of α-T was performed by taking into account what was already known about thiophene. Thiophene resembles benzene as an aromatic compound, although it is generally more reactive and less stable. Electrophilic substitution at the C-5 and/or C-5" positions of α-T would be consistent with that of unsubstituted thiophene which usually directs electrophiles to the C-2 position almost exclusively[143].

Electrophilic substitution of α-T invariably gave two products, the C-5 and the C-5, C-5" substituted derivatives. In some cases the yield of the desired product could be optimized by altering reaction conditions. The NMR (section 2.5) showed that the thiophene ring furthest removed from the substituent in a C-5 substituted compound has chemical shifts and coupling constants that are almost identical to the unsubstituted molecule. From our results, it seems reasonable to suggest that for electrophilic attack, there is little difference between derivatives already substituted at C-5 and α-T.

Purification of the synthesized analogues and derivatives was particularly important as the compounds were to be biologically tested. Mixtures of thiophene derivatives can sometimes be separated by distillation while α-T and its related compounds which are usually solid, were separated when possible by column chromatography. With substituents like CH_3 or halogen, the C-5, and C-5, C-5" substituted products were inseparable by usual methods and required HPLC with reverse phase columns.

2.4.1 Halogenation of α-Terthienyl

Bromination of α-T was first attempted using bromine in $CHCl_3$ and stirring overnight. This reaction yielded a reddish oil which by TLC and by the MS was a complex mixture of α-T, mono, di and trisubstituted products. A phase transfer bromination method developed by Olah[144] was also performed on α-T using calcium carbonate, hydrogen bromide, H_2O_2 and Triton X as a surfactant in CCl_4. Separation by column chromatography yielded products brominated from one to four times. The major products were the 5-brominated derivative in 32% yield and the 5,5"-dibrominated derivative in 23% yield.

N-bromosuccinimide (NBS) has been used for bromination of thiophene[143] in good yield and it proved to be the reagent of choice for brominating α-T. After refluxing α-T and NBS for 10 h, HPLC analysis revealed the major products of the reaction to be the 5-bromo (57%) and the 5,5"-dibromo (26%) derivatives. The high percentage of the dibrominated product was unexpected as just over one equivalent of NBS was used. NBS was a better brominating agent for α-T when compared to the other methods (see above), probably because NBS provides a low steady state concentration of Br_2, the attacking species.

The same brominating method was used on 2,2"-bithienyl with similar results. Column chromatography revealed that the major product was 5-bromo-2,2'-bithienyl in 54% yield, with the 5,5'-dibromo product present in only 14% yield. The brominations of α-T and bithienyl with NBS were not optimized.

The brominated products of α-T were almost inseparable by column chromatography. TLC in hexane showed that the brominated products were less polar than the parent compound with the dibrominated product closest to the solvent front (R_f=0.7), the mono derivative next at R_f=0.65 and α-T at R_f=0.6.

Iodination of α-T was achieved in moderate yields using HgO and I_2 in $CHCl_3$[145]. The iodination of thiophene with HgO[146] or HNO_3[147] and I_2 also occurs in good yield. The function of HgO is to oxidize I_2 to a better electrophile[148]. The exact nature of the electrophilic species responsible for iodination is uncertain, perhaps I^+ or I_3^+. The HgO must be added in small aliquots to the reaction flask; adding the oxidizing agent in one aliquot greatly reduces the yield. The iodination can be performed in different solvents (benzene, CCl_4 and $CHCl_3$) and the yield of the monoiodinated product was diminished when excess I_2 was added. The additional iodine may react with HgO forming HgI_2 immediately and preventing the formation of the active electrophile.

This method of iodination produced the 5-iodo derivative in 45% yield. The 5,5"-diiodo derivative was prepared in 24% yield by using two equivalents of the reagents. Both compounds were insoluble or sparingly soluble in common laboratory solvents. The diiodo derivative was insoluble in chlorinated solvents, sparingly soluble in benzene, hexane and alcoholic solvents. The monoiodinated product was slightly more soluble and crystallization from hot hexanes was possible. One solvent in which the diiodo derivative was soluble was CS_2. The purification of these compounds was both by crystallization

and by column chromatography. Elemental analysis of these derivatives consistently gave larger amounts of I_2 than the calculated value. These results could be due to additional iodine being complexed with the product.

Another method of iodination[149] was used involving the preparation of the mercuric chloride salt of α-T to which iodine was added. Based on previous work with thiophene[143], it was expected that mercuric chloride would add at the C-5 and/or C-5" positions of α-T, however our results do not bear this out. Addition of a saturated $HgCl_2$ solution precipitated the yellow mercuric salt which, after filtration and treatment with I_2, gave a mixture of α-T and the 5-iodinated derivative, with no diiodinated product formed. Even after lengthy heating, the formation of the mercuric chloride salt was never quantitative, and after treatment with iodine, always resulted in mixtures of starting material and 5-iodo-α-T. This iodinating method did not seem to offer any advantages over the HgO/I_2 iodination method previously used on α-T and the mixture obtained was not further purified.

Rossi *et al.*[149] used the mercuric chloride iodination method on 2,2'-bithienyl, and report isolating only the 5-iodo derivative by distillation in 85% yield. Repetition of this reaction and capillary GC analysis of the reaction mixture after treatment with I_2, indicated that the monoiodinated product was formed in 66% yield, and the 5,5'-diiodinated product in 11.4% yield. A small amount of starting material was also present.

2.4.2 Methylation of α-Terthienyl

The 5-methyl derivative of α-T was of interest for some biological tests and photochemical studies. It is also a naturally occurring compound isolated from *T. erecta*[7]. Radioactive labeling of this compound to perform incorporation experiments may elucidate key parts of the biosynthetic pathway to α-T (see section 1.3.2).

Our initial attempt to prepare the methyl derivative was by Grignard-Wurtz coupling between 5-iodo-α-terthienyl and methyl magnesium iodide in ether using Ni[dppp]Cl_2 as catalyst. After work-up, a red-brown oil was obtained which contained a small amount of the product by GC analysis. Two other products were isolated by column chromatography which appeared to be polymeric in nature.

Our next attempt to introduce a methyl group was by preparing the lithio-derivative of α-T followed by treatment with methyl iodide. The heteroatom in the thiophene ring makes lithiation regioselective at the position ortho to the sulfur atom. Recent work on lithiation of 2,5-dibromothiophene[150] and 3-(methylthio)-thiophene[151], the latter by abstraction of a proton or by halogen metal exchange, showed the importance of the ring sulfur in directing lithiation.

The lithiation of α-T was first performed in ether at RT, and the reaction mixture refluxed for 24 h after the addition of methyl iodide. Column chromatography of the reaction mixture isolated the major product of the reaction, a ruby-red solid which was not the methyl derivative and was not further identified.

Changing the reaction conditions (Table 6A) by stirring the reaction mixture for 24 h at RT instead of refluxing, resulted in a mixture of products which was analyzed by HPLC. The major product was the 5-methyl derivative (65%), along with α-T and 5,5"-dimethyl-α-terthienyl. It was not possible to separate this mixture by conventional methods of liquid chromatography. However, this mixture could be separated by reverse phase HPLC in small quantities (100mg). The presence of the dimethyl derivative was surprising as exactly one equivalent of butyllithium had been added.

Similar results were reported by Kagan *et al.*[152] using lithiumdiisopropylamide (LDA) as the lithiating reagent in the preparation of the 5-carboxyl derivative of α-T. The addition of CO_2 to α-T which had been lithiated with one equivalent of LDA resulted in a mixture of 5 and 5,5"-carboxylic acids. The yield of the 5-carboxylic acid was increased by adding 0.5 equivalents of LDA. Although this increased the amount of the monosubstituted product, half the reaction mixture was unreacted starting material. α-T and the acid derivative are easily separated by column chromatography - this is not the case for α-T and the 5-methyl derivative.

The lithiation of α-T was repeated at various temperatures in THF to try and improve the yield of the mono methyl derivative. The yield of 5-methyl-α-terthienyl was optimized by performing the reaction in THF at -78 °C and then allowing the reaction to warm up to room temperature (see Table 6C). For entry A, B and C, α-T was treated with one equivalent of butyllithium and two equivalents of methyl iodide. 5,5"-Dimethyl-α-terthienyl (Table 6D) was prepared

in 82% yield by adding two equivalents of butyllithium and three equivalents of CH_3I to α-T in THF at -50 °C. Treatment of the lithium salt with ethyl iodide or bromide did not yield the ethylated product.

Table 6. HPLC Analysis[a] of Reaction Mixtures[b] using Different Reaction Conditions to Methylate α-Terthienyl

Entry	Conditions	α-T	5-CH_3-α-T	5,5"-CH_3-α-T
A	Diethyl ether, RT	12%	65%	11.5%
B	THF, -50°C	8%	60%	30%
C	THF, -78°C	7%	69%	23%
D	THF, -50°C	0.2%	16%	82%

(a) HPLC was performed using an MCH-5NCAP reverse phase column with UV detection at 400 nm. The eluent was 5% H_2O/ CH_3OH.

(b) Reaction mixtures were pre-purified on silica gel eluted with petroleum ether.

2.4.3 Nitro and Cyano Derivatives

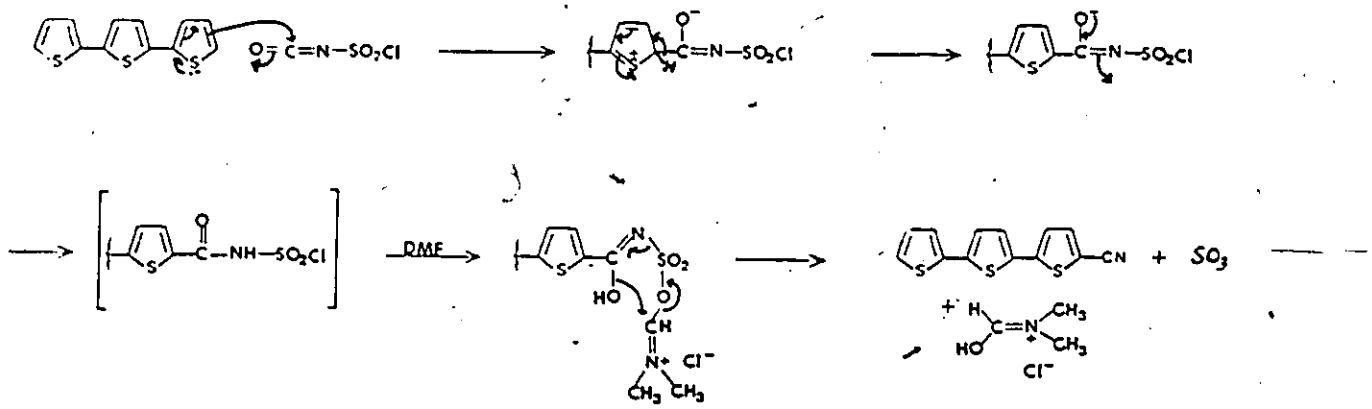
Thiophene can be nitrated by using HNO_3 in acetic anhydride[143] or the nitration conditions employed for α-T[153]. The 5-nitro derivative was obtained in 36% yield using HNO_3 in sulfuric acid. Capillary GC analysis of the brick-red compound separated by liquid chromatography revealed an impurity, which was not evident by TLC or MS. The capillary gas chromatogram showed two peaks, one at

15.35 min with a peak area of 20.5%, and another at 16.33 min with a peak area of 79.5%.

The ^1H NMR also showed that the separated mixture contained 20% of another compound. There was considerable overlap between the 5-nitro derivative and the impurity. However, four doublets can be clearly observed, two of which are upfield from the main envelope of thiophenic protons. These doublets are located at δ 7.61 and δ 7.47 with J values of 5 Hz and 3 Hz respectively. These are typical J values for a thiophenic compound. The upfield shift for these protons could be due to a nitro substituent. The other two doublets observed in the spectrum are at δ 7.17 and δ 7.15, with J values of 3 Hz and 1 Hz respectively. It is possible that this compound may be the 4-nitro-derivative of α-T. The ^1H NMR does not confirm this assignment definitively. The presence of this isomer could explain why the impurity was undetected by MS as well as the difficulty in separating the two compounds by liquid chromatography. The nitration of thiophene using mild conditions gives a small percentage of the 3-nitro compound[143]. GC-MS was attempted to try and identify the unknown, but the sample polymerized or degraded on the 50 m column. The mixture of 5-nitro-α-terthienyl and the impurity was not further purified.

The preparation of the cyano derivative of α-T was modeled on that used for thiophene[154]. Treatment of α-T with chlorosulfonyl isocyanate (CSI) generates the chlorosulfonyl derivative of α-T which precipitated as a yellow solid. Treatment with dimethylformamide (DMF) yielded the 5-cyano derivative of α-T in 58% yield, comparable to the cyanation of thiophene which occurred in

66% yield[154]. The 5,5"-dicyano derivative was prepared in 70% yield (capillary GC analysis) by treating α-T with four equivalents of CSI. The other 30% was the 5-cyano derivative, identified by its retention time.



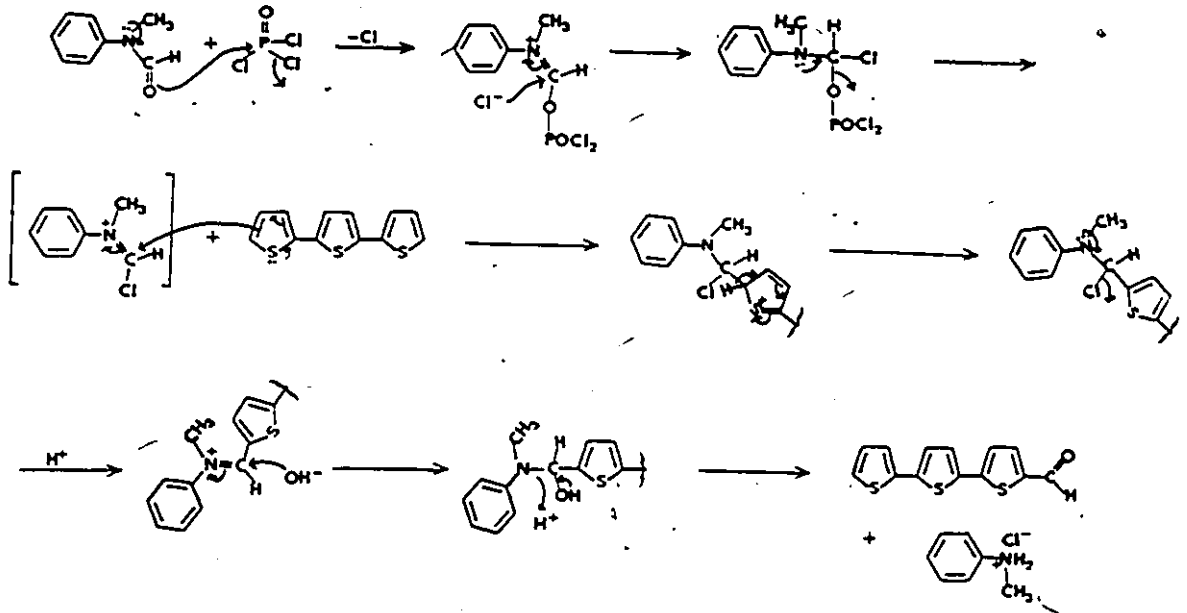
Scheme 21.

The mechanism (Scheme 21) of this reaction was proposed by Lohaus[155] and involves the displacement of the chlorine on the tautomer of the chlorosulfonyl intermediate forming a six membered transition state which falls apart to liberate the cyanated compound, SO_3, and DMF as the water soluble salt.

2.4.4 Formyl and Amide Derivatives

The 5-formyl derivative of α-T was prepared by the Vilsmeier reaction with N-methylformanilide and phosphorus oxychloride ($POCl_3$) (Scheme 22). This reaction had been performed previously on thiophene[156] resulting in a 71% yield of the 2-formylated derivative. The electrophile was formed first, and the α-T dissolved in CH_2Cl_2, added at RT and stirred for 24 h. Pouring the reaction mixture into dilute hydrochloric acid decomposed the $POCl_3$ and removed

the N-methylaniline as the hydrochloride, yielding 5-formyl-α-T in 85% yield. Separation of the aldehyde derivative is facile using flash chromatography with $CHCl_3$ as eluent or recrystallization from benzene for larger scale purification. Recently, Nakayama[157] reported the synthesis of this compound in 75% yield using the same reaction but different conditions. The reaction was performed in dimethylformamide (DMF) with $POCl_3$ at 70 °C for 1 h. The solvent used was also DMF.



Scheme 22.

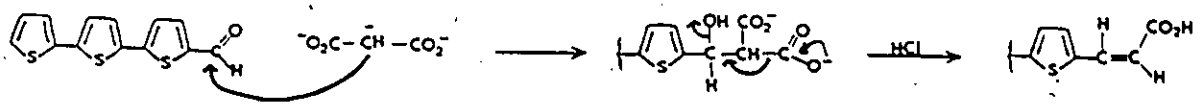
The 5-formyl derivative of α-T is a naturally occurring compound isolated from *T. erecta*[7]. Radioactive labeling studies will be performed with this compound as it is one of the last compounds postulated to occur before α-T in the biosynthetic pathway (see Scheme 6). The aldehyde functional group on the C-5 position of α-T provides an entry for many other derivatives formed by Wittig reactions or condensation reactions. It also provides a means of

preparing the methyl derivative by reduction and eliminates the problems associated with separation of mono and dimethylated products. Attempts to oxidize the 5-aldehyde of α-T to the acid failed with a variety of reagents[152].

The 5-amide of α-T was prepared by treating the 5-cyano derivative of α-T with NaOH in ethanol. Column chromatography of the crude product isolated a yellow solid (71% yield) which was mainly the amide with a small amount of the completely hydrolyzed product, 5-carboxy-α-T. The acid was not detected by ^{1}H NMR or MS but was observed on TLC. Column chromatography was difficult due to the insolubility of the 5-amide derivative and the 5-carboxylic acid derivative. Neither compound has been isolated from a plant source. The 5-carboxy derivative is postulated to be the last compound before α-T in the biosynthetic pathway (Scheme 6). Due to the insolubility of the acid in most solvents, the fact that it has not been detected during a plant isolation/extraction does not necessarily mean that it was not present.

2.4.5 Acrylic Acid and Alcohol Derivatives

The acrylic acid derivative of α-T was prepared[158] by a condensation reaction between 5-formyl-α-terthienyl and malonic acid in pyridine with piperidine as catalyst. This is the Doebner modification[158] of the Knoevenagel reaction. The reaction is similar to the Perkin reaction which usually uses the potassium or sodium salt of the acid as the base. After nucleophilic attack on the carbonyl group of 5-formyl-α-T, decarboxylation and elimination occur, perhaps as a single concerted process, (Scheme 23) to give the E isomer[159]. This may be catalyzed by the pyridinium ion which can decarboxylate arylidenemalonic acids[160].



Scheme 23.

The major product of this reaction with 5-formyl α-T was the E isomer isolated in 47% yield. ^{1}H NMR was used to identify the isomers formed. The vinylic protons for the major product were doublets with a coupling constant of 14 Hz, which is usually the value associated with protons in a trans arrangement. A small amount of the Z isomer was also isolated as a red powder. For both isomers, the carboxylic proton was located at δ 1.04 as a sharp doublet with a coupling constant of 6 Hz. The OH proton may be coupled to the closest vinylic proton or to the solvent (acetone-d_6). Both isomers were sparingly soluble in $CDCl_3$.

Grignard reagents have been used as ring opening nucleophiles[161], as have organocopper reagents[162] and alkyllithiums[163]. The alkyllithiums have been shown to be superior to the Grignard reagents, yielding more of the direct product. This type of reaction has been known to occur with metalated thiophene[143]. The lithium salt of α-T was treated with ethylene oxide resulting in a mixture of many products, one of which was the alcohol resulting from ring opening. This mixture of products could not be purified easily. The 5-(2-hydroxyethyl)-α-terthienyl was separated by column chromatography and identified by ^{1}H NMR and MS. Like α-T, this substance photodecomposed, turning

brown on TLC when exposed to light for a few hours. This reaction did not occur in high yield and was not optimized.

2.5 ^{1}H NMR of α-TERTHIENYL DERIVATIVES

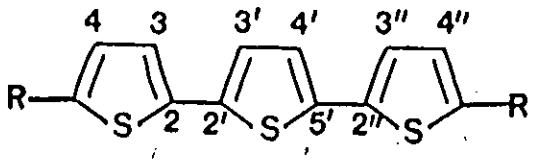
^{1}H NMR is an effective method to determine the position of substituents on the α-T molecule and the effect of these substituents on other parts of the molecule. It also gives an indication of the electron donating or electron withdrawing ability of particular substituents.

The ^{1}H NMR spectra of the derivatives were often complicated because of overlapping signals. Assignment of protons was sometimes difficult due to overlap with the $CDCl_3$ solvent at δ 7.24 ppm or other ring protons which were all contained within one ppm for any derivative. Solubility problems with $CDCl_3$ as solvent occurred for some of the derivatives and either acetone-d_6 or dimethylsulfoxide (DMSO-d_6) had to be used.

As expected, the coupling constants for the derivatives are similar to that of thiophene and are not dramatically altered by differing substituents. For the disubstituted derivatives (Table 7), $J_{3,4} = J_{3",4"}$ were between 3.6 and 4.0 Hz. The shielding effect of the methyl group on the C-4 proton can be observed as a downfield shift of 0.37 ppm. The electron withdrawing cyano group had the opposite effect on the same position shifting the proton upfield by 0.47 ppm.

The monosubstituted derivatives (Table 8) are characterized by doublets of doublets at the unsubstituted end. This pattern is due to the long range coupling between the protons at C-3" and C-5" of about 1.0 Hz. The thiophene

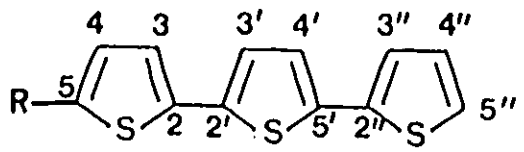
Table 7. ^1H NMR Data for Disubstituted Derivatives of α-Terthienyl*.



Compound	Solvent	(ppm)				Coupling Constants (Hz)		
		5,5''	4,4''	3,3''	3',4'	$J_{4,5}=J_{4'',5''}$	$J_{3,4}=J_{3'',4''}$	$J_{3,5}=J_{3'',5''}$
R=H	$CDCl_3$	7.20	7.01	7.16	7.06	5.2	3.6	1.0
R=H	DMSO-d_6	7.53	7.11	7.34	7.27	5.2	3.6	1.0
R=Br	$CDCl_3$		-0.12	-0.21	-0.09		3.6	
R=CH_3	$CDCl_3$		-0.37	-0.24	-0.12		4.0	
R=I	$CDCl_3$		-0.15	+0.02	-0.02		3.6	
R=CN	DMSO-d_6		+0.47	+0.64	+0.35		4.0	

* The chemical shift values for the standard, α-Terthienyl in a particular solvent are as given, and the values for the derivatives are reported as the difference in chemical shift from the standard.

Table 8. ^1H NMR Data for Monosubstituted Derivatives of α-Terthienyl*.



Compound	Solvent	(ppm)							Coupling Constants (Hz)				
		4	3	3'	4'	3"	4"	5"	$J_{3,4}$	$J_{3',4'}$	$J_{3",4"}$	$J_{4",5"}$	$J_{3",5"}$
R=H	$CDCl_3$	7.01	7.16	7.06	7.06	7.16	7.01	7.20			3.6	5.4	1.0
R=H	DMSO-d_6	7.11	7.34	7.27	7.27	7.34	7.11	7.53			3.6	5.4	1.0
R=H	Acetone-d_6	7.08	7.29	7.21	7.21	7.29	7.08	7.43			3.6	5.1	1.0
R=I	DMSO-d_6	-0.01	+0.02	+0.01	+0.01	+0.04	+0.03	+0.03	3.6	3.6	3.6	5.4	1.0
R=Br	$CDCl_3$	-0.12	-0.21	-0.07	-0.01	-0.01	-0.01	+0.01	3.6	3.6	3.6	5.4	<1.0
R=CH_3	$CDCl_3$	-0.36	-0.22	-0.09	-0.04	-0.03	-0.31	-0.01	3.2	3.2	3.2	5.2	<1.0
R=CHO	Acetone-d_6	+0.85	+0.01	+0.27	+0.28	+0.09	+0.04	+0.07	3.6	3.6	4.2	5.4	<1.0
R=NO_2	$CDCl_3$	+0.82	-0.11	+0.20	+0.06	+0.06	+0.03	+0.08	4.0	3.0	3.0	5.2	<1.0
R=CN	Acetone-d_6	+0.73	+0.01	+0.25	+0.21	+0.09	+0.04	+0.07	3.5	3.7	3.5	5.5	<1.0
R=$(CH_2)_2OH$	$CDCl_3$	-0.24	-0.11	-0.06	-0.07	-0.02	-0.01	0.00	3.6	2.4	3.6	5.2	<1.0

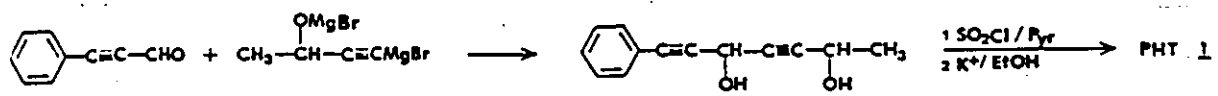
* The chemical shift values for the standard, α-Terthienyl in a particular solvent are as given, and the values for the derivatives are reported as the difference in chemical shift from the standard.

ring furthest removed from the substituent was the least affected by it and shows little change either in chemical shift or in coupling constants from that of the parent molecule. In some cases (X=I, Br, CH_3, CHO), the C-3' and C-4' protons are almost interchangeable with chemical shifts differing by only 0.05 ppm. For these protons the assignment was based on the electronic nature of the substituent. For an electron donating group (X= CH_3), the proton closest to the substituent in the second ring was considered slightly more shielded and was therefore assigned the lower chemical shift. The proton located at C-4 was usually identified easily as the substituent effects were greatest in this position. The changes in chemical shift were dramatic with the functions CHO or NO_2 giving upfield shifts of 0.85 and 0.82 ppm respectively. A corresponding downfield shift (0.36 ppm) was observed with the methyl substituent. The J values of the protons at C-4 and C-3 as well as between C-3' and C-4' were often identical in the monosubstituted derivatives.

