PHYTOCHEMICAL INVESTIGATIONS OF
BLUMEA OBLIQUA

Thesis submitted
for
the Fulfilment of the Degree of

DOCTOR OF PHILOSOPHY

by

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In the Name of Allah the Most Beneficent, the Most Merciful.
DEDICATED TO
MY LOVING PARENTS,
BROTHER AND SISTERS
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SUMMARY

Phytochemical investigations of the aerial parts of *Blumea obliqua* are presented in this thesis. Pet.-ether soluble part of the methanolic extract of the aerial parts of *Blumea obliqua* has yielded nine bithiophene acetylenes (85-93). Compounds 85-88 are new natural products. The compound 93 has been mentioned in one of the Bohlmann’s papers but no physical or chemical data are available anywhere in the literature. The rest of the bithiophenes are isolated for the first time from *Blumea obliqua*. Some known compounds (94-97) have also been isolated, 97 is being reported for the first time from *B. obliqua*.

Bithiophene Acetylenes from *Blumea obliqua*

5-Methyl-5'-{4-(3-methyl-1-oxobutoxy)-1-butylnyl}-2,2'-bithiophene (85)


5'-Hydroxymethyl-5-(butyl-3-en-1-yn)-2,2'-bithiophene isovaleroyl ester (86)


5'-Methyl-5-(3-hydroxy-4-isovaleroyl-1-butylnyl)-2,2'-bithiophene (87)

*Phytochemistry*, 42, 733 (1996)
Methylene-bis-[5-(3-butene-1-ylnyl)-2,2'-bithiophene] (88)  

5'-Methyl-[5-(4-acetoxy-1-butynyl)-2,2'-bithiophene (89)  
*Phytochemistry*, 42, 733 (1996)

5'-Methyl-5-(4-hydroxy-1-butynyl)-2,2'-bithiophene (90)  
*Phytochemistry*, 42, 733 (1996)

5'-Acetomethyl-5-(3-butene-1-ylnyl)-2,2'-bithiophene (91)  
*Phytochemistry*, 42, 733 (1996)

5'-Hydroxymethyl-5-(3-butene-1-ylnyl)-2,2'-bithiophene (92)  
5-[(3-Butene-1-ynyl)[2,2'-bithiophene]-5'-carboxaldehyde (93)

1.0 GENERAL INTRODUCTION
1.0 General Introduction

Plants are vital for the existence of animal life in the universe. Plants alone, among living forms, are capable of capturing minute quantity of solar energy and through photosynthesis transform elementary substances into complex molecules. They not only synthesize food necessary for the life of man, but also manufacture different chemicals essential for human health.

The relationship between man and plants has been very close throughout the development of human civilization. It is presumed that man learnt the art of curing human ailments from animals. He observed that when deer was shot with an arrow it ate a certain kind of grass that softened the tissue and loosened the arrow from its body. It was also found that the sick dogs searched for certain grasses to produce purgation. By trial and error, primitive man acquired the basic biological knowledge that was useful in determining which plants possessed medicinal value and which were poisonous or dangerous. These observations were handed down to him from generation to generation. The healing powers of certain herbs, roots and juices of plants might have been discovered by accidents but once their attributions were learnt, their importance was established.

With the rise and fall of great civilizations, the medical knowledge of man did not fade into oblivion, but became improved in its applicability. However, some observers are inclined to believe that traditional methods lead to the modern practice. This is not entirely the case. In the dynamics of medical history there is always a cycle in the evolution of ideas, where ancient medicine never dies but keeps on evolving into new forms, as the concept of diseases and the methods of treatment are deeply rooted in cultural ideas, religious dogmas and environmental factors. In fact, traditional medicine is inter-linked with the nature of man.
The knowledge of drugs goes back to prehistoric times. Records of ancient civilizations show that a considerable number of drugs, utilized in modern practice, were already used in one form or another in ancient times. The Babylonians, Egyptians, Chinese, Greeks, Romans and the people of sub-continent of India and Pakistan had developed their respective materia medica, characterized by the local customs and methods.

Chen Nung [1] in China and Sekhet Enanch [2] in Egypt were the first to peruse studies in the medicinal uses of herbs around 3000 BC, for the treatment of various human ailments. The other earlier known references of medicine are in "Rigvedas" (4500-1600 BC) and the Ayurvedas (2500-600 BC).

There is a pharmacopoeia-like compilation in Chinese tradition called "Peutsao" or "the great herbal" (about 1500 BC), containing thousands of prescriptions. The famous Egyptian materia medica "Papyrus Ebers", dating back to about sixteenth century BC, is among the early manuscripts or literature pertaining to pharmacy and medicine which contains a chapter on remedial agents and methods for compounding.

The actual history of medicine and pharmacy begins from Hippocrates [3], "The father of medicine" (460 BC) and Theophrastus [4] (287-370 BC). Hippocrates reported nearly 400 samples as medicine substances. However, the most significant pharmacological compilation of the Greeks was the authoritative text of Discordies [2]. After him, Pliny The Elder (23-79 AD) wrote "Natural History" in 37 volumes. Galen wrote some 30 books on pharmacology beside "Galenicals", his medical formulae [5]. After Galen, the work of early Greek physicians were transferred to Romans and then to Muslims.

In the Muslim period of civilization we find a treasure of valuable medical knowledge. The great physician and philosopher Bu-Ali Sina (Avicenna,
908-1037 AD) has described 700 herbal drugs in his famous book “Qanun fi al Tibb”, the principles of medicine [6].

The source of Indian medicine are derived from “Rigvedas” and “Ayurvedas”. They are mainly based on the use of drugs of plant origin. The Ayurvedic system of medicine is mainly attributed to Charaka [7] and Sushruta [8], who cited about 700 medicinal plants. The Muslim rulers introduced their traditional system of medicine in India and incorporated it in the native Ayurvedic medicine. This mixture is known as Unani medicine or Eastern medicine.

Higher plants have been the source of medicinal agents since earliest times, and today they continue to play a dominant role in the primary health care of about 80% of the world population [9]. Natural products, and medicinal agents derived therefrom, are also an essential feature in the health care system of the remaining 20% of the population residing mainly in developed countries, with more than 50% of all drugs in clinical use having a natural product origin [10]. Of the world’s 25 best-selling pharmaceutical agents, 12 are natural product-derived [11]. Natural products continue to play an important role in drug discovery programs of the pharmaceutical industry and other research organizations [12-14]. Research into the chemical and biological properties of natural products over the past two centuries has not only yielded drugs for the treatment of human ailments, but has provided the stimulus for the development of modern synthetic organic chemistry, and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents. Numerous phytomedicines are registered and extensively used in Europe, and more than 600 botanical items have been recognized in various editions of the United State Pharmacopoeia [15], in spite of legislative ban on some of the marketable items as drugs.
Of the 119 plant-derived drugs commonly in use in one or more countries, 74 were discovered as a result of chemical studies directed at the isolation of the active constituents of plants used in traditional medicine [9]. Well known examples include the cardiac glycosides from *Digitalis purpurea* L., the antihypertensive agent and tranquilizer, reserpine, from the East Indian snakeweed, *Rauwolfia serpentina* L. Bentham ex Kurz, The antimalarial agent, quinine, from *Cinchona* sp. and the analgesics, codeine and morphine, from *Papaver somniferum* L. [10]. Secondary metabolites isolated from medicinal plants have also served as precursors or models for the preparation of effective agents through semi-syntheses or lead-based total syntheses. Examples include the anticancer agent, etoposide, a semi-synthetic derivative of epipodophyllotoxin isolated from *Podophyllum* sp. [16], and anticholinergic drugs modeled on the belladonna alkaloids (e.g. atropine) isolated from *Atropa belladonna* L. and other medicinal plant species [9].

Very little is known about the secondary metabolites of the estimated 250,000 currently known higher plant species. This is particularly true for tropical flora, which constitute over 60% of this estimated number [10, 17]. Even less is known about the far more abundant (though taxonomically relatively unexplored) insect and microbial worlds [17], as well as the biologically rich and enormously diverse marine environment [18]. Considering that the 119 drugs were isolated from only about 90 plant species [9], the potential for drug discovery from plants and other natural sources are enormous.

Although the long-established traditional medicinal systems, such as existing in China and India, have recorded much of their knowledge, including the use of many medicinal plants; in written text, ethnobotanists and anthropologists have expressed alarm at the rapid loss of the knowledge of the traditional healers, particularly among the indigenous groups in the Neotropics [17]. Before the late 1980s, the developed world displayed little interest in such indigenous
knowledge, and minimal effort was expended to assist indigenous communities in preserving their unique knowledge and traditions. With the resurgence of interest in the screening of plants and other natural resources for potential medicinal properties, western research organizations are beginning to place greater value on such knowledge [19].

Research in this direction has been greatly facilitated by the use of modern physico-chemical techniques of isolation and structure elucidation. In this connection particular attention has been paid to studies involving correlation of structure and biological activity on selected pharmacologically active constituents. Such phytochemical screening of medicinal plants has served the dual purpose of discovering new therapeutic agents and providing precedence for chemotherapeutic studies directed towards the synthesis of drugs modeled on the structure of natural products. Moreover, these studies promote work on the correlation of the chemical structure and pharmacological activity through functional variations in the active components of the plant material.

Sometimes the isolation of pure compounds is also helpful to plant taxonomists. Now-a-days many taxonomists are interested in the distribution of secondary metabolites in plants. Certain types of compounds are restricted to some particular classes or genera and are regarded as taxonomic markers. Numerous examples exist where problems concerning the position of a genus or a species can not be solved by classical morphological methods. Chemical data about the constituents of a plant provide one possible additional method in taxonomy.
2.0 INTRODUCTION
2.1 Naturally Occurring Acetylenic Thiophenes and Related Compounds

Sulfur is indispensable for life. Some organic sulfur compounds, like Coenzyme A, thiamine, thioctic acid, biotin, cysteine, cystine and methionine, are found universally in living organisms. Plants from algae to angiosperms also contain sulfur compounds of restricted distribution. The term natural sulfur compound is commonly understood to exclude compounds like sulfate esters and imply the presence of carbon-sulfur bond.

Among the numerous sulfur compounds encountered in higher plants, acetylenic thiophenes form a well defined group, compact in origin and behavior. Their natural occurrence is greatly limited. Polycetylenes are found in a dozen or more families of higher plants but are more common and varied in the largest family Asteraceae. Except a few thiophenes from Basidiomycetes, it is exclusively the Asteraceae which yielded acetylenic thiophenes so far.

Acetylenic thiophenes are classified as “polyacetylenes” although some of them, e.g. α-terthiienyl, do not have any acetylenic bond. However, their biogenesis has showed them of acetylenic origin. During biosynthesis they have consumed two alternate acetylenic bonds for the synthesis of the thiophene ring(s) [20]. Now it is well established that two alternate acetylenic bonds are used in the synthesis of each ring of α-terthiophene, and all those thiophenes which have used up some or all of the available diacetylenic groupings (\(-C≡C-\ C≡C-\)) are regarded as “polyacetylenes”.

Isolation of α-terthiophene from the petals of marigold (Tagetes erecta) in 1947 was the first report on naturally occurring thiophenes [21]. In 1958, the second acetylenic thiophene from higher plants, 2-phenyl-5-(1-propynyl)-thiophene, was isolated from Coreopsis grandiflora [22]. Since then a large
number of acetylenic thiophenes have been isolated from various plants of the Asteraceae. Birkinshaw isolated a C\(_4\)-acetylenic thiophene, Junipal, from Daedalea juniperina (Basidiomycetes) in 1955 [23]. This was the first report on the occurrence of acetylenic thiophene in Basidiomycetes. In 1969 Curtis also isolated junipal and its derivatives from the same fungus [24]. Acetylenes have been found in many families of higher plants but they occur regularly in only seven of them, namely, Asteraceae (Compositae), Araliaceae, Campanulaceae, Olacaceae, Pittosporaceae, Santalaceae and Umbelliferae [20]. The Asteraceae is the interesting subject of chemical investigations for polyacetylenes and most of the naturally occurring acetylenes have been isolated from this family. Acetylenes are present in all tribes of and the structural variations among these compounds are extremely wide. The tribes Inuleae, Anthemideae, Arctiteae, Astereae, Cynareae, Helenieae and Heliantheae are especially rich in acetylenes [20,25].

There are eighteen other families of higher plants and some genera of Basidiomycetes which have yielded acetylenes [20], however, these acetylenes are different in structure from those found in the Asteraceae. The Basidiomycetes acetylenes and the acetylenes of all but one of the families of higher plants are predominantly aliphatic, by contrast, the Asteraceae is crowded with “curled up” acetylenes, i.e. compounds which are aromatic, furanoid, thiophenic or spiroketal [26]. Within the thirteen tribes of Asteraceae, the occurrence of such cyclized acetylenes is very irregular [20].

A number of polyacetylenic compounds from Asteraceae are physiologically very active against various organisms but their physiological importance within the plant is still unknown. What is known with certainty is that the type of compounds varies in different parts of the plant, i.e. roots, leaves, stem and flower and in a number of cases with the season. In those cases where labeled polyacetylenes have been studied, they have been rapidly metabolized with a
half life of 1-2 days [26]. Polycetylenes of the Asteraceae are stored in resin canals which are present in the eleven tribes of the subfamily Tubulifloraee. These canals are colored red owing to structural elements unknown elsewhere, the 1,2-dithiadiene 1, which have also been written as cis endedithiones 2. It has been shown that they are derived from polycetylenes [26]. These pigments lose sulfur easily and pass into the corresponding thiophenes 3 (Fig. 1) [27, 28].

\[
\begin{align*}
R-C\equiv C-C\equiv C-R' & \xrightarrow{[H_2S]} R-SH\text{HS}R' \\
R-S-S-R' & \xrightarrow{[-H]} R-S\text{S}R' \\
R-S-S-R' & \xrightarrow{[-S]} R-S\text{S}R'
\end{align*}
\]

*Fig 1: Formation of dithiadienes and thiophenes in the Asteraceae.*

Similarly, some thiols, thioethers and thioesters present in the Asteraceae have been shown to be of acetylenic origin [20]. A novel compound containing sulfur in a four membered ring, thietanone 4, has also been found in the Asteraceae (Arctodideae) and it has been shown that they are derived from acetylenic precursor (Fig. 2) [20].
In the family Asteraceae, acetylenic thiophenes are present as monothiophenes, bithiophenes or trithiophenes. Trithiophenes have been reported from four, bithiophenes from six and monothiophenes from not less than nine of the thirteen tribes of the Asteraceae [26]. The occurrence of mono-, bi- and trithiophenes in the various tribes of the Asteraceae have been tabulated in Table 2.1.

The tribe Inuleae is characteristic for the presence of polyacetylenes and their thiophenic derivatives and they are the most promising constituents to examine for any chemotaxonomic program [29]. However, polyacetylenes do not as yet seem to add a great deal to the present classification because only a small percentage of the species and genera have been examined.
Bohlmann et al. [20] have reported the result of a survey of 108 species, representing all the Bentham subtribes of Inuleae; for polyacetylenes. The results are summarized in Table 2.2. The simple tridecapentaynene (a) was present in almost all the subtribes. The two related structures, dihydro compound (b) and alcohol (c) were also found in many subtribes. Monothiophene derivatives (d) were present in three groups while bithiophene derivatives (e) were found only in Buphthalminae.
Table 2.2: Distribution of polyacetylenes and their derivatives in Inuleae.

<table>
<thead>
<tr>
<th>Bentham subtribe</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tarchonanthinae</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>+</td>
<td>---</td>
</tr>
<tr>
<td>Plucheinae</td>
<td>+</td>
<td>---</td>
<td>---</td>
<td>+</td>
<td>---</td>
</tr>
<tr>
<td>Inuleae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Filagininae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Gnaphalininae</td>
<td>+</td>
<td>---</td>
<td>+</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Agianthinae</td>
<td>+</td>
<td>---</td>
<td>+</td>
<td>+</td>
<td>---</td>
</tr>
<tr>
<td>Relhaninae</td>
<td>+</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Athrixinae</td>
<td>+</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Buphthalmininae</td>
<td>+</td>
<td>+</td>
<td>---</td>
<td>---</td>
<td>+</td>
</tr>
</tbody>
</table>

\[a = \text{Me}(\text{C} = \text{C}_3\text{H}_2) = \text{CH}_2, \quad b = \text{Me} \text{Cl} = \text{CH} (\text{C} = \text{C}_3\text{H}_2) = \text{CH}_2, \quad c = \text{Me}(\text{C} = \text{C}_3\text{Cl} = \text{CH} (\text{CH}_2)_4 \text{OH}\]

The table shows data till 1973 when many subtribes were not sufficiently explored, however, one can get an idea that thiophenes are present only in those genera which are known to contain polyacetylenes. The subtribe Tarchonanthinae may be an exception, but isolation of monothiophenes suggests for an acetylenic precursor for this compound.
2.2 Biosynthesis of Naturally Occurring Thiophenes

All thiophenes, except Jumipal and its derivatives, are found only in the members of the family Asteraceae. Among the various tribes of Asteraceae, thiophenes are distributed in Aractoteae, Cynareae, Heliantheae, Heleniumae, Imuleae, Anthemideae, Vernonieae, Eupatorae and Senecioneae [20,25]. Thiophenes were considered to be derived from polyacetylenes by the time the first naturally occurring thiophene, α-terthienyl, was isolated [21]. Challenger and Holmes [30] showed good judgment in suggesting, as the aftermath of their studies on the formation of thiophenes from acetylene and boiling sulfur, that:

"It may be more than a coincidence that the only incidence so far recorded of the occurrence of a true thiophene derivative in plants should be found in a family so many members of which contain polyacetylenes. The α-terthienyl may arise by interaction of hydrogen sulfide with a straight-chain compound containing an acetylenic olefinic system......Other reactions such as oxidation, decarboxylation, or dehydrogenation might be involved. It could be urged that a long-chain paraffin or fatty acid might serve equally well as the starting point. Ring closure would, however, undoubtedly be facilitated by the presence of olefinic and acetylenic linkages."

Later it was confirmed that α-terthienyl was of acetylenic origin [20]. As it is not possible for the isoprenoid skeleton of the carotenoids to form polyacetylenes, the only possible precursor of a straight-chain polyacetylene may be a straight chain fatty acid. They may have been originated by head-to-tail condensation of a single sequence of acetate and malonate units. The chain may be straight or in cyclized form near the other end to a phenyl group. This pathway closely resembles the biosynthesis of fatty acids.
All the acetylenes present in the members of the family Asteraceae follow the same biosynthetic route [20], and are assumed to be derived from C₁₈ fatty acids through the sequence, oleic acid (5) → linoleic acid (6) → crepenyonic acid (7). Crepenyonic acid is an acetylene isolated for the first time from the seed oil of Crepis foetida L (Asteraceae) [20, 31]. Many precursor incorporation studies have confirmed this assumption [20, 32-34] and further that they are built up of acetate and malonate units [20, 35-36].

\[
\text{CH}_3(\text{CH}_2)_7\text{CH}═\text{CH}(\text{CH}_2)_7—\text{COOH}
\]

5

\[
\text{CH}_3(\text{CH}_2)_4\text{CH}═\text{CHCH}_2\text{CH}═\text{CH}(\text{CH}_2)_7—\text{COOH}
\]

6

\[
\text{CH}_3(\text{CH}_2)_4\text{C}═\text{CCH}_2\text{CH}═\text{CH}(\text{CH}_2)_7—\text{COOH}
\]

7

Biosynthesis of saturated fatty acids from acetate is well delineated and the conversion of stearic acid into oleic acid with its 9 (Z), 10-double bond takes place in the presence of molecular oxygen, NADPH and a stereospecific enzyme system [37-38]. Bloch [39] has established by precursor incorporation studies that the dehydrogenasis takes place by stereospecific removal of first 9-pro-\text{R} hydrogen and then of the 10-pro-\text{R} hydrogen. There is at present not much known of the biological mechanism by which unsaturation is introduced in the series of acids (5 → 7). By analogy with the formation of double bond in oleic acid, the reaction involved could be enzymatically catalyzed oxidative dehydrogenation. Such a mode of synthesis tallies well with the range of structures found among the C₁₈ acetylenic acids, and further more, some of the same acids occur simultaneously in polyacetylene producing plants and microorganisms.
Haigh et al. [40] had shown that crepenyic acid (7) was formed from oleyl-Co enzyme A or oleic acid (5). If the Co-A derivative was an intermediate, it was likely that the first step was hydrolysis to free acid which was then oxidatively dehydrogenated to crepenyic acid. The participating enzyme system was not known, but contained probably Mg$^{2+}$ and Cu$^{2+}$. The mechanism did not involve free linoleic acid, although the intermediate may have been enzyme bound linoleic acid [40]. Bu'Lock [33] has found that oleic and linoleic acids are precursors of crepenyic acid in Tricholoma grammapodium. Incorporation studies have also shown that both linoleic and crepenyic acids are the precursors of a considerable number of polyacetylenes [20, 33-34, 41].

Proceeding onwards to polyacetylenes from crepenyic acid or a similar compound the usual transformations are chain shortening from the C-methyl group, dehydrogenation and oxygenation. Thus a C$_{16}$ or a C$_{18}$ fatty acid can yield a polyacetylene with the common chain length of 10 to 14 carbon atoms.

Various types of acetylenes are present in Asteraceae with chain length of eight to eighteen carbon atoms. Some alkanamides with chain length of four to six carbon atoms are also of acetylenic origin [42].

The C$_{18}$ acetylenes are present in the Asteraceae in the form of C$_{18}$ acetylenic aldehydes, alcohols and acetates [42-44]. These compounds are formed as a result of dehydrogenation, oxidation and acetylation of crepenyic acid.

The entire range of C$_{17}$ acetylenes can be derived from oleic acid via crepenyic acid and dehydrocrepenyic acid (8) (Scheme-1). Incorporation studies have shown that these are biosynthesized from dehydrocrepenyic acid (8) by β-oxidation and further oxidation and dehydrogenation steps [20]. Dehydrocrepenyic acid itself has not been isolated from higher plants until present and a reasonable explanation for this seems to be its rapid conversion.
Scheme 1: A possible biosynthesis of some C_{17} acetylenes from Asteraceae.
to further intermediates [20]. The general biosynthetic route for some of the C₁₇ acetylenes found in the Asteraceae is outlined in Scheme-1 [43].

Most of the C₁₆ acetylenes are probably formed from C₁₈ acetylenes by α-oxidation followed by β-oxidation and further dehydrogenation and oxidation [20, 42].

Structural analysis of C₁₅ acetylenes isolated from the Asteraceae revealed that most of them might be related biogenetically to the C₁₇ acetylenes and in many cases structural features of C₁₇ acetylenes are recognizable in C₁₅ series [20]. On the basis of these facts it can be concluded that the biogenetic pathway for the formation of C₁₅ acetylenes is the chain shortening of C₁₇ acetylenes by β-oxidation [20].

The C₁₄ acetylenes are biosynthesized from C₁₈ acetylenes by double β-oxidation [20]. A double β-oxidation of C₁₈ triynic acid 9, followed by reduction, gave compound 10 (Scheme-2). Compound 10 is an important precursor in the biosynthesis of C₁₄ triynes and its role has been verified by feeding experiments [20, 45]. Biosynthesis of C₁₄ acetylenes with an enediyne-diene chromophore follow another route probably starting with the C₁₈ acetylenic acid II (Scheme-2).

The C₁₃ acetylenes are the most widespread acetylenes and their four types are very common i.e., straight chain-, aromatic-, thiophenic- and O-heterocyclic acetylenes. It has been shown by feeding experiments that the multiplicity of the C₁₃ acetylenic hydrocarbons from various tribes of Asteraceae could be reduced to a simple biosynthetic scheme starting from two C₁₄ alcohols, 12 and 13 [20, 42, 46].
\[
\text{CH}_3\text{CH} = \text{CH}(\text{C} \equiv \text{C})_2(\text{CH} = \text{CH})_2(\text{CH}_2)_3\text{OH}
\]

12

\[
\text{CH}_3(\text{C} \equiv \text{C})_3(\text{CH} = \text{CH})_2(\text{CH}_2)_3\text{OH}
\]

13

The resulting C\textsubscript{13} hydrocarbons are themselves precursors for other compounds such as C\textsubscript{12} and C\textsubscript{13} thiophenes and C\textsubscript{13} dithio-, thioether-, sulfoxide- and sulfone acetylenes \cite{20}. The main biosynthetic pathway for C\textsubscript{13} acetylenes formed from 12 is shown in Scheme-3.

The C\textsubscript{12} and C\textsubscript{11} acetylenes are biosynthesized from C\textsubscript{13} acetylenes by oxidative degradation of carbon atom(s) \cite{20, 25}. The C\textsubscript{10} acetylenes are formed from C\textsubscript{18} acetylenes by four times \(\beta\)-oxidation, which has been verified by feeding experiments on some of these compounds \cite{20} and dehydromatricaria ester is supposed to be a precursor in the biosynthesis of C\textsubscript{8} acetylenes.

Although the biosynthetic routes of polyacetylenes are well documented but little work has been done on the biosynthesis of naturally occurring thiophenes. This may be, at least in part, due to the probable experimental difficulties, one might face while studying the biosynthetic pathways of thiophene acetylenes as they are often unstable molecules and thus the isolation or even the detection of all the presumed intermediates is some times difficult. The possible influence of work-up procedures on the yields of isolated components has been recently investigated with thin layer chromatography on silica gel, a standard analytical and preparative procedure in natural products analyses. The recovery of a bi- and a trithiophene placed on silica gel plates ranged between 77\% and 97\% immediately after their application \cite{47}. When the extraction was
Scheme 2: A possible biosynthetic pathway for some C_{14} acetylenes from Asteraceae with a triyne-diene and ene-diyne-diene chromophore.
Scheme 3: A possible biosynthetic scheme for some C₁₃ acetylenes from Asteraceae.
performed 24 hours after the application, the recovery ranged from 43 to 74% for the trithiophene but only 18 to 57% for the bithiophene. This study strongly suggested that more labile compounds are likely to be even more readily affected by the "mild" silica gel chromatography and this procedure may yield unreliable quantitative data. In the same study admixture of several substances with silica gel significantly protected the thiophenes from degradation. Although the degradation of thiophenes was attributed to light-dependent reactions, experiments did not clearly support this explanation, for example by comparing the degradation of sample kept in light with controls kept in the dark.

Another study investigated how the handling of the seedlings of *Tagetes patula* affected the detection of bithiophenes [48]. The seedlings of the same age were (a) deep-frozen (-80°C), (b) freeze-dried, (c) dried at 40°C in the dark, (d) dried at 40°C in the presence of incandescent light, or (e) dried at 105°C in the dark. Analysis of the extract by HPLC showed that procedure (a) led to the highest amounts of extractable components. As expected, drying at high temperature was the most deleterious. Surprisingly, the amount of a bithiophene alcohol detected per mg of dry weight was the lowest in procedure (a), the values being in the ratio of 1, 1.6, 2.7, 2.4 and 1.9, respectively, in going from (a) to (e). It may be concluded that the same bithiophene alcohol was, at least partially, an artifact of the isolation procedure. In the same study, exposure of the crude plant extracts to day light and to near-ultraviolet light resulted in the time-dependent change in the concentration of the components. Irradiation greatly reduced the HPLC peak intensities. At least half of the compound got destroyed after three hour exposure to day light and almost all was disappeared after 40 minutes exposure to UV light. Again, the concentration of the bithiophene alcohol was greater after day light exposure and did not greatly diminish upon UV treatment.
With a few exceptions, the number of carbon atoms present in thiophenes occurring in the Asteraceae range from ten to fourteen. The steps involved in their biosynthesis are the synthesis of a basic polyacetylene skeleton, introduction of sulfur atoms which are then incorporated into thiophene rings and finally the minor side chain adjustments. These may include reduction, oxidation, formation of epoxides, conversion of the latter into diols, esterification of hydroxyl groups, decarboxylation, etc. It is still not clear whether the losses in the $C_{18}$ precursor of the basic acetylene skeleton occurs before or after the formation of the thiophene ring.

Incorporation of sulfur in the acetylenic backbone have been studied by various workers. Schulte [49] has showed that 14 may be prepared in good yield by the addition of $H_2S$ to the dehydromatricaria ester in weakly alkaline medium (pH 8-10).

![Diagram of compound 14]

*In vitro* synthesis of the alkyl thiophene was even possible if cysteine or glutathione was used instead of $H_2S$ [50]. Schulte has also shown by feeding experiments that *in vivo* formation of the thiophene rings takes place by formal addition of $H_2S$ to diyne groupings [51]. The incorporation of sulfur into the thiophene ring is yet to be explained clearly, however, it has been shown that both inorganic and organic precursors could be incorporated into thiophene ring i.e., $Na_2SO_4$ [52], methionine [52] and cystein [53]. Both approaches were preparatively useful for obtaining $S^{35}$ labeled products used in feeding experiments, but $Na_2SO_4$ was incorporated more efficiently than methionine.
when administered through the roots. In contrast, NaHS was not incorporated [52].

Such experiments may not provide a true picture of the biosynthesis, because they combine at least two variables, namely, the uptake and transport of the precursor to the site(s) of biosynthesis, and the incorporation of a usable form of sulfur into the final thiophenes. The low incorporation of NaHS may owe as much to its toxicity to the plants [54] as to the biosynthesis itself. Palaszek felt that cystein was clearly the precursor of the sulfur atom in some bi- and trithiophenes [53]. As suggested by Challenger and Homes [30] the reaction of Na$_2$S and 1,3-butadiyne to yield a thiophene can easily be carried out in vitro, though, enough data are not available on the reaction in vivo. However, it has been urged that two separate steps may be involved in this addition, unlike the easy laboratory cyclization process which directly produces a thiophene derivative. The first step would produce an enyne species which was then cyclized to a thiophene in the second step. Such an intermediate may have been trapped as its methyl thioether as shown in Scheme-4 for *Flaveria repanda* [55]. However, a 1,4-dithione may also be formed by addition of a

\[
\begin{align*}
  \text{CH}_3- & \text{(C=C)}_5 \text{CT=C=CHT} \\
  \text{(F. repanda)} \\
  & \\
  \text{CH}_3- & \text{(C=C)}_2 \text{C=CH-(C=C)}_2 \text{CT=C=CHT} \\
  \text{SCH}_3 & \\
  \end{align*}
\]

*Scheme 4:* Sulfur introduction into an acyclic polynconeylene in *F. repanda.*
second equivalent of H₂S onto an adjacent triple bond. Oxidative cyclization of the enolic form of 1,4-butadithione could lead to the anti-aromatic six
membered ring product possessing two adjacent sulfur atoms (a 1,2-dithiin).
Such structures, named as thiarubrenes because of their red color, have been
found only in the Asteraceae and only in the genera Ambrosia, Chaenctis,
Eriophyllum and Rudbeckia. When heated or irradiated, thiarubrenes lose one
sulfur atom and produce thiophenes. The mechanism of this formal extrusion
reaction has not been elucidated, however, one possibility involves the
intermediacy of the isomeric enedithione. Alternatively, the loss of sulfur may
occur through a 1,1-dioxide formed by oxidation, followed by loss of SO₂.
Whether 1,2-dithiins are intermediates in the biosynthesis of thiophenes in
those genera where they have been found is not known, and their intermediacy
in the biosynthesis of thiophenes in the other genera has not been specifically
eliminated. Bohlmann and Bresinsky have suggested that H₂S₂ rather than H₂S
may be the sulfur introducing agent [56]. Thus a double addition reaction of a
thiol moiety onto two alternate acetylene bonds would give a 1,2-dithiin, which
would then lose one sulfur atom to produce the thiophene ring (Scheme-5).

An alternative cyclization which forms a six membered ring (a 6-endo-dig
process) could be a cyclization to form a five membered ring (a 5-exo-dig
process). Neither process is disfavored in Baldwin’s classification [57]. When
the reaction of a 1,3-butadiyne with Na₂S₂ was performed in the laboratory, it
produced a thiophene in addition to the five membered ring disulfide from the
5-exo-dig reaction (Scheme-6).