2.6 SYNTHESIS OF PHENYLHEPTATRIYNE AND ACETYLENIC COMPOUNDS

2.6.1 Previous Syntheses of Phenylheptatriyne

Soon after the isolation of PHT from the plant *C. grandiflora*[2], this compound was synthesized (Scheme 24) in order to confirm the proposed structure. The 1-phenylhepta-1,4-diyne-3,6-diol was first prepared by reacting the di-Grignard reagent of but-1-yn-3-ol with phenylpropargyl-aldehyde. The dichloride was then synthesized and converted by alcoholic potassium to the triyne. The final yield was not given for this multistep synthetic route which gave rise to various side products and leaves room for improvement[2].



Scheme 24.

Coupling reactions have become one of the best methods of synthesizing polyacetylenes. Modifying the acetylenes first and then coupling the two synthons together minimizes further reactions with potentially explosive substrates. Many different types of coupling reactions have been developed for this purpose[164].

The most common type of symmetrical coupling reaction is the Glaser coupling procedure,[164,165] which involves the coupling of two terminal alkynes via the cuprous derivative in the presence of NH_4OH. The reaction also uses an oxidant which is typically air, O_2 or H_2O_2. This reaction is unsuitable for unsymmetrical type coupling procedures. When two different alkynes are used, the reaction yields the three possible diynes. This problem of synthesizing unsymmetrical diynes was solved by a coupling reaction discovered by Cadiot and Chodkiewicz[100].

The Cadiot-Chodkiewicz coupling reaction joins a bromoacetylene and an ethynyl compound in high yield. The reaction is copper catalyzed and is performed in the presence of base. The general reaction is as follows:

$$R\text{-}C{\equiv}C\text{-}H + Cu(I) \longrightarrow R\text{-}C{\equiv}C\text{-}Cu + H^+$$

$$R\text{-}C{\equiv}C\text{-}Cu + Br\text{-}C{\equiv}C\text{-}R' \longrightarrow R\text{-}(C{\equiv}C)_2\text{-}R' + CuBr$$

Hydroxylamine hydrochloride functions as the reducing agent and is added as required generating a low concentration of Cu(I) from $CuCl_2$. The primary amine removes liberated acid from the reaction and may assist in forming a complex with the cuprous derivative. The bromoalkyne does not react with the cuprous ions at such low concentrations, but may form a complex with the triple bond. Cadiot-Chodkiewicz coupling reactions[100,164] are performed in a variety of solvents such as water, alcoholic solvents, ether, THF, or DMF. The best results are obtained when the ethynyl compound and the cuprous derivative that is formed are soluble in the reaction medium. The bromoalkyne is added in the same solvent. This reaction is of broad scope as almost any functional group on either alkyne will be tolerated.

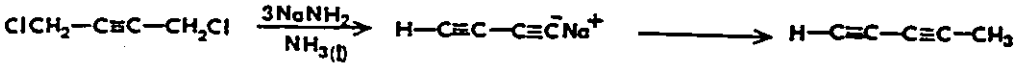
Homo coupling of the bromoalkyne is the only unwanted side reaction which occurs for this type of coupling reaction. This side reaction can be minimized by maintaining only a catalytic quantity of Cu(I) by adding sufficient reducing agent. Slow addition of the bromoalkyne and low reaction temperatures also reduce the yield of this product.

The Cadiot-Chodkiewicz coupling procedure has become the method of choice for unsymmetrical coupling of alkynes, and many polyacetylenes have been prepared using this procedure[164]. In testing the scope of the Cadiot-Chodkiewicz coupling reaction, PHT was synthesized in 91% yield from pentadiyne and bromophenylacetylene[166]. The coupling of bromopentadiyne and phenylacetylene[167] has also been performed resulting in a decreased yield of PHT (67%).

2.6.2 Synthesis of 1-Phenylhepta-1,3,5-triyne

The first attempt to synthesize PHT involved the coupling reactions between bromopropiolic acid and phenylacetylene using the Cadiot-Chodkiewicz procedure[100,164]. The acid could then be cleaved[168] using activated copper bronze in dioxane and the phenyl diyne recoupled with bromopropiolic acid. Bromopropiolic acid adds one acetylene unit each time, with the carboxylate group functioning as a protecting group. The methylation of the phenylhexatriyne was to be the final step to PHT. This route contained too many steps and one major flaw - isolated phenylhexatriyne has been reported to explode[169] even when stored at 0 °C in the absence of air.

The convergent synthesis of Cadiot and Chodkiewicz to synthesize PHT[166] was the best route available for preparing PHT safely and in high yield. Bromophenylacetylene was prepared immediately before the coupling reaction by treating phenylacetylene with NaOBr (section 2.6.3). The pentadiyne was prepared by treating 1,4-dichlorobutyne with three equivalents of $NaNH_2$ and methylating the sodium diacetylide in situ (Scheme 25).



Scheme 25.

Pentadiyne was distilled from decalin without heat, using the vacuum pump, into a specially designed trap which was cooled by an acetone/CO_2 bath. The sharp peak at

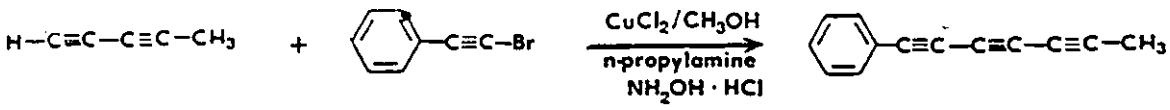
3300 cm^{-1} in the IR of the decalin due to the terminal diyne, was used to determine when the distillation of the pentadiyne was complete. Distillation from decalin is a common method of isolating potentially explosive acetylenes[170]. Pentadiyne was a clear liquid after distillation which quickly turned yellow due to polymerization products when kept in the freezer (-15 °C) or at room temperature for a few minutes. Polymerization reactions were almost eliminated by storing the product in an acetone/CO_2 bath. By-products formed in the reaction and identified by GC-MS were dimethylbutadiyne and butadiyne.

Earlier work on the physical properties of pentadiyne has not been consistent[171-174]. Jones *et al.* have compiled the previous syntheses of pentadiyne and provided explanations for the reported data.

The coupling between pentadiyne and bromophenylacetylene produced PHT in 84% yield after chromatography (Scheme 26). The primary amine used was propylamine instead of the ethylamine used previously[166], and did not alter the yield of the reaction appreciably. There was no evidence of diphenylbutadiyne, the homo coupled product. PHT was identified by TLC comparison to a naturally occurring sample and by its mass spectrum. The UV spectrum of synthetic PHT was identical to the spectrum obtained for naturally occurring PHT. Storage of PHT in a small amount of petroleum ether and wrapped in tin foil minimized polymerization and photochemical reactions.

The PHT prepared was used to conduct biological toxicity tests and to study the photochemistry of this naturally occurring triyne[175]. This synthetic route produced 1.3 g of PHT, a large quantity compared to the

small amounts obtained previously by isolation from plant sources (see section 1.2).



Scheme 26.

2.6.3 Bromination of Terminal Acetylenes

The bromination of the terminal alkynes was performed using the hypobromite procedure[100,176] and can be represented as follows:

The bromination occurs faster with alkynes soluble in the alkaline hypobromite, but insoluble alkynes may be brominated by stirring the reaction mixture vigorously and for long reaction times. The greater acidity of the ethynyl hydrogen, also shortens the reaction time. This difference is evident in the reaction time of pentadiyne and phenylacetylene. Both brominations occur quantitatively, the phenylacetylene reaction required 6 h and the pentadiyne only 0.5 h. After stirring the pentadiyne for 9 h other reaction products were observed by TLC and MS including dibrominated butadiyne.

Bromination was easily monitored by analyzing the IR spectrum at various reaction times. The characteristic bands at approximately 3300 and 2100 cm^{-1} which are due to the terminal alkyne disappear, as the band for the disubstituted alkyne appears at 2200 cm^{-1}. Generally, purification of the brominated product was not necessary as the reactions proceeded in high yield. The product was extracted with CH_2Cl_2 and the solvent removed under reduced pressure without heat. Heating during the removal of solvent or during distillation may cause the bromoacetylene to decompose violently, even under reduced pressure[164]. For this reason, bromoacetylenes are usually used in the crude state.

The bromination of propiolic acid was initially performed in the same fashion as for the previous alkynes, except for preparing and adding the sodium salt of the acid to the hypobromite. After acidification of the reaction mixture and continuous extraction with CH_2Cl_2 or ether, little product was recovered.

The inverse addition was suggested by Chodkiewicz[100] for bromination of oxygen containing terminal alkynes. When the hypobromite was added to the salt of the acid, the brominated product was obtained in 34% yield. The brominated acid was very soluble in aqueous solution, and was isolated as an oil from ether. When the extracting solvent was changed to CH_2Cl_2, the product was isolated as pale yellow crystals. The brominated acid was sparingly soluble in petroleum ether and pentane but soluble in both ether and CH_2Cl_2 and very soluble in water.

Commercon *et al.*[177] have also synthesized bromopropiolic acid using the sodium hypobromite procedure

and describe the product which was extracted from aqueous solution with pentane, as very insoluble in water. The analytical data for this compound consisted of an IR spectrum, one proton signal in the NMR and the melting point. This data suggests that the product was the brominated acid. This does not explain the discrepancy in solubility between the product they isolated and the bromopropiolic acid we prepared.

2.6.4 Coupling Reactions with $Ni[dppp]Cl_2$

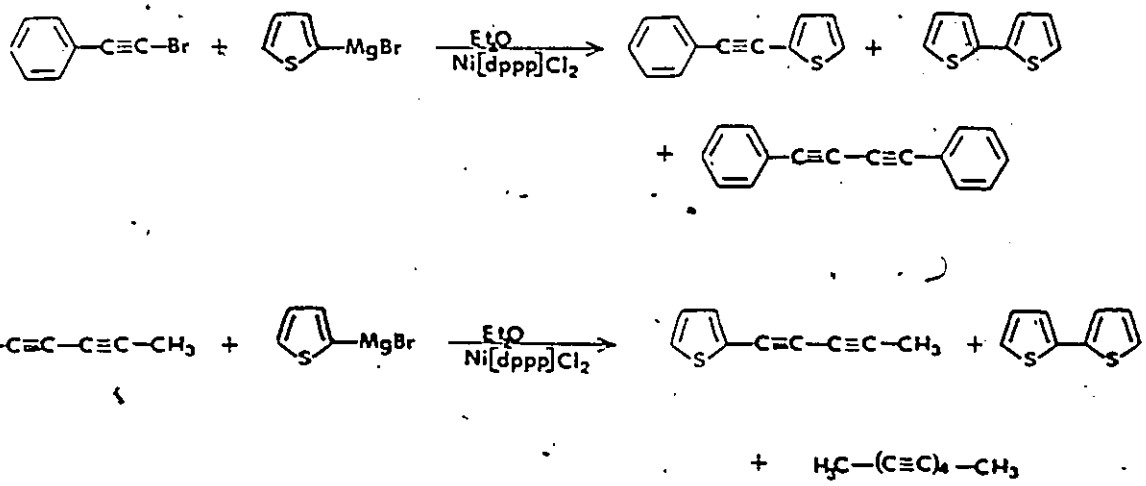
Recent interest in thiophene and acetylene derivatives isolated from natural sources has stimulated the search for a direct route to couple thiophenes and acetylenes. Initially reactions of this sort were performed by coupling aryl halides with cuprous acetylides[178-180]. The reaction between 2-iodo-furan or 2-iodo-thiophene with a cuprous acetylide[181] occurs in good yield. The reaction was performed with boiling pyridine as solvent in an inert atmosphere with a small amount of cupric ion. While this coupling reaction was useful, a few problems were also evident. A by-product was always formed in significant quantities in the reaction mixture where the iodine in the C-2 position of the heterocycle is replaced by hydrogen[181]. Also, the scope of the reaction is sometimes limited by the drastic reaction conditions, and by difficulties in preparing cuprous acetylides. Copper(I) acetylides have been known to explode[182]. Further development of this type of cross-coupling reaction has led to palladium catalyzed reactions[183-186].

Monosubstituted alkynes were coupled with aryl, heterocyclic or vinylic halides using a Pd(II) triphenyl-phosphine complex as catalyst in the presence of a basic

amine[183,184]. Acetylenic side chains have been introduced into 2-iodo or 4-iodo-pyrimidines in very good to excellent yields[187] using Pd(II) as catalyst. Cross-coupling reactions catalyzed by Pd(II) complexes can sometimes also be catalyzed by Ni(II) complexes[128]. Several reactions were performed using Ni[dppp]Cl_2 as catalyst to couple thienyl magnesium bromide with a brominated alkyne. Kumada *et al.* reported one example of such a reaction[102], with no yield given and without any discussion except to say that haloacetylenes undergo coupling with the Ni(II) catalyst very easily.

The reaction between 1-bromopenta-1,3-diyne and thienyl magnesium bromide using the nickel(II) phosphine catalyst produced the novel product, 1-(2-thienyl)-pentadiyne in 29% yield (Scheme 27). Column chromatography effected a partial separation of the reaction mixture; part of the product was isolated in pure form, while the rest was contaminated by the homo-coupled products 2,2'-bithienyl and 1,10-dimethyltetryne, which were identified by MS. By TLC, these products were located at R_f=0.5 as a long spot which turned blue at the top (bithienyl), violet in the center (perhaps the tetryne), and brown at the bottom (1-[2-thienyl]-pentadiyne) after spraying with H_2SO_4 and heating the TLC plate briefly.

The coupling of 1-bromo-2-phenylacetylene with thienyl magnesium bromide using the Ni(II) catalyst was an exothermic reaction; which yielded the desired product, 2-(2-thienyl)-1-phenylacetylene (Scheme 27). However, the homo-coupled products 2,2'-bithienyl and 1,4-diphenyl-butadiyne, were also present in the reaction mixture.



Scheme 27.

Column chromatography on alumina with petroleum ether or hexane as eluent effected only partial separation. Reverse phase HPLC would be required to separate the product completely from 1,4-diphenylbutadiyne.

The results of these preliminary reactions were encouraging as the desired product was formed in both cases. However, the by-products formed in these acetylene/thiophene coupling reactions reduce the yields and produce complex reaction mixtures which are difficult to separate. At the time these preliminary reactions were being performed, a similar type of reaction with the same target molecules was reported in the literature[149].

Rossi *et al.* synthesized the naturally occurring 5-(3-buten-1-ynyl)-2,2'-bithienyl (Scheme 6, 17), and some related compounds using a palladium catalyzed cross-coupling method[149]. This method couples aryl halides and

heterocycles with ethynyl Grignard reagents in good yield using tetrakis (triphenylphosphine) palladium ($[PPh_3]_4Pd$) as catalyst. Many heterocyclic acetylene derivatives have been prepared including 2-(2-thienyl)-1-phenylacetylene in 85% yield. This product was synthesized in higher yield using the Pd(II) catalyst and without the homo coupled side products that were present when Ni(II) was used as the catalyst.

EXPERIMENTAL

3.1 GENERAL

Melting points were determined on a Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian XL-300 NMR spectrometer using deuterochloroform as solvent and tetramethylsilane as the internal standard. If other solvents were used, they will be specified. UV spectra were obtained on a Hewlett Packard 8451A diode array spectrophotometer. IR spectra were recorded on a Perkin Elmer 783 Infrared spectrometer as a mixture of sample and KBr in the form of a pellet unless otherwise indicated. Mass Spectra were taken on a VG Analytical 7070E mass spectrometer equipped with a Dani 3800 Gas Chromatograph for GC-MS. The HPLC was done using a Varian Vista 5500 HPLC using a UV detector and coupled to a Shimadru C-R34 recorder. The type of column will be specified for each compound purified in this manner.

Thin layer chromatography was performed on either Baker-Flex pre-coated silica gel 1B2-F plates or Baker pre-coated glass plates. The reverse phase plates used were Whatman KC18f. The silica gel for liquid chromatography was purchased from Terrochem Laboratories, flash chromatography type with 20-435 micron particle size.

All solvents were distilled before use and solvents used for the Grignard reaction were anhydrous. Tetrahydrofuran (THF) was distilled over sodium and benzophenone. Mallinckrodt anhydrous diethyl ether was used undistilled. The petroleum ether used was of the boiling range 35-65 °C.

The magnesium metal used for the Grignard reactions was obtained from the Fisher Scientific Company and specifically intended for Grignard reactions. The magnesium was used as received.

3.1.1 General Procedure for the Grignard-Wurtz Coupling Reaction

This coupling procedure was developed by Kumada and Tamao *et al*[103,104]. Magnesium turnings (2.2 eq.) and anhydrous diethyl ether were placed in a three-necked flask, equipped with a reflux condenser, an inlet tube for passing a current of nitrogen and an additional funnel. The reaction was started by adding a small crystal of iodine while a solution of 2-bromothiophene (2 eq.) in anhydrous diethyl ether was slowly added dropwise under nitrogen with magnetic stirring. The mixture was stirred for 45 minutes after the addition of the 2-bromothiophene was complete.

The solution of 2-thienyl magnesium bromide was poured into a separatory funnel and added dropwise with stirring under nitrogen to the appropriate brominated substrate (1 eq.) in anhydrous diethyl ether. Dichloro-[1,3-bis(diphenylphosphinopropane)]-nickel, Ni[dppp]Cl_2, (trace) was added as catalyst. The mixture was stirred for 24 h at RT and then worked up with with 3N HCl. The diethyl ether layer was separated and washed with water. It was then dried over $MgSO_4$ and the solvent removed under reduced pressure. Crystallization, liquid chromatography or HPLC were used to purify the coupled product.

3.1.2 General Procedure For Selection of Grignard Solvent

In a three-necked flask magnesium (0.3 g, 12.0 mM), a crystal of iodine and solvent (10 ml) were placed. To this, 2-bromothiophene (0.87 ml, 9.0 mM) in the selected solvent was added through the dropping funnel. The reaction was stirred under a nitrogen flow for 1 h at RT. A trace of ethyl iodide was added and heat was applied only when necessary. All glassware was oven dried and the solvents were distilled prior to use.

The Grignard reagent was then slowly added to the $Ni[dppp]Cl_2$ catalyst (10 mg) and 2,5-dibromothiophene (0.75 ml, 4.0 mM) in the selected solvent (20 ml). The reaction mixture was stirred at RT for 24 h before work-up. At this time, 3N HCl (20 ml) was added dropwise and the two solvent layers separated. The ether layer was washed with water, dried over $MgSO_4$ and the solvent removed under reduced pressure.

The residue was chromatographed on flash silica (50 g), with petroleum ether (500 ml) as eluant.

3.2 PREPARATION OF 2,2':5',2"-TERTHIENYL IN VARIOUS SOLVENTS

α-T was prepared using the general procedure as described above and was used as starting material for the synthesis of its derivatives. The reaction product isolated after purification had the following characteristics; pale yellow needles, mp 91-92 °C (lit.[9] mp 94-95.5 °C); m/z 248 (M^+); ^1H NMR ($CDCl_3$), δ 7.20 (dd, 2H, J = 5.2, 1.0 Hz, 5,5"H), 7.01 (dd, 2H, J = 3.6, 5.2 Hz, 4,4"H), 7.16 (dd,

2H, J = 3.6, 1.0 Hz, 3,3"H), 7.06 (s, 2H, 3',4'H); Found C, 59.97; H, 3.11; S, 38.47; $C_{12}H_8S_3$ requires C, 58.06; H, 3.22; S, 38.71.

Diethyl Ether

Anhydrous diethyl ether was used undistilled. The Grignard reagent formed immediately at RT. The coupling reaction was stirred for 25 h.

n-Propyl Ether

n-Propyl ether was distilled from sodium and benzophenone immediately before use. The Grignard reagent formed easily at RT. The coupling reaction mixture was stirred 22 h before work-up.

n-Butyl Ether

The n-butyl ether was distilled from sodium onto molecular sieves before use. The Grignard began after adding 2 drops of ethyl iodide and stirring at RT. The coupling reaction was stirred for 24 h.

Diisopropyl Ether

The diisopropyl ether was distilled from sodium and benzophenone onto molecular sieves. The Grignard reagent would not form even with 4 h reflux and the addition of ethyl iodide.

Dimethoxyethane

Dimethoxyethane was distilled from sodium onto molecular sieves before use. After adding a few drops of ethyl iodide and 2 ml of anhydrous ether, the reaction was

stirred for 3 h at RT. Since the Grignard reagent had not formed, it was necessary to reflux the mixture for 3 h at which time the reaction occurred. The light brown colored Grignard reagent was not very soluble in dimethoxyethane. The coupling reaction was stirred for 24 h.

Tetrahydrofuran

The tetrahydrofuran used was distilled from sodium and benzophenone. The Grignard reagent was formed almost immediately. The coupling reaction was stirred for 26 h.

Tetrahydrofuran and Diethyl Ether

The Grignard reagent of 2-bromothiophene was prepared in tetrahydrofuran (20 ml) as in the previous example. It was added to 2,5-dibromothiophene and catalyst in tetrahydrofuran (15 ml) and diethyl ether (5 ml). The reaction was stirred for 24 h.

Dioxane

The dioxane was distilled from sodium and benzophenone onto molecular sieves. Difficulties were encountered in the formation of the Grignard reagent. The mixture was refluxed for 20 h with 4 drops of ethyl iodide, but no reaction occurred.

3.2.1 Large Scale Purification of A-Terthienyl

Soxhlet extraction of a crude A-T/ silica gel mixture with hexane as solvent proved to be an excellent method of purifying A-T, especially for gram scale quantities. The crude product (10 g) was mixed with an equal amount of silica gel and placed in a soxhlet

extraction thimble. Glass wool was inserted at the top of the thimble to prevent the silica gel/α-T mixture from rising during the initial wetting. The mixture was then continuously extracted with hexane (400 ml) for 24 h. On cooling, α-T crystallized from the hexane as a bright yellow solid. The product purity using this procedure was >99% using capillary GC.

3.2.2 Preparation of Dichloro[1,3-bis(diphenylphosphino)-propane]-Nickel(II), ($Ni[dppp]Cl_2$)

The nickel catalyst was prepared using a known preparation from the literature[188]. A solution of nickel(II) chloride hexahydrate (2.2 g, 9.0 mM) in a 5:2 mixture of 2-propanol and methanol (40 ml) was added to a hot solution of 1,3-bis(diphenylphosphino)propane (3.25 g, 7.8 mM) in 2-propanol (35 ml). A reddish brown precipitate formed almost immediately. The mixture was heated for 1 h and then brought to RT. The product was filtered off, washed with methanol and dried under vacuum. The red complex was formed in almost quantitative yield.

3.3 PREPARATION OF ANALOGUES OF α-TERTHIENYL

3.3.1 2,6-(Di-2-Thienyl)-Pyridine

The procedure for the following coupling reactions was based on the Kumada Grignard-Wurtz coupling[103,104]. 2-Bromothiophene (5 ml, 0.046 m) in anhydrous ether (25 ml) was added to magnesium turnings (1.2 g, 0.05 m) and a trace of iodine in diethyl ether (40 ml). It was stirred for one hour and then added to 2,6-dibromopyridine (4.5 g, 0.019 m) and nickel catalyst in diethyl ether (30 ml). The reaction

was stirred for 24 h at RT. After work-up, dark brown crystals (4 g, 86% crude yield) were isolated.

The crystals (2.15 g) were chromatographed on flash silica with 4 lbs of pressure. Petroleum ether was the eluant. A pale yellow crystalline solid (1.89 g, 76% yield) was separated; mp 79-80 °C (lit.[104] mp 78-79 °C); m/z 243 (M^+); ^1H NMR (Acetone-d_6) δ 7.78 (dd, 2H, J = <1, 4 Hz, 5,5"H), 7.16 (dd, 2H, J = 4, 6 Hz, 4,4"H), 7.57, (dd, 2H, J = <1, 6 Hz, 3,3"H), 7.71 (dd, 2H, J = 7, 1 Hz, 3',5'H), 7.83 (dd, 1H, J = <1, 7 Hz, 4'H); Found: C, 64.36; H, 3.81; S, 26.02; N, 5.78. $C_{13}H_9NS_2$ requires C, 64.2; H, 3.7; S, 26.3; N, 5.80.

3.3.2 1,3-(Di-2-Thienyl)-Benzene

The Grignard reagent was prepared by adding 2-bromothiophene (8.15 g, 0.05 m) in diethyl ether (10 ml) to magnesium turnings (1.22 g, 0.05 m) and iodine (trace) in diethyl ether (15 ml). After 1 hr., the Grignard was slowly added to 1,3-dibromobenzene (5.0 g, 0.021 m) and nickel catalyst in diethyl ether (20 ml). Work-up after 24 h afforded a residue which crystallized from hot hexanes yielding a green solid (540 mg, 10% crude yield). The green solid (190 mg) was purified by column chromatography using petroleum ether as the eluant. A light green crystalline solid (136 mg, 7.6% yield) was separated; mp 83-84 °C; m/z 242 (M^+); ^1H NMR ($CDCl_3$) δ 7.35 (dd, 2H, J = <1, 3 Hz, 5,5"H), 7.085 (dd, 2H, J = 5, 3 Hz, 4,4"H), 7.29 (dd, 2H, J = <1, 5 Hz, 3,3"H), 7.81 (t, 1H, J = <1 Hz, 2'H), 7.38 (dd, 1H, J = 8 Hz, 5'H), 7.51 (ddd, 2H, J = <1, 2, 8 Hz, 6',4'H); Found C, 69.22; H, 4.04; S, 26.09; $C_{14}H_{10}S_2$ requires C, 69.22; H, 4.1; S, 26.4.