Although the authors concluded that the disulfide addition reaction was not a
likely mode of synthesis for thiarubrenes in vivo [56, this conclusion requires
additional experimental support since the thiophene isolated from the reaction
in vitro may actually indicate that the desired thiarubrene had been formed, but
had decomposed under the reaction conditions.
Scheme 5: Formal routes to thiophenes from 1,3-butadiynes and 1,2-dithiins.

Scheme 6: The reaction of Na$_2$S$_2$ with a 1,3-butadiyne
In vitro addition of H$_2$S to diynes in weakly basic medium, forming thiophenes, was studied by Schulte [58] who found in particular that the dehydromatricaria ester 15, \textit{(cis and trans)} reacts at the triple bond closest to the ester carbonyl to give thiophene 16 both in \textit{cis} and \textit{trans} forms.

\[
\text{CH}_3\text{--(C=\text{C})}_3\text{--CH=CH--COOCH}_3 \quad \xrightarrow{\text{in vitro or in vivo}} \quad (\text{Chrysanthemum vulgare})
\]

\[
\text{cis and trans}
\]

\[
\text{H}_3\text{C--C=\text{C}--}\text{\frown}--\text{CH=CH--COOCH}_3
\]

\[
\text{16}
\]

\[
\text{cis and trans}
\]

Glutathione could also act as a sulfur source in dilute alkalies with \textit{trans} dehydromatricaria acid, furnishing the \textit{cis} and \textit{trans} acids corresponding to 16 [50]. Further more \textit{trans} dehydromatricaria ester was converted to 16 by \textit{Chrysanthemum vulgare} [59]. In \textit{Anthemis nobilis}, however, the addition to the \textit{cis} and \textit{trans} 2-$^{14}$C dehydromatricaria methyl ester 15 involved the terminal and central triple bonds and gave \textit{cis} and \textit{trans} thiophene 17, respectively [60].

\[
\text{CH}_3\text{--(C=\text{C})}_3\text{--CH=CH--COOCH}_3 \quad \xrightarrow{\text{in vivo}} \quad (\text{Anthemis nobilis})
\]

\[
\text{cis and trans}
\]

\[
\text{H}_3\text{C--C=\text{C}--CH=CH--COOCH}_3
\]

\[
\text{17}
\]

\[
\text{cis and trans}
\]

It has been established by Bohlmann [25] that dehydromatricaria esters were the precursors of most of the C$_{10}$ monothiophenes and these thiophenes were isolated from the plants of the tribe \textit{Anthemideae} where \textit{cis} and \textit{trans}
dehydromatricaria esters are widespread [20]. Addition of \( \text{H}_2\text{S} \) or its biochemical equivalent to the cis and trans forms of dehydromatricaria ester (15) can yield four types of monothiophenes 16 (cis), 16 (trans), 17 (cis) and 17 (trans) (Scheme-7) and these have been isolated from several members of the Anthemideae [20]. Oxidative elimination of C-10, a reaction that is very common in naturally occurring thiophenes, can also give two more thiophenes 18 (cis) and 18 (trans) (Scheme-7) [61]. Free carboxylic groups of 17 (cis) and 17 (trans) can attack C-4 acetylenic bond to give a lactone 19 [62]. Further hydrogenation and/or oxidation steps can yield a variety of other monothiophenes [25]. Similarly, addition of \( \text{H}_2\text{S} \) to the isomeric matricaria esters 20 and 21 can give monothiophenes 22 and 23 [25].

\[
\text{MeCH=CH(C\equiv C)_2CH=CHCO}_2\text{Me} \quad \xrightarrow{\text{trans}} \quad \text{MeCH=CH} \begin{array}{c}
\text{cis} \\
\text{cis}
\end{array}
\text{CH=CH-CO}_2\text{Me}
\]

20 \hspace{1cm} \text{cis} \hspace{1cm} \text{trans} \hspace{1cm} 21 \hspace{1cm} \text{cis} \hspace{1cm} \text{cis} \hspace{1cm} 22 \hspace{1cm} \text{cis} \hspace{1cm} \text{trans} \hspace{1cm} 23 \hspace{1cm} \text{cis} \hspace{1cm} \text{cis}

The tetrayne and pentayne hydrocarbons have also been established as precursors of a variety of mono-, bi- and trithiophenes. Feeding experiments have also confirmed that most of the C\(_{14}\) monothiophenes are derived from the widespread tridecapentaynenone 24 [42] (Scheme-8). Addition of one equivalent \( \text{H}_2\text{S} \) would lead to 25 and 26 which have been isolated from many species of the Asteraceae [20]. Both compounds are further transformed to a large number of derivatives either by the opening or the reductive cleavage of the epoxide ring (Scheme-8). A few monothiophenes are also derived from C\(_{13}\) triynes and the compound 31 seems to be a precursor [25] (Scheme-9). Oxidation of 31 can
Scheme 8: Biosynthetic path to some of the C13 monothiophenes present in the Asteraceae.
yield 32, which upon elimination of H$_2$O leads to a furan, a compound isolated from the different genera of the tribe Anthemideae [25]. The formal addition of H$_2$S then yields 33. Similarly, dehydration of 32 leads to 34 which upon addition of H$_2$S can give 35. Some of the naturally occurring monothiophenes are derived from acetylenic compounds with a C$_{14}$ chain, and it is obvious that the precursor of 37 is the triyne 13 [25] (Scheme-10). Addition of H$_2$S would lead to 36 which by oxidative decarboxylation can be transformed to 37. Allylic oxidation of 13 followed by elimination of H$_2$O would lead to 39 which
on hydrogenation, addition of \( \text{H}_2\text{S} \) and oxidative decarboxylation can lead to thiophenes 40 and 41. The reduction of ketone 40 can also lead to 42.

**Scheme 10**: Biosynthetic pathway of monothiophenes from \( \text{C}_{14} \) acetylenes in the Asteraceae.
The tetrayne and pentayne hydrocarbons have been established as precursors of a variety of thiophenes including bi- and trithiophenes. It has been shown by feeding experiments that a tritiated ene-tetrayne-ene 43 in *Bidens connatus* furnished the tritiated monothiophene 44 by addition to the central diyne system and the respective tritiated bithiophene 45.

\[
\text{CH}_3-\text{CH}=\text{CH}-(\text{C}≡\text{C})_4\text{CT}=\text{CHT} \quad \text{in vivo} \quad (\text{Bidens connatus})
\]

\[
\begin{array}{c}
\text{CH}_3-\text{CH}=\text{CH}-\text{C}≡\text{C} \quad \begin{array}{c}
\text{C}≡\text{C} \quad - \quad \text{CT}=\text{CHT}
\end{array}
\end{array}
\]

Lack of a triple bond in the bithiophene 45 also confirms that it has originated from a tetrayne. As outlined in Scheme-7, tridecapentaynelene (24) can be transformed to 25 and 26 by the addition of H$_2$S or its biochemical equivalent, addition of another molecule of H$_2$S on the diyne units of these monothiophenes would lead to the bithiophenes 46 and 47, respectively [25] (Scheme-11). These compounds have been isolated from many species of the Asteraceae, most of them belonging to the tribe Heliantheae [20]. Further reactions like hydrogenation, oxidation, oxidative decarboxylation and addition of HCl could lead to a number of compounds. Several thiophenes are most probably formed via tridecahexayne 48, which may have been originated from ene-pentayne 24 [25] (Scheme-12). Addition of two equivalents of H$_2$S leads to 49, 50 and 51. A further possibility, 1,4- and 9,12- addition leading to 52, was
realized in the adduct 54 which was isolated from *Berkheyra species* and was obviously derived from 53 (Scheme-12).

![Chemical structure](image)

Scheme 11: Biosynthesis of bithiophenes from monothiophenes.
Scheme 12: Biosynthesis of some bithiophenes from Asteraceae.
In vivo studies has been performed to search for the biosynthesis of bithiophenes from pentaynes. The tritiated ene-pentayne 55 had been converted to 56 and its oxidation product, the acetate 57, in Buphthalmum salicifolium [63].

\[
\begin{align*}
\text{CH}_3-(\text{C}≡\text{C})_5\text{CT}≡\text{CHT} & \xrightarrow{\text{in vivo}} \text{CH}_3-\begin{array}{c}
\text{S} \\
\text{S}
\end{array}-\begin{array}{c}
\text{S} \\
\text{S}
\end{array}-\text{C}≡\text{C}−\text{CT}≡\text{CHT}
\end{align*}
\]

55 \hspace{1cm} 56

\[
\begin{align*}
\text{AcO-CH}_2-\begin{array}{c}
\text{S} \\
\text{S}
\end{array}-\begin{array}{c}
\text{S} \\
\text{S}
\end{array}-\text{C}≡\text{C}−\text{CT}≡\text{CHT} & \hspace{1cm} +
\end{align*}
\]

57

In Echinops sphaerocephalus the tritiated ene-pentayne 55 was efficiently transformed to 58 and also to the glycol (59), the chlorohydrin acetate (60) and

\[
\begin{align*}
\text{CH}_3-(\text{C}≡\text{C})_5\text{CT}≡\text{CHT} & \xrightarrow{\text{in vivo}} \text{CH}_3-\begin{array}{c}
\text{S} \\
\text{S}
\end{array}-\begin{array}{c}
\text{S} \\
\text{S}
\end{array}-\text{C}≡\text{C}−\text{CT}≡\text{CHT} \\
+ \hspace{1cm} +
\end{align*}
\]

55 \hspace{1cm} 58

\[
\begin{align*}
\text{CH}_3-\begin{array}{c}
\text{S} \\
\text{S}
\end{array}-\begin{array}{c}
\text{S} \\
\text{S}
\end{array}-\text{C}≡\text{C}−\text{CT}−\text{CHT} & \hspace{1cm} + \\
\text{OH} & \hspace{1cm} \text{OH}
\end{align*}
\]

59

\[
\begin{align*}
\text{CH}_3-(\text{C}≡\text{C})_2\text{S}-\text{C}≡\text{C}−\text{CTCl}−\text{CHTOCOCH}_3 & \hspace{1cm} +
\end{align*}
\]

60

\[
\begin{align*}
\text{CH}_3-\begin{array}{c}
\text{S} \\
\text{S}
\end{array}-\begin{array}{c}
\text{S} \\
\text{S}
\end{array} & \hspace{1cm} \text{T}
\end{align*}
\]

61
a trithiophene (61). In all these products, except in 60, the terminal methyl group of the ene-pentayne was removed by oxidation [55].

The radioactivity in the trithiophene formed from 55 was entirely displaced by Friedel-Craft's acylation of both free α positions, so all the 2-tritium had been lost during conversion to 61. It proved that the double bond of the ene-pentayne was dehydrogenated before attachment of sulfur to the 1 position. This was further confirmed by the fact that the bithiophene 62 was not converted to α-terthienyl (63) in *Tagetes patula* [64].

\[
\begin{align*}
\text{62} & \\
\text{in vivo} & \quad (T. \text{patula})
\end{align*}
\]

Results of the feeding experiments [63, 55] with radioactively labeled ene-pentayne allowed the ordering of the compounds into a hypothetical biogenetic pathway shown in Scheme-13 [65].

Isolation and characterization of the enzymes involved in the biosynthesis of thiophenes provides the ultimate touch to the formulation of biosynthetic pathways. This field is still in its infancy. Nothing is known about the enzymology of the pathway(s) leading to bi- and trithiophenes, or about their regulation. Enzymes involved in the side chain modification of bithiophenes with a four carbon side chain were the first to be investigated.
Scheme 13: Proposed biosynthetic pathway to α-terthienyl (63) from one-pentayne in Asteraceae.
A highly specific 5-(4-acetoxy-1-butynyl)-2,2'-bithiophene acetate esterase, occurring in the aerial parts of *Tagetes patula* was partially purified [66]. It catalyses the reaction shown in Scheme-14, but it could not be decided whether this enzyme was involved in the degradative or a biosynthetic step.

\[
\begin{align*}
\text{Scheme 14: Enzyme action of 5-(4-acetoxy-1-butynyl)-2,2'-bithiophene : acetate esterase.}
\end{align*}
\]

Further careful analysis of seedlings of *T. patula* revealed the presence of a diacetate congener. An enzyme system, different from the esterase of Scheme-14, which hydrolyzes the diacetate to a diol was recognized and partially purified (Scheme-15) [67].

\[
\begin{align*}
\text{Scheme 15: Enzymatic conversion of 5-(3,4-diacetoxy-1-butynyl)12,2'-bithiophene to diol.}
\end{align*}
\]
The kinetic data implicated cooperation of two enzymes. Interestingly, the activity of this enzyme system was found to change over time in hypocotyls, with the highest activity in the youngest plants. The enzyme activity remained constant in the roots [67]. Subsequent studies on *T. patula* seedlings also identified acetyl-CoA hydroxybutynyl-bithiophene O-acetyltransferases, enzymes involved in the reverse steps of acetylation of the monohydroxy alcohol or the diol [68]. These enzymatic studies were reviewed by Sutfeld [69] who suggested that some of the thiophene constituents of *T. patula*, such as the alcohols and their esters, actively participate in the biochemical developments of the plants, while others are end products. His observation that some bi- and trithiophenes occur almost exclusively in senescing tissues like fading cotyledons, flower heads or in ripening achenes led him to suggest that these components were end products.

Similar studies were reported by Tosi *et al.* [70] who used HPLC, TLC and HPTLC techniques to determine the distribution of thiophene derivatives at thirteen stages between germination and senescence. Flowering parts contained the highest total amount of thiophenes, while the concentration of the other constituents varied with age. They concluded that bithiophene synthesis takes place mainly in the roots and that trithiophene synthesis takes place mainly in the leaves. No enzymes associated with the early stages of the biosynthesis of bi- and trithiophenes have so far been purified or characterized.
2.3 Biological Properties of Thiophene Acetylenes

Certain chemicals, such as psoralens, when applied to human skin cause damage in the presence of long range UV light, UV-A (300-400 nm), but not in the dark [71]. The compounds which exhibit this character are called phototoxic. Thiophene acetylenes have been found to be phototoxic and their toxicity extends to a wide range of organisms including fungi, bacteria, membrane containing viruses, nematodes, insect larvae and mammalian cells.

2.3.1 Phototoxicity Towards Bacteria and Fungi

It was discovered by Fowlks that psoralens which were phototoxic to human skin were also phototoxic to bacteria [72]. Daniels screened various plants for phototoxicity against Candida albicans [73]. The method used by Daniels was relatively simple and involved placing small parts of plant material on agar plates spread with living C. albicans and incubating the plates in the dark or under long range UV light. Diffusion of the active compounds from the parts of the plant into the medium and subsequent irradiation killed the cells in the vicinity of these compounds. The zones of inhibition were not observed in the dark. A positive phototoxic test was observed for the achenes of Marigold (Tagetes patula). Later it was investigated that the compounds responsible for phototoxicity to C. albicans in T. patula were α-terthienyl (63) and 5-(3-butene-1-ynyl)-2,2'-bithiophene [74].

\[ \text{α-Terthienyl (63)} \]

It was further shown that the phototoxicity of some species of the genera Chrysanthemum, Cirsium [75], Dahlia, Solidago and some other species of the
Asteraceae [76] could be ascribed to polyacetylenic compounds. A suppression of growth of *C. albicans* was also observed by some plants rich in polyacetylenes and thiophenes without UV light, showing that these compounds also exhibit some antibiotic activity even in the dark [76]. Bacteriostatic, as well as fungistatic activity of simple polyacetylenes have been clearly demonstrated [77] and the notorious unstable tridecapentaynone, CH$_3$-(C≡C)$_5$-CH=CH$_2$, has been shown an effective antibiotic against a number of microorganisms including *C. albicans* [77]. Daniels method was also combined with chromatography and the minimum amount of α-terthiienyl (63) which inhibited growth of *C. albicans*, *E. coli* B/r and *E. coli* B$_{t-1}$ was found to be between 1 and 5 μg per spot of the TLC plate placed in contact with the agar surface of the cooled petri dishes spread with the above mentioned organisms [78]. Bithiophenes were also proved phototoxic to *E. coli* as well as *Saccharomyces cerevisiae* [79-81].

Bi- and trithiophenes were also found to be toxic to a number of phytopathogens and other filamentous fungi [82]. *Alternaria alternata*, *Aspergillus niger*, *Cladosporium variabile*, *Colletotrichum* sp., *Rhizopus nigricans*, *Pythium aphanidermatum*, and *Saprolegnia* sp. have shown complex effects. They did show some toxicity in dark controls, however, their activity has very much increased when irradiated with near-UV light. *A. alternata* and *C. variabile* were found to be less sensitive to α-terthiienyl (63), both in dark and in near UV light. This may be due to protection by the dark pigmentation in these organisms.

The plants of genus *Bidens* are known to contain photosensitizing constituents. Fungi isolated from these plants [83-84] were independently tested for photosensitivity to a trithiophene, α-terthiienyl (63), and to several polyacetylenes found in these plants. The growth of *Sporobolomyces*
shibatanus, S. salmonicolor, Cryptococcus albidus, C. laurentii, Cladosporium cladosporioides, and Aureobasidium pullulans was found to be inhibited by α-terthienyl in the presence of near-UV light but not in the dark. The growth of Rhizopus mucilaginosa was affected both in the dark and in the UV light. Sporobolomyces roseus and Epicoccum purpureascens were not affected by α-terthienyl at all. This study showed that the organisms did not acquire any special resistance from contact with their usual photosensitizer-containing hosts.

A comparative study of phototoxicity of α-terthienyl and simple polyines against various fungi, bacteria and viruses has been reported. α-Terthienyl was found to be a potent antibiotic than its derivatives, however, some gram negative bacteria showed resistance. Certain fungi showed ultrastructural modifications within the hyphae when treated with α-terthienyl followed by UV-A irradiation [86]. α-Terthienyl caused damage to the membrane of the nucleus, mitochondria and endoplasmic reticula of the various fungi tested, only in the presence of UV-A light. No important cytological modifications were noted in the dark controls.

Earlier studies on the phototoxicity of α-terthienyl revealed that the presence of oxygen was not necessary for the activity of α-terthienyl [87] However, it was proved later with liquid crystals of E. coli and Saccharomyces cerevisiae [88] that many of the results claimed in the former study could not have been based on the actual experiments, because complete deoxygenation of the liquid cultures was not achieved. Subsequent studies in this field proved that α-terthienyl is phototoxic only in the presence of oxygen [89-91].

Detailed mechanistic informations were obtained from the inactivation of E. coli by α-terthienyl. Gel electrophoresis revealed cross-linking of soluble
proteins of *E. coli* when treated with α-terthienyl followed by UV-A irradiation [92]. A number of strains were used to probe for possible DNA damage. For example, *rec* mutants of *E. coli* K12 have much reduced ability to repair damaged sites on DNA. Both wild type and mutant strains showed the same size inhibition zones in Daniels test after photosensitization with α-terthienyl, suggesting that DNA repair is not an important factor in protecting *E. coli* irradiated with this compound [88]. Strains of *E. coli* having mutations in either the excision repair mechanism or the recombination repair mechanism were studied in liquid cultures. No differences were found in the kinetics of inactivation of these strains and no mutations to histidine independence were detected [93]. All these results showed that DNA was not an important target for phototoxicity of α-terthienyl towards bacteria and fungi *in vivo*. Experiments with a genetically modified strain of *E. coli* [94] also supported the evidence that α-terthienyl irradiated with UV light principally attacks the membrane of the bacterial cell with the generation of singlet oxygen.

The relative phototoxicity of different thiophenes in different organisms is also variable. For example, yeast and gram-negative bacteria, having different cell wall structures, could react differently to thiophene derivatives [95]. It has been suggested that the difference in cell wall and cell membrane may be responsible for the difference in the observed phototoxicity. It has also been observed that eukaryotic cells and viruses show a different spectrum of responses to thiophenes [96]. Based on these results, it is reasonable to expect that many bacteria and fungi will be found to be sensitive to photosensitizing treatments with bi- and trithiophenes. There are some exceptions which may be due to unusual membrane structures and/or by the fact that they may accumulate pigments which either act as optical filters or as singlet oxygen quenchers.
The difficulties in predicting the phototoxicity of α-terthienyl in bacteria and fungi are clearly illustrated by the work of Daub on cercosporin [97]. This pigment is capable of generating singlet oxygen as well as superoxide anion photochemically, just like α-terthienyl. It is produced in high concentrations by a fungus which is unaffected by its presence, even in the presence of light. Yeast, particularly *S. cerevisiae*, are resistant, but *Neurospora crassa* and several *Aspergillus* species are not. It is interesting that several related mycelial fungi which are also plant pathogens (for example *Alternaria*, *Fusarium*, *Colletotrichum*, and *Verticillium* species) were not affected by cercosporin.

![Cercosporin](image)

However, the study confirmed, in the case of fungi, that the presence of carotenoids (singlet oxygen quenchers) and the composition of the cell wall were important in conferring resistance to organisms.

### 2.3.2 Effect of Thiophene Acetylenes on DNA

Most of the biological properties of thiophenes in mammalian cells or cell components have been studied with α-terthienyl as the agent and it was shown that this substance has a very low level of toxicity in rats and mice and does little damage to DNA [98-99]. α-Terthienyl did not cause the formation of interstrand cross-links in calf thymus DNA [100]. Another study showed that
α-terthienyl did not cause chromosome aberrations or sister chromatid exchanges in cultured mammalian cells [101] when irradiated with UV-A. However, it did affect DNA repair in cultured human fibroblasts [102]. The anaerobic irradiation of calf thymus DNA in the presence of radioactive α-terthienyl led to a slight but measurable level of incorporation of radioactivity into the DNA [103]. However, no mutations were observed in *Escherichia coli* upon UV irradiation in the presence of α-terthienyl [93]. When plasmid pBR322 DNA was irradiated in the presence of α-terthienyl, the nicking of supercoiled into relaxed circular DNA was observe in which one strand of a double stranded circular DNA was cleaved in a time dependent and concentration dependent manner even in the absence of oxygen [104]. Furthermore, aerobic irradiation in the presence of histidine, a good singlet oxygen quencher, led to enhanced DNA damage. It proved that singlet oxygen reactions cannot be the only important processes in the photobiocchemistry of α-terthienyl. This was further supported by the fact that superoxide dismutase, catalase and the antioxidant BHT had no effect on the photosensitized cleavage of pBR322 [104].

2.3.3 Antiviral Properties of Thiophene Acetylenes

Antiviral properties of naturally occurring thiophenes and of many synthetic analogs have extensively been investigated [96, 105-109]. These studies followed the discovery that phenylheptatriyne, found in many species of the Asteraceae, modified murine cytomegalovirus (MCMV) in the presence of near-UV radiation. Acetylenic thiophenes were tested for activity against two viruses with membranes, MCMV, a double stranded DNA herpes virus which replicates in the nucleus of mouse cells, and Sindbis virus (SV), a single-stranded RNA virus which replicates in many types of animal cells. The compounds were also tested on viruses without membranes i.e., phage T4 and
fish infectious pancreatic necrosis virus (IPNV). Antiviral activity was observed only with ultraviolet activation. After treatment, the virus penetrated the mouse cells normally and the viral DNA entered the nucleus, viral genes were not expressed and the virus therefore did not replicate [110]. α-terthienyl has shown potent antiviral activity but the activity was virus dependent. Its ratios for decreasing virus infectivity by 99% were 1:65:400 for SV, MCMV and T₄ viruses, respectively [111]. The MCM virus treated with α-terthienyl in the presence of near-UV penetrated mouse cells normally but failed to replicate [107].

Naturally occurring bithiophenes have also shown antiviral activity, less potent than α-terthienyl against SV but more potent against MCMV [112]. However, the activity of bithiophenes was related to the nature of side chain onto the thiophenic ring [113]. The key structural features of the most active compounds were the possession of a conjugated linear configuration of 2 or 3 thiophene rings and a substituent with an acetylenic linkage [95]. The thiophenic compounds having 1-alkynyl groups linked to a thiophene in the α-position or to an α-terthienyl appeared to act on eukaryotic membrane via fatty acids [85] with the generation of singlet oxygen. It has been suggested that in addition to lipids, some viral proteins may also be the targets for these compounds [85]. It is well established that \(^{1}\text{O}_2\) in aqueous solutions is an intermediate in a number of photodynamic reactions since it reacts rapidly with histidine, tryptophan and methionine [114-115].

2.3.4 Nematocidal Activity of Thiophene Acetylenes

The resistance of Tagetes species to root-knot nematodes (Meloidogyne species) was observed as early as 1938 [116]. Now the plants of the Tegetes are well known for the occurrence of thiophene acetylenes. It has been found as a result of experiments that the cultivation of one crop of marigolds (Tagetes) in
soil infested with *Pratylenchus penetrans* reduced the nematode population to a level which allowed *Narcissus* bulbs grown in that soil not to suffer from root-rot damage normally inflicted by the root-knot nematodes [117].

It was further observed that the ethanolic extract of the plant had *in vitro* activity against nematodes such as *Ditylenchus dipsaci* and *Anguina tritici*, and larvae of *Heteroda rostochiensis, Pratylenchus penetrans*, and *Panagrellus redivivus* [118], however, the juice of the plant was ineffective against cysts of the potato root eelworm. The nematocidal activity of a bithiophene from *T. erecta*, 5-(3-buten-1-ynyl)-2,2'-bithiophene, was found to be lower than that of α-terthienyl against *Ditylenchus dipsaci* and *Anguina tritici* but high against *Panagrellus redivivus* [119]. Plants of the family Asteraceae were later screened extensively for suppressing effect on *P. penetrans*. Many species, including many of the plants belonging to genera known to contain thiophene constituents; e.g. *Berkhaya, Didelta, Eclipta, Echinops, Flaveria, Gaillardia,* and *Tagetes* have shown suppressing activity [120-122]. These results strongly indicated a correlation between chemical composition and activity against *P. penetrans*. However, Marigolds were not active against certain kinds of nematodes. *T. minuta*, rich in thiophene acetylenes, has also shown significant nematocidal activity against *Meloidogyne javanica*, the tobacco root eelworm [123]. α-Terthienyl was found to be more active than a bithiophene in a comparative study against *M. incognita* [124].

Mechanistic studies have been performed for UV-activated nematocidal activity of thiophene acetylenes. The wavelength range of the activity of α-terthienyl was found to be in the near UV and the extent of the activity was related to the wavelength of irradiation [125]. It has been proved that α-terthienyl was a singlet oxygen sensitizer and could inactivate enzymes, particularly glucose-6-dehydrogenase and acetylcholine esterase, and that the nematode *ApheLENCHUS*
avenae, which can live in the absence of oxygen, was killed by \( \alpha \)-terthiienyl and ultraviolet light only in the presence of oxygen [126-127]. The dependence of light for the nematocidal activity of \( \alpha \)-terthiienyl was also confirmed in experiments on adult Caenorhabditis elegans [128].

Direct generation of singlet oxygen may not be the only route for the phototoxicity of \( \alpha \)-terthiienyl in vivo, since transmission of light through plants roots can be discounted [129]. Gommer and Bakker proposed a model for the generation of electronically excited \( \alpha \)-terthiienyl in the absence of light through energy transfer from an electronically excited molecule formed as the result of an enzymatic reaction [117]. The enzymatic reaction utilized to demonstrate the concept was the oxidation of indoleacetic acid with horseradish peroxidase. This reaction involves formation of a dioxetane intermediate which decomposes thermally to produce an electronically excited aldehyde. The latter is quenched by the added \( \alpha \)-terthiienyl which thus becomes electronically excited and can now react exactly as if it had been generated photochemically. A second energy transfer, this time from \( \alpha \)-terthiienyl to \( O_2 \), then produces singlet oxygen or superoxide anion with their usual toxic reactions. The proposed mechanism was further supported by the fact that peroxidase activity in Tagetes roots increased markedly in plants infected with P. penetrans [117].

Although the location of thiophene derivatives in intact roots has not been determined accurately, sulfur distribution has been analyzed using a micro particle-induced X-ray emission technique [130]. The lowest concentration was found in the epidermis and the highest in the endodermis, providing an evidence that nematocidal thiophenes were concentrated in the endodermis [131]. Using a continuous root exudate trapping system and mass spectrometric analysis, undisturbed roots of Tagetes patula were proved to release \( \alpha \)-terthiienyl and other bithiophene components [132].
2.3.5 Effect of Thiophene Acetylenes on Mosquitoes

The potential for effective use of photosensitizing agents against malaria vectors was recognized by Barbieri in 1928 [133]. The phototoxicity of \( \alpha \)-terthienyl was tested against various species of \textit{Aedes} mosquitoes namely, \textit{Aedes aegypti} [128, 134-140], \textit{A. intrudens} [99, 141-142], \textit{A. atropalpus} [98-99, 143], \textit{A. tritaeniorhynchus} [135], \textit{A. epactius} [136], as well as with \textit{Culex quinquefasciatus} [135, 144]. The levels of toxicity have been impressive enough to encourage patenting the use of \( \alpha \)-terthienyl [145], and to search for possible field control of malaria vectors using crude plant extracts rich in \( \alpha \)-terthienyl [144], although little is known on phototoxic reactions in \textit{Anopheles} species except for \textit{Anopheles stephensi} [136] and \textit{A. gambiae} [146]. Even the crushed root segments of \textit{Tagetes patula} have produced phototoxic response [128].

Both artificial UV light and sunlight can generate toxicity but, as determined by using filters, the UV portion of the solar spectrum is required for activity [128]. It has been suggested that \( \alpha \)-terthienyl itself, rather than a structurally different metabolite, was responsible for phototoxicity as its action spectrum (\( \sim 350 \) nm) was similar to its absorption spectrum (\( \lambda_{\text{MAX}} 350 \) nm) [134].

In a comparative study photo-activated \( \alpha \)-terthienyl was found to be less active than Dursban, an organophosphorous compound, but more active than DDT or malathion, however without light, \( \alpha \)-terthienyl was much less toxic [134]. Studies with \textit{A. aegypti} showed that strains resistant to Dieldrin and DDT were as sensitive to the phototoxic effects of \( \alpha \)-terthienyl as the wild type [140].

\( \alpha \)-Terthienyl has also been tested against a malaria vector with fourth-instar \textit{Anopheles gambiae} [146] and was found to possess potent larvicidal properties. It has also shown larvicidal activity against the larvae of blackfly [128] and
fruitfly [138]. Ovicidal activity of UV-activated α-terthienyl has been demonstrated with α-terthienyl in A. aegypti mosquitoes, this effect was discovered in the fruitfly Drosophila melanogaster [147]. Eggs placed over a filter paper treated with α-terthienyl failed to hatch after they had been irradiated with near UV light.

The effect of topical applications of α-terthienyl to the larvae of some insects has also been reported [49, 148-51]. Topical application of α-terthienyl to larvae of the tobacco hornworm Manduca sexta was followed 24 hours later by irradiation with UV light. Tissue necrosis occurred at the application sites, and pupae showed morphological abnormalities and irregular melanization [149]. Larvae fed on a diet containing α-terthienyl were also irradiated and it was found that increased doses of α-terthienyl and irradiation resulted in delayed pupation, reduced pupal weights, increased occurrences of abnormal pupae, and decreased emergence of adult moths. At the highest ingested dose of α-terthienyl (50 μg per g of larval weight, followed by irradiation for 4 hours) pupation was delayed about 50%, and there was no subsequent adult emergence.

The effect of dietary α-terthienyl was also tested on larvae of the European corn borer, Ostrinia nubilalis [151], under conditions using solar light simulating lamps with small UV intensity. The survival to pupation and adult emergence in O. nubilalis was significantly reduced. At the highest concentration used (100 μg/g body weight), the mean time to pupation was increased by 30-52% and both the pupation and adult emergence were 71% of the controls. It was also observed that O. nubilalis tended to avoid the effect of photosensitization by burrowing into diet or spinning silk [95].
Although it has been assumed that the photoinsecticidal activity of \( \alpha \)-terthienyl is related to its ability to generate singlet oxygen, no direct experimental support has so far been obtained. One major obstacle is the difficulty of proving that oxygen itself is involved in the toxic reactions as the insects cannot survive anaerobic control experiments, even in the dark.