3.3.3 1,4-(Di-2-Thienyl)-Benzene

2-Bromothiophene (22.5 g, 0.15 m) in diethyl ether (150 ml) was added to magnesium turnings (4.0 g, 0.15 m) and iodine (trace) in diethyl ether (25 ml). The Grignard reagent was added to 1,4-dibromobenzene (13 g, 0.055 m) and nickel catalyst in diethyl ether (25 ml). The reaction mixture was refluxed under nitrogen for 10 h and at RT for another 36 h. The reaction was worked up in the usual fashion. The crude residue was dissolved in hot hexanes (75 ml) and filtered to remove insoluble material. After evaporation of the hexane to 40 ml and cooling, orange plate-like crystals (1.0 g) separated. An orange solid (4.9 g) was also obtained by filtering the water layer and washing the water layer with benzene (44% crude yield). 1,4-(Di-2-thienyl)-benzene (0.66 g) was further purified by column chromotography using 1.5% diethyl ether/petroleum ether as eluant. Bright yellow crystals (153 mg) were isolated. The remainder of the product was recrystallized from hot benzene affording yellow-orange crystals (1.9 g, 15% yield); mp 207-208 °C, (lit.[140] mp 208 °C); m/z 242 (M^+); ^1H NMR ($CDCL_3$) δ 7.28 (dd, 2H, J = 3.6, 1.2 Hz, 5,5"H), 7.04 (dd, 2H, J = 3.06, 5.4 Hz, 4,4"H), 7.23 (dd, 2H, J = 5.4, 1.2 Hz, 3,3"H), 7.56 (s, 4H, 2',3',5',6'H); Found: C, 69.29; H, 4.03; S, 26.33; $C_{14}H_{10}S_2$ requires C, 69.42; H, 4.03; S, 26.33.

3.3.4 1-(2-Thienyl)-Naphthalene

2-Bromothiophene (1.45 ml, 15.0 mM) in diethyl ether (20 ml) was added slowly to magnesium turnings (0.48 g, 20.0 mM) and iodine (trace) in diethyl ether (10 ml). It was stirred for 0.5 h before being added to 1-bromo-naphthalene (2.07 g, 10.0 mM) and nickel catalyst in diethyl

ether (25 ml). The coupling reaction was stirred for 24 h. The reaction was worked up in the usual way yielding a brown oil (2.49 g). The brown oil was purified by column chromatography. Flash silica (104 g) eluted with petroleum ether (600 ml) and increased to 5% diethyl ether/petroleum ether separated a clear pale green oil (1.50 g, 71% yield); m/z 210 (M^+); ^1H NMR ($CDCl_3$) δ 7.17 (dd, 1H, J = 3.2, 5.6 Hz), 7.23 (dd, 1H, J = <1, 3.2 Hz), 7.41 (dd, 1H, J = <1, 5.6 Hz), 7.45 (d, 1H, J = 4.8 Hz), 7.47 (d, 1H, J = 4.8 Hz), 7.48 (d, 1H, J = 8 Hz), 7.55 (dd, 1H, J = <1, 8 Hz), 7.83 (d, 1H, J = 8 Hz), 7.88 (dd, 1H, J = 3.2, 4.8 Hz), 8.20 (dd, 1H, J = 5.6, 4.8 Hz); Found: C, 80.26; H, 4.91; S, 15.24; $C_{14}H_{10}S$ requires C, 80.00; H, 4.76; S, 15.24.

3.3.5 2-(2-Thienyl)-Naphthalene

2-(2-Thienyl)-naphthalene was prepared in the same manner as for the 1-thienyl-naphthalene derivative, with the addition of the Grignard reagent to 2-bromonaphthalene (2.07 g, 0.01 m). After work-up, a brown solid (2.39 g) was isolated. Column chromatography yielded starting material, 2-bromonaphthalene (630 mg) and the product as a white crystalline solid (1.13 g, 73% yield based on recovered starting material); mp 103-104 °C (lit.[189] mp 104-105 °C); m/z 210 (M^+); ^1H NMR ($CDCl_3$) δ 7.17 (dd, 1H, J = 5.6, 3.5 Hz), 7.30 (dd, 1H, J = <1, 5.6 Hz), 7.42 (dd, 1H, J = <1, 5 Hz), 7.45 (dd, 1H, J = <1, 7 Hz), 7.46 (dd, 1H, J = 1, 4 Hz), 7.73 (dd, 1H, J = 1, 8 Hz), 7.79 (dd, 1H, J = 1, 8 Hz), 7.81 (s, 1H), 7.83 (s, 1H), 8.03 (d, 1H, J = <1 Hz); Found: C, 79.75; H, 4.80; S, 15.09. $C_{14}H_{10}S$ requires C, 80.0; H, 4.76; S, 15.24.

3.4 SYNTHESIS OF α-TERTHIENYL DERIVATIVES

3.4.1 5-Methyl-2,2':5',2"-Terthienyl

α-Terthienyl (1.0 g, 4.0 mM) was dissolved in dry THF (75 ml) and cooled to -78 °C via an acetone/CO_2 bath. All glassware was oven dried and the butyllithium titrated before use. The reaction was carried out under nitrogen. Butyllithium (2.08 M, 1.92 ml, 4.0 mM) was added to the cooled solution dropwise over 15 min. The yellow lithium salt formed and precipitated out of solution. The mixture was stirred for 0.5 h at which time methyl iodide (0.5 ml, 8.0 mM) was slowly added. The reaction was stirred for 2 h at -78 °C and then allowed to warm up to RT. At -30 °C, the lithium salt rapidly disappeared leaving a clear orange solution. After stirring at RT for 24 h, the reaction was worked up with 3N HCl. Methylene chloride (50 ml) was added and the organic layer separated and washed with water. It was then dried over $MgSO_4$ and solvent removed by rotary evaporation, yielding a brown-green solid (1.12 g).

The crude product was chromatographed with silica (52 g) using petroleum ether as the eluant. A bright yellow solid (0.93 g) was isolated and determined by reverse phase TLC (90% ethanol/ 10% H_2O) to be a mixture of α-T and the mono- and dimethylated products. The products were separated by HPLC using an MCH-5NCAP column (analytical) purchased from Varian and eluting with 5% H_2O/ 95% CH_3OH. The product ratio of the reaction mixture using integrated peak areas was α-T -7%, 5-methyl-2,2':5'2"-terthienyl -69%, and 5,5"-dimethyl-2,2':5',2"-terthienyl -23%. Preparative HPLC using 2% H_2O/ 98% CH_3OH as eluant was performed using a Serva ODS 100 polyol column (22 mm x 500 mm, with 5 micron particle size). mp 94.5-95.5 °C (lit.[9] 93-94.5 °C); m/z 262

(M^+); ^1H NMR ($CDCl_3$) δ 2.46 (d, 3H, J = <1 Hz, 5-C$\underline{H}_3$), 6.65 (m, 1H, J = <1, 3.2 Hz, 4$\underline{H}$), 6.94 (d, 1H, J = 3.2 Hz, 3$\underline{H}$), 6.97 (d, 1H, J = 3.2 Hz, 3'$\underline{H}$), 7.02 (d, 1H, J = 3.2 Hz, 4'$\underline{H}$), 7.13 (dd, 1H, J = 1, 3.2 Hz, 3"$\underline{H}$), 6.70 (dd, 1H, J = 3.2, 5.2 Hz, 4"$\underline{H}$), 7.19 (dd, 1H, J = <1, 5.2 Hz, 5"$\underline{H}$).

3.4.2 5,5"-Dimethyl-2,2':5',2"-Terthienyl

The procedure for synthesizing the dimethylated derivative was similar to that of 5-methyl-α-terthienyl so only the modifications will be mentioned here. The butyllithium (2.02 M, 3.96 ml, 8.0 mM) was slowly added to the solution of α-terthienyl (1.0 g, 4.0 mM) in tetrahydrofuran (75 ml) which was kept between -50 and -40 °C in an acetone/CO_2 bath. The mixture was stirred at this temperature for 45 min at which time methyl iodide (0.75 ml, 12.0 mM) was slowly added. It was stired for a further 20 min and allowed to warm up to RT. At 4 °C, most of the lithium salt had disappeared leaving an almost clear orange solution. After work-up, a green solid was isolated (1.23 g).

Liquid chromatography yielded a bright yellow solid (0.90 g) which showed by reverse phase TLC (90% ethanol/ 10% water), a mixture of mono and dimethylated α-terthienyl. HPLC using the MCH-5NCAP reverse phase analytical column by Varian and eluting with 5% H_2O/ 95% CH_3OH showed the mixture to be 5- methyl-2,2':5',2"-terthienyl -16%, and 5,5"-dimethyl-2,2':5',2"-terthienyl - 82%. Preparative HPLC using the Serva ODS 100 polyol column (22 mm x 500 mm, 5 micron particle size) and eluting with 2% H_2O/ 98% CH_3OH yielded a yellow-orange solid. mp 96-97 °C, (lit.[9] mp 98-99 °C); m/z 276 (M^+); ^1H NMR ($CDCl_3$) δ 2.46 (s, 6H, 5-C$\underline{H}_3$, 5'-C$\underline{H}_3$), 6.64 (m, 2H, J = <1, 4 Hz, 4,4"$\underline{H}$), 6.92 (d, 2H, J = 4 Hz, 3,3"$\underline{H}$), 6.94 (s, 2H, 3',4'$\underline{H}$); Found: C,

60.34; H, 4.36; S, 35.04. $C_{14}H_{12}S_3$ requires: C, 60.87; H, 4.34; S, 34.78.

3.4.3 5-Iodo-2,2':5'2"-Terthienyl

The iodination method used was based on a previous preparation of iodothiophene in 72% yield[145]. α-Terthienyl (1.00 g, 4.0 mM) was dissolved in $CHCl_3$ (50 ml), and I_2 (1.19 g, 4.5 mM) was added. HgO (0.72 g, 3.3 mM) was added in small portions over 3 h. The mixture was left to stir at RT for 24 h. At this time TLC (petroleum ether eluant) showed the presence of starting material, and the 5-iodo derivative of α-T. The HgI_2 was filtered off and the solution washed with sodium thiosulfate (300 ml, 10%). The $CHCl_3$ was then dried over $MgSO_4$ and removed under reduced pressure yielding a brown solid (2.8 g).

The product was dissolved in hexane, and the insoluble material filtered off (220 mg). It was mostly the 5-iodinated product mp 128-130 °C. On cooling, 5-iodo-α-terthienyl (342 mg) crystallized from the hexane as a tan-yellow powder. A second crop of product (116 mg) with a small amount of starting material present was also recovered. 5-Iodo-2,2':5',2"-terthienyl was crystallized in 45% yield. Some of the crystallized product was purified by column chromatography using flash silica gel and petroleum ether as eluant; mp 138-139 °C; m/z 374 (M^+); ^1H NMR (DMSO-d_6) δ 7.10 (d, 1H, J = 3.6 Hz, 4H), 7.36 (d, 1H, J = 3.6 Hz, 3H), 7.28 (m, 2H, 4',3'H), 7.38 (dd, 1H, J = 3.6, 1 Hz, 3"H), 7.14 (dd, 1H, J = 3.6, 5.4 Hz, 4"H), 7.56 (dd, 1H, J = 5.4, 1 Hz, 5"H).

3.4.4 5,5"-Diiodo-2,2':5',2"-Terthienyl

α-Terthienyl (2.48 g, 10.0 mM), HgO (3.0 g, 13.8 mM), and I_2 (4.36 g, 17.0 mM), were added to $CHCl_3$ (75 ml) and the reaction stirred for 18 h at RT. TLC (petroleum ether eluant) showed the presence of α-terthienyl so further HgO (1.0 g, 4.6 mM) and I_2 (2.0 g, 7.9 mM) were added and the reaction stirred for 24 h. Filtration of HgI_2 and removal of benzene on a rotary evaporator afforded a pale brown solid. Hot benzene was added to the solid and the undissolved material, the diiodinated product, was filtered off (1.2 g, 24% yield); mp 188-190 °C; m/z 500 (M^+); ^1H NMR ($CDCl_3$) δ 6.86 (d, 2H, J = 3.6 Hz, 4,4"H), 7.18 (d, 2H, J = 3.6 Hz, 3,3"H), 7.04 (s, 2H, 3',4'H).

3.4.5 5-Bromo- and 5,5"-Dibromo-2,2':5',2"-Terthienyl

α-Terthienyl (2.46 g, 0.01 m) and N-bromo-succinimide (NBS) (1.96 g, 0.011 m) were dissolved in CCl_4 (50 ml) and refluxed for 10 h. The succinimide floated on top of the solution and the solution turned from yellow to green. The imide was filtered off and the filtrate removed under reduced pressure yielding a green solid (4.2 g). By TLC the product was a mixture of monobrominated and dibrominated α-terthienyl with very little unreacted starting material.

Liquid chromatography yielded only partial separation, so the yield was determined by HPLC using a Beckman ODS DP5M (6 mm ID, 25 cm) reverse phase column and eluting with acetonitrile. The reaction mixture was determined to be 5-bromo-α-terthienyl -57% and 5,5"-dibromo-α-terthienyl -26%. For the monobrominated derivative: mp 136-137.5 °C ; m/z 326,328 (M^+); ^1H NMR ($CDCl_3$) δ 6.89 (d,

1H, J = 3.6 Hz, 4H), 6.95 (d, 1H, J = 3.6 Hz, 3H), 6.99 (d, 1H, J = 3.6 Hz, 3'H), 7.05 (d, 1H, J = 3.6 Hz, 4'H), 7.15 (dd, 1H, J = 3.6, <1 Hz, 3"H), 7.00 (dd, 1H, J = 5.4, 3.6 Hz, 4"H), 7.21 (dd, 1H, J = 5.4, <1 Hz, 5"H). Found C, 44.17; H, 2.10; S, 29.45; Br, 24.59. $C_{12}H_7S_3Br$ requires C, 44.03; H, 2.14; S, 29.36; Br, 24.5. For the dibrominated derivative: mp 156-157 °C (lit.[9] 160-161 °C); m/z 404,406,408 (M^+); ^1H NMR ($CDCl_3$) δ 6.89 (d, 2H, J = 3.6 Hz, 4,4"H), 6.96 (d, 2H, J = 3.6 Hz, 3,3"H), 6.97 (s, 2H, 3',4'H).

3.4.6 5-Cyano-2,2':5',2"-Terthienyl

The cyanation method used was previously tried on thiophene with good results and was considered a general method suitable for compounds which readily undergo electrophilic substitution[154]. To a solution of α-terthienyl (2.46 g, 0.01 m) in methylene chloride (20 ml), was added chlorosulfonyl isocyanate (1.75 ml, 0.02 m) in methylene chloride (15 ml). A yellow solid separated almost at once. The reaction mixture was stirred for 2 h and left overnight. Dimethylformamide (5.0 ml, 0.064 m) was added to the ice-cold reaction mixture dropwise. Addition of water (40 ml) precipitated a yellow solid insoluble in methylene chloride. The latter was removed in vacuo and the yellowish green solid (2.0 g) was collected in a Buchner funnel and washed with water. Recrystallization from the minimum amount of boiling ethanol-benzene (1:1) afforded 1.6 g of product (58.6 % crude yield).

The recrystallized material (500 mg) was chromatographed on flash silica (110 g), eluted with 3% ethyl acetate/ hexane and gradually increased to 15% ethyl acetate. A bright yellow solid (234 mg, 27.4% yield) was

isolated. mp 101-105 °C, m/z 273 (M^+); ^1H NMR (Acetone-d_6) δ 7.81 (d, 1H, J = 3.5 Hz, 4H), 7.30 (d, 1H, J = 3.5 Hz, 3H), 7.42 (d, 1H, J = 3.7 Hz, 4'H), 7.46 (d, 1H, J = 3.7 Hz, 3'H), 7.38 (dd, 1H, J = <1, 3.5 Hz, 3"H), 7.12 (dd, 1H, J = 3.5, 5.5 Hz, 4"H), 7.50 (dd, 1H, J = <1, 5.5 Hz, 5"H); Found C, 57.45; H, 2.87; S, 34.49; N, 4.89; $C_{13}H_7S_3N$ requires C, 57.10; H, 2.56; S, 35.10; N, 5.13.

3.4.7 5,5"-Dicyano-2,2':5',2"-Terthienyl

α-Terthienyl (2.46 g, 0.01 m) was treated with chlorosulfonyl isocyanate (3.5 ml, 0.04 m) in methylene chloride as described in the preparation of 5-cyano-2,2':5',2"-terthienyl. Work-up as in that procedure gave crude product (2.4 g, 56% crude yield). Capilliary GC analysis of the product showed that it was composed of the 5-cyano product (30%) and the 5,5"-dicyano product (70%). mp 165-170 °C, contracting around 140 °C ; m/z 298 (M^+); ^1H NMR (DMSO-d_6) δ7.98 (d, 2H, J = 4 Hz, 4,4"H), 7.62 (s, 2H, 3',4'H), 7.58 (d, 2H, J = 4 Hz, 3,3"H).

3.4.8 5-Nitro-2,2':5',2"-Terthienyl

The procedure[153] used to synthesize 2-nitro-thiophene was the method chosen to nitrate α-T. To a solution of α-terthienyl (1.24 g, 5.0 mM) in methylene chloride (10 ml) with acetic anhydride (10 ml) at 5 °C was added with stirring, 3.5 ml of a solution composed of fuming nitric acid (3.0 ml) in glacial acetic acid (30 ml). The reaction mixture which became slightly warm was stirred at RT for 6 h. It was then poured into water (60 ml) and stirred for 30 min to hydrolyse the acetic anhydride. The reddish-brown precipitate (1.3 g) obtained after removing methylene chloride in vacuo was collected in a Büchner

funnel and washed with water: mp 128-130 °C, shrinking at 124 °C. TLC (20% ethyl acetate/hexane) showed the product to be a mixture of mono-and dinitro-α-terthienyl.

Part of the mixture (300 mg) was separated by liquid chromatography using flash silica (76 g) and eluting with hexane gradually increasing to 5% ethyl acetate/ hexane. A red-brown solid (110 mg, 36% yield) was isolated and was determined by capillary GC to be the mono-nitro derivative (80%) and 20% of another compound, possibly an isomer. mp 139-141 °C; m/z 293 (M^+); For the 5-nitro-derivative: ^{1}H NMR ($CDCl_3$) δ 7.83 (d, 1H, J = 4.0 Hz, 4H), 7.05 (d, 1H, J = 4.0 Hz, 3H), 7.12 (d, 1H, J = 3.0 Hz, 4'H), 7.26 (d, 1H, J = 3.0 Hz, 3'H), 7.22 (dd, 1H, J = <1, 3.0 Hz, 3"H), 7.04 (dd, 1H, J = 3.0, 5.2 Hz, 4"H), 7.28 (dd, 1H, J = <1, 5.2 Hz, 5"H).

3.4.9 5-Formyl-2,2':5',2"-Terthienyl

The procedure used to make the aldehyde of α-T was based on a method of preparing the aldehyde of thiophene in good yield[156]. N-methylformanilide (0.52 ml, 4.4 mM) and phosphorus oxychloride (0.37 ml, 4.0 mM) were mixed and left to stand for 15 min to allow the reagent to form. A solution of α-terthienyl (1.0 g, 4.0 mM) in methylene chloride (10 ml) was added with stirring. There was no appreciable rise in temperature. The reaction mixture was stirred for 24 h at RT before pouring it into HCl (50 ml) and stirring for 1 h to remove dimethylaniline as the hydrochloride. The methylene chloride extract was freed of solvent in vacuo leaving an orange solid (1.33 g) as residue. Flash column chromatography with $CHCl_3$ as eluant yielded an orange product (0.93 g, 85% yield); mp 135-136 °C (lit.[26] mp 135-136 °C); m/z 276 (M^+); ^{1}H NMR (Acetone-d_6)

δ 9.92 (s, 1H, 5-CHO), 7.93 (d, 1H, J = 3.6 Hz, 4H), 7.30 (d, 1H, J = 3.6 Hz, 3H), 7.49 (d, 1H, J = 3.6 Hz, 3'H), 7.48 (d, 1H, J = 3.6 Hz, 4'H), 7.12 (dd, 1H, J = 4.2, 5.4 Hz, 4"H), 7.50 (dd, 1H, J = <1, 5.4 Hz, 5"H), 7.38 (dd, 1H, J = <1, 4.2 Hz, 3"H); Found: C, 56.03; H, 2.87; S, 34.7. $C_{13}H_8S_3O$ requires C, 56.50; H, 2.89; S, 34.8.

3.4.10 5-(2-Hydroxyethyl)-2,2':5,2"-Terthienyl)

The lithium salt of α-terthienyl was prepared by adding, under nitrogen, butyllithium (2.0 M, 2.0 ml, 4.0 mM) via a syringe and septum to a stirred solution of α-terthienyl (1.00 g, 4.0 mM) in anhydrous THF (50 ml) cooled to -40 °C in a dry ice bath. After 1 h of stirring at -20 °C, excess ethylene oxide was bubbled through the yellow suspension of the lithium salt for 10 min. The reaction mixture was allowed to come to RT, stirred for 20 h and acidified with 3N HCl. THF was removed under vacuum and the residual oil was taken up in diethyl ether. Evaporation of the dried diethyl ether solution under vacuum left a green solid (1.1 g). Recrystallization from ethanol and then hexane gave green crystals (0.50 g, 43% crude yield). Column chromatography (ethyl acetate/ hexane eluent) was used to purify a small amount of the α-ethanol derivative : mp 85-87 °C; ^{1}H NMR ($CDCl_3$) δ 3.87 (t, 2H, J = 6.0 Hz, 5-CH_2OH), 3.02 (t, 2H, J = 6.0 Hz, 5-CH_2-CH_2OH), 6.77 (dt, 1H, J = <1, 3.6 Hz, 4H), 7.05 (d, 1H, J = 3.6 Hz, 3H), 6.99 (d, 1H, J = 2.4 Hz, 3'H), 7.00 (d, 1H, J = 2.4 Hz, 4'H), 7.14 (dd, 1H, J = <1, 3.6 Hz, 3"H), 7.00 (dd, 1H, J = 3.6, 5.2 Hz, 4"H), 7.20 (dd, 1H, J = <1, 5.2 Hz, 5"H).

3.4.11 5-(2,2':5',2"-Terthienyl)-Amide

5-Cyano-α-terthienyl (100 mg, 0.36 mM) was dissolved in ethanol (15 ml) and added to an aqueous NaOH solution (10 ml, 6.8 mM, 0.68 M). The reaction mixture was stirred for 1 h and then heated for 4 h at 70 °C. At this time the starting material was not detected by TLC (ethyl acetate/ hexane 1:5). Dilute acid was added precipitating the amide (0.97 g). The crude product was purified by column chromatography using silica gel and eluting with hexane/ acetone, increasing the amount of acetone gradually to 100%. The 5-amide was isolated as a yellow powder (75 mg, 71.6% yield) with a small quantity of the 5-carboxy derivative, which was detected by TLC; mp 225 °C shrinking and then charring to 240° C; m/s 291 (M^+); IR (KBr pellet) 3165, 3390 cm^{-1} (NH_2 stretching, broad), 1680 cm^{-1} (C=O stretching, very strong), 1390 cm^{-1} (C-N stretching, very strong); ^1H NMR (acetone-d_6) δ 7.67 (d, 1H, J = 3.5 Hz, 4H), 7.46 (dd, 1H, J = <1.0, 5.0 Hz, 5"H), 7.32-7.34 (m, 4H), 7.29 (d, 1H, J = 3.5 Hz, 3'H), 7.25 (d, 1H, J = 3.5, 4'H), 7.10 (dd, 1H, J = 3.5, 5.0 Hz, 4"H).

3.4.13 5-(2,2':5',2"-Terthienyl)-Acrylic Acid

The following procedure was based on the Perkin reaction which was used to prepare β-piperonylacrylic acid[158]. A mixture of 5-formyl-α-terthienyl (2.7 g, 0.01 m), malonic acid (2.0 g, 0.019 m) and piperidine (0.1 ml) in pyridine (20 ml) was heated until there was no evolution of CO_2 through a bubble counter on top of the reflux condenser. This took 2 h. The cold reaction mixture was then poured into 2N HCl (75 ml). The orange precipitate was collected on a Büchner funnel and washed copiously with water. It was digested with dilute sodium hydroxide in

ethanol (50 ml, 25%). Insoluble material was collected on a filter and was treated as described later. Acidification of the yellow filtrate precipitated a red solid (100 mg), mp 190-191 °C with decomposition, probably evolution of CO_2. Both the yellow precipitate and the red precipitate gave the same MS ion m/z 318 (M^+).

The insoluble product was digested with hot dilute HCl and filtered off, It amounted to 2.0 g and melted at 230 °C. Recrystallization from glacial acetic acid (200 ml) resulted in a yellow product melting at 220-225 °C with decomposition. The yield was 47% (1.5 g) of the E isomer; m/z 318 (M^+); ^1H NMR (Acetone-d_6) δ 1.04 (d, 1H, J = 6.0 Hz, $CO_2\underline{H}$), 6.21 (d, 1H, J = 14 Hz, CH=C$\underline{H}$-CO_2H), 7.10 (dd, 1H, J = 5.0, 3.0 Hz, 4"$\underline{H}$), 7.26 (d, 1H, J = 3.0 Hz), 7.33-7.35 (m, 3H), 7.43 (d, 1H, J = 3.0 Hz), 7.47 (dd, 1H, J = 1.0, 5.0 Hz, 5"$\underline{H}$) 7.77 (dd, 1H, J = <1.0, 14 Hz, C$\underline{H}$=CH-CO_2H). The red solid was the Z isomer; ^1H NMR (Acetone-d_6) δ 1.04 (d, 1H, J = 6.0 Hz, $CO_2\underline{H}$), 7.12 (dd, 1H, J = 3.0, 5.0 Hz, 4"$\underline{H}$), 7.31 (d, 1H, J = 3.0 Hz), 7.38 (dd, 1H, J = 1.0, 3.0 Hz, 3"H), 7.45-7.79 (m, 4H), 7.80 (dd, 1H, J = 3.0, 1.0 Hz, C$\underline{H}$=CH-CO_2H), 8.32 (d, 1H, J = <1.0 Hz, CH=C$\underline{H}$-CO2H).

3.5 PREPARATION OF PHENYLHEPTATRIYNE AND ACETYLENIC COMPOUNDS

3.5.1 1-Bromo-2-Phenylacetylene

The brominations of the acetylenic substrates were done using the hypobromite method first developed by Strauss *et al.*[176].

Hypobromite was prepared by adding bromine (1.7 ml, 0.034 m) to aqueous NaOH (5.3 M, 15 ml, 0.079 m) which

was cooled to 5 °C. Nitrogen was introduced into the flask before slowly adding phenylacetylene (2.02 g, 0.02 m) dissolved in dimethoxyethane (8 ml). After stirring the reaction mixture for 5 h at RT, it was extracted with diethyl ether (6 x 50 ml) and the organic layer dried with $MgSO_4$. The solvent was then removed under reduced pressure with the bath temperature not exceeding 30 °C. Any remaining solvent was removed using the vacuum pump. An IR (neat) of the orange oil showed a strong sharp peak at 2200 cm^{-1}, characteristic of a disubstituted acetylene. The absence of the monosubstituted acetylene band due to starting material at 2110 cm^{-1} indicated that the reaction was essentially quantitative. GC-MS showed one peak with m/z 180, 182 (M^+).

3.5.2 1,4-Dichloro-2-Butyne

The procedure follwed was a known procedure from the literature[170]. 2-Butyne-1,4-diol (172.22 g, 2.0 m) was placed in a three-necked two litre flask fitted with an additional funnel, thermometer and drying tube leading to a neutralizing NaOH solution. To this, pyridine (15 ml) was added and the flask cooled to -35 °C via an acetone/ CO_2 bath. Thionyl chloride (380 ml, 5.2 m) was pre-cooled to -40 °C and added slowly to the diol over 2 h. After the addition, the reaction temperature was slowly brought to 0 °C . This took 2 h. At this time the butynediol was consumed and a brown liquid remained. An ice bath was employed to slowly bring the reaction from 0 °C to RT. The reaction was left to stand for 18 h at which time the gases were sucked out using an aspirator. When most of the gases had been removed, the temperature was raised to 40 °C for 4 h. The reaction mixture was distilled yielding a clear

pale yellow liquid (111 ml, 57% yield) bp 60 °C/ 12 mm. The IR (neat) spectrum contained sharp medium peaks at 2990, 2950 cm^{-1} with smaller peaks at 2320, 2690, 2840 cm^{-1}, identical to a commercial sample.

3.5.3 Pentadiyne

The preparation of pentadiyne was from a large scale procedure published by Brandsma[170]. The apparatus used for this experiment was thoroughly dried before condensing NH_3 (500 ml) into a one litre three-necked flask. An acetone/ CO_2 bath was used throughout. With nitrogen slowly flowing through the apparatus, commercial $NaNH_2$ (25.5 g, 0.65 m) was added to the NH_3. The temperature of the bath was then brought to between -40 - -50 °C and dichlorobutyne (20 ml, 0.204 m) was added by syringe over a 2 h period. The reaction was stirred for 15 min and any NH_3 that evaporated during the addition was replaced. After stirring another 15 min, pre-cooled CH_3I (12.75 ml, 0.204 m) was added by syringe over a 25 min period. The reaction mixture was stirred for 20 min and the temperature of the bath was brought to -30 °C. Decalin (125 ml) was added and the reaction mixture poured into a two-litre Erlenmeyer flask containing crushed ice and decalin (50 ml). Using a separatory funnel the decalin layer was saved and the aqueous layer reextracted with decalin (2 x 100 ml). The decalin was dried with $MgSO_4$ and then distilled without heat using the vacuum pump (2 mm). The pentadiyne (8 ml, 45% yield) was collected in an acetone/ CO_2 trap specifically constructed to trap volatile products. The IR (neat) of the clear liquid showed a sharp peak at 3300 cm^{-1} which is characteristic of a terminal acetylene. All of the peaks in the spectrum were small as the sample volatilized very

quickly. The decalin contained the same intense peak at 3300 cm^{-1} before distillation. m/z 64 (M^+) with the following cracking pattern 64, 63(-H), 62(-H), 61(-H), 60(-H) in decreasing intensity.

Dimethylbutadiyne, a side product from this reaction was characterized by GC-MS; m/z 78 (M^+) with the following cracking pattern, 78, 63($-CH_3$), 51. The IR (nujol) showed small sharp peaks at 2040, 2150, and 2210 cm^{-1} indicating a disubstituted acetylene.

A small amount of butadiyne was also detected by GC-MS; m/z 50 (M^+) with the cracking pattern 50, 49(-H), 48(-H).

3.5.4 1-Phenylhepta-1,3,5-Triyne

PHT has previously been prepared using this method, during an examination of the scope of the Cadiot-Chodkiewicz cross-coupling reaction[166].

$CuCl_2$ (9.9 mg, 0.074 mM) was placed in a three-necked flask (50 ml) equipped with a dropping funnel, thermometer and an inlet for nitrogen. To this was added propylamine (1.7 ml, 20.5 mM) HPLC grade methanol (5 ml) and $NH_2OH \cdot HCl$ (45.5 mg, 0.65 mM). The ethynyl compound, pentadiyne, (1.1 ml, 0.78 g, 12.2 mM) slightly yellow from polymerization, was then added to the flask immediately forming a soluble red copper acetylide. 1-Bromo-2-phenyl-acetylene (1.69 g, 9.4 mM) was solubilized in methanol (5 ml) and added slowly to the reaction flask. The reaction was kept at 20 °C under nitrogen and vigorously stirred for 6.5 h. KCN (0.5 M, 15.5 ml, 7.75 mM) was added to stop the reaction. The mixture was then extracted with ether (6 x

20 ml) and the solvent removed under reduced pressure yielding a red-brown oil (3-4 ml). The only spot on TLC past the baseline (100% pentane rf=0.6) of the reaction mixture was identical to that of isolated naturally occurring PHT. The UV spectrum of the reaction mixture was also identical to that of the natural compound with absorbances at 332, 312, 292, 274, 250, 240 and 223 nm. Flash column chromatography eluting with petroleum ether at 6 lbs pressure yielded PHT in the first fraction as a colorless oil (1.3 g, 84% yield). The IR (methylene chloride solution) showed sharp intense peaks at 2300, 2980, and 3040 cm^{-1}, with a less intense peak at 2220 cm^{-1}. m/z 164 (M^+). PHT was stored as an ether solution to reduce polymerization.

3.5.5 Bromopropiolic Acid

Propiolic acid (2.01 g, 28.8 mM) was treated with cold aqueous NaOH (1.8 M, 16 ml, 28.8 mM) to prepare the salt. Hypobromite was prepared from aqueous NaOH (4.3 M, 20 ml, 86.0 m M) and bromine (1.54 ml, 30 mM) and cooled to 0 °C. The hypobromite was added to the salt with cooling and vigorous stirring. The temperature rose to 15 °C. The mixture was stirred for 3 h under nitrogen. The clear yellow liquid was then acidified to ph=2 with cold H_2SO_4 (40%). The reaction mixture was then continuously extracted with methylene chloride for 16.5 h. The evaporation of the methylene chloride yielded crude bromopropiolic acid as pale yellow crystals (1.51 g, 35.4% yield); mp 79-85 °C (lit.[176] mp 84-86 °C); m/z 104, 106 ($-CO_2$) as the base peak with the next prominent peaks 148, 150 (M^+) and 131, 133 (-OH). When ether was used as the extractant, the acid was isolated as an oil. The IR (neat) showed a strong sharp

peak at 2200 cm^{-1} characteristic of a disubstituted acetylene.

3.5.6 1-Bromopenta-1,3-diyne

Hypobromite was prepared by adding bromine (3.63 ml, 0.071 m) to an aqueous NaOH solution (8.0M, 20.0 ml, 0.16 m). The reaction was done under a nitrogen atmosphere, at 20 °C with vigorous stirring. Pentadiyne (2.5 ml, 0.029 m) was then added to the hypobromite via a syringe. The reaction was stirred for 30 min before extraction with petroleum ether (5 x 25 ml). TLC (100% hexane) showed one spot rf=0.6. The petroleum ether was dried over MgSO4 and removed under reduced pressure. The product was a yellow orange oil (3.13 g, 76% yield). In the IR (neat) spectrum, two intense peaks at 2140 and 2240 cm^{-1} with a smaller peak at 2040 cm^{-1} characterized the product. m/z 63 (-Br) was the base peak with the parent ion being the next prominent peaks at m/z 142, 144.

When the same reaction was stirred for 9 h, the mixture was a dark brown color and another compound, 1,4-dibromobutadiyne, was detected by GC-MS and IR. m/z 206, 208, 210 (M^+). The IR showed an additional peak in the acetylene region at 2120 cm^{-1}.

3.5.7 1-(2-Thienyl)-Penta-1,3-diyne

Coupling of acetylene derivatives to thienyl magnesium bromide was performed using the Kumada[103,104] cross-coupling procedure. 2-Thienyl magnesium bromide was prepared from 2-bromothiophene (3.7 g, 0.023 m) and magnesium (0.55 g, 0.023 m) in anhydrous ether (20 ml). The Grignard reagent was added to 1-bromopenta-1,3-diyne (2.4 g, 0.017 m)

in ether (10 ml) and Ni[dppp]Cl_2 (trace) under a nitrogen atmosphere with vigorous stirring. The reaction was immediate and within a few minutes the solution was dark brown. TLC (100% hexane) after 1 h and 24 h showed three new products which were distinguished by their color after spraying with H_2SO_4. They were located at R_f= 0.4-0.5 as an elongated spot. After hydrolysing with 2N HCl and extracting with ether, a dark brown oil (2.20 g) was isolated.

The reaction mixture was flash chromatographed on silica gel with petroleum ether (100%) as the eluant. The mixture was very complex with 7 different products. By MS, some of these products were identified as the homo-coupled Grignard reagent, bithienyl m/z 166 (M^+) and homo-coupled pentadiyne, deca-(2,4,6,8)-tetryne m/z 126 (M^+). Some of the desired product was isolated by column chromatography (flash silica gel eluted with petroleum ether) as a yellow oil (410 mg, 29% yield) and identified as 1-(2-thienyl)-pentadiyne. m/z 146 (M^+). The IR showed two large sharp peaks at 2150 and 2240 cm^{-1}, a feature which is characteristic of umsymmetrical disubstituted acetylenes. ^{1}H NMR ($CDCl_3$) δ 6.98 (dd, 1H, J = 3.6, 5.6 Hz, 4H), 7.26 (dd, 1H, J = 1.0, 5.6 Hz, 5H), 7.28 (dd, 1H, J = 1.0, 3.6 Hz, 3H), 2.02 (s, 3H, 2-CH_3).

3.5.8 2-(2-Thienyl)-1-Phenylacetylene

The Kumada procedure[103,104] was also used in the following reaction. The Grignard reagent was formed from 2-bromothiophene (2.5 ml, 0.025 m) and magnesium (0.6 g, 0.025 m) in anhydrous diethyl ether (20 ml). It was then added to 1-bromophenylacetylene (2.0 g, 0.011 m) and nickel catalyst (trace) in ether (30 ml). The mixture stirred

21 h before work-up with 3N HCl. After extraction and solvent removal, a dark brown oil (3.26 g) was obtained. GC-MS showed the major product to be the unsymmetrical coupled product, 2-(2-thienyl)-phenylacetylene with bithienyl as a significant impurity.

Column chromatography was carried out on part of the oil (500 mg) using alumina (activity 1) with petroleum ether as eluant. Yellowish crystals (0.054 g) were isolated that by MS showed the product m/z 184 (M^+ -100%) and an impurity m/z 202 which was 1,4-diphenylbutadiyne; mp 48-49 °C (lit.[190] mp 50-51); ^1H NMR (DMSO-d_6) δ 7.12 (dd, 1H, J = 5.0, 3.5 Hz), 7.40- 7.43 (m, 4H), 7.51-7.55 (m, 2H), 7.66 (dd, 1H, J = <1.0, 5.0 Hz).

PHOTOCHEMISTRY OF α-TERTHIENYL AND RELATED COMPOUNDS

4.1 INTRODUCTION

The potent toxicity of α-T to a broad range of organisms (see section 1.4.4) including insects and microorganisms has been shown to be due to a photodynamic mechanism involving singlet oxygen[40,47]. PHT, a closely related compound is thought to effect its toxicity by a dual mechanism involving both singlet oxygen production and a radical based pathway[58]. These compounds are natural photosensitizers found in the plant family Asteraceae where they may be used to exploit the environment for defence purposes. Molecular oxygen, a ground state triplet, is easily sensitized by a compound with a higher energy triplet to form singlet oxygen, (23 kcal relative to the ground state[191]), a highly reactive species which when formed in a biological system causes oxidative damage which leads to cell death.

The long-lived triplet states of α-T[192] and PHT[175] have been examined and fully characterized since the production of singlet oxygen occurs by energy transfer via these excited state species. By elucidating the photophysical processes, it may be possible to predict whether a specific derivative or analogue will enhance or decrease phototoxicity. The photochemical data, when combined with other physical properties and biological activities, may also provide information that can be used to design the structure of a more efficient phototoxin.

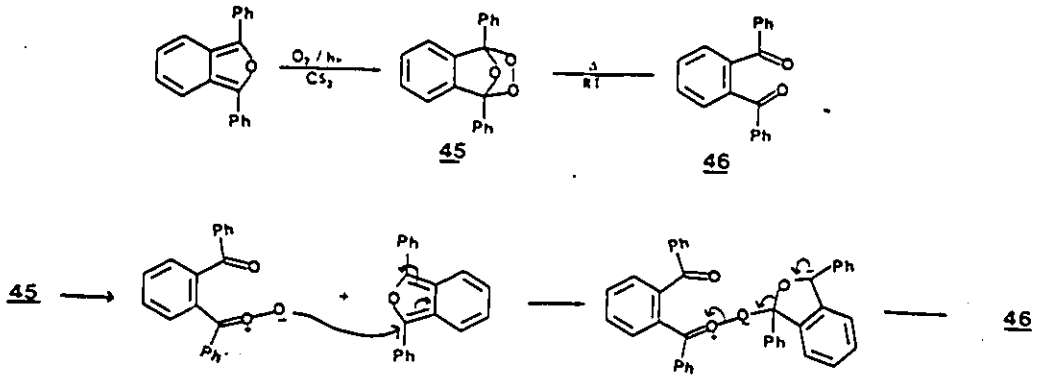
The relationship between biological activity and photochemical[58,193] or physical properties such as chemical

structure[140-142] or lipophilicity[85] have been studied. A positive correlation between lipophilicity, measured as octanol-water partition coefficients, and toxicity was reported for a set of thiophenic derivatives. The photochemical studies related the relative amount of singlet oxygen produced by a number of compounds including α-T and PHT, to biological toxicity. In one of these studies[193], both a-T and PHT were almost twice as efficient at photosensitizing adamantylidene adamantane as the reference compound, methylene blue. While these results are useful within the set of compounds studied, comparisons with other studies are difficult since the quantum yield of singlet oxygen was not determined.

The efficiency of these compounds as sensitizers is a fundamental factor in their toxicity, and it was considered crucial to find the quantum yields of singlet oxygen generation. The trends from the analysis of these results with mortality data obtained for mosquito larvae and the cornborer will also be discussed. Partition coefficients for the set of compounds studied were obtained for an octanol-water system, and will be used to assess polarity differences.

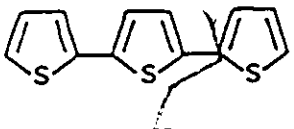
A laser flash photolysis method developed by Chattopadhyay *et al.* was used to determine the efficiency of singlet oxygen generation of the sensitizers[194]. This method measures the depletion of DPBF (1,3-diphenyl-isobenzofuran), due to irreversible reaction with the singlet oxygen produced. DPBF reacts with singlet oxygen by initially forming an endoperoxide (Scheme 28) which has been isolated at low temperatures[195]. It then decomposes by a mechanism which is not well established to the diphenyl

ketone. One suggested pathway involves an intermediate carbonyl oxide which forms a dimer with DPBF and rearranges to give the diphenyl ketone[195,196].

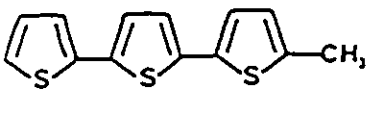


Scheme 28.

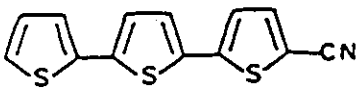
The equation (Eqn 1) derived by Chattopadhyay *et al.*, which relates the quantum yield of singlet oxygen to the photoprocesses involved, requires that the triplet states of the sensitizers be fully characterized. This equation, which will be described in detail elsewhere (section 4.2.4) includes the triplet lifetime value of the sensitizers as well as their rate of quenching with molecular oxygen. The sensitizers are shown with the acronym used for this work in Scheme 29. The triplet states of α-T[192,197], DTB[192], and DBrα-T[197] have already been studied using laser flash spectroscopy, however few or no photochemical data were available for the other sensitizers.



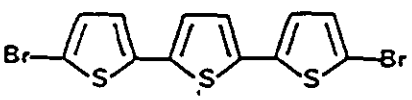
α-T



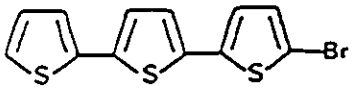
Mα-T



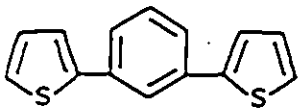
CNα-T



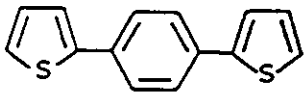
DBrα-T



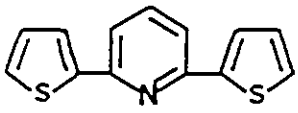
Brα-T



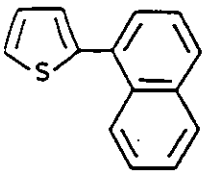
DTB



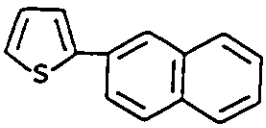
BTP



PYT



ONP



TNP

Scheme 29.

For this reason, the triplet states of the compounds we studied will be described first.

4.2 RESULTS AND DISCUSSION

On excitation with a pulse from a nitrogen laser (337.1 nm), α-T and its derivatives form very short-lived singlet states. The singlet lifetime for α-T and the 5-iodo or 5,5"-diiodo derivatives, was reported by Evans *et al.*[192] as <1 ns. The singlet lifetime of the analogue DTB, reported by the same workers, is 6.0 ± 0.2 ns. The singlet undergoes rapid intersystem crossing to the triplet excited state, the species responsible for sensitizing molecular oxygen.

4.2.1 Transient Spectroscopy

Nitrogen saturated samples of α-T, derivatives and analogues produced strong transient signals after laser excitation (337.1 nm). These transients were identified as the triplet excited states, based on the long lifetimes, quenching studies and photochemical results[192,197] previously obtained for α-T and some closely related derivatives. Figure 2 shows the triplet decay for Mα-T (0.38 mM, CH_3OH solvent) at full laser power. The first order kinetic fit for the decay had a correlation coefficient of 0.9955. Generally the triplet decay for the sensitizers showed mostly first order kinetics although at higher laser doses and for concentrated samples, second order contributions were sometimes observed. This may be due to triplet-triplet annihilation, a process represented by the following equation:

$$^3\alpha\text{-T} + {}^3\alpha\text{-T} \longrightarrow {}^1\alpha\text{-T} + {}^0\alpha\text{-T}$$

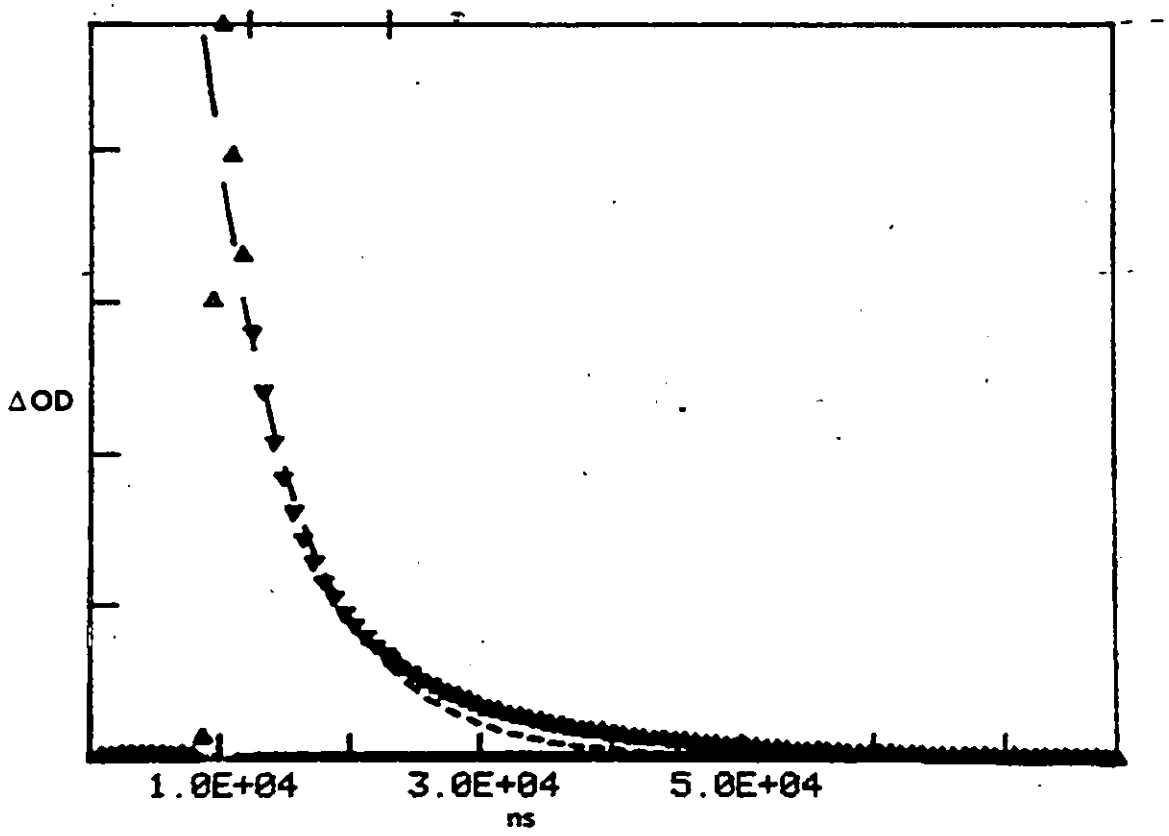


Figure 2. Trace recorded at 460 nm following 337 nm excitation of a 0.38 mM solution of Mα-T in methanol at full laser power.

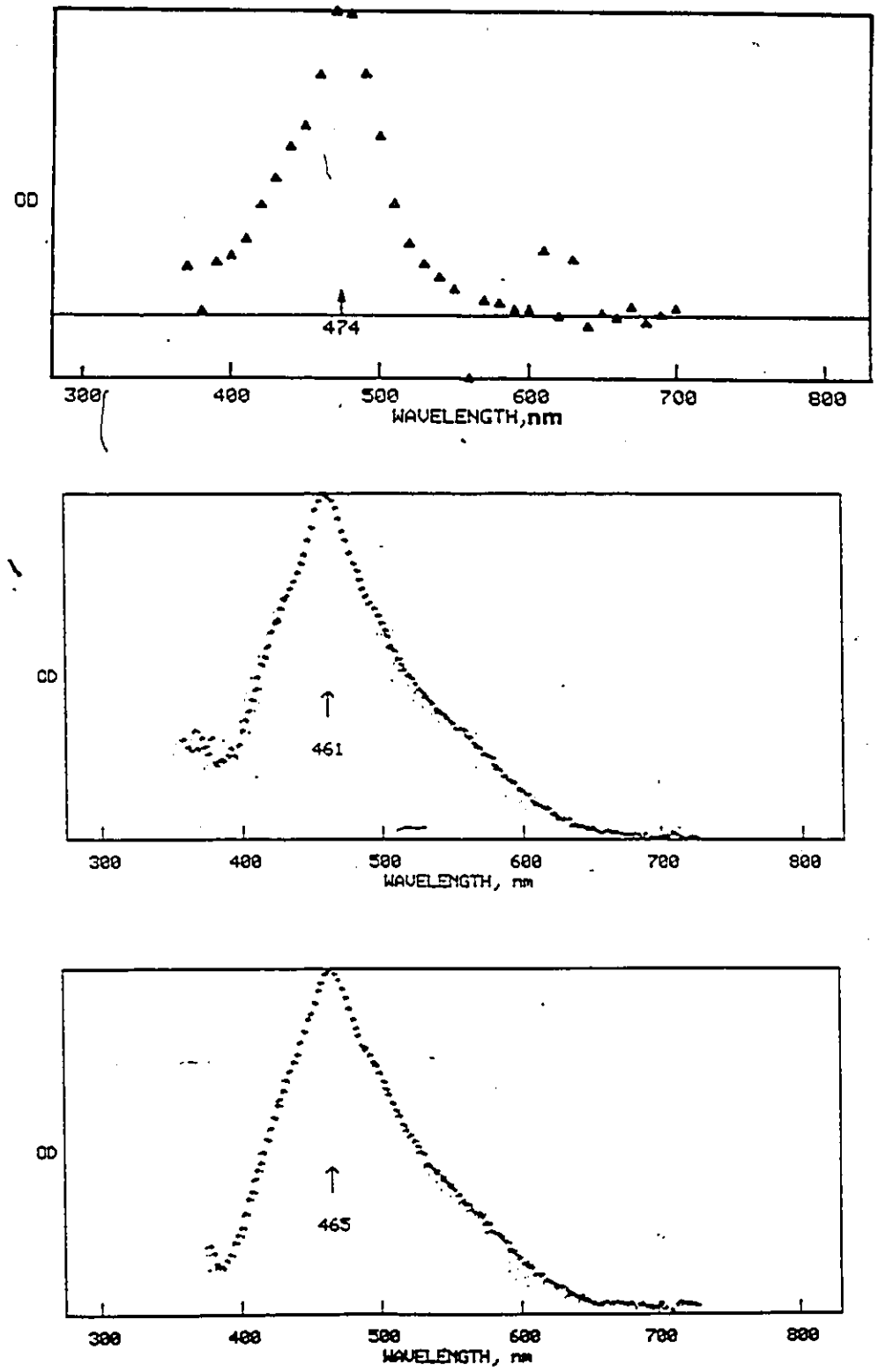


Figure 3. Triplet-triplet absorption spectra of ONP (A), Mα-T (B) and Brα-T (C) in methanol under a nitrogen atmosphere.