At the molecular level, little is known about the target(s) of the lethal photosensitized reactions of \( \alpha \)-terthienyl in insects. It has been reported that \( \alpha \)-terthienyl inactivated the enzyme acetylcholinesterase in larvae of the mosquito \textit{A. aegypti} [139]. After confirming that pure acetylcholinesterase rapidly decreased in activity, \textit{in vitro}, upon irradiation (an oxygen-dependent process), the total soluble enzyme activity was tested in larvae irradiated for various lengths of time after initial incubation in the presence of \( \alpha \)-terthienyl. A gradual decrease in enzyme activity was observed. About 40\% of the initial enzyme activity was lost at the point where all the larvae had died. Many other enzymes in the larvae were probably inactivated as a result of photosensitized treatment and the decrease of acetylcholinesterase activity may not be the sole direct cause of the death of the larvae.
### 2.4 Bithiophene Acetylenes in Plants

The first naturally occurring bithiophene was isolated from *Bidens radiata* in 1961 [152]. Since then a large number of bithiophene acetylenes have been isolated from the family Asteraceae. In most naturally occurring bithiophenes the two thiophene rings are directly attached to each other but in a few cases they are separated by two or four carbon atoms. Naturally occurring bithiophene acetylenes isolated from plants so far (December 1997), have been tabulated in Table 2.3.

**Table 2.3:** Naturally occurring bithiophene acetylenes in plants

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>Source</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Compound 1" /></td>
<td><em>Tagetes</em> spp., <em>Echinops</em> spp., <em>Berkheya</em> spp., <em>Culnumia</em> spp., <em>Dyssodia</em> spp., <em>Flaveria</em> spp. and other plant spp.</td>
<td>153</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Compound 2" /></td>
<td><em>Berkheya</em> spp.</td>
<td>154</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Compound 3" /></td>
<td><em>Berkheya</em> spp.</td>
<td>155</td>
</tr>
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<td>4</td>
<td><img src="image4" alt="Compound 4" /></td>
<td><em>Berkheya cerninopsis</em> and <em>Tagetes erecta</em>.</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Chemical Structure</td>
<td>Molecular Weight</td>
<td>Natural Sources</td>
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<td>------------------</td>
<td>--------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>5</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>C\text{$<em>{13}$}H$</em>{8}$S$_{2}$</td>
<td><strong>Dyssodia papposa</strong>, <em>Cullumia squarrosa</em> and <em>Vigniera stenoloba.</em></td>
</tr>
<tr>
<td>6</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>C\text{$<em>{12}$}H$</em>{10}$O$<em>{2}$S$</em>{2}$</td>
<td><em>Tagetes</em> spp., <em>Echinops</em> spp., <em>Berkheya</em> spp., <em>Cullumia</em> spp., <em>Dyssodia</em> spp., <em>Porophyllum</em> spp. and other plant spp.</td>
</tr>
<tr>
<td>7</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>C\text{$<em>{14}$}H$</em>{12}$O$<em>{2}$S$</em>{2}$</td>
<td><em>Tagetes</em> spp., <em>Echinops</em> spp., <em>Berkheya</em> spp., <em>Dyssodia</em> spp., and other plant spp.</td>
</tr>
<tr>
<td>8</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>C\text{$<em>{17}$}H$</em>{18}$O$<em>{2}$S$</em>{2}$</td>
<td><em>Haploesthes greggii.</em></td>
</tr>
<tr>
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<td>C\text{$<em>{12}$}H$</em>{10}$O$<em>{2}$S$</em>{2}$</td>
<td><em>Berkheya</em> spp., <em>Echinops</em> spp., <em>Flaveria</em> spp. and other plant spp.</td>
</tr>
<tr>
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<td><img src="image6" alt="Chemical Structure" /></td>
<td>C\text{$<em>{14}$}H$</em>{12}$O$<em>{3}$S$</em>{2}$</td>
<td><em>Berkheya</em> spp., <em>Echinops</em> spp., <em>Cullumia</em> spp., <em>Dyssodia</em> spp., <em>Porophyllum</em> spp. and other plant spp.</td>
</tr>
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<td>11</td>
<td><img src="image7" alt="Chemical Structure" /></td>
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<td>Molecular Weight</td>
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<td>Molecular Weight</td>
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<td><img src="image" alt="Structure 39" /></td>
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<td>264.369</td>
</tr>
<tr>
<td>40</td>
<td><img src="image" alt="Structure 40" /></td>
<td>C_{13}H_{14}O_{2}S_{2}</td>
<td>290.406</td>
</tr>
<tr>
<td>41</td>
<td><img src="image" alt="Structure 41" /></td>
<td>C_{13}H_{16}O_{4}S_{2}</td>
<td>348.443</td>
</tr>
<tr>
<td>42</td>
<td><img src="image" alt="Structure 42" /></td>
<td>C_{23}H_{28}O_{4}S_{2}</td>
<td>432.604</td>
</tr>
<tr>
<td>43</td>
<td><img src="image" alt="Structure 43" /></td>
<td>C_{23}H_{26}O_{4}S_{2}</td>
<td>430.588</td>
</tr>
<tr>
<td>44</td>
<td><img src="image" alt="Structure 44" /></td>
<td>C_{23}H_{26}O_{4}S_{2}</td>
<td>430.588</td>
</tr>
<tr>
<td>No.</td>
<td>Structure</td>
<td>Formula</td>
<td>MW</td>
</tr>
<tr>
<td>-----</td>
<td>-----------</td>
<td>---------</td>
<td>-----</td>
</tr>
<tr>
<td>45</td>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>C₁₀H₈O₂S₂</td>
<td>208.305</td>
</tr>
<tr>
<td>46</td>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>C₁₃H₁₂S₂</td>
<td>232.370</td>
</tr>
<tr>
<td>47</td>
<td><img src="image3.png" alt="Structure 3" /></td>
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<td>232.370</td>
</tr>
<tr>
<td>48</td>
<td><img src="image4.png" alt="Structure 4" /></td>
<td>C₁₃H₈S₂</td>
<td>228.338</td>
</tr>
<tr>
<td>49</td>
<td><img src="image5.png" alt="Structure 5" /></td>
<td>C₁₃H₈O₂S₂</td>
<td>244.338</td>
</tr>
<tr>
<td>50</td>
<td><img src="image6.png" alt="Structure 6" /></td>
<td>C₁₅H₁₀O₂S₂</td>
<td>286.375</td>
</tr>
<tr>
<td>51</td>
<td><img src="image7.png" alt="Structure 7" /></td>
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<td>242.322</td>
</tr>
<tr>
<td>No.</td>
<td>Structure</td>
<td>Formula</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>-----</td>
<td>-----------</td>
<td>---------</td>
<td>------------------</td>
</tr>
<tr>
<td>52</td>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>C_{11}H_{6}OS_{2}</td>
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</tr>
<tr>
<td>53</td>
<td><img src="image2.png" alt="Structure 2" /></td>
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</tr>
<tr>
<td>54</td>
<td><img src="image3.png" alt="Structure 3" /></td>
<td>C_{13}H_{10}O_{2}S_{2}</td>
<td>262.353</td>
</tr>
<tr>
<td>55</td>
<td><img src="image4.png" alt="Structure 4" /></td>
<td>C_{12}H_{8}OS_{2}</td>
<td>232.327</td>
</tr>
<tr>
<td>56</td>
<td><img src="image5.png" alt="Structure 5" /></td>
<td>C_{12}H_{10}OS_{2}</td>
<td>234.342</td>
</tr>
<tr>
<td>57</td>
<td><img src="image6.png" alt="Structure 6" /></td>
<td>C_{14}H_{12}O_{2}S_{2}</td>
<td>276.380</td>
</tr>
<tr>
<td>Page</td>
<td>Chemical Structure</td>
<td>Description</td>
<td>MW</td>
</tr>
<tr>
<td>------</td>
<td>-------------------</td>
<td>-------------</td>
<td>-----</td>
</tr>
<tr>
<td>58</td>
<td><img src="image1.png" alt="Image" /></td>
<td>Centurea sphaerocephala spp. polyacantha.</td>
<td>250.342</td>
</tr>
<tr>
<td>59</td>
<td><img src="image2.png" alt="Image" /></td>
<td>Berkheya carduoides.</td>
<td>262.420</td>
</tr>
<tr>
<td>60</td>
<td><img src="image3.png" alt="Image" /></td>
<td>Berkheya carduoides.</td>
<td>262.420</td>
</tr>
<tr>
<td>61</td>
<td><img src="image4.png" alt="Image" /></td>
<td>Berkheya barbata.</td>
<td>230.311</td>
</tr>
</tbody>
</table>
Dimers of bithiophenes, tetrathiophenes, have also been reported from *Cardopatium corymbosum*, *Echinops bonnaticus* and *E. ritrol* [43, 173].
2.5 Literature Survey of Genus *Blumea*

The chemistry of *Blumea* constituents has been of some interest for at least 75 years but many of its plants have not been much investigated. The essential oil was the first product to be studied [174]. In this initial study, *d*-carvotanacetone, *l*-tetrahydrocarvone, a mixture of butyric, isobutyric and *n*-octanoic acids and an unidentified phenol were isolated. In 1940, Bose and Dutt [175] reported the isolation of a flavonol, erianthin, from *B. eriantha* which was 5,7-dihydroxy-3,3',4',6,8-pentamethoxy flavone. Later in 1968 its structure was revised by Bose *et al.* [176] and it was confirmed that erianthin was quercetagetin pentamethyl ether, 5-hydroxy-3,3',4',6,7-pentamethoxy flavone 64.

In 1969 Bohlmann and Zedro reported the presence of a polyacetylene, tridecapentaynene, an acetylenic monothiophene, 65 and a coniferyl alcohol, 66 from *B. lacera* [177].

![Image of chemical structures](image-url)
Pal et al. isolated campestrol from the aerial parts of *B. lacera* [178]. α-Spinasterol was isolated from the pet.-ether extract of *B. myriocephala* and a saponin from the alcoholic extract of this plant showed a hemolytic effect on human blood [179]. *l*-Borneol [180], xanthoxylin [180], coumarins and triterpenes [180], myristic acid [181], fenchone [181] and 1,8-cineol [181] have also been reported from *Blumea* species. Rao et al. [182] reported β-sitosterol, 5-hydroxy-3,6,7,3′,4′-pentamethoxy flavone 64, 5,3′,4′-trihydroxy-3,6,7-trimethoxy flavone 67 and an unidentified flavone from the leaves of *B. lacera*.

*B. wightiana* was reported to contain carvotanacetone derivatives 68 and 69 [183].
In 1981, Ruangrungsi et al. isolated two flavonoids, (2\,R,\,3\,R)-dihydroquercetin-4'-methyl ether 70 and (2R, 3R)-dihydroquercetin-4',7-dimethyl ether 71, from the leaves of *B. balsamifera* [184].

Some sesquiterpene lactones 72 and a series of ring A-hydroxylated isosalantolactone derivatives 73 were isolated from *B. densiflora* [185].
In 1985, Bohlmann et al. reported the isolation of α-humulene, caryophylene, squalene and minute amounts of cauthemone derivatives 74 [186] from the aerial parts of *B. alata*. The same year, antispasmodic principal, cryptomeridiol was isolated from the ethanolic extract of the leaves of *B. balsamifera* [187].

A series of 6-hydroxyflavonols, 6-hydroxy-3,5,7,4'-tetramethoxy flavone, 6,2',5'-trihydroxy-3,5,7-trimethoxy flavone, 6,5'-dihydroxy-3,5,7,2'-tetramethoxy flavone and 6-hydroxy-3,5,7,2',5'-pentamethoxy flavone have been isolated from *B. malcomii* [188], but later it was confirmed that they were 5-hydroxy-3,6,7,4'-tetramethoxy flavone and 3,6,7-, 3,6,7,3'- and 3,6,7,3',4'-methyl ethers of quercetagetin, respectively [189].
One report described the isolation of an acetylenic monothiophene derivative, amplectol 75 along with taraxasteryl acetate, dihydrosterculic acid and sesquiterpene, ferutinine 76 [190] from B. ampectens.

\[ 
\begin{align*}
\text{H}_3\text{C} & \quad \text{S} \quad \text{C} \equiv \text{C} \quad \text{C} \equiv \text{C} \quad \text{H} \equiv \text{C} \quad \text{H} \equiv \text{C} \quad \text{H} \equiv \text{C} \quad \text{H} \equiv \text{C} \\
& \quad \text{OH} \quad \text{OH}
\end{align*}
\]

75

In 1988, blumea lactones A 77, B 78 and C 79 were reported from B. balsamifera [191] and they were shown to possess cancerostatic activities [192].
A trihydroxymethoxy dihydroflavone was isolated from *B. balsamifera* by Lin *et al.* in 1989 [193]. In the same year β-sitosterol, α-taraxasterol, α-taraxasteryl acetate, β-amyrin, β-amyrin acetate and epigenin were reported from *B. obliqua* [194].

Blumea lactone C, deacetyl blumea lactone C, 80 and deoxy blumea lactone C 81 have been isolated from *B. arfakiana* along with stigmasterol, β-eudesmol and four graniline esters 82 [195].
Barun and Sharma reported the presence of unusual flavones in *B. balsamifera* [196]. A triterpenoid glycoside, 19-α-hydroxyurs-12-ene-24, 28-dicarboxylate 3-O-β-D-xylopyranoside 83 and a prenylated phenol glycoside, 2-isoprenyl-5-isopropylphenol 4-O-β-D-xylopyranoside 84 have also been isolated from the petrol extract of the whole plant of *B. lacera* [197].

In 1996, Deng *et al.* [198] reported isolation of two flavonoids from *B. balsamifera* which were identified as 3,5,3'-trihydroxy-7,4-dimethoxy flavone and 3,5,3',4-tetrahydroxy-7-methoxy flavone.
3.0 PRESENT WORK
3.1 Selection of Plant

The Inuleae is a cosmopolitan tribe of the Asteraceae and the plants of this tribe have been used for medicinal purposes for ages. Three of the best known medicinal plants of the tribe are elecampane, *Inula helenium* (for the treatment of chest diseases), fleabane, *Pulicaria dysenterica* (used in herbal remedies) and cat's foot, *Antennaria dioica* (for throat infections). Leaves of *Inula* and *Pulicaria* species have also been employed as insecticidal sources [29].

In spite of the practical interest in plants of Inuleae, the chemistry of the tribe has not been extensively explored. However, polyacetylenes have been reported from many plants of this tribe of the Asteraceae [20]. Thiophene acetylenes are characteristic chemical constituents of the Inuleae but the genus *Blumea* has not been enough explored for polyacetylenes and so far bithiophene acetylenes have not been isolated from this genus.

Considering the biological importance of thiophene acetylenes and their role as taxonomic marker we selected *Blumea obliqua* as a representative of Inuleae for the investigation of thiophene acetylenes and other chemical constituents.
3.2 Botanical Description of *Blumea obliqua*

The genus *Blumea* belongs to the family Asteraceae and is placed in tribe Inuleae [199]. This genus consists of sixteen species out of which only seven are found in Pakistan [199, 200]. *Blumea obliqua* (L.) Druce (syn. *Erigeron obliquum* L. Mant.; *Blumea amplexentis* DC.) is an annual herb that grows in sandy or clayey areas especially near the sea in dry regions of Pakistan and India [200]. It has been reported from Karachi University and Mangopir area in Karachi [200]. The plant is 3-45 cm tall; stem erect, much branched, terete, covered with spreading pilose hairs. Leaves 0.5-6 x 0.3-2.5 cm; the lower ones oblong-lanceolate or oblanceolate-spathulate, attenuate at the base, obtuse or sub-acute at the apex; upper leaves ovate-oblong, sessile, cordate, rounded or auriculate, semi-amplexicaul at the base, acute or obtuse at the apex, margins entire or coarsely dentate, sparsely or densely pubescent on both surfaces. Capitula campanulate or urceolate-campanulate, 4 mm in diameter, few or numerous, each solitary on peduncles 1-5 cm long from the axils of the upper leaves. Phyllaries 4-seriate, linear-lanceolate, 3-5 mm long, strongly whitish pubescent on lobes. Receptacle glabrous. Achenes dark brown, oblong-elliptic, somewhat compressed, unribbed, c. 0.5 mm long, puberulous. Pappus yellowish-white, 3-4 mm long [199].
3.3 Medicinal Significance of *Blumea obliqua*

*Blumea obliqua* has not been much investigated for medicinal properties. It is occasionally used as sudorific and the juice of its leaves is considered to be insect repellent [201]. Many plants of genus *Blumea* are used in folk medicine for the treatment of various ailments. A decoction of *B. balsamifera* is used for dropsy and as a vasodilator, sedative and hypotensor. The leaves of *B. balsamifera* are also considered to have stomachic, expectorant, antispasmodic and sudorific properties. They are also prescribed for leucorrhea, rubbed on scabies, applied to head for migraine, put in the nose to stop nose bleed and are also considered as a diuretic, stimulative, and at the same time palliative [202]. The powder of the roots of *B. balsamifera* is considered as a stomachic, and that of the bark ground and mixed with whey, is a valuable remedy for piles [203]. Some of its preparations are used as tonic, as aphrodisiac and to prevent the hairs turning white or falling off [203].