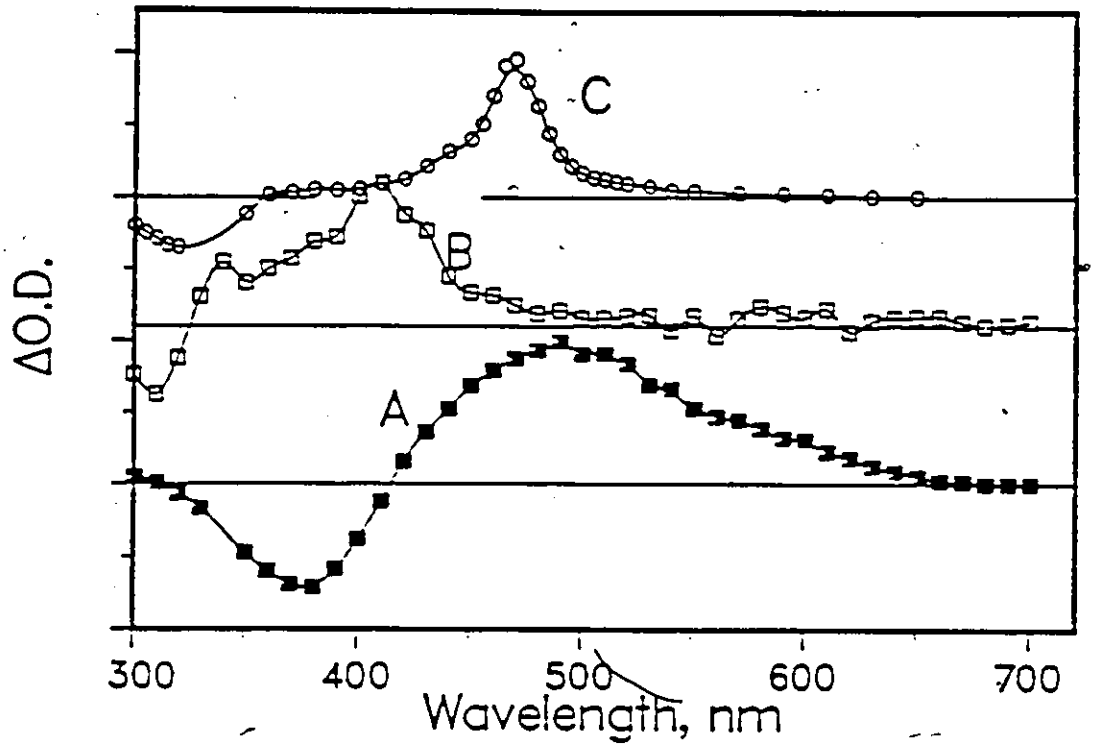


Figure 4. Triplet-triplet absorption spectra of CNα-T (A), TNP (B) and BTP (C) in methanol under a nitrogen atmosphere.

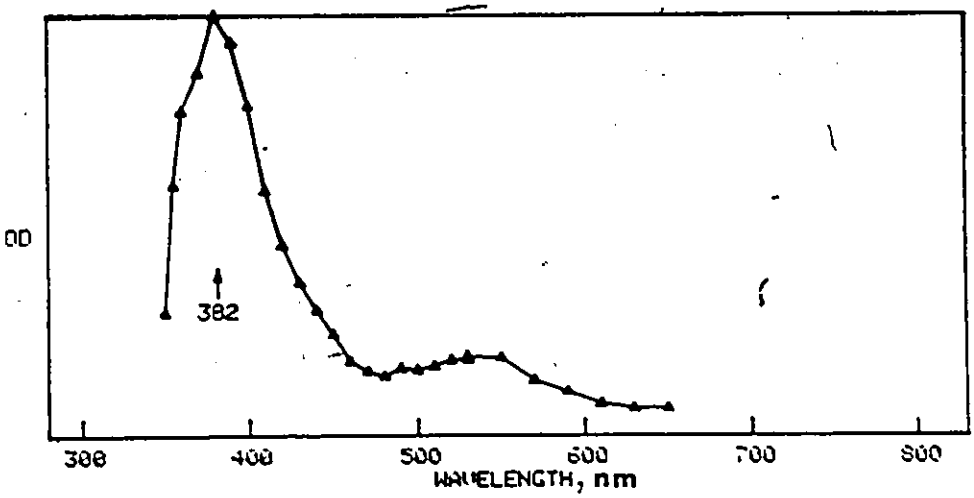


Figure 5. Triplet-triplet absorption spectrum of PYT in methanol under a nitrogen atmosphere.

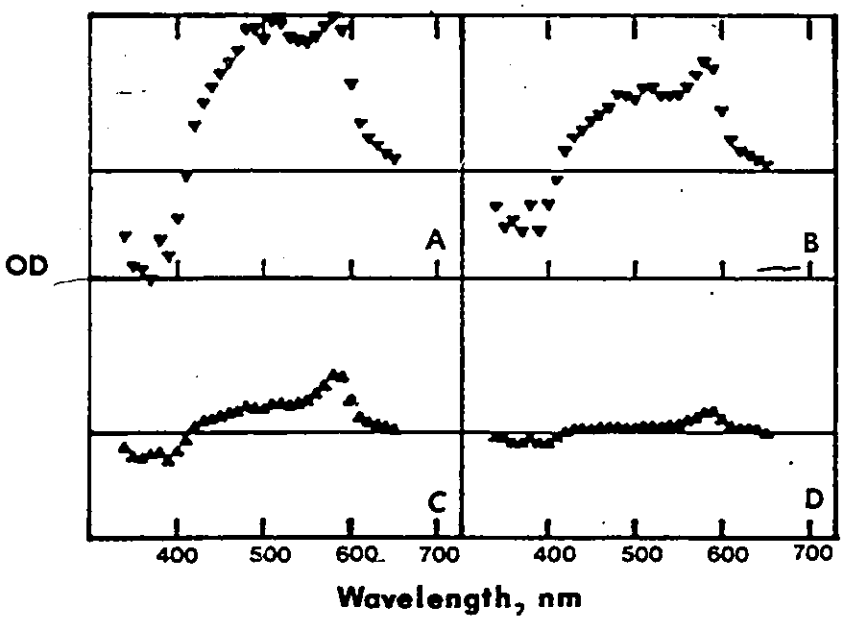


Figure 6. Time dependent triplet-triplet spectra of DBrα-T in methanol under a nitrogen atmosphere. Window A shows the spectrum immediately after laser excitation. The time after excitation (337.1 nm) is progressively longer for the other windows, with window D being about 20 μs.

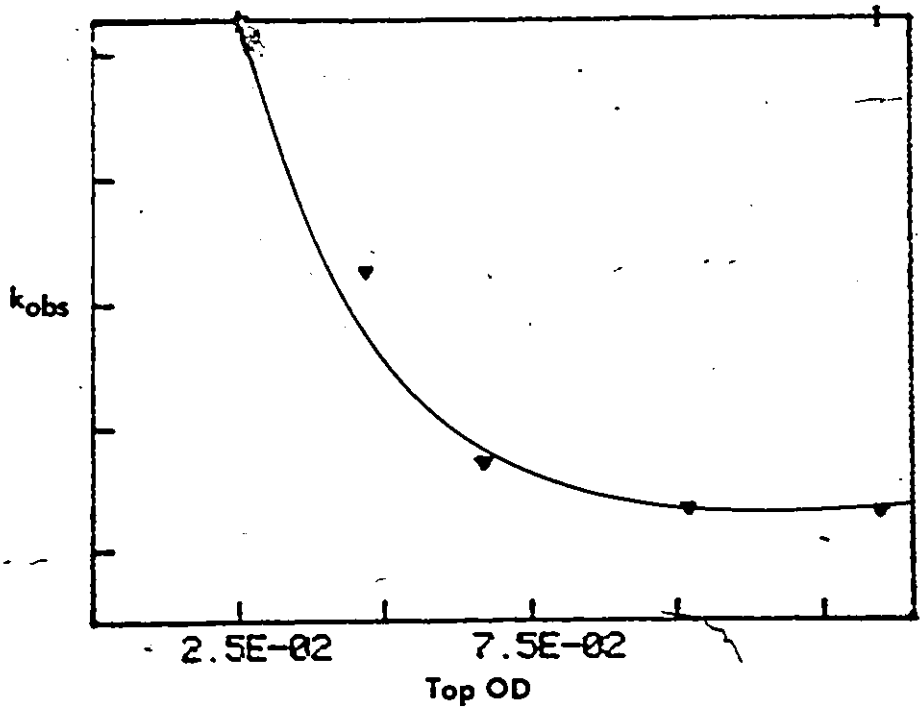


Figure 7. Variation in triplet decay rate as a function of laser dose (as represented by the maximum observed triplet optical density, termed the Top OD) for DBrα-T (0.084 mM) in $CDCl_3$. The sample was monitored at 460 nm and was under a nitrogen atmosphere.

The triplet-triplet absorption spectra (Figures 3-6) recorded for the sensitizers were obtained using pulses from either a nitrogen (337.1 nm) or excimer (308 nm) laser. The absorption spectrum of the triplet transient is recorded over a series of wavelengths and at different time delays

Table 9. Triplet- Triplet Absorption Characteristics of α-T, Derivatives and Analogues.

Substrate[a]	λ_{max}[c], nm	$\lambda_{isobestic}$[c], nm
α-T[d]	450	338
Mα-T	461	-
CNα-T	493	415
DBrα-T[b]	500,590	410
Brα-T	465	-
DTB[d]	390,610	360
BTP	467	355
PYT	380,550	-
ONP	474	310
TNP	408	-

(a) in methanol
(b) in $CDCl_3$
(c) ±2 nm
(d) Reference 192.

after laser excitation. The point in the triplet-triplet spectrum where the change in optical density (OD) between the excited state and the ground state is equal to zero is

called the isobestic point. The triplet-triplet maxima of the sensitizers and the isobestic points are listed in Table 9.

The time dependent triplet-triplet spectra of the sensitizers indicated that the excited triplets represented one transient species, that is, only one transient absorption spectrum was seen from 0 to 20 μs after the laser pulse. The only exception was DBrα-T which showed at least two different transients (Figure 6). The triplet maximum for compounds containing an intact α-T chromophore differ very little with the maxima located between 450-465 nm. The CNα-T compound containing a substituent which extends the conjugation of the chromophore has a triplet maximum occurring at a longer wavelength (493 nm). Of the analogues, in terms of both structure and triplet-triplet maximum, BTP contains the closest chromophore to that of α-T. For PYT and DTB, two bands are observed in the triplet-triplet spectrum, both due to the same transient.

In our experiments with DBrα-T it was discovered that there was a spectral dependence on laser dose. At least two transient species were being formed (Figure 6), one at 500 nm and another longer-lived species at 590 nm. This latter species can be observed at 100 μs and slightly at 300 μs. Initially, it was suspected that the brominated derivative of α-T was liberating halogen as was previously reported[198] for the iodinated derivatives[192]. The iodine produced by cleavage of the C-I bond in 5,5"-diiodo-α-T after laser excitation, had been detected by forming an iodine/benzene complex which has an absorption maximum of 490 nm. On addition of benzene to a sample of DBrα-T, the Br/benzene complex expected to be observed at 540 nm[201] was

not formed. The sample DBrα-T, colorless and clear before laser experiments, was of a tan color afterwards, perhaps indicating the presence of Br_2 in solution. The power dependence plot for DBrα-T is shown in Figure 7. The non-linearity of the plot could indicate two-photon processes.

4.2.2 Triplet Lifetimes

The triplet lifetimes were determined by laser flash photolysis and attenuating the laser dose with neutral density filters (in the 15% to 100% range). The lifetimes at "zero" laser dose were obtained through extrapolation. This procedure was followed to eliminate triplet-triplet annihilation contributions as well as two-photon processes. The rate data may incorporate some contribution from self-quenching, which could be eliminated by extrapolation to zero concentration. However, it was preferred to determine the lifetimes under conditions similar to those used for the singlet oxygen experiments. The rate of self quenching[192] for α-T in methanol was reported to be $k_{sq} = 5 \times 10^9\ M^{-1}\ s^{-1}$ and can be described by the following equation:

$$\alpha\text{-}T^* + \alpha\text{-}T \longrightarrow 2\alpha\text{-}T$$

The triplet lifetimes of the substrates were obtained in methanol or $CDCl_3$, at the concentrations used in the singlet oxygen studies.

Figure 8 illustrates the plots obtained for triplet decay rate versus laser dose for ONP and TNP. The laser dose was represented by the Top OD, which is the maximum triplet optical density monitored at the triplet-triplet absorption maximum of the compound for a particular laser intensity. The intensity was altered by changing the

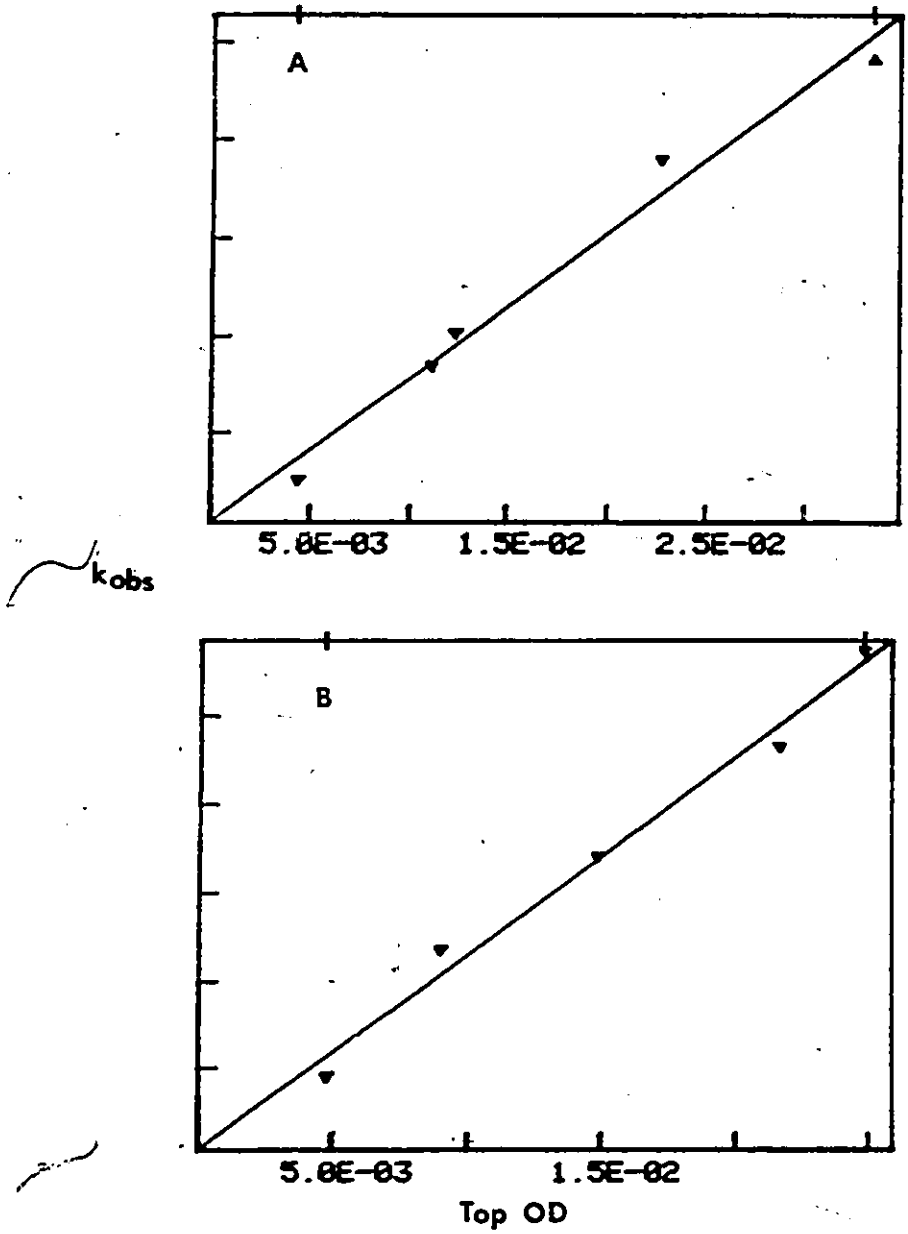


Figure 8. Variation in triplet decay rate as a function of laser dose (as represented by the maximum observed triplet optical density, termed the Top OD) in methanol under a nitrogen atmosphere for ONP (A) and TNP (B). ONP was monitored at 470 nm and TNP at 410 nm.

neutral density filter. This value was determined before significant triplet decay took place. The triplet lifetime is the reciprocal of the intercept obtained from the plot ($\tau = 1/k_o$).

Table 10. Triplet Lifetime Data for the Substates Examined.

Substrate	λ_{max}, nm	τ_T, μs	[substrate], mM	Solvent
α-T[a]	450	30	(0)	methanol
α-T[b]	460	57	(low)	ethanol
α-T	450	19	0.24	$CDCl_3$
Mα-T	461	12	0.38	methanol
CNα-T	493	13	0.01	methanol
CNα-T	493	8.5	0.39	methanol
DBrα-T	460	22	(c)	ethanol
DBrα-T	460[d]	13	0.084	$CDCl_3$
Brα-T	465	11.5	0.35	methanol
DTB[a]	390	31	(0)	methanol
BTP	467	20	0.41	methanol
BTP	467	24	0.19	$CDCl_3$
PYT	550	23.8	0.01	methanol
ONP	474	39	0.097	methanol
TNP	408	33	0.052	methanol

(a) Reference 192, extrapolated to zero laser dose and zero concentration.

(b) Reference 197.

(c) At OD<1 at the laser wavelength.

(d) Lifetime value measured at the λ_{max} reported in Reference 197.

The triplet lifetimes of the compounds and the values reported by other investigators are shown in Table 10. The triplet lifetimes for the sensitizers examined were concentration dependent, having shorter lifetimes at higher concentrations. They were all long lived triplets and their lifetimes ranged between 8.0 and 40.0 μs.

4.2.3 Oxygen Quenching Studies

Quenching experiments with oxygen were carried out for all of the analogues and most of the derivatives. The ground state triplet of molecular oxygen can quench sensitizers with excitation energies as low as 23 kcal/mole to produce singlet oxygen.

The rate constant for oxygen quenching (k_q) can be obtained by plotting the rate constant for triplet decay (k_{exp}) as a function of quencher concentration ([Q]), oxygen in this case, i.e.,

$$k_{exp} = k_o + k_q[Q]$$

where k_o is the constant for triplet decay in the absence of quencher, ($k_o = \tau_T^{-1}$). The concentration of oxygen was changed by purging the sample with different N_2-O_2 mixtures.

The k_q values obtained for the samples are reported in Table 11. The solubility of oxygen in methanol[199] was taken as 1.02×10^{-2} M (under 1 atm partial pressure). A spin statistical treatment of triplet quenching by oxygen suggests that 1/9 of the encounters between the sensitizer and oxygen can lead to energy transfer[200]. Oxygen quenching for most of the sensitizers was very rapid and of the order 1/9 the diffusion controlled rate, suggesting that energy transfer was the major

mechanism for quenching the triplet excited state. Diffusion controlled energy transfer occurs at a rate of 1.8×10^{10} M^{-1} s^{-1} in methanol. CNα-T is quenched at a faster rate than the other sensitizers however, the error associated with this value is particularly high. The errors in this experiment are largely due to the preparation of the N_2-O_2 mixtures.

Table 11. Rate Constants Obtained for Oxygen Quenching.

Sensitizer[a]	$k_q \times 10^{-9}$ [c] (M^{-1} s^{-1})	λ_{mon} nm [d]
PYT	2.7 ± 0.3	390
BTP	3.3 ± 0.5	465
CNα-T	6.9 ± 2.0	500
ONP	4.3 ± 0.7	475
TNP	3.6 ± 0.7	410
α-T[b]	3.3 ± 0.2	450
DTB[b]	1.5 ± 0.4	385

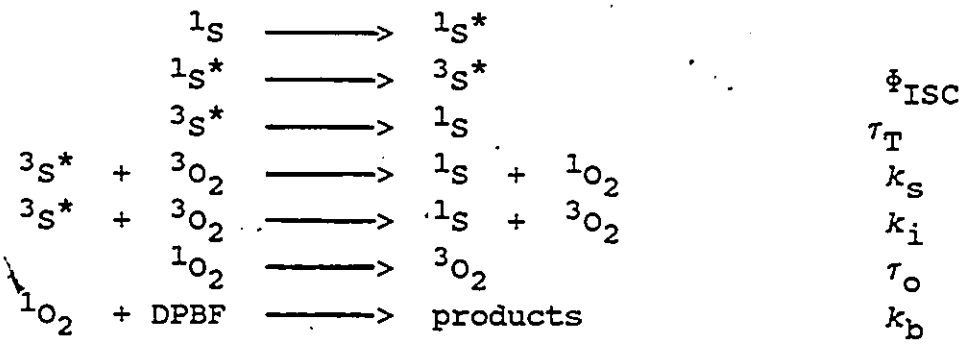
(a) in CH_3OH
(b) From reference 192.
(c) errors as $\pm 2\sigma$
(d) λ_{laser} = 337.1 nm

Lack of sufficient material did not permit this experiment for the methylated or brominated derivatives of α-T. The rate constants for oxygen quenching of the mono- and diiodinated derivatives[192] have values similar to that

of α-T. It is likely that the triplet states from the other derivatives are also efficiently quenched by oxygen.

4.2.4 Introduction: Singlet Oxygen Quantum yields

The method of Chattopadhyay *et al.*[194] was used to determine the singlet oxygen quantum yields of α-T and related compounds using 1,3-diphenylisobenzofuran (DPBF) as the singlet oxygen monitor. Laser excitation (337.1 nm) of the sensitizer (S), α-T for example, produces a short-lived singlet (<1.0 ns)[192] which undergoes intersystem crossing to a low-lying triplet state. The sensitizer triplet can then be quenched by ground state molecular oxygen as discussed above, to produce singlet oxygen. Other processes may compete to decrease the efficiency of the oxygen quenching reaction, such as fluorescence from the singlet, or decay of the triplet to the ground state. The singlet oxygen produced can decay to the ground state or be quenched by DPBF. The latter process leads to irreversible depletion of DPBF.



Scheme 30.

Monitoring the depletion of DPBF in solution provides a method of measuring the amount of singlet oxygen

produced by the sensitizer (S). This method assumes that DBPF is not a physical quencher i.e. by collisional processes, of singlet oxygen. The processes associated with this experiment and the symbols used to describe them are summarized in Scheme 30.

k^T_q is the sum of k_s and k_i and k^D_{obs} is the pseudo first order rate constant for bleaching of DPBF. τ_T^{-1} and τ_o^{-1} are the inverse of the triplet lifetime of the sensitizer and singlet oxygen respectively. The following equation based on these photochemical processes was derived by Chattopadhyay *et al.* to relate the experimental data to singlet oxygen quantum yield.

$$\Phi_\Delta = \Phi^A_{ISC} \left(\frac{-\Delta(OD)^{\lambda_1}_\infty}{\Delta(OD)^{\lambda_2}_0} \right) \frac{\epsilon^{\lambda_2}_A}{\epsilon^{\lambda_1}_{DPBF}} \frac{k^T_q[^3O_2] + \tau_T^{-1}}{k^T_q[^3O_2]} \cdot \frac{k^D_{obs}}{k^D_{obs} - \tau_0^{-1}}$$

$\Delta(OD)$ signifies the changes in optical density at λ_1, the wavelength where the DPBF was monitored and λ_2, the wavelength where the benzophenone triplets were monitored. λ_1 for DPBF was 440 nm, where it absorbs strongly in the ground state[194]. The actinometer, benzophenone was monitored at its triplet-triplet absorption maximum,[201] 525 nm. The extinction coefficient for benzophenone (ϵ_A) is 7640 M^{-1} cm^{-1} at 525 nm and the reported[202] quantum yield for formation of the triplet (Φ_{ISC}) is 1.0. The molar extinction coefficient for DPBF was 10,780 M^{-1} cm^{-1} at 440 nm. The symbol Φ_Δ represents the quantum yield of

singlet oxygen generation under conditions where all triplets are quenched by oxygen and where every singlet oxygen molecule decays by reaction with DPBF.