Juice of *B. eriantha* is administered as a carminative and a warm infusion is given as a sudorific in catarrhal affections and cold and is considered to be diuretic and emmenagogue [203]. *B. lacera* is used to treat skin diseases. It is also employed as an insectifuge, vermifuge and to treat bronchitis [202]. The fresh root held in the mouth is said to relieve dryness [203]. The plant is a useful anthelmintic and is an invaluable remedy in Tinea Tarsi. The juice of leaves mixed with black pepper, is given in bleeding piles [203].

A decoction of *B. lanceolata* is considered to be a remedy for malaria, influenza and beriberi. The plant is sudorific and is recommended to treat bronchitis, aphthae and asthma. Leaves are boiled and applied as a poultice on rheumatic parts [202]. A decoction of the roots of *B. chinensis* is drunk to relieve colic. In Indonesia the plant is used in bath for the treatment of beriberi [203].
3.4 RESULTS AND DISCUSSION
The compounds isolated in the pure form from *Blumea obliqua* are discussed below. The method of isolation is described in detail in the Experimental Section.

3.4.1 *5-Methyl-5’-{4-(3-methyl-1-oxobutoxy)-1-butylnyl}-2,2’-bithiophene* (85):

Compound 85 was isolated from the pet-ether soluble part of the methanolic extract of *Blumea obliqua* by column chromatography followed by prep. TLC.

The electron impact mass spectrum (EIMS) showed a molecular ion peak [M⁺] at *m/z* 332.06. A strong [M+2]⁺ peak at *m/z* 334.06 was also observed in the EIMS which was found to be 11.1% of the molecular ion peak. This indicated the presence of sulfur in the compound. Peak matching of the molecular ion peak showed the exact *m/z* value of 332.0902 which corresponded to the molecular formula C₁₉H₂₀O₂S₂, suggesting nine degrees of unsaturation. The IR spectrum (CCl₄) displayed intense absorption peaks at 2850 (C–H), 2300 (C≡C) and 1717 cm⁻¹. This indicated the presence of acetylenic bond and an
ester carbonyl in the compound. The UV spectrum (Et₂O) showed an absorption maxima at 338.8 nm (ε 26726). Both the ε and the absorption maxima values were in agreement with the presence of a 2,2’-bithiophene acetylene chromophore [25].

The ¹H-NMR spectrum (CDCl₃, 300 MHz) of 85 exhibited a doublet of six protons at 8 0.97, a multiplet of one proton at 8 2.12, a doublet of two protons at 8 2.22, a doublet of three protons at 8 2.48, two triplets of two protons each at 8 2.78 and 4.24, a doublet of quartet of one proton at 8 6.64 and three doublets of one proton each at 8 6.88, 6.93 and 6.99. The doublet of six protons at 8 0.97 with a coupling constant of 6.5 Hz was due to two secondary methyl groups which were geminal to each other. In the COSY-45° spectrum these methyls showed correlation with signal at 8 2.12 which corresponded to a multiplet of a single proton. This proton was again correlated to a signal at 8 2.22 which represented a two protons doublet with a coupling constant of 6.5 Hz. The signals at 8 22.4, 25.8 and 43.4 in the ¹³C-NMR (CDCl₃, 75 MHz, see Table 3.1) were assigned to C-4a, C-3a and C-2a, respectively. These assignments were supported by the DEPT analysis which showed that they were for the methyl, methine and the methylene signals, respectively, and were confirmed by the HMQC spectrum. In the HMBC spectrum C-4a was correlated to C-3a which in turn was correlated to C-2a. A correlation of C-2a was observed with a quaternary carbon at 8 172.8. As the IR spectrum has revealed the presence of an ester carbonyl, C-2a might be attached to the carbonyl carbon of the ester carbonyl. A loss of 102 a.m.u. (isovaleric acid) from the molecular ion peak in the EIMS also confirmed this assumption. The loss of isovaleric acid leaves the rest of the molecule with a molecular mass of m/z 230 which appeared as the base peak in the EIMS. The high resolution electron impact mass spectrum (HREIMS) of this fragment gave the exact value
of $m/z$ 230.0186 which corresponded to a formula of $C_{13}H_{10}S_2$. These findings helped to elucidate a partial structure shown in 85 a.

A pair of triplets of two protons each at $\delta$ 2.78 ($J=6.7$ Hz) and 4.24 ($J=6.7$ Hz) in the $^1$H-NMR indicated the presence of two adjacent methylene groups. These protons showed strong correlation in the COSY-45° spectrum which also indicated that they constitute an independent spin system which was not further correlated to any other proton. These signals were due to H-3" and H-4" protons respectively. Thus another structural unit can be drawn as 85 b.

The UV spectrum was in agreement with a 2,2'-bithiophene acetylene skeleton. The presence of two sulfur atoms in the molecular formula, four methine signals in the aromatic region of the $^1$H-NMR and -C≡C- stretching in the IR also supported this skeleton. The $^1$H-NMR exhibited three doublets at $\delta$ 6.88 (1H, $J=3.7$ Hz), 6.93 (1H, $J=3.5$ Hz) and 6.99 (1H, $J=3.7$ Hz) which were
assigned to H-3, H-3' and H-4 protons, respectively. The H-4' proton showed a doublet of quartet (J=1.0 and 3.5 Hz). The three proton doublet which appeared at 8 2.48 (J=1.0 Hz) was assigned to the methyl group attached to C-5'. In COSY-45° spectrum, H-3 was correlated to H-4 while H-4' exhibited interactions with H-3' and with the ring methyl protons (H-6'). These assignments were fully supported by 13C-NMR (Table 3.1) and were confirmed by HMQC and HMBC spectrum. These values were in accordance with the values reported in the literature for these type of compounds [25, 204]. This completed another structural unit presented in 85 c.

\[
\text{Partial structure } 85\text{c}
\]

The HMBC spectrum showed that structural unit 85 c is attached to 85 b. This gave an extended structure 85 d which resulted in the complete structure 85 on attachment of the isovaleroxy moiety to the methylene group.

\[
\text{Partial structure } 85\text{d}
\]
The COSY-45° interactions are presented in 85 e and the HMBC correlations are shown in 85 f. This compound is found to be a new natural product.
3.4.1.1 Biological Activities of Compound 85

Antifungal and antibacterial activity:

Compound 85 was evaluated for antifungal and antibacterial activities by photometric microtiter broth dilution method [205]. It was found to possess potent antifungal activity against various human, animal and plant pathogens like *Microsporum canis*, *Pleurotus ostreatus*, *Alternaria solani*, *Curvularia lunata*, *Epidermophyton floccosum*, *Trichophyton schoenleinii*, *T. longifusis*, *Pseudallescheria boydii*, *Candida albicans*, *Trichophyton mentagrophytes*, *T. simii*, *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani*. The MIC values against most of these strains are shown in Table 3.2. The table also shows the MIC values of 85 against various bacteria.

Antileishmanial activity:

*In vitro* antileishmanial activity [206] of 85 was performed against *Leishmania major*. The compound was quite active against *L. major* promastigotes showing IC$_{100}$ of 50 μg/ml. Pentamidine was used as reference compound which showed IC$_{100}$ of 100 μg/ml under similar conditions.
Table 3.1: $^1\text{H}/^{13}\text{C}$-NMR connectivities (HMOC) for compound 85 in CDCl$_3$.

<table>
<thead>
<tr>
<th>Carbon No</th>
<th>Chemical Shift (δ)</th>
<th>Multiplicity (DEPT)</th>
<th>$^1\text{H}/^{13}\text{C}$ Connectivity (J=Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>138.6</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>122.5</td>
<td>CH</td>
<td>6.88 (d, J=3.7)</td>
</tr>
<tr>
<td>4</td>
<td>132.5</td>
<td>CH</td>
<td>6.99 (d, J=3.7)</td>
</tr>
<tr>
<td>5</td>
<td>121.6</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>134.5</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3'</td>
<td>124.3</td>
<td>CH</td>
<td>6.93 (d, J=3.5)</td>
</tr>
<tr>
<td>4'</td>
<td>126.0</td>
<td>CH</td>
<td>6.64 (dq, J=1.0, 3.5)</td>
</tr>
<tr>
<td>5'</td>
<td>139.8</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>6'</td>
<td>15.3</td>
<td>CH$_3$</td>
<td>2.48 (d, J=1.0)</td>
</tr>
<tr>
<td>1''</td>
<td>75.3</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2''</td>
<td>90.5</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3''</td>
<td>20.4</td>
<td>CH$_2$</td>
<td>2.78 (t, J=6.7)</td>
</tr>
<tr>
<td>4''</td>
<td>60.6</td>
<td>CH$_2$</td>
<td>4.24 (t, J=6.7)</td>
</tr>
<tr>
<td>1a</td>
<td>172.8</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2a</td>
<td>43.4</td>
<td>CH$_2$</td>
<td>2.22 (d, J=6.5)</td>
</tr>
<tr>
<td>3a</td>
<td>25.8</td>
<td>CH</td>
<td>2.12 (m)</td>
</tr>
<tr>
<td>4a</td>
<td>22.4</td>
<td>2 x CH$_3$</td>
<td>0.97 (d, J=6.5)</td>
</tr>
</tbody>
</table>
Table 3.2: MIC values of 85 against tested fungi and bacteria.

<table>
<thead>
<tr>
<th>Name of Fungi</th>
<th>MIC (µg/100µl)</th>
<th>Name of Bacteria</th>
<th>MIC (µg/100µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichophyton schoenleinii</em></td>
<td>90</td>
<td><em>Bacillus cereus</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Trichophyton longifuses</em></td>
<td>90</td>
<td><em>Corynebacterium diptheriae</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Pseudallescheria boydii</em></td>
<td>95</td>
<td><em>Escherichia coli</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>100</td>
<td><em>Shigella sonii</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>100</td>
<td><em>Proteus mirabilis</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Trichophyton simii</em></td>
<td>100</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Fusarium solani</em></td>
<td>100</td>
<td><em>Salmonella typhi</em></td>
<td>200</td>
</tr>
<tr>
<td><em>Macrophomina phaseolina</em></td>
<td>100</td>
<td><em>Shigella boydii</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Rhizoctonia solani.</em></td>
<td>100</td>
<td><em>Staphylococcus aureus</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Microsporum canis</em></td>
<td>90</td>
<td><em>Streptococcus pyogenes</em></td>
<td>150</td>
</tr>
</tbody>
</table>
Proposed Biosynthesis of
$5'$-Methyl-5-[4-(3-methyl-1-oxobutaxy)-1-hutnyl]-2,2'-bithiophene (85).

\[
\begin{align*}
CH_3-(C\equiv C)_5-CH=CH_2 & \quad 24 \\
\xrightarrow{[H_2S]} & \\
CH_3-(C\equiv C)_2-S-C=C-CH=CH_2 & \\
\xrightarrow{[H_2S]} & \\
\begin{array}{c}
\text{Cyclic Structure} \\
CH_3-S-S-C=C-CH=CH_2
\end{array} & \\
\xrightarrow{[O], [Esterification]} & \\
\begin{array}{c}
\text{Cyclic Structure} \\
CH_3-S-S-C=C-CH_2-CH_2-O-C-CH_2-C-H
\end{array} & \quad 85
\end{align*}
\]
3.4.2 5'-Hydroxymethyl-5-(butyl-3-en-1-yn)-2,2'-bithiophene isovaleroxy ester (86):

Compound 86 was isolated from the pet.-ether soluble part of the methanolic extract of *Blumea obliqua* by repeated column chromatography and prep. TLC.

![Chemical Structure of Compound 86](image)

The mass spectrum displayed the molecular ion peak [M⁺] at m/z 329.7 with a strong [M+2]⁺ peak at m/z 331.7 which was 11.1% of the molecular ion peak, indicating the presence of sulfur in the compound. The high resolution mass measurement of the molecular ion peak afforded the exact mass at m/z 330.0750 which was in agreement with the molecular formula C₁₅H₁₈O₂S₂. Thus ten degrees of unsaturation were present in the compound.

The IR spectrum (CCl₄) showed intense absorptions at 2840 (C—H), 2175 (C≡C) and 1725 (ester carbonyl) cm⁻¹. This indicated the presence of acetylenic linkage and an ester carbonyl function in the compound. The UV spectrum (Et₂O) exhibited an intense absorption maxima at 346.8 nm (ε 27,620). Both the UV and absorption maximum values indicated the presence of a 2,2'-bithiophene acetylene chromophore in the compound [25].
The $^1$H-NMR spectrum of 86 (CDCl$_3$, 500 MHz) showed a 6H doublet at δ 0.94 with a coupling constant of 7.0 Hz, a 1H multiplet at δ 2.12 and a 2H doublet at δ 2.24 with a coupling constant of 7.0 Hz. These findings coupled with the indication of an ester carbonyl moiety in the IR spectrum and a loss of 101 a.m.u. (isovalerate) from the molecular ion peak confirmed the presence of an isovaleroxy function and this partial structure is shown in 86 a.

\[
\text{Partial structure "86 a"}
\]

In aromatic region, the $^1$H-NMR exhibited four doublets of one proton each at δ 6.96, 7.00, 7.01 and 7.07 and each one with a coupling constant of 3.5 Hz. These values were respectively assigned to H-4', H-3, H-3' and H-4 of a 2,2'-bithiophene acetylene system. The presence of two sulfur in the molecular formula, —C≡C— stretching in the IR spectrum and the absorption behavior in the UV spectrum also suggested a bithiophene acetylene group in the compound. So the presence of a 2,2'-bithiophene acetylene system was confirmed and this structural unit was shown as 86 b. A singlet of 2H at δ 5.20 instead of a 3H doublet at δ 2.48 as in the case of compound 85 proposed that a methylene instead of a methyl group was present in compound 86 at 6' position. Similarly, three doublet of doublets of 1H each at δ 5.54 (J=1.5, 11.0 Hz), 5.72 (J=1.5, 17.5 Hz) and 6.01 (J=11.0, 17.5 Hz) indicated that an end vinyl group was present instead of two adjacent methylenes as in the case of
compound 85. These values were assigned to H-4" (cis), H-4" (trans) and to H-3" protons, respectively. This segment of the structure could be drawn as 86 c.

The complete structure of the compound could be deduced if 86 c was affixed to the acetylenic end and 86 a to the methylene end of the partial structure 86 b.

Two dimensional NMR measurements (COSY-45° and J-resolved) were carried out to investigate the structure. The coupling interactions of various protons were established through the COSY-45° spectrum while the multiplicities of the protons signals were determined from the 2D J-resolved spectrum. The connectivities between H-3 and H-4 and H-3' and H-4' could be seen in the COSY-45° spectrum of compound 86. Similarly, H-3a showed cross peaks with
H-2a and H-4a. The H-4'' (cis) and H-4'' (trans) protons were correlated to each other and also to the H-3'' proton. The complete COSY-45° interactions are shown in 86 d.

The broad band 13C-NMR spectrum of compound 86 exhibited the presence of all the eighteen carbon atoms. The multiplicities of the various carbons were confirmed by employing DEPT pulse sequence with the last polarization pulse angles at 135°, 90° and 45°. The broad band and DEPT analysis indicated the presence of seven quaternary, six methine, three methylene and two methyl carbons.