The solvent used for the experiments was $CDCl_3$, in which singlet oxygen has a lifetime of 300 μs[41]. This long lifetime allowed simplification of the last term of equation 1 to unity as the values of k_{obs} were around 6 x 10^5 s^{-1}. The last term of the equation was also unity as the value of $k_q[^3O_2] >>> \tau_T^{-1}$ (for α-T $k_q[^3O_2]$ = 3 x 10^9 M^{-1} s^{-1}). With these modifications and using the intersystem crossing quantum yield of benzophenone as 1.0[202], the equation used under our experimental conditions was:

$$\Phi_\Delta = \left(\frac{-\Delta(OD)_\infty^{\lambda_1}}{\Delta(OD)_0^{\lambda_2}}\right) \cdot \frac{\epsilon_A^{\lambda_2}}{\epsilon_{DPBF}^{\lambda_1}}$$

4.2.5 Singlet Oxygen Quantum Yields

As well as using the simplified equation (Eqn 2), several modifications were implemented to improve the procedure. Chattopadhyay *et al.* reported that DPBF absorbed a significant portion of the laser photons (337.1 nm)[194]. Besides having DPBF absorb some of the photons rather than the sensitizer, this also made it possible that direct excitation of the furan could contribute to the observed bleaching. This problem was alleviated by performing laser flash experiments with a blank containing the same amount of

DPBF in the absence of sensitizer. The appropriate correction was then calculated taking into account the fraction of light absorbed by DPBF is different when the sensitizer is present. This type of correction does not account for the conditions of laser photolysis experiments, in which the volume irradiated (and therefore the overlap between excitation and monitoring beams) is a function of the optical density of the solution. This is especially true for the conditions employed in this study with relatively high concentrations of DPBF.

The blank used in our experiments contained DPBF in the same concentration as used in the sensitizer sample, and o-hydroxybenzophenone (OHBP). OHBP is photostable, its photochemistry well characterized and involving rapid and reversible proton transfer[203]. OHBP is added in order to optically match the blank with that of the sample containing the sensitizer and DPBF. In other words, the absorption of OHBP effectively substitutes for the thiophenic compound. In this way the correction used in our study was a direct value and not a calculated one such as that used by Chattopadhyay *et al.*.

A flow system was used in order to avoid DPBF depletion which was clearly evident when using static samples of small optical path lengths. The optical path length of the flow-through cell was 0.03 cm, which optimized the overlap between the excitation and the monitoring beam. This optical path length allowed for a sufficient amount of DPBF to be used for bleaching experiments while keeping the solutions transparent at the monitoring wavelength.

DPBF has a triplet energy[191] of 34 kcal mol^{-1} and so may quench the triplet of the sensitizer in competition

with molecular oxygen. This process would yield DPBF triplets which could then be quenched by molecular oxygen to generate singlet oxygen, causing an error to occur in the final quantum yield measurement. This type of error would be unlikely to occur under the conditions used for the bleaching experiments, with the concentration of oxygen[204] in the solutions approximately 1.16×10^{-2} M as compared to that of DPBF which was used at concentrations < 1.0 mM. With such low concentrations of DPBF, oxygen would be expected to quench at least 90% of the substrate triplets.

This method assumes that singlet oxygen is not reacting with the ground state of the sensitizers. Oxidation of alkyl and phenyl substituted thiophenes with photochemically generated singlet oxygen has been reported[205] via the endoperoxide affording 2-buten-1,4-diones. Information regarding the reaction of α-T derivatives and analogues with singlet oxygen is currently not available. The pseudo first order rate constant (k_b) for the reaction of singlet oxygen with DPBF was measured under the experimental conditions ($CDCl_3$, oxygen saturation) and determined to be 4×10^8 M^{-1} s^{-1}. This rate constant is similar to previously reported values[206]. Considering this fast rate constant and the concentrations of DPBF and sensitizer, reactions between singlet oxygen and the ground state sensitizer are unlikely to occur.

In order to show that this was the case, experiments were performed using twice (1.62 mM) and half (0.4 mM) the concentration of DPBF, with α-T as the sensitizer. If a portion of the singlet oxygen generated was being depleted by reaction with the substrate, it was expected that the dilute system would give a higher quantum

yield of singlet oxygen. The differences between the two values was less than 10% with the dilute system having the lower quantum yield, contrary to expectations if this type of error was occurring. These experiments showed that DPBF was the only important scavenger of singlet oxygen under our conditions.

The data from a complete laser experiment are shown in Figure 9 with α-T as the sensitizer. The bleaching of DPBF for the blank containing OHBP (B) and for the sample with DPBF and α-T were monitored at 440 nm. The insert in this same figure shows the triplet decay of the actinometer, benzophenone, at its triplet maximum (525 nm) for a solution of matched optical density (OD_{337} = 1.16 for an optical path length of 0.03 cm) with the previous two samples in the figure. The three decay traces shown were obtained at full laser power.

The data for each sample were extrapolated to "zero laser dose" and usually involved at least ten experiments per sample. The plots of signal intensity (bleaching or absorption) vs. laser dose are shown in Figure 10 for the sensitizer sample, the DPBF blank and the actinometer. The reasons for non-linearity in plots of this type are numerous, but commonly are due to partial depletion of one of the reagents, possibly DPBF in our case, or competition of a reaction intermediate for the absorption of the exciting beam.

The values used in equation 2 were not the measured Δ(OD) but the slopes at the origin of the power dependence plots as in Figure 10 for example. α-T was included as a separate experiment each time samples were

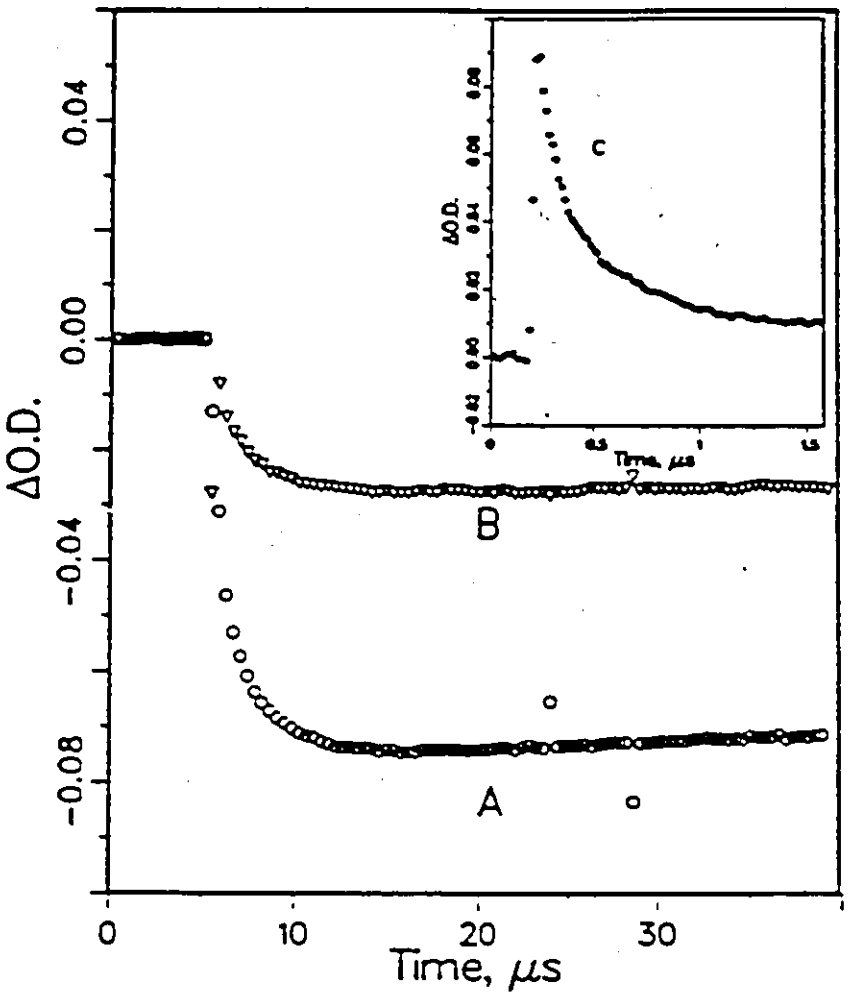


Figure 9. Bleaching observed at 400 nm for 1.64 mM α-T and 0.85 mM DPBF (trace A) and for the same concentration of DPBF in the presence of sufficient OHBP to match the optical absorption with sample A (trace B). The insert shows a benzophenone trace monitored at 525 nm (OD_{337}=1.16 for an optical path length of 0.03 cm).

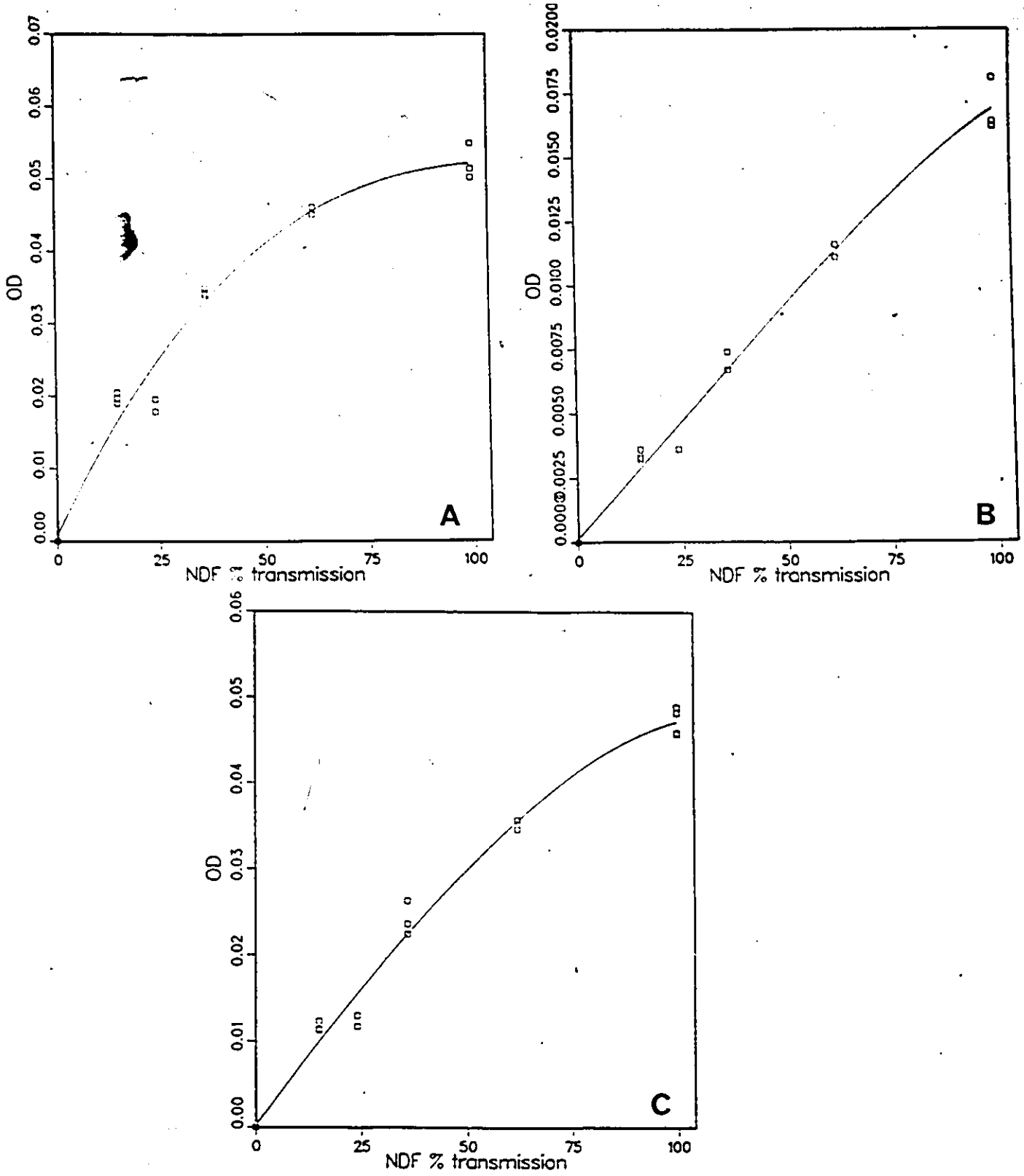


Figure 10. Mutiple regression plots of the changes in transient optical density as a function of the relative laser dose for 11.8 mM ONP and 0.82 mM DPBF (A), the DPBF/OHBP standard (B) and the corresponding benzophenone actinometer(C).

tested, providing an indication of the reliability of the method from day to day on the laser facility. As well, the singlet oxygen generating efficiency for acridine and benzophenone was determined under the same experimental conditions employed for the sensitizers. The quantum yield obtained for acridine was 0.95 and is in agreement with previously determined values[207,208] indicating the high efficiency of this compound in generating singlet oxygen.

Table 12. Singlet Oxygen Generating Efficiencies in $CDCl_3$ at Room Temperature.

Substrate	Φ_Δ	[substrate], mM
acridine	0.95	1.74
benzophenone	0.55	306
α-T	0.86	1.6
Mα-T	0.93	0.53
CNα-T	0.86	0.61
DBrα-T	0.69	1.8
Brα-T	0.70	1.7
BTP	0.69	0.083
DTB	0.75	9.9
PYT	0.53	5.6
ONP	0.76	15.6
TNP	0.63	11.7

Previous quantum yield values obtained for benzophenone range between 0.12 to 0.90[209] with the current "best" value

of 0.29 obtained in benzene. With no agreement in the literature, the quantum yield of 0.55 obtained using the present method does support the modest efficiency of benzophenone as a sensitizer.

The singlet oxygen quantum yields determined for α-T derivatives and analogues are summarized in Table 12. The error in these values is probably ±0.15, although statistical analysis suggests smaller errors. The relative errors are probably ±0.10. These results vary by less than a factor of two from the highest to the lowest quantum yield, indicating that all the sensitizers were efficient generators of singlet oxygen.

In a similar study by Reyftmann *et al.*[197], the quantum yield of DPBF bleaching was determined for a few substrates including α-T and DBrα-T. Intersystem crossing quantum yield values of 0.20 and 0.35, respectively, were also obtained for the same substrates. This is in conflict with the singlet oxygen generating efficiency of these substrates which we have measured as 0.86 and 0.69. The intersystem crossing quantum yield should be equal to or higher than the quantum yield of singlet oxygen formation.

Faced with a large discrepancy in results, other types of mechanisms were examined that could account for a high quantum yield of singlet oxygen generation and a low intersystem crossing quantum yield. These results could be explained either by considering additional triplet formation or singlet oxygen generation from another process. Additional triplet could be formed by oxygen-catalysed

intersystem crossing[210] from the singlet excited state as shown below.

$$^1\alpha\text{-T} + {}^3O_2 \longrightarrow {}^3\alpha\text{-T} + {}^3O_2$$

Also, singlet oxygen could arise from direct quenching of the excited singlet state of the sensitizer with oxygen[191].

In order to test these possibilities, a fluorescence quenching experiment was performed on α-T in $CDCl_3$. The solution was excited at 352 nm and monitored at 426 nm. Saturation of this solution with oxygen resulted in less than a 5% decrease in the luminescence intensity in comparison to a sample purged with nitrogen. Thus, the excited singlet state of α-T is not quenched by oxygen under these conditions and the singlet oxygen that is generated by the sensitizer must arise via the excited triplet state. It also follows that for these substrates, the intersystem crossing quantum yield is the same for an oxygen or nitrogen saturated solution. The intersystem crossing quantum yields reported by Reyftmann *et al.* must be reviewed at least for the substrates in common with this work and perhaps the methodolgy for obtaining these results improved.

4.3 STRUCTURE-FUNCTION RELATIONSHIPS

4.3.1 Relationship Between Chemical Structure and Biological Activity

Studies of structure-function relationships of biologically active molecules are undertaken to probe many aspects which contribute to activity, including the features of the chemical structure that are necessary for a compound to be active. Structure-function relationships may provide the information necessary to successfully alter the

structure of a molecule, making a more active compound or one with desirable characteristics such as stability.

Synthesis of various closely related derivatives and analogues of pyrethrin, and examination of how the chemical structure affects biological activity has been successful in developing new, potent pyrethroid insecticides[211]. This was the approach initially used for investigating the naturally occurring phototoxin α-T and a series of synthetic analogues and derivatives.

The lead compound of this study α-T, was altered chemically by substitution in either the C-5 or C-5 and C-5" positions by bromine, iodine, methyl or nitrile. The effect of methylation at the C-3'and C-4' positions in the center ring was also examined. A series of analogues were prepared by replacing the center thiophene ring with either a phenyl or a pyridine ring. The thienyl-naphthalene compounds represent the most structurally different analogues that were prepared and tested.

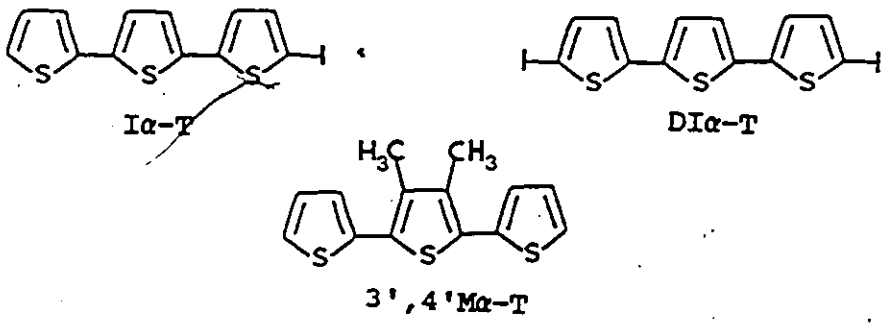
The biological tests were carried out in the laboratory of Dr J.T. Arnason at the University of Ottawa. The person(s) involved in a specific biological test will be mentioned in the text.

The series of compounds described above were tested against mosquito larvae (*Aedes atropalpus*) and their LC_{50} (lethal concentration for 50% mortality) values determined (Table 13). These tests were carried out by Cornelius Berg and France Duval. The mosquito larvae were treated with 4 h near-UV irradiation from a bank of 4

Table 13. The LC_{50} Values for a Series of Compounds Against Mosquito Larvae (*A. atropalpus*) After Treatment With Near-UV.

Compound	LC_{50} (ppm)
Mα-T	0.0153
α-T	0.0275
CNα-T	0.0258
ONP	0.3296
DTB	0.060
Brα-T	0.436
BTP	(b)
DBrα-T	1.7
TNP	0.1829
PYT[a]	0.261
Iα-T[a]	30.7
DIα-T[a]	>1.0
3',4'Mα-T[a]	0.164

(a) Previously published, reference 212.
(b) See text.



Scheme 31.

blacklight blue tubes (4 W/m^2) immediately following addition of the test compound, then held in the dark for a further 20 h. No mortality was seen in the controls. The acronyms and the structures of the last three compounds not previously shown are in Scheme 31.

The biological activity of the test compounds varies widely, even among the closest structurally related derivatives. The most potent larvicide was Mα-T followed closely by CNα-T and α-T. With the present small sample of derivatives tested thus far, the influence of an electron donating group or withdrawing group in the C-5 position remains unclear, particularly since the two most potent larvicides in this study represent a derivative from each of the two categories. Natural selection may account for the excellent activity of α-T and Mα-T both naturally occurring compounds.

The larvicidal activity of 3',4'Mα-T was an order of magnitude less than that of α-T. In the halogenated series of derivatives, the brominated derivatives were more toxic than the iodoinated derivatives while in both cases the monosubstituted derivatives were superior to the disubstituted derivatives. The most toxic of this series, Brα-T, was 16 times less active than α-T.

The phenyl analogues BTP and DTP were both very active and showed significant toxicity in the absence of UV light, perhaps indicating an alternative non-photosensitizing mechanism. DTB was equally toxic in the presence or absence of light. BTP showed no toxicity toward the larvae within the 24 h for the LC_{50} tests at all concentrations. However, 48 h after the end of the test period 100% mortality was observed at all concentrations.

The naphthalene analogs and the pyridine analogue PYT, were active and all around 10 times less toxic than α-T.

To summarize the results above, the best larvicides retained the terthienyl skeleton and were mono-substituted rather than disubstituted. Methylation in the C-5 position was more favorable than in the C-3' and C-4' position. All of the analogues were active and showed toxicity in the dark but were less toxic than α-T under the test conditions.

Similar results were reported by Uhlenbroek *et al.*[140] for a series of bithienyl and terthienyl compounds which were tested for nematicidal activity against *Heterodera rostochiensis*. All of the compounds with high activity in this study could be considered derivatives of 2,2'-bithienyl. The necessity of the intact bithienyl unit was illustrated by comparing the biological results for BTP (as named in our study) and 5-phenyl-2,2'-bithienyl. The latter compound was highly active while the former was considered inactive. Nematicidal activity was enhanced by methylating 2,2'-bithienyl or α-T at C-5 while methyl in other positions reduced activity. Mono substitution in the C-5 position produced a more active derivative than disubstitution in the C-5 and C-5" position of α-T or the C-5 and C-5' position of 2,2'-bithienyl.

While the results of Uhlenbroek *et al.* are in good agreement with our findings for mosquito larvae, some differences in the two studies must be raised. The biological activity was determined from an *in vitro* test on a nematode rather than an *in vivo* test on an insect. As well, there are no indications from the experimental on how much light the samples received, since at this time the

light enhanced activity of these compounds was unknown. This is particularly important because some of the bithienyl derivatives from this study have been reported to operate via a photodynamic type II mechanism similar to α-T[142]. Another problem associated with assessing the results of this study is that they are qualitative. In testing 5-methyl and 5,5"-dimethyl-α-T, a "high activity" rating was obtained for both. However, in the text it is revealed that the dimethylated derivative is "slightly" less active.

A more recent study by Kagan *et al.*[141] examined the phototoxicity of derivatives of α-T and 1,4-butadiynes against five target organisms including the larvae of the mosquito *Aedes aegypti*. The results of this study were also qualitative, a + indicating toxicity and a - indicating inactivity. Two of their test compounds, α-T and PYT were common to this work and were both toxic to the mosquito larvae. α-T was toxic to the other four target organisms which included microorganisms and the fruitfly *Drosophila melanogaster*.

4.3.2 Contribution of Physical Properties to Biological Activity

Investigatation into the physical properties that could be considered contributing factors in the toxicity of α-T and related compounds was undertaken. The toxicity of α-T is due to the production of singlet oxygen as α-T operates as a photodynamic type II sensitizer *in vitro* and *in vivo*. Previous attempts correlating biological activity with relative singlet oxygen production for a series of compounds have been only partly successful[58,193]. The results are useful for the set of compounds studied but comparison to other studies is difficult. The singlet

oxygen quantum yields for α-T and some related compounds have been described (section 4.2.5).

The quantum yields of singlet oxygen for all of the test compounds was very high, showing they all were efficient producers of singlet oxygen. There is a modest negative correlation (-0.40) between the singlet oxygen quantum yields of the test compounds and the LC_{50} data for mosquitoe larvae. A higher correlation (-0.69) was obtained by eliminating the two least toxic compounds (DBrα-T and Brα-T) and plotting the LC_{50} data for mosquito larvae against the log of the quantum yield (Figure 11). 47% of the variation in toxicity can be explained by the quantum yield of singlet oxygen. The correlation coefficient clearly indicates that this is not the only factor involved in the toxicity.

The location of a phototoxin in a cell affects the toxicity it can exert against a target organism[213]. The location depends partially on the lipophilicity of the compound. Lipophilicity is an important factor in the toxicity of α-T, as the membrane is its site of action[62,73,74]. A positive correlation between lipophilicity and toxicity has previously been described for a series of naturally occurring acetylenic and thiophenic compounds including α-T[85]. The compounds with the highest lipophilicity may be expected to have enhanced toxicity, with all other physical factors being equal.

The lipophilicity of the compounds is represented by the partition coefficient (log P)[214] and was determined for an octanol-water system by Francesca Campos. Known standards were run on a Reverse Phase-C_{18} Ultrasphere column with acetonitrile-water (65:35) as eluent and UV detection

at 254 nm. The linear correlation of log P (partition coefficient) versus log k'(capacity factor based on the retention time) for the known compounds was 0.98. The derivatives and analogues were then run under the same conditions in order to obtain their retention times. With this information log P could be calculated using the following equation.

$$\log P = 2.14 + 3.83 \log k'$$

The partition coefficients (PC) are shown in Table 14 and show little variation. All of the compounds tested were highly lipophilic. A correlation of -0.82 was obtained for the plot of LC_{50} mortality data for mosquito larvae versus log PC (Figure 12). Therefore, the lipophilicity accounts for 67% of the variation in toxicity.

Table 14. Partition Coefficients for α-T and Related Compounds for an Octanol-Water System.

Compound	PC (log P)	Compound	PC (log P)
Mα-T	6.16	Brα-T	6.97
α-T	5.41	BTP	5.31
CNα-T	5.14	TNP	4.79
ONP	4.71	PYT	4.28
DTB	5.31		

The biological data for mortality toward neonates of the European cornborer (*Ostrinia nubilalis*) are presented in Table 14. These contact toxicity tests were performed by Natalie Donskov. The compounds to be tested were applied to

the inside of a vial in acetone and the solvent allowed to evaporate. The neonates were then inserted into the vial and irradiated for 2 h with near-UV light from a bank of 4 blacklight blue tubes (4 W/m^2). A diet cube was added to the vial and the neonates kept in the dark for 20 h at which time the percent mortality was determined. Not more than 10% mortality was found in the controls.

Table 15. Percent Mortality of α-T and Related Compounds Toward the Cornborer, With or Without UV Light Treatment.