The proton carbon connectivities (1H/13C) were established with the help of the HMQC experiments (Table 3.3). The chemical shift of the various carbons were assigned on the basis of the HMBC spectrum and were in agreement with the values reported in the literature for these type of compounds [25, 204]. The HMBC correlations were shown in 86 e. This compound is a new natural product.
3.4.2.1 Antifungal Activity of Compound 86

The antifungal activity of compound 86 was determined by the tube dilution method [207]. It was found active against *Microsporum canis*, *Pleurotus ostreatus*, *Alternaria solanii*, *Curvularia lunata* and *Epidermophyton floccosum*. The activity was highly potent against *Epidermophyton floccosum* and *Pleurotus ostreatus* and the MIC values for both these strains were found to be 200 µg/ml as compared to griseofulvin which showed a value of 250 µg/ml for both these fungi.
Table 3.3: $^1$H/$^{13}$C-NMR connectivities (HMOC) for compound 86 in CDCl$_3$

<table>
<thead>
<tr>
<th>Carbon No</th>
<th>Chemical Shift ($\delta$)</th>
<th>Multiplicity (DEPT)</th>
<th>$^1$H/$^{13}$C Connectivity ($J$=Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>138.6</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>123.7</td>
<td>CH</td>
<td>7.01* (d, $J$=3.5)</td>
</tr>
<tr>
<td>4</td>
<td>132.8</td>
<td>CH</td>
<td>7.07 (d, $J$=3.5)</td>
</tr>
<tr>
<td>5</td>
<td>122.2</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>137.9</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3'</td>
<td>123.7</td>
<td>CH</td>
<td>7.00* (d, $J$=3.5)</td>
</tr>
<tr>
<td>4'</td>
<td>128.8</td>
<td>CH</td>
<td>6.96 (d, $J$=3.5)</td>
</tr>
<tr>
<td>5'</td>
<td>138.0</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>6'</td>
<td>60.2</td>
<td>CH$_2$</td>
<td>5.2 (s)</td>
</tr>
<tr>
<td>1''</td>
<td>83.1</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2''</td>
<td>93.1</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3''</td>
<td>116.8</td>
<td>CH</td>
<td>6.01 (dd, $J$=11.0, 17.5)</td>
</tr>
<tr>
<td>4''</td>
<td>127.0</td>
<td>CH$_2$</td>
<td>5.54 (dd, $J$=1.5, 11.0) (cis)</td>
</tr>
<tr>
<td>4'''</td>
<td>127.0</td>
<td>CH$_2$</td>
<td>5.72 (dd, $J$=1.5, 17.5) (trans)</td>
</tr>
<tr>
<td>1a</td>
<td>172.2</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2a</td>
<td>43.2</td>
<td>CH$_2$</td>
<td>2.21 (d, $J$=7.0)</td>
</tr>
<tr>
<td>3a</td>
<td>25.7</td>
<td>CH</td>
<td>2.12 (m)</td>
</tr>
<tr>
<td>4a</td>
<td>22.4</td>
<td>2 x CH$_3$</td>
<td>0.94 (d, $J$=7.0)</td>
</tr>
</tbody>
</table>

*Assignments may be interchanged.
Proposed Biosynthesis of

5′-Hydroxymethyl-5-(butyl-3-ene-1-yne)-2,2′-bithiophene isovaleroyl ester (86).

\[ \text{CH}_3\text{(C} \equiv \text{C})_5\text{CH} \equiv \text{CH}_2 \]

\[ \xrightarrow{[\text{H}_2\text{S}]} \]

\[ \text{CH}_3\text{(C} \equiv \text{C})_2\text{S} \text{C} \equiv \text{C} \text{C} \text{C} \text{CH} \equiv \text{CH}_2 \]

\[ \xrightarrow{[\text{H}_2\text{S}]} \]

\[ \text{CH}_3\text{S} \text{S} \text{C} \equiv \text{C} \text{C} \text{C} \text{C} \text{CH} \equiv \text{CH}_2 \]

\[ \xrightarrow{[\text{O}], [\text{Esterification}]} \]

\[ \text{CH}_3\text{CH} \equiv \text{CH}_2\text{C} \equiv \text{O} \text{H}_2\text{C} \text{S} \text{S} \text{C} \equiv \text{C} \text{C} \text{C} \text{C} \text{CH} \equiv \text{CH}_2 \]

86
3.4.3 5'-Methyl-5-(3-hydroxy-4-isovaleroxy-1-butynyl)-2,2'bitiophene (87):

The column chromatography of the pet.-ether soluble part of the methanolic extract of *Blumea obliqua* yielded 87 in semi-purified form which was purified by prep. T.L.C.

![Chemical Structure of 87](image)

The FDMS showed a molecular ion peak [M'] at *m/z* 348. Peak matching of this peak resulted in the exact value of *m/z* 348.0851 which agrees to the molecular formula C_{16}H_{20}O_{3}S_{2}. The formula indicated ten degrees of unsaturation in the compound. The UV spectrum showed a characteristic pattern of 2,2'-bitiophene acetylene [25] with an absorption maximum at 338.0 nm.

The $^1$H-NMR spectrum (CDCl$_3$, 300 MHz) featured a doublet of six protons at $\delta$ 0.96 with a coupling constant of 6.5 Hz. This was due to two secondary methyl groups which were geminal to each other. The spectrum also exhibited a 1H multiplet at $\delta$ 2.12 and a 2H doublet at $\delta$ 2.26 with a coupling constant of 6.5 Hz. These values tallied well with an isovaleroxy unit as observed in compound 85 and 86 and were assigned to H-1a, H-2a and H-3a which correspond to the methyl, methine and methylene protons, respectively. In
aromatic region the spectrum showed the same pattern as that of compound 85. A 1H doublet of quartet at δ 6.66 (J=3.5, 1.0 Hz) and three doublets at δ 6.91 (1H, J=3.8 Hz), 6.97 (1H, J=3.5 Hz) and 7.08 (1H, J=3.8 Hz) confirmed a 2,2'-bithiophene function. These values were respectively given to H-4', H-3, H-3' and H-4 protons. A 3H doublet at δ 2.46 (J=1.0 Hz) was assigned to H-6' protons. A 2H doublet at δ 4.29 (J=5.5 Hz) and a 1H broad triplet at δ 4.81 were assigned to H-4'' and H-3'' protons respectively. A downfield shift for H-3'' proton as compared to the H-3'' proton in compound 85 (δ 2.78), established the presence a hydroxy substituent at position 3''. This was shown in partial structure 87 a.

![Partial structure 87 a]

The assignments of the various protons were confirmed by COSY-45° spectra and these interactions were shown in 87 b.

The 13C-NMR spectrum (broad band and DEPT) of 87 exhibited resonance for three methyl, two methylene, six methine and seven quaternary carbon atoms. Their 13C chemical shifts (Table 3.4) were assigned according to the literature values for similar type of compounds [25, 204]. The compound is found to be a new natural product.
3.4.3.1 Antifungal and Antibacterial Activities:

The antifungal and antibacterial activities of compound 87 were evaluated by photometric microtiter broth dilution method [205]. It was found to possess potent antifungal and antibacterial activities against various human, animal and plant pathogens. The MIC values for *Microsporum canis*, *Trichophyton schoenleinii*, *T. longifusus*, *Pseudallescheria boydii*, *Candida albicans*, *Trichophyton mentagrophytes*, *T. simii*, *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* are shown in Table 3.5. Table 3.5 also shows the MIC values of compound 87 against various bacteria tested.
Table 3.4: $^{1}H/^{13}C$-NMR connectivities (HMOC) for compound 87 in CDCl₃

<table>
<thead>
<tr>
<th>Carbon No</th>
<th>Chemical Shift (δ)</th>
<th>Multiplicity (DEPT)</th>
<th>$^{1}H/^{13}C$ Connectivity (J=Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>139.4</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>122.6</td>
<td>CH</td>
<td>6.91 (d, $J=3.8$)</td>
</tr>
<tr>
<td>4</td>
<td>133.6</td>
<td>CH</td>
<td>7.08 (d, $J=3.8$)</td>
</tr>
<tr>
<td>5</td>
<td>122.1</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>140.0</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3'</td>
<td>124.4</td>
<td>CH</td>
<td>6.97 (d, $J=3.5$)</td>
</tr>
<tr>
<td>4'</td>
<td>126.2</td>
<td>CH</td>
<td>6.65 (dq, $J=1.0, 3.5$)</td>
</tr>
<tr>
<td>5'</td>
<td>140.2</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>6'</td>
<td>14.0</td>
<td>CH₃</td>
<td>2.46 (d, $J=1.0$)</td>
</tr>
<tr>
<td>1''</td>
<td>79.7</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2''</td>
<td>90.4</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3''</td>
<td>65.0</td>
<td>CH</td>
<td>4.82 (t, $J=5.0$)</td>
</tr>
<tr>
<td>4''</td>
<td>61.8</td>
<td>CH₂</td>
<td>4.29 (d, $J=5.0$)</td>
</tr>
<tr>
<td>1a</td>
<td>173.0</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2a</td>
<td>43.2</td>
<td>CH₂</td>
<td>2.26 (d, $J=6.5$)</td>
</tr>
<tr>
<td>3a</td>
<td>24.8</td>
<td>CH</td>
<td>2.15 (m)</td>
</tr>
<tr>
<td>4a</td>
<td>22.3</td>
<td>2 x CH₃</td>
<td>0.96 (d, $J=6.5$)</td>
</tr>
</tbody>
</table>
Table 3.5: MIC values of compound 87 against tested fungi and bacteria.

<table>
<thead>
<tr>
<th>Name of Fungi</th>
<th>MIC (µg/100µl)</th>
<th>Name of Bacteria</th>
<th>MIC (µg/100µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichophyton schoenleinii</em></td>
<td>100</td>
<td><em>Bacillus cereus</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Trichophyton longifuses</em></td>
<td>100</td>
<td><em>Corynebacterium diptheriae</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Pseudallescheria boydii</em></td>
<td>90</td>
<td><em>Escherichia coli</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>100</td>
<td><em>Shigella sonii</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>100</td>
<td><em>Proteus mirabilis</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Trichophyton sinnii</em></td>
<td>90</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Fusarium solani</em></td>
<td>100</td>
<td><em>Salmonella typhi</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Macrophomina phaseolina</em></td>
<td>150</td>
<td><em>Shigella boydii</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Rhizoctonia solani.</em></td>
<td>100</td>
<td><em>Streptococcus pyogenes</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Microsporum canis</em></td>
<td>50</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
Proposed Biosynthesis of

\[ 5'-\text{Methyl-5-(3-hydroxy-4-isovaleroxy-1-butynyl)-2,2'-bithiophene (87)} \]

\[
\begin{align*}
\text{CH}_3-(C\equiv C)_5-\text{CH}=\text{CH}_2 & \quad 24 \\
& [\text{H}_2\text{S}] \\
\text{CH}_3-(C\equiv C)_2-\text{S}-\text{S}-C\equiv C-\text{CH}=\text{CH}_2 & \\
& [\text{H}_2\text{S}] \\
\text{CH}_3-\text{S}-\text{S}-\text{C}\equiv C-\text{CH}=\text{CH}_2 & \\
& [\text{H}_2\text{O}] \\
\text{CH}_3-\text{S}-\text{S}-\text{C}\equiv C-\text{CH}-\text{CH}_3 & \\
& [\text{O}, \text{Esterification}] \\
\text{CH}_3-\text{S}-\text{S}-\text{C}\equiv C-\text{CH}_2-\text{O}-\text{C}-\text{CH}_2-\text{C}-\text{CH}_3
\end{align*}
\]
3.4.4 *Methylene-bis-[5-(3-buten-1-yl)-2,2′-bithiophene]* (88):

Compound 88 was isolated from the pet.-ether soluble part of the methanolic extract of *Blumea obliqua*. The compound was very labile when exposed to daylight, and changes from colorless to greenish and then to blackish when kept in chloroform for long time (24 hours).

![Chemical structure of 88](image)

The electron impact mass spectrum (EI MS) showed a molecular ion peak [M+] at m/z at 444.2 with a strong [M+2]⁺ peak at m/z 446.4 (14.2% of the [M⁺]), indicating the presence of sulfur. The high resolution electron impact mass spectrum (HREIMS) of the molecular ion peak resulted in the exact value of m/z 444.0146 which was analyzed for the molecular formula C₂₃H₁₆S₄, suggesting eighteen double bond equivalents.

The IR spectrum displayed intense absorption peaks at 2900 (C–H) and 2175 (C≡C) cm⁻¹. The UV (MeOH) spectrum was very informative which exhibited
absorption maximum at 347.6 nm indicating the presence of a 2,2'-bithiophene
diyne chromophore conjugated with a double bond [25].

The $^1$H-NMR (CDCl$_3$, 400 MHz) exhibited three doublet of doublets at $\delta$ 5.52
(2H, J=1.9, 11.2 Hz), 5.72 (2H, J=1.9, 17.5 Hz) and 6.01 (2H, J=11.2, 17.5 Hz). These values indicated the presence of an end vinyl group. The integration of 2H for each of these values suggested that two symmetrical end vinyl groups were present in the compound. These signals were assigned to H-9 (cis),
H-9 (trans) and H-8 protons, respectively. In the aromatic region the spectrum exhibited signals at $\delta$ 6.80 (2H, d, J=3.6 Hz), 6.95 (2H, d, J=3.8 Hz), 7.00 (2H, d, J=3.6 Hz) and 7.06 (2H, d, J=3.8 Hz). These were the characteristic signals for the protons of 5,5'- substituted 2,2'-bithiophene ring system, however, the integration of two protons for each value suggested the presence of two symmetrical bithiophene rings. These values were assigned to H-4', H-3, H-3' and H-4 protons, respectively. A 2H singlet at $\delta$ 4.27 was attributed to H-6' protons.

The $^1$H-NMR assignments were confirmed by COSY-45° spectrum which showed correlations between H-8/H9(cis), H-8/H-9(trans), H-9(cis)/H-9(trans),
H-3/H-4 and H-3'/H-4'protons. The H-6' was also correlated to H-4' due to allylic coupling. The COSY-45° interactions are shown in 88 a. The HOHAHA spectrum also exhibited that H-6' was correlated to H-4'and H-3'.

These findings suggested that 88 was a dimer of 88 b and 88 c. One of them ,
88 b, has also been isolated by us from this plant while 88 c is abundantly
found in Asteraceae. The compound 88 might have been formed as a result of
an enzyme catalyzed condensation reaction of 88 b and 88 c. As the plant
material was not subjected to acid or base and was not exposed to heat, we
must conclude that it was present in the fresh plant. Similar type of dimeric
compounds have also been reported from *Echinops bannaticus* and *E. ritrol* [43] and from *Cardapatum corymbosum* [173].

\[ \text{88 a} \]

\[ \text{88 b} \]

\[ \text{88 c} \]
The $^{13}$C-NMR exhibited signals for thirteen carbons (Table 3.6), and twelve of them were part of the dimeric units and thus represented twenty four carbon atoms. The broad band spectrum and the DEPT experiment showed the presence of two methylene, five methine and six quaternary carbons. The signals in the low field (δ 121.6, 123.2, 124.0, 126.3, 132.7, 135.8, 139.1 and 142.5) were characteristic of the 2,2'-bithiophene skeleton and were assigned to C-5, C-3', C-3, C-4', C-4, C-2', C-2 and C-5', respectively. The acetylenic carbons C-6 and C-7 appeared at δ 83.6 and 92.9, respectively. The central methylene resonated at δ 30.7 while the signals for the olefinic carbons appeared at δ 116.9 (C-8) and 126.8 (C-9). The proton-carbon ($^1$H/$^{13}$C) connectivities were established with the help of HMQC experiment (Table 3.6) while the chemical shifts of the various carbons were established on the basis of the HMBC spectrum. The complete HMBC correlations are presented in 88 d. This novel tetrathiophene is a new natural product.
Table 3.6: $^{13}$C-NMR data and $^{1}H/^{13}$C connectivities (HMQC) for compound 88.