Compound[a]	0.01 mg/ml		0.1 mg/ml	
	-UV	+UV	-UV	+UV
Mα-T	35	100	44	100
α-T	68	100	80	100
CNα-T	22	15	28	32
ONP	78	54	100	100
DTB	32	32	54	50
Brα-T	40	65	46	66
BTP	16	14	30	26
DBrα-T	25	32	30	48
TNP	38	54	60	62
PYT	30	26	44	38

(a) The compounds are listed in the order of decreasing quantum yield of singlet oxygen.

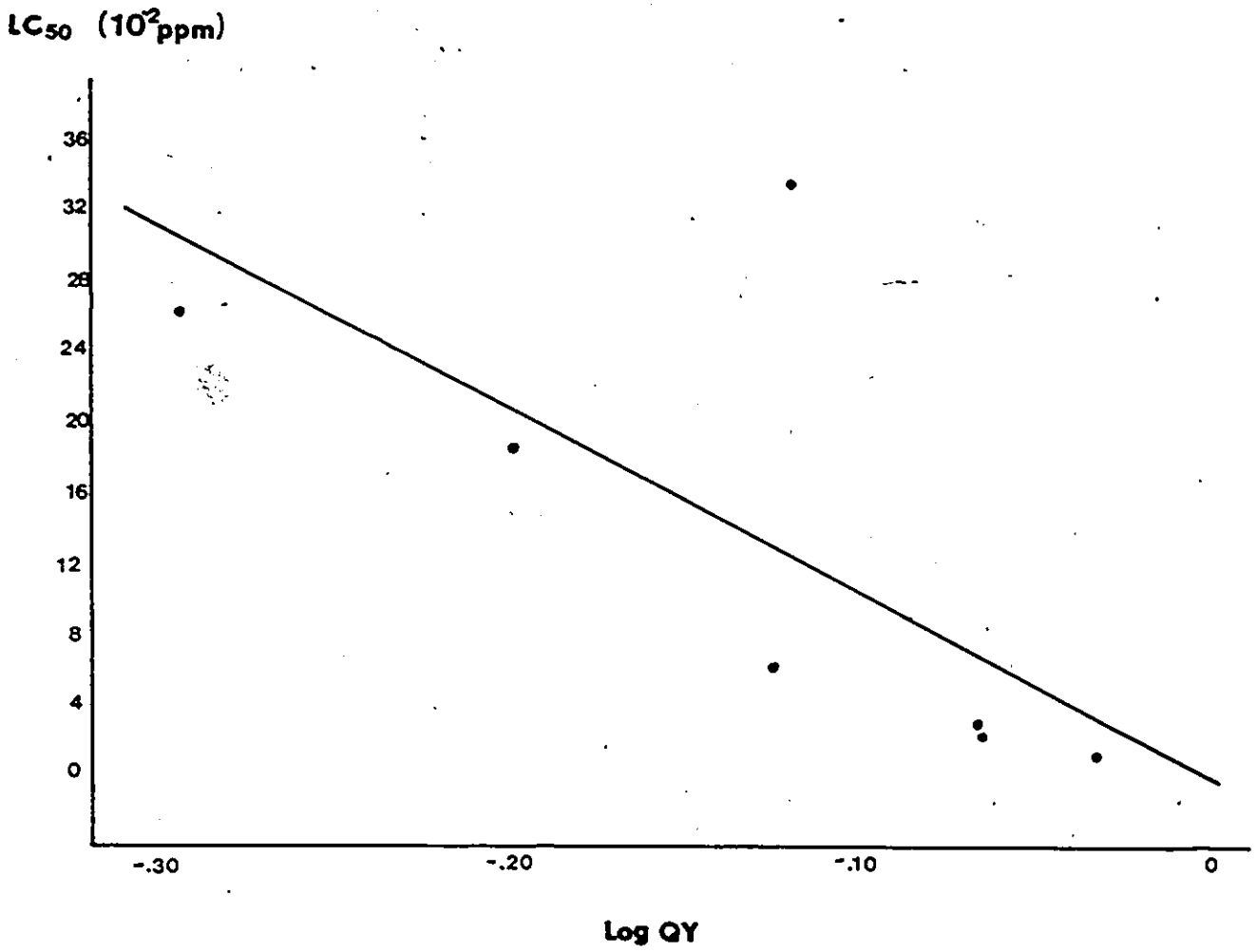


Figure 11. Plot of LC_{50} values for a series of α-T derivatives and analogues, versus the log of their quantum yield of singlet oxygen generation.

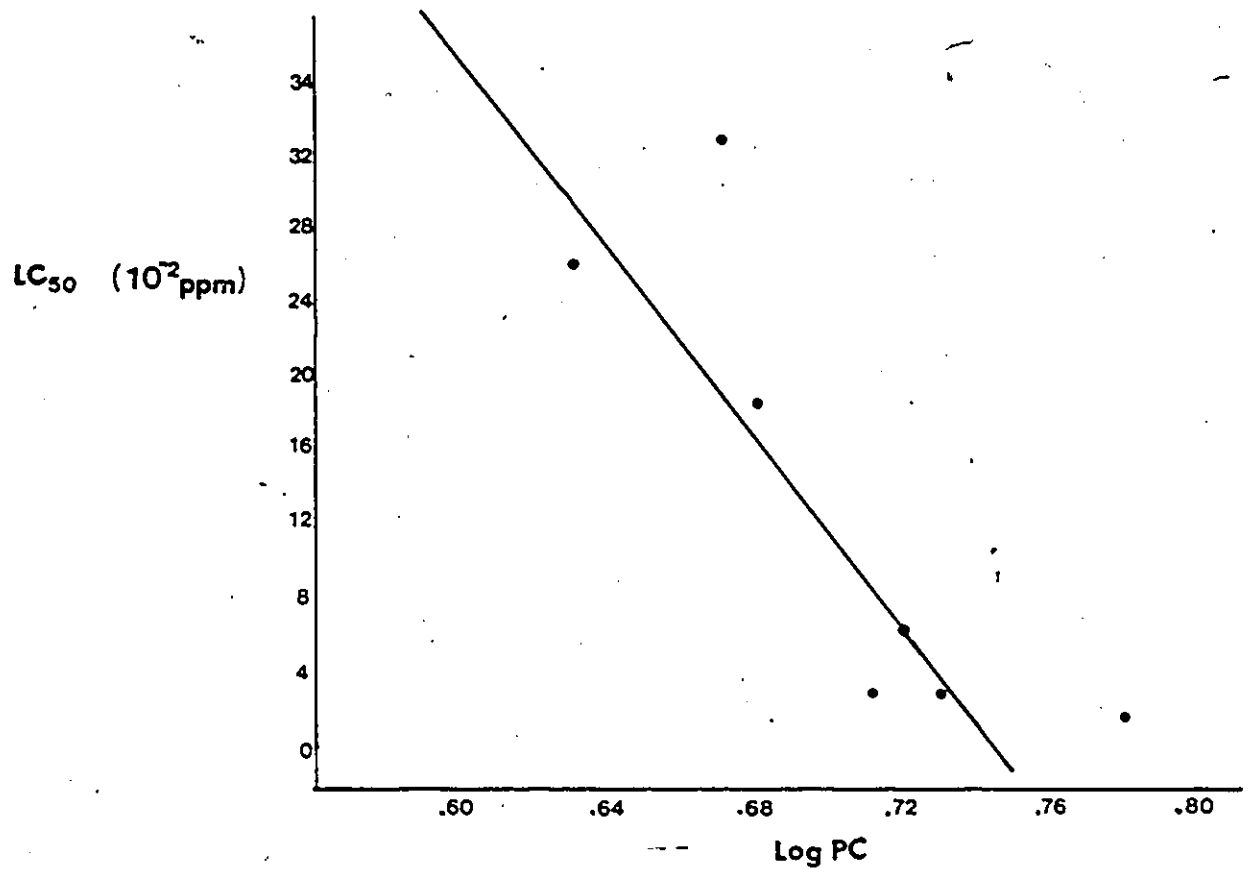


Figure 12. Plot of LC_{50} values for a series of α-T derivatives and analogues, versus the log of their partition coefficient.

100% mortality of the cornborer after UV treatment with 0.1 mg/ml of test compound was observed for 3 of the 4 derivatives with the highest quantum yields of singlet oxygen. The reason why CNα-T is not as toxic to the cornborer when its quantum yield of singlet oxygen and its partition coefficient are similar to that of α-T is not obvious. For the analogues (ONP, TNP, PYT, BTP and DTB), toxicity toward the cornborer shows little or no enhancement in the presence of UV light. This could point to a non-sensitizing mechanism of toxicity based on structural differences. The cornborer may not be the best choice as a target organism due to its ability to metabolize and excrete α-T within 6 h. Thus the differences in toxicity may be a function of how easily the insect is able to metabolize the test compound. This data are not as reliable as the data obtained for mosquito larvae and were not further analyzed.

In conclusion, there is evidently a contribution of both the singlet oxygen generating ability of the compounds and their partition coefficients to the toxicity of the α-T derivatives and analogues toward mosquito larvae. All of the compounds tested were good singlet oxygen generators and highly lipophilic. The results also indicate that other factors are involved which may include the *in vivo* site of action due to the different chemical functionalities and the availability of oxygen at these sites. A definitive establishment of a toxicity model will require further study. The non-sensitizing toxic mechanism of the analogues also requires further research and may yield a potent conventional pesticide.

4.4 EXPERIMENTAL

4.4.1 General

UV spectra were recorded before and after laser experiments to ensure photoconversion was kept to acceptable levels (< 20%). Solvents were of the highest commercial purity available and were used without further purification.

The UV spectra were obtained using a Hewlett-Packard 8451 diode array spectrophotometer. Luminescence studies were carried out with a Perkin Elmer LS5 spectrofluorimeter equipped with a PE3600 data station.

4.4.2 Quenching Studies

Oxygen quenching studies were performed in methanol solutions at various oxygen concentrations. The solutions were saturated with known N_2-O_2 mixtures prepared using a Matheson model 610 gas mixer. The gases used were Linde products of 99+% purity.

For the quenching studies k_{obs} was the rate of decay of the triplet recorded at or near the triplet-triplet absorption maximum of the sample. Quenching data were treated by fitting to a first order kinetic expression.

4.4.3 Singlet Oxygen Quantum Yields

The compounds used in this study were synthesized and purified as described in Chapter 3. The procedure followed for determination of singlet oxygen quantum yields was based on a method devised by Chattopadhyay *et al.*[194]. A flow-through apparatus was used consisting of a cell with an optical path length of 0.03 cm equipped with an inlet for

continuous gas flow. The flow rate of the sample was controlled using a Cole-Parmer Masterflex pump system and was usually around 2 ml/ min. The samples (OD between 0.8 - 1.2 at the laser wavelength), one consisting of the sensitizer plus 1,3-diphenylisobenzofuran (DPBF), an optically matched sample containing o-hydroxybenzophenone (OHBP) and DPBF were excited by pulses from a nitrogen laser (337.1 nm) and the bleaching of DPBF monitored at 440 nm. Both of these samples contained the same concentration of DBPF (0.82 mM) and were continuously saturated with oxygen (Ultra pure). A third optically matched sample, benzophenone, was monitored at its triplet maximum 525 nm and served as the actinometer. This sample was continuously purged with nitrogen. The solvent used for each sample was $CDCl_3$. The bleaching of DPBF or the monitoring of the actinometer were extrapolated to "zero" laser dose by using neutral density filters (15-100%) to attenuate the dose.

Chloroform-d 99.8% isotopic purity was purchased from Merck Sharp and Dohme and was used as received. Benzophenone, acridine and o-hydroxybenzophenone were purified by recrystallization from ethanol. 1,3-diphenylisobenzofuran, purchased from Aldrich, was purified by recrystallization from methanol.

4.4.4 The Laser System

The laser system used for the flash photolysis experiments is located at the National Research Council of Canada in Ottawa, and is set up as shown in Figure 13. Detailed descriptions of this laser facility are also found in the literature[215].

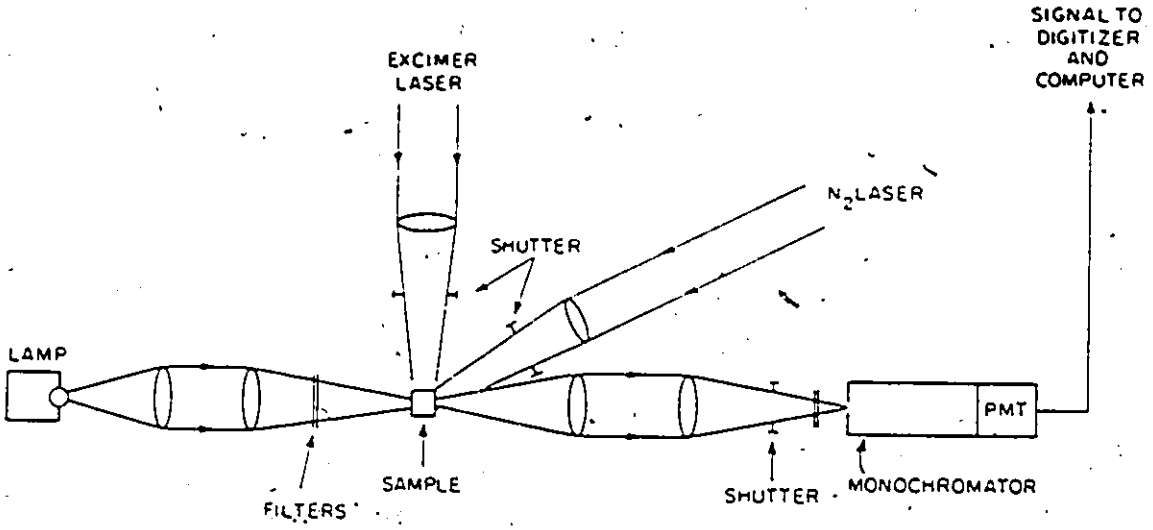


Figure 13. Diagram of the laser facility at the National Research Council of Canada.

While a flow system was necessary for the singlet oxygen experiments, static samples were used for determining the triplet-triplet absorption spectra, the oxygen quenching rate constants and some of the triplet lifetime data. Samples were usually contained in 3 X 7mm^2 or 7 X 7 mm^2 cells made of Supracil tubing which held 1 ml or 2 ml of sample respectively. Samples were purged with oxygen free nitrogen by bubbling the gas into the sample via a syringe for 10 min and sealing the tube. The septa used to seal the sample tube were previously soaked in benzene for two days to remove impurities that might later leach out into the sample. Concentrations of solutions ranged from 1 - 10 x 10^{-5} M unless otherwise specified.

A molectron UV-24 nitrogen laser is used to excite the sample, emitting a pulse at 337.1 nm with $\leq$10 mJ energy per pulse for a pulse duration of 10 ns. The repetition rate of the pulses is 1 Hz which ensured reproducible laser pulses. The laser beam is set at a 20° angle to the monitoring beam to optimize the signal/(pulse energy) ratio. Neutral density filters are used to attenuate the dose. The monitoring lamp is a pulsed 200 Watt xenon lamp in which the beam is increased by a factor of 20-100 during a few milliseconds. The monitoring beam after concentration passes through the sample and then is concentrated again before entering the PRA-B204 monochromator. An RCA-4840 photomultiplier (PMT) is the detector which generates the electrical signal.

The PMT signal is initially captured by an R-7912 Tektronix transient digitizer. The digitizer contains a local memory in which each trace, the various settings and experimental information are stored. The monitor displays

each trace and the memory is read by a PDP 11/23 computer. The computer controlled the equipment, provided suitable storage, processing and hard-copy facilities.

The data generated from a laser flash photolysis experiment are presented as an array of data points obtained as a function of time representing the change in optical density between the ground state sample and the excited species formed after a laser pulse. The data from each experiment are given a file name and saved on a floppy disc from which it can be recalled for further processing. A photocopy of the trace which includes the conditions of the experiment can also be provided from a 4631 Tektronix hard-copy unit.

Usually an experiment is carried out with an average of 10 laser shots per wavelength. The signal shots are alternated with correction shots, rather than independently accumulating each one. The two types of corrections used to enhance data quality are called baseline or fluorescence corrections. The baseline correction minimizes the noise associated with the laser and the monitoring beam, and corrects for changes in the intensity of the latter. The fluorescence correction minimizes the fluorescence from the sample as well as the contributions from other photon sources such as phosphorescence or scattered laser light.

The PDP 11/23 computer activates various shutters which are used to prevent unnecessary irradiation of the sample by either the laser or the monitoring beam. Computer control also allows the corrections previously described to be carried out easily. For a baseline correction, the

computer activates all units except the shutter controlling the laser beam.

For some of the triplet-triplet absorption spectra, the laser pulses were from a lumonics TE 860-2 excimer laser operated with Xe-HCl-He mixtures. The samples were contained in 7 x 7 mm^2 cells of supracil tubing holding 4 ml of sample. The pulses emitted were at 308 nm, for 5 ns duration and of <60 mJ energy per pulse. The excimer laser beam (Figure 13) is located at a 90° angle to the monitoring beam. This laser was used with an EG & G model 1420 optical multichannel analyzer (OMA), which measures the triplet-triplet absorption of a transient over the wavelength range desired, for a specific time delay after excitation.

GENERAL CONCLUSIONS

Increasing insect resistance to current pesticides has pointed out the necessity for a cooperative effort in all areas of pesticide management. More than 500 species of insects have formed some type of immunity to insecticides, some strains with resistance to almost everything available in the chemical arsenal[216]. Besides further research on the diversity of factors influencing resistance which includes genetic, physiologic, and ecological aspects, development of new pesticides is a priority.

The naturally occurring phototoxin α-T, is an alternative to the type of pesticides currently available. The mechanism of action is completely different from the neurotoxins that have made insects resistant. As α-T is in an early stage of its development as a commercial pesticide, little is known about how easily insects would develop resistance. However, the current belief that α-T is a defense compound[88] used by the plant family Asteraceae suggests that it may be less hazardous to the environment. The research to date supports this view. α-T photodegrades quickly, with a half-life of about 4 h[81], into products which are nontoxic to the target organism[42]. Acceptable levels of mortality to nontarget organisms have also recently been reported[82].

This work has described a large scale synthesis of α-T by a Grignard-Wurtz reaction using a nickel(II) phosphine catalyst. This one-pot synthesis is cost efficient and produces the product in 75-80% yield. The large quantities of α-T synthesized in this manner have

enabled field trials to be carried out and these have confirmed the potential of α-T as a commercial larvacide.

The synthesis of derivatives of α-T and analogues was undertaken to determine their biological activity, and to examine how chemical structure affected the activity. All of the compounds synthesized were biologically active, with two compounds (Mα-T, CNα-T) being more potent than α-T. Some of the analogues showed activity in the dark as well as the light indicating a non-photosensitizing mechanism. Future synthetic work to put a sugar moiety or amino acid in the C-5 position of α-T could produce a water soluble derivative. The effect of a water soluble derivative may be completely different, as it would not be located on the surface of the water like α-T. Particular sugars could be used which are specific to insect enzymes[217]. Substituents that have improved the potency of pyrethrin, such as the vinyldichloride group, added in the C-5 position of α-T could also produce a more potent insecticide[211].

The derivatives prepared in this work will also be used to study the biosynthesis of α-T which is still ambiguous. Tritium labeling of the 5-methyl and the 5-formyl derivative of α-T is currently in progress for feeding studies to resolve the final biosynthetic steps to α-T.

The photochemical work on the analogues and derivatives prepared confirmed the photodynamic type II mechanism of toxicity. The singlet oxygen quantum yields indicated that these compounds are extremely potent singlet oxygen generators. Determining the singlet oxygen quantum yields of these sensitizers in lipid bilayers may give

interesting results and is one step closer to the site of action *in vivo*.

The quantum yields of singlet oxygen generation and the partiton coefficients were used to begin to understand structure-function relationships for these compounds. The amount of singlet oxygen produced by a compound correlates with the observed toxicity toward mosquito larvae. Compounds with high quantum yields have lower LC_{50} values. A negative correlation also existed between the lipophilicity and LC_{50} values. Most of these data are based on a set of 10 points at maximum and this thesis describes only the preliminary results. By gathering and correlating the data on a larger set of α-terthienyl derivatives, it may be possible to establish a definitive toxicity model.

CLAIMS TO ORIGINAL RESEARCH

1. The Grignard-Wurtz reaction for preparing α-terthienyl was examined for various ether solvents with complete quantitative analysis and identification of all reaction products.

2. The singlet oxygen quantum yields were determined for the following compounds:

2,2':5',2"-Terthienyl
5-Methyl-2,2':5',2"-terthienyl
5-Cyano-2,2':5',2"-terthienyl
5,5"-Dibromo-2,2':5',2"-terthienyl
5-Bromo-2,2':5',2"-terthienyl
1,3-(Di-2-thienyl)-benzene
1,4-(Di-2-thienyl)-benzene
2,6-(Di-2-thienyl)-pyridine
1-(2-Thienyl)-naphthalene
2-(2-Thienyl)-naphthalene

3. The following new compounds were synthesized:

1,3-(Di-2-thienyl)-benzene
5-Iodo- and 5,5"-Diiodo-2,2':5',2"-terthienyl
5-Cyano and 5,5"-Dicyano-2,2':5',2"-terthienyl
5-Nitro-2,2':5',2"-terthienyl
5-(2-Hydroxyethyl)-2,2':5',2"-terthienyl
5-(2,2':5',2"-Terthienyl)-amide
5-(2,2':5',2"-Terthienyl)-acrylic acid
1-(2-Thienyl)-penta-1,3-diyne

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS WORK

1. Mac Eachern, A.M., Morand, P., Arnason, J.T., Kaminski, J., Leitch, L.C. and Philogene, B..
Synthesis and Activity of a Heterocyclic Plant Phototoxin.
Tenth International Congress of Heterocyclic Chemistry, Aug. 1985, University of Waterloo, Ontario, Canada.

2. Arnason, J.T., Philogene, B.J.R., Berg, C., Mac Eachern, A., Kaminski, J., Leitch, L.C., Morand, P. and Lam, J..
Phototoxicity of naturally occurring and synthetic thiophene and acetylene analogues to mosquito larvae.
Phytochem., 1986, 25, 1609.

3. Evans, C., Weir, D., Scaiano, J.C., Mac Eachern, A., Arnason, J.T., Morand, P., Hollebone, B., Leitch, L.C. and Philogene, B.J.R..
Photochemistry of the botanical α-terthienyl and some related compounds.
Photochem. Photobiol., 1986, 44, 441.

4. Morand, P., Arnason, J.T., Philogene, B.J.R., Mac Eachern, A.M., Leitch, L.C., and Kaminski, J..
Phototoxic Compounds for use as Insect Control Agents.
U.S. Patent Application, Filed Aug. 14, 1986.

5. Morand, P., Mac Eachern, A.M., Leitch, L.C., Arnason, J.T..
Process for the Synthesis of Alpha-Terthienyl.
Can. Patent Application, Filed July 15, 1986.

6. Mac Eachern, A.M., Leitch, L.C., Morand, P., Arnason J.T., Philogene, B.J.R., Scaiano, J.C., Weir, D., and Evans, C..
Structure-Activity Relationships for Derivatives and Analogues of the Phototoxin, Alpha-Terthienyl.
The Sixth International Congress of Pesticide Chemistry, Aug., 1986, Ottawa, Ontario, Canada.

7. Scaiano, J.C., Mac Eachern, A., Arnason, J.T., Morand, P., and Weir, D..
Singlet Oxygen Generating Efficiency of α-Terthienyl and Some of its Synthetic Analogues.
Photochem. Photobiol., 1987, in press.

REFERENCES

1. Jones, E.R.H.and Thaller, V., "*The Chemistry of the Carbon-Carbon Triple Bond*", **1978**, 621, (Ed. by Patai, S.), John Wiley and Sons, Toronto.

2. Sorensen, J.S. and Sorensen N.A., *Acta. Chem. Scand.*, **1958**, 12, 765.

3. Sorensen, N.A., *Pure Appl. Chem.*, **1961**, *2*, 569.

4. Zechmeister, L. and Sease, J.W., *J. Am. Chem. Soc.*, **1947**, *69*, 273.

5. Birkinshaw, J.H. and Chaplen, P., *Biochem. J.*, **1955**, *60*, 255.

6. Challenger, F. and Holmes J.L., *J. Chem. Soc.*, **1953**, 1857.

7. Bohlmann, F., Burkhardt, T. and Zdero, C.,"*Naturally Occurring Acetylenes*", **1973**, Academic Press: London and New York.

8. Rawls, R.L., *C. & E.N.*, **1986**, Sept. 22, 21.

9. Sease, J.W. and Zechmeister, L., *J. Am. Chem. Soc.*, **1947**, 69, 270.

10. Groneman, A.F., Posthumus, M.A., Tuninstra, L.G.M.T. and Traag, W.A., *Anal. Chem. Acta.*, **1984**, 163, 43.

11. Downum, K.R., Niel, D.J. and Rodriquez, E., *Biochem. Syst. Ecol.*, **1985**, 13, 109.

12. Downum, K.R. and Towers, G.H.N., *J. Nat. Prod.*, **1983**, 46, 98.

13. Morris, G.A., *Magn. Reson. Chem.*, **1986**, 24, 371.

14. Bu'lock, J.D., Allport, D.C. and Turner, W.B., *J. Chem. Soc.*, **1961**, 1654.

15. Bohlmann, F., *Prog. Chem. Nat. Prod.*, **1967**, *25*, 1.

16. Goodwin, T.W. and Mercer, E.I.,"*Introduction to Plant Biochemistry*", 1983, Chapter 8, 273, Pergammon Press: New York.

17. Vance, E.,and Vance, J.E.,"*Biochemistry of Lipids and Membranes*", 1985, The Benjamin/Cumming Publishing Company Inc: Ontario.

18. Bohlmann, F., Bonnet, H. and Jente, R., *Chem. Ber.*, 1968, *101*, 855.

19. Bohlmann, F., Jente, R., Lucas, W., Laser, J. and Schulz, H., *Chem. Ber*, 1967, *100*, 3183.

20. Bohlmann, F. and Skuballa, W., *Chem. Ber.*, 1973, *106*, 497.

21. Bohlmann, F. and Sewita, A., *Chem. Ber.*, 1975, *108*, 515.

22. Schulte, K.E., Henke, G., Rucker, G. and Foerster, S., *Tetrahedron*, 1968, *24*, 1899.

23. Jente, R., Olatunji, G.A. and Bosold, F., *J. Phytochem.*, 1981, *20*, 2169.

24. Schulte, K.E. and Foerster, S., *Tetrahedron Lett.*, 1966, 773.

25. Bohlmann, F., Grenz, M., Wotschokowsky, M. and Berger, E., *Chem. Ber.*, 1967, *100*, 2518.

26. Bohlmann, F. and Zdero, C., *Chem. Ber.*, 1970, *103*, 834.

27. Sutfield, R. and Towers, G.H.N., *J. Phytochem.*, 1982, *21*, 277.

28. Pensl, R. and Sutfield, R., *Z. Naturforsch.*, 1985, *40c*, 3.

29. Schulte, K.E., Reisch, J., Horner, L., *Chem. Ber.*, 1962, *95*, 1943.

30. Kagan, J. and Arora, S.K., *J. Org. Chem.*, 1983, *48*, 4317.

31. Schulte, K.E., Rucker, G. and Meinders, W., *Tetrahedron Lett.*, 1965, 659.

32. Schuetz, R.D., Waggoner, T.B. and Byerrum, R.U., "*Biochemistry*", 1965, 4, 436.

33. Bohlmann, F. and Zdero, C, "*Chemistry of Heterocyclic Compounds*", 1985, (Ed. by Weissberger, A. and Taylor E.C.), J. Wiley and Sons Inc., New York, 261-323.

34. Uhlenbroek, J.H. and Bijloo, J.D., *Rec. Trav. Chim. Pay. Bas.*, 1958, 77, 1004.

35. Uhlenbroek, J.H. and Bijloo, J.D., *Rec. Trav. Chim. Pay. Bas.*, 1959, 78, 382.

36. Gommers, F., *Nematologia*, 1972, 18, 458.

37. Gommers, F. and Geerligs, J., *Nematologia*, 1973, 19, 389.

38. Chan, G.F.Q., Towers, G.H.N. and Mitchell, J.C., *Phytochem.*, 1975, 14, 2295.

39. Pathak, M.A., Kramer, D.M. and Fitzpatrick, T.B.,"*In Sunlight and Man*", 1974, University of Tokyo Press, Tokyo.

40. Bakker, J. and Gommers, F., *J. Biol. Chem.*, 1979, 254, 1841.

41. Merkel, P.B., Nilsson, R. and Kearns, D.R., *J. Am. Chem. Soc.*, 1972, 94, 1030.

42. Kagan, J., Gabriel, R. and Reed, S.A., *Photochem. Photobiol.*, 1980, 31, 465.

43. Reyftman, J.P., Kagan, J., Santus, R. and Morliere, P., *Photochem. Photobiol.*, 1985, 41, 1.

44. Kagan, J. and Gabriel, R., *Experientia*, 1980, 36, 587.

45. Kagan, J., Gabriel, R. and Singh, S.P., *Photochem. Photobiol.*, 1980, 32, 607.

46. Kagan, J., Gabriel, R. and Singh, S.P., *Experientia*, 1981, 37, 80.

47. Arnason, T., Chan, G.F.Q., Wat, C.-K., Downum, K. and Towers, G.H.N., *Photochem. Photobiol.*, 1981, 33, 821.

48. Kobayashi, K. and Ito, T., *Photochem. Photobiol.*, **1976**, 23, 21.

49. Wat, C.K., Mac Rae, W.D., Yamamoto, E., Towers, G.H.N. and Lam, J., *J. Ethnopharmacol.*, **1980**, 2, 279.

50. Kulig, M.J. and Smith, T.L., *J. Org. Chem.*, **1973**, 38, 3639.

51. Mac Rae, W.D., Chan, G.F.Q., Wat, C.K., Towers, G.H.N. and Lam, J., *Experientia*, **1980**, 36, 1096.

52. Downum, K.R., Hancock, R.E.W. and Towers, G.H.N., *Photochem. Photobiol.*, **1982**, 36, 517.

53. Wat, C.K., Biswas, R.K., Graham, E.A., Bohm, L., Towers, G.H.N., and Waygood, E.R., *J. Nat. Prod.*, **1977**, 42, 103.

54. Wat, C.K., Mac Rae, W.D., Yamamoto, E., Towers, G.H.N. and Lam, J., *Photochem. Photobiol.*, **1980**, 32, 167.

55. Arnason, T., Wat, C.K., Downum, K., Yamamoto, E., Graham, E. and Towers, G.H.N., *Can. J. Microbiol.*, **1980**, 26, 698.

56. Calloman, J.H. and Ramsay, D.A., *Can. J. Phys.*, **1957**, 35, 129.

57. Poole, C.P. and Anderson, R.S., *J. Chem. Phys.*, **1959**, 31, 346.

58. Mc Lachlan, D., Arnason, T. and Lam, J., *Photochem. Photobiol.*, **1984**, 39, 177.

59. Kagan, J., Tadema-Wielandt, K., Chan, G., Dhawan, S.N., Jaworsky, J., Prakash, I. and Arora, S.K., *Photochem. Photobiol.*, **1984**, 39, 465.

60. Camm, E.L., Towers, G.H.N. and Mitchell, J.C., *Phytochem.*, **1975**, 14, 2007.

61. Downum, K.R., Hancock, R.E.W. and Towers, G.H.N., *Photobiochem. Photobiophysics*, **1983**, 6, 145.

62. Downum, K.R., Hancock, R.E.W. and Towers, G.H.N., *Photochem. Photobiol.*, **1982**, 36, 517.

63. DiCosmo, F., Towers, G.H.N. and Lam, J., *Pest. Sci.*, **1982**, 13, 589.

64. Gommers, F.J., Bakker, J. and Wynberg, H., *Photochem. Photobiol.*, **1982**, 35, 615.

65. Wat, C.K., Prasad, S.K., Graham, E.A., Partington, S., Arnason, T. and Towers, G.H.N., *Biochem. Syst. Ecol.*, **1981**, 9, 59.

66. Arnason, T., Swain, T., Wat, C.K., Graham, E.A., Partington, S. and Towers, G.H.N., *Biochem. Syst. Ecol.*, **1981**, 9, 63.

67. Kagan, J. and Chan, G., *Experentia*, **1983**, 39, 402.

68. Kagan, J., Chan, G., Dhawan, S.N., Arora, S.K. and Prokash, I., *J. Nat. Prod.*, **1983**, 46, 646.

69. Downum, K.R., Rosenthal, G.A. and Towers, G.H.N., *Pest. Biochem. Physiol.*, **1984**, 22, 104.

70. Champagne, D.E., Arnason, J.T., Philogene, B.J.R., Morand, P. and Lam, J., *J. Chem. Ecol.*, **1986**, 12, 835.

71. Arnason, T., Wat, C.-K., Graham, E.A., Downum, K., Yamamoto, E. and Towers, G.H.N., *Can. J. Microbiol.*, **1980**, 26, 698.

72. Campbell, G., Lambert, J.D.H., Arnason, T. and Towers, G.H.N., *J. Chem. Ecol.*, **1982**, 8, 961.

73. Yamamoto, E., Wat, C.K., Mac Rae, W.D. and Towers, G.H.N., *FEBS Lett*, **1979**, 107, 135.

74. Wat, C.-K., Mac Rae, W.D., Yamamoto, E., Towers, G.H.N. and Lam, J., *Photochem. Photobiol.*, **1980**, 32, 167.

75. Arnason, T., Stein, J.R., Graham, E., Wat, C.K., Towers, G.H.N. and Lam, J., *Can. J. Bot.*, **1981**, 59, 54.

76. Towers, G.H.N., Wat, C.-K., Graham, E.A., Bandoni, R., Chan, G. and Lam, J., *Lloydia*, **1977**, 40, 487.

77. Graham, K., Graham, E.A. and Towers, G.H.N., *Can. J. Zool.*, **1980**, 58, 1955.

78. Warren, R.A.J., Hudson, J.B., Downum, K., Graham, E.A., Norton, R. and Towers, G.H.N., *Photobiochem. Photobiophys.*, **1980**, 1, 385.

79. Hudson, J.B., Graham, E.A. and Towers, G.H.N., *Photochem. Photobiol.*, **1982**, 36, 181.

80. Mac Rae, W.D., Irwin, D.A.J., Bisalputra, T. and Towers, G.H.N., *Photochem. Photobiophys.*, **1980**, 1, 309.

81. Philogene, B.J.R., Arnason, J.T., Berg, C.W., Duval, F., Champagne, D., Taylor, R.G., Leitch, L.C. and Morand, P., *J. Econ. Entomol.*, **1985**, 78, 121.

82. Philogene, B.J.R., Arnason, J.T., Berg, C.W., Duval, F. and Morand, P., *J. Chem. Ecol.*, **1986**, 12, 893.

83. Mc Lachlan, D., Arnason, J.T., Philogene, B.J.R. and Champagne, D., *Experientia*, **1982**, 38, 1061.

84. Doleiden, F.H., FahrenHoltz, S.R., Lamola, A.A. and Trozzolo, A.M., *Photochem. Photobiol.*, **1974**, 20, 519.

85. Mc Lachlan, D., Arnason, T. and Lam, J., *Biochem. Syst. Ecol.*, **1986**, 14, 17.

86. Tuveson, R.W., Berenbaum, M.R. and Heininger, E.E., *J. Chem. Ecol.*, **1986**, 12, 933.

87. Giese, A., *Photophysiology*, **1971**, 6, 77.

88. Towers, G.H.N. *Can. J. Bot.*, **1984**, 62, 2900.

89. Kourany, E., Thesis, University of Ottawa, **1986**.

90. Downum, K.R., *ACS Symp. Ser.*, **1986**, 296, 197.

91. Downum, K.R., Rodriguez, E., *J. Chem. Ecol.*, **1986**, 12, 823.

92. Steinkopf, W., von Petersdorff, H.J. and Gording, R., *Ann. Chim.*, **1937**, 527, 272.

93. Harvey J., US 3086854, *Chem. Abstr.*, **1959**, 59, 11430d.

94. Kagan, J. and Arora, S.K., *Heterocycles*, **1983**, 20, 1941.

95. Asano, T., Ito, S., Saito, N. and Hatakeda, K., *Heterocycles*, **1977**, 6, 317.

96. Wynberg, H. and Metselaar, *Synth. Commun*, **1984**, 14, 1.

97. Beny, J.P. and Kagan, J., *J. Label. Comp.. Radiopharma.*, **1982**, XIX, 313.

98. Bestmann, H.J. and Schaper, W., *Tetrahedron Lett.*, **1979**, 243.

99. Kagan, J. and Arora, S.K., *Tetrahedron Lett.*, **1983**, 24, 4043.

100. Chodkiewicz, W., *Ann. Chim.*, **1957**, 13, 819.

101. Kumada, M., Tamao, K. and Sumitani, K., *Org. Synth.*, **1978**, 58, 127.

102. Kumada, M., *Pure Appl. Chem.*, **1980**, 52, 669.

103. Tamao, S.K., Sumitani, K., Kiso, Y., Zembayashi, M., Fujioka, A., Kodama, S., Nakajima, I., Minato, A. and Kumada, M., *Bull. Chem. Soc. Jpn.*, **1976**, 49, 1958.

104. Tamao, S.K., Kodama, S., Nakajima, I. and Kumada, M., *Tetrahedron*, **1982**, 38, 3347.

105. Tamao K., Sumitani, K. and Kumada, M., *J. Am. Chem. Soc.*, **1972**, 94, 4374.

106. Corriu, R.J.P. and Masse, J.P., *J. Chem.Soc. Chem. Commun.*, **1972**, 144.

107. Adamczyk, M., Watt, D.S. and Netzel, D.A., *J. Org. Chem.*, **1984**, 49, 4226.

108. Minato, A., Tamao, K., Suzuki, K. and Kumada, M., *Tetrahedron Lett.*, **1980**, 21, 4017.

109. Wenkert, E., Fernandes, J.B., Michelotti, E.L. and Swindell, C.S., *Synth. Commun.*, **1983**, 701.

110. Sugimura, H. and Takei, H., *Bull. Chem. Soc. Jpn.*, **1985**, 58, 664.

111. Wenkert, E., Leftin, M.H. and Michelotti, L., *J. Chem. Soc. Chem. Commun.*, **1984**, 617.

112. Tiecco, M. and Tingoli, M., *J. Org. Chem.*, **1985**, 50, 3828.

113. Colomer, E., Corriu, R.J.P. and Meunier, B., *J. Organomet. Chem.*, **1974**, 71, 197.

114. Fraser, P.J., Roper, W.R. and Stone, F.G.A., *J. Chem. Soc. Dalton Trans.*, **1974**, 102.

115. Kochi, J.R., "*Organometallic Mechanisms and Catalysis*", **1978**, 293-398, Academic Press, New York.

116. Tsou, T.T. and Kochi, J.K., *J. Am. Chem. Soc.*, **1979**, 101, 7547.

117. Smith, G. and Kochi, J.K., *J. Organomet. Chem.*, **1980**, 198, 199.

118. Babudri, F., Florio, S., Ronzini, L. and Aresta, M., *Tetrahedron*, **1983**, 39, 1515.

119. Hayashi, T., Konishi, M., Yokota, K. and Kumada, M., *Chem. Lett.*, **1980**, 767.

120. Hayashi, T., Tajika, M., Tamao,K. and Kumada, M., *J. Am. Chem. Soc.*, **1976**, 98, 3718.

121. Hayashi, T., Fukushima, M., Konishi, M. and Kumada, M., *Tetrahedron Lett.*, **1980**, 21, 79.

122. Molander, G.A., Rahn, B.J., Shubert, D.C. and Bonde, S.E., *Tetrahedron Lett.*, **1983**, 24, 5449.

123. Tamura, M. and Kochi, J.K., *J. Am. Chem. Soc.*, **1971**, 93, 1483.

124. Kochi, J.K., *Acc. Chem. Res.*, **1974**, 7, 351.

125. Derguini-Boumechal, F. and Linstrumelle, G., *Tetrahedron Lett.*, **1976**, 3225.

126. Tamura, M. and Kochi, J.K., *J. Organometal. Chem.*, **1972**, 42, 205.

127. Murahashi, S., Yamamura, M., Vanagisawa, K., Mita, N. and Kondo, K., *J. Org. Chem.*, **1979**, 44, 2408.

128. Negishi, E., *Acc. Chem. Res.*, **1982**, 15, 340.

129. Baba, S. and Negishi, E., *J. Am. Chem. Soc.*, **1976**, 98, 6729.

130. March, J., "*Advanced Organic Chemistry*", 1985, John Wiley and Sons Inc., New York.

131. Parris, G.E. and Ashby, E.C., *J. Am. Chem. Soc.*, 1971, 93, 1206.

132. Kharash, M.S. and Reinmuth, D., "*Grignard Reactions of Nonmetallic Substances*", 1954, Prentice Hall Inc., New York.

133. Tamborski, C. and Moore, G.J., *J. Organometal. Chem.*, 1971, 26, 153.

134. Zakharkin, I., Okhlobystin, O.Y. and Bilevitch, K.A., *J. Organometal. Chem.*, 1964, 2, 309.

135. Jayasuriya, N. and Kagan, J., *Heterocycles*, 1986, 24, 2901.

136. Carpita, A., Rossi, R. and Veracini C.A., *Tetrahedron*, 1985, 41, 1919.

137. Garcia, F.J., Yamamoto, E., Abramowski, Z., Downum, K. and Towers G.H.N., *Photochem. Photobiol.*, 1984, 39, 521.

138. Montheard, J.P. and Dubois, J.C. *J. Heterocycl. Chem.*, 1985, 22, 719.

139. Ribereau, P.Queguiner, G. and Pastour, P. *Bull. Soc. Chim. Fr.*, 1972, 1581.

140. Uhlenbroek, J.H. and Bijloo, J.D., *Rec. Trav. Chim. Pay. Bas.*, 1960, 79, 1181.

141. Kagan, J., Beny, J.P., Chan, G., Dhawan, S.N., Jaworski, J.A., Kagan, E.D., Kassner, P.D., Murphy, M. and Rogers, J.A., *Insect Sci. Application*, 1983, 4, 377.

142. Gommers, F.G., Bakker, J. and Wynberg, H., *Photochem. Photobiol.*, 1982, 35, 615.

143. Acheson, R. M., "*An Introduction to the Chemistry of Heterocyclic Compounds*", 1976, John Wiley and Sons, New York.

144. Ho, T.-L., Gupta, B.G.B. and Olah, G.A., *Synthesis*, 1977, 676.

145. Minnis, W., *Org Synth. Coll. Vol. II*, 357.

146. Hartough, H.D., "*The Chemistry of Heterocyclic Compounds. Thiophene and its derivatives*", **1952**, 500, Interscience Publishers Inc., New York.

147. Butler, A.R. and Sanderson, A.P., *J. Chem. Soc. Perkin Trans. 2*, **1974**, 1214.

148. Butler, A.R., *J. Chem. Educ.*, **1971**, 48, 508.

149. Rossi, R., Carpita, A. and Lezzi, A., *Tetrahedron*, **1984**, 40, 2773.

150. Taylor, E.C. and Vogel, D.E., *J. Org. Chem.*, **1985**, 50, 1002.

151. van Pham, C., Macomber, R.S., Mark, Jr. H.B. and Zimmer, H., *J. Org. Chem.*, **1984**, 49, 5250.

152. Kagan, J., Arora, S.K. and Ustunol, A., *J. Org. Chem.*, **1983**, 48, 4076.

153. Babasinian, V.S., *Org. Synth. Coll. Vol. II*, 466.

154. Lohaus, G., *Org. Synth.*, **1970**, 50, 52.

155. Lohaus, G., *Chem. Ber.*, **1967**, 100, 2719.

156. Weston, A.W. and Micheals, Jr., R.T., *Org. Synth.*, **1951**, 31, 108.

157. Nakayama, J., Murabayashi, S. and Hoshino, M., *Heterocycles*, **1986**, 24, 2639.

158. Johnson, J.R., *Org.React.*, **1942**, 1, 210.

159. House, O.H., "*Modern Synthetic Reactions*", **1972**, W.A. Benjamin Inc., California, U.S.A..

160. Corey, E.J. and Fraenkel, G., *J. Am. Chem. Soc.*, **1953**, 75, 1168.

161. Anderson, R.J., *J. Am. Chem. Soc.*, **1970**, 92, 4978.

162. Herr, R.W., Wieland, D.M. and Johnson, C.R., *J. Am. Chem. Soc.*, **1970**, 92, 3813.

163. Herr, R.W., and Johnson, C.R., *J. Am. Chem. Soc.*, 1970, 92, 4979.

164. Eglinton, G. and Mc Crae, W., *Adv. Org. Chem.*, 1963, 4, 225.

165. Glaser, C., *Ann.*, 1870, 154, 137.

166. Meier, J., Chodkiewicz, W., Cadiot, P. and Williemart, A., *Compt. Rend.*, 1957, 245, 1634.

167. Schulte, K.E., Reisch, J. and Horner, L., *Chem. Ber.*, 1942, 95, 1942.

168. Bohlmann, F., Sucrow, W. and Queck, I., *Chem. Ber.*, 1961, 94, 948.

169. Armitage, J.B., Entwistle, N., Jones, E.R.H. and Whiting, M.C., *J. Chem. Soc.*, 1954, 147.

170. Brandsma, L., "*Preparative Acetylene Chemistry*", 1971, Elsevier Publishing Company, New York.

171. Prevost, C., *Ann. Chim.*, 1928, 10, 356.

172. Schlubach, H.H., Wolf, V., Justus, W. and Kohncke, C.H., *Liebigs Ann.Chem.*, 1950, 568, 141.

173. Armitage, J.B., Jones, E.R.H. and Whiting, M.C., *J. Chem. Soc.*, 1952, 1993.

174. Jones, E.R.H. and Whiting, M.C., *J. Chem. Soc.* 1953, 3317.

175. Wier, D., Scaiano, J.C.,Arnason, J.T. and Evans, C., *Photochem. Photobiol.*, 1985, 42, 223.

176. Straus, F., Kollek, L. and Heyn, W., *Ber.*, 1930, 1868.

177. Commercon, A., Normant, J.F. and Villieras J., *Tetrahedron*, 1980, 36, 1215.

178. Stephans, R.D. and Castro, C.E., *J. Org. Chem.*, 1963, 28, 3313.

179. Burdon, J., Coe, P.L., March, C.R. and Tatlow, *J. Chem. Soc. Chem. Commun.*, 1967, 1259.

180. Atkinson, R.E., Curtis, R.F., Jones, D.M. and Taylor, J.A., *J. Chem. Soc. Chem. Commun.*, **1967**, 718.

181. Atkinson, R.E., Curtis, R.F., and Philips, G.T., *Chem. Ind.*, **1964**, 2101.

182. Curtis, R.F. and Taylor, J.A., *J. Chem. Soc.*, **1971**, 186.

183. Cassar, L., *J. Organometal. Chem.*, **1975**, 93, 253.

184. Dieck, H.A. and Heck, F.R., *J. Organometal. Chem.*, **1975**, 93, 259.

185. Songashira, K., Tohuda, Y. and Hagihara, N., *Tetrahedron Lett.*, **1975**, 4467.

186. Oshawa, A., Abe, Y. and Igeta, H., *Chem. Lett.*, **1979**, 241.

187. Edo, K., Yamanaka, H. and Sakamoto, T., *Heterocycles*, **1978**, 9, 271.

188. Van Heck, G.R. and Dew Horrocks, Jr., W., *Inorg. Chem.*, **1966**, 5, 1968.

189. Wynberg, H., Van Driel, H., Kellogg, R.M. and Buter, J., *J. Am. Chem. Soc.*, **1967**, 89, 3487.

190. Carpita, A., Lezzi, A. and Rossi, R., *Synth. Commun.*, **1982**, 571.

191. Turro, N.J., "*Modern Molecular Photochemistry*", **1978**, The Benjamin/Cummings Publishing Co. Inc., London, England.

192. Evans, C., Weir, D., Scaiano, J.C., Mac Eachern, A., Arnason, J.T., Morand, P., Hollebone, B., Leitch, L.C. and Philogene, B.J.R., *Photochem. Photobiol.*, **1986**, 44, 441.

193. Kagan, J., Prakash, I., Dhawan, S.N. and Jaworski, J.A., *Photobiochem. Photobiophys.*, **1984**, 8, 25.

194. Chattopadhyay, S.K., Kumar, C.V. and Das, P.K., *J. Photochem.*, **1984**, 24, 1.

195. Wasserman, H.H. and Murray, R.W.," *Singlet Oxygen*", **1979**, 435, Academic Press, New York.

196. Murray, R.W. and Higley, P.D., *J. Am. Chem. Soc.*, **1974**, 96, 3330.

197. Reyftmann, J.P., Kagan, J., Santus, R. and Morliere, P., *Photochem Photobiol.*, **1985**, 41, 1.

198. Scaiano, J.C. and Stewart, L.C., *J. Am. Chem. Soc.*, **1983**, 105, 3609.

199. Wilhelm, E., and Battino, R., *Chem. Rev.*, **1973**, 73.

200. Kearns, D.R., *Chem. Rev.*, **1971**, 71, 395.

201. Carmicheal, I. and Hug, G.L., *J. Phys. Chem. Ref. Data*, **1986**, 15, 1.

202. Lamola, A.A., Hammond, G.S., *J. Chem. Phys.*, **1965**, 43, 2129.

203. Scaiano, J.C., *Chem. Phys. Lett.*, **1982**, 92, 97.

204. Battino, R.," *Solubility Data Series. Oxygen and Ozone*", **1981**, 7, Pergamon Press, Oxford.

205. Benders, P.H., Reinhoudt D.N. and Trompenaars, W.P.,"*The Chemistry of Heterocyclic Compounds: Thiophene and its Derivatives*", **1985**, 705, (Ed. by Gronowitz, S.), John Wiley and Sons, Toronto, Canada.

206. Wilkinson, F. and Brummer, J.G., *J. Phys. Chem. Ref. Data.*, **1981**, 10, 809.

207. Gorman, A.A., Lovering, G. and Rodgers, M.A.J., *J. Am. Chem. Soc.*, **1978**, 100, 4521.

208. Gorman, A.A., Gould, I.R. and Hamblett, I., *Tetrahedron Lett.*, **1980**, 21, 1087.

209. Stevens, B., Marsh, K.L., and Barltrop, J.A., *J. Phys. Chem.*, **1981**, 85, 3079.

210. Hoytink, G.J., *Acc. Chem. Res.*, **1969**, 2, 114.

211. Elliot, M.," *Natural Products for Innovative Pest Management in the Tropics*", **1984**, Whitehead, D., Ed., Pergamon Press, New York.

212. Arnason, J.T., Philogene, B.J.R., Berg, C., Mac Eachern, A., Kaminski, J., Leitch, L.C., Morand, P. and Lam, J., *Phytochem.*, **1986**, 25, 1609.

213. Ito, T., *Photochem. Photobiol.*, **1977**, 25, 47.

214. Ellgehausen, H., D'Hondt, C., and Fuerer, R., *Pest. Sci.*, **1981**, 12, 219.

215. Scaiano, J. C., *J. Am. Chem. Soc.*, **1980**, 102, 7747.

216. Krieger, J., *C. & E.N.*, **1987**, March 2.

217. Evans, S.V., Fellows, L.E., Shing, T.K.M. and Fleet, G.W.J., *J. Phytochem.*, **1985**, 24, 1953.