<table>
<thead>
<tr>
<th>Position</th>
<th>Chemical Shift (δ)</th>
<th>Multiplicity (DEPT)</th>
<th>$^{1}H/^{13}$C Connectivity (J=Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>139.1</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>124.0</td>
<td>CH</td>
<td>6.95 (d, J=3.8)</td>
</tr>
<tr>
<td>4</td>
<td>132.7</td>
<td>CH</td>
<td>7.06 (d, J=3.8)</td>
</tr>
<tr>
<td>5</td>
<td>121.6</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>83.2</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>92.9</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>116.9</td>
<td>CH</td>
<td>6.01 (dd, J=11.2, 17.5)</td>
</tr>
<tr>
<td>9</td>
<td>126.8</td>
<td>CH$_2$</td>
<td>5.52 (dd, J=1.9, 11.2) (cis)</td>
</tr>
<tr>
<td></td>
<td>126.8</td>
<td>CH$_2$</td>
<td>5.72 (dd, J=1.9, 17.5) (trans)</td>
</tr>
<tr>
<td>2'</td>
<td>135.8</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>3'</td>
<td>123.2</td>
<td>CH</td>
<td>7.00 (d, J=3.6)</td>
</tr>
<tr>
<td>4'</td>
<td>126.3</td>
<td>CH</td>
<td>6.80 (d, J=3.6)</td>
</tr>
<tr>
<td>5'</td>
<td>142.5</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>6'</td>
<td>30.7</td>
<td>CH$_2$</td>
<td>4.27 (s)</td>
</tr>
</tbody>
</table>
Proposed biosynthesis of

*Methylene-bis-[5-(3-butene-1-ynyl)]-2,2'-bithiophene* (88).

\[
\begin{align*}
\text{CH}_3 &\quad (\text{C} \equiv \text{C})_2 \text{CH} = \text{CH}_2 & 24 \\
&\quad [\text{H}_2\text{S}] \\
\text{CH}_3 &\quad (\text{C} \equiv \text{C})_2 \text{C} \equiv \text{C} \text{CH} = \text{CH}_2 & \\
&\quad [\text{H}_2\text{S}] \\
\text{CH}_3 &\quad \text{S} \quad \text{S} \quad \text{C} \equiv \text{C} \text{CH} = \text{CH}_2 & \\
&\quad [\text{O}] \\
&\quad \text{HOOC} \quad \text{S} \quad \text{S} \quad \text{C} \equiv \text{C} \text{CH} = \text{CH}_2 & \\
&\quad \text{HOH}_2\text{C} \quad \text{S} \quad \text{S} \quad \text{C} \equiv \text{C} \text{CH} = \text{CH}_2 & -[\text{CO}_2] \\
&\quad \text{S} \quad \text{S} \quad \text{C} \equiv \text{C} \text{CH} = \text{CH}_2 & -[\text{H}_2\text{O}] \\
\text{H}_2\text{C} &\quad \text{S} \quad \text{S} \quad \text{C} \equiv \text{C} \text{CH} = \text{CH}_2 & 88
\end{align*}
\]
3.4.5 5'-Methyl-15-(4-acetoxy-1-butynyl)-2,2'-bithiopine (89):

Compound 89 was isolated from the pet.-ether soluble part of the methanolic extract of *Blumea obliqua*.

\[
\begin{array}{c}
\text{CH}_3 \\
\text{S} \\
\text{CH}_3 \\
\text{S} \\
\text{C}=\text{C} \text{-CH}_2 \text{-CH}_2 \text{-O} \text{-C} \text{-CH}_3 \\
\end{array}
\]

89

The electron impact mass spectrum (EIMS) showed a molecular ion peak \([M^+]\) at \(m/z\) 289.9 with a \([M+2]^+\) peak at \(m/z\) 291.9 which was 10.6% of the molecular ion peak. Thus the presence of sulfur was indicated in the compound. The high resolution electron impact mass spectrum (HREIMS) of \([M^+]\) gave an exact value of 290.0409 corresponding to the molecular formula \(C_{13}H_{14}O_2S_2\), suggesting nine degrees of unsaturation.

The UV spectrum (Et_2O) displayed absorption maximum at 339.2 nm which was characteristic of the presence of a 2,2'-bithiophene acetylene chromophore [25].

The \(^1\)H-NMR featured a 3H singlet at \(\delta \) 2.08 and a 3H doublet at \(\delta \) 2.46 with a coupling constant of 0.8 Hz. These values were assigned to the acetoxy protons (H-2a) and the protons of the ring methyl group (H-6'), respectively. The doublet of the ring methyl protons (H-6') was due to the allylic coupling to H-4' proton. The two triplets of two protons at \(\delta \) 2.76 and 4.22 each with a
A 1H doublet of quartet at 6.64 (J=3.5, 1.0 Hz) and three doublets of one proton each at \( \delta \) 6.88 (J=3.7 Hz), 6.93 (J=3.5 Hz) and 6.99 (J=3.9 Hz) were characteristic of 2,2'-bithiophene ring protons and were assigned to H-4', H-3, H-3' and H-4 protons, respectively. These assignments were further confirmed by COSY-45° spectrum. Strong correlations were observed for H-3'' and H-4'' protons. The H-3 proton was correlated to H-4 while H-4' showed interactions both with H-3' and H-6' protons. The COSY-45° interactions are shown in 89 a.

The \(^{13}\)C-NMR was very conclusive for the confirmation of the structure. It exhibited signals for twelve carbons (three quaternary carbons did not appear) (Table 3.7). The multiplicity of the various carbons were confirmed with the help of the DEPT experiment and the proton-carbon connectivities (\(^1\)H/\(^{13}\)C) were established with the help of the HMQC spectrum. The chemical shifts of the various carbons corresponded well with the values reported in the literature.
for these type of compounds [25, 204]. The compound has previously been isolated [162] but this is its first report from *B. obliqua*. 
Table 3.7: $^1$H/$^{13}$C-NMR connectivities (HMOC) for compound 89 in CDCl$_3$.

<table>
<thead>
<tr>
<th>Carbon No</th>
<th>Chemical Shift ($\delta$)</th>
<th>Multiplicity (DEPT)</th>
<th>$^1$H/$^{13}$C Connectivity ($J$=Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>*</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>122.5</td>
<td>CH</td>
<td>6.88 (d, $J$=3.7)</td>
</tr>
<tr>
<td>4</td>
<td>132.5</td>
<td>CH</td>
<td>6.99 (d, $J$=3.7)</td>
</tr>
<tr>
<td>5</td>
<td>121.1</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>*</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>3'</td>
<td>124.0</td>
<td>CH</td>
<td>6.93 (d, $J$=3.5)</td>
</tr>
<tr>
<td>4'</td>
<td>126.0</td>
<td>CH</td>
<td>6.64 (dq, $J$=0.8, 3.5)</td>
</tr>
<tr>
<td>5'</td>
<td>140.0</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>6'</td>
<td>14.1</td>
<td>CH$_3$</td>
<td>2.48 (d, $J$=0.8)</td>
</tr>
<tr>
<td>1''</td>
<td>77.5</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2''</td>
<td>90.5</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3''</td>
<td>22.7</td>
<td>CH$_2$</td>
<td>2.76 (t, $J$=6.9)</td>
</tr>
<tr>
<td>4''</td>
<td>62.1</td>
<td>CH$_2$</td>
<td>4.22 (t, $J$=6.9)</td>
</tr>
<tr>
<td>1a</td>
<td>*</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>2a</td>
<td>20.9</td>
<td>CH$_3$</td>
<td>2.08 (s)</td>
</tr>
</tbody>
</table>

* = Carbon did not appear.
Proposed biosynthesis of

5'-Methyl-5-(4-acetoxy-1-hexynyl)-2,2'-bithiophene (89).

\[
\begin{align*}
\text{CH}_3 & \text{--(C\equiv C)_2--CH=CH}_2 & 24 \\
\text{[H}_2\text{S]} & \\
\text{CH}_3 & \text{--(C\equiv C)}_2\text{--S--C\equiv C--CH=CH}_2 \\
\text{[H}_2\text{S]} & \\
\text{CH}_3\text{--S--S--C\equiv C--CH=CH}_2 & \\
\text{[O], [Esterification]} & \\
\text{CH}_3\text{--S--S--C\equiv C--CH}_2\text{--CH}_2\text{--O--C--CH}_3 &
\end{align*}
\]
3.4.6 5'-Methyl-5-(4-hydroxy-1-butyyl)-2,2'-bithiophene (90):

Column chromatography of the pet.-ether soluble part of the methanolic extract of *Blumea obliqua* yielded 90 in the semi-purified form which was purified by prep. TLC.

![Chemical Structure of 5'-Methyl-5-(4-hydroxy-1-butyyl)-2,2'-bithiophene (90)](image)

The FDMS exhibited the molecular ion peak [M⁺] at m/z 247.8. The peak matching of [M⁺] corresponded to the exact mass of m/z 248.0324 which agreed well with the molecular formula C₁₃H₁₂O₂S₂ with eight degrees of unsaturation.

The IR spectrum (CHCl₃) displayed intense absorption peaks at 3630 (OH) and 2860 (C—H) cm⁻¹ indicating the presence of a hydroxyl function and the (C—H) of the thiophene rings. The UV spectrum (MeOH) showed an absorption maximum at 322.0 nm.

The ¹H-NMR (CDCl₃, 500 MHz) was very conclusive in elucidating the structure and exhibited diagnostic signals of 2,2'-bithiophene ring system at 6 6.64 (1H, dq, J=1.1, 3.5 Hz), 6.89 (1H, d, J=3.8 Hz), 6.93 (1H, d, J=3.5 Hz) and 7.01 (1H, d, J=3.8 Hz). These values were assigned to H-4', H-3, H-3' and
H-4 protons, respectively. A pair of triplets at δ 2.70 and 3.80, each with a coupling constant of 6.2 Hz, was attributed to H-3′′ and H-4′′ methylene protons, respectively. A down field shift in the chemical shift of H-4′′ proton was due to the attachment of the hydroxyl group to C-4′′. The ring methyl protons (H-6′) resonated at δ 2.46 as a doublet with a coupling constant of 1.1 Hz, due to the allylic coupling with H-4′ proton. The spectroscopic data of 90 was similar to that reported in the literature for this compound [162]. The compound has been reported earlier [162] but isolated from B. obliqua for the first time.
Proposed biosynthesis of

5'-Methyl-5-(4-hydroxy-1-butynyl)-2,2'-bithiophene (90).

\[ \text{CH}_3-(\text{C}≡\text{C})_5-\text{CH}=\text{CH}_2 \rightarrow \text{CH}_3-(\text{C}≡\text{C})_2-\text{S}-\text{C≡C-CH}=\text{CH}_2 \rightarrow \text{CH}_3-\text{S}-\text{S}-\text{C≡C-CH}=\text{CH}_2 \rightarrow \text{CH}_3-\text{S}-\text{S}-\text{C≡C-CH}≡\text{CH}_2-\text{CH}_2\text{-OH} \]
3.4.7 5'-Acetomethyl-5-(3-buten-1-ynyl)-2,2'-bithiophene (91):

Compound 91 was isolated from the pect.-ether soluble part of the methanolic extract of *Blumea obliqua* by repeated column chromatography and prep. TLC.

![Chemical Structure](image)

The electron impact mass spectrum (EIMS) showed a molecular ion peak [M⁺] at m/z 287.8 and an [M+2]⁺ peak at m/z 289.8 (11.6% of the [M⁺]) indicating the presence of sulfur in the compound. The high resolution electron impact mass spectrum (HREIMS) of the molecular ion peak resulted in the exact value of m/z 288.0277 which corresponded well with the molecular formula C₁₅H₁₂O₂S₂. The molecular formula showed ten degrees of unsaturation in the compound.

The ¹H-NMR spectrum exhibited a singlet of three protons at δ 2.08 which was assigned to H-2a protons. The loss of 59 amu from the molecular ion peak also confirmed the presence of an acetate moiety. A singlet of two protons at δ 5.19 was assigned to the methylene protons at position 6'. Three doublets of doublet δ 5.54 (J=1.9, 11.3 Hz), 5.72 (J=1.9, 17.5 Hz) and 6.01 (J=11.3, 17.5 Hz) were characteristic of an end vinyl group and were due to H-9 (cis), H-9 (trans) and
H-8 protons, respectively. In the aromatic region the spectrum exhibited four doublets of one proton each at δ 6.97, (J=3.6 Hz), 7.00 (J=3.8 Hz), 7.02 (J=3.6 Hz) and 7.07 (J=3.8 Hz) which were the diagnostic signals of the presence of a 5,5'-substituted 2,2'-bithiophene ring system. These values were assigned to H-4', H-3, H-3' and H-4 protons, respectively. These assignments were also confirmed with the help of COSY-45° which showed correlations between H-3'/H-4', H-3/H-4, H-8/H-9 (cis), H-8/H-9 (trans) and H-9 (cis)/H-9 (trans). The COSY-45° interactions are shown in fig 91 a.

The 13C-NMR spectrum (broad band) featured all the fifteen carbon atoms. The multiplicity of the various carbon atoms were established with the help of the broad band and DEPT experiments which confirmed the presence of one methyl, two methylenes, five methines and seven quaternary carbon atoms. The signals at δ 83.0 and 93.1 were due to acetylenic carbon atoms and were assigned to C-6 and C-7, respectively. The proton-carbon connectivities (1H/13C) were established with the help of the HMQC spectrum and the chemical shifts (Table 3.8) for the various carbon atoms were assigned on the
basis of the HMBC spectrum. These values were in accordance with the values reported in the literature for similar type of compounds [25, 204]. The HMBC correlations are presented in 91 b.

This compound has previously been isolated from many species of the Asteraceae [153] but this is its first report from *Blumea obliqua*. 
Table 3.8: $^{1}H/^{13}C$-NMR connectivities (HMOC) for compound 91 in CDCl$_3$

<table>
<thead>
<tr>
<th>Carbon No</th>
<th>Chemical Shift (δ)</th>
<th>Multiplicity (DEPT)</th>
<th>$^{1}H/^{13}C$ Connectivity (J=Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>138.1</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>123.7</td>
<td>CH</td>
<td>7.00 (d, J=3.8)</td>
</tr>
<tr>
<td>4</td>
<td>132.8</td>
<td>CH</td>
<td>7.07 (d, J=3.8)</td>
</tr>
<tr>
<td>5</td>
<td>122.2</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>138.6</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>3'</td>
<td>123.7</td>
<td>CH</td>
<td>7.02 (d, J=3.6)</td>
</tr>
<tr>
<td>4'</td>
<td>129.0</td>
<td>CH</td>
<td>6.97 (d, J=3.6)</td>
</tr>
<tr>
<td>5'</td>
<td>137.6</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>6'</td>
<td>60.5</td>
<td>CH$_2$</td>
<td>5.19 (s)</td>
</tr>
<tr>
<td>6</td>
<td>83.0</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>93.1</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>116.8</td>
<td>CH</td>
<td>6.01 (dd, J=11.3, 17.5)</td>
</tr>
<tr>
<td>9</td>
<td>127.1</td>
<td>CH$_2$</td>
<td>5.54 (dd, J=1.9, 11.3) (cis)</td>
</tr>
<tr>
<td>9</td>
<td>127.1</td>
<td>CH$_2$</td>
<td>5.72 (dd, J=1.9, 17.5) (trans)</td>
</tr>
<tr>
<td>1a</td>
<td>172.0</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>2a</td>
<td>20.9</td>
<td>CH$_3$</td>
<td>2.08 (s)</td>
</tr>
</tbody>
</table>
Proposed biosynthesis of

5′-Acetomethyl-5-(3-butene-1-ynyl)-2,2′-bithiophene (91).

\[
\begin{align*}
\text{CH}_3-(C≡C)_5-CH=CH_2 & \quad 24 \\
& \xrightarrow{[H_2S]} \\
\text{CH}_3-(C≡C)_2-\text{S} & \quad \text{C≡C-CH=CH}_2 \\
& \xrightarrow{[H_2S]} \\
\text{CH}_3 & \quad \text{S} & \quad \text{S} & \quad \text{C≡C-CH=CH}_2 \\
& \xrightarrow{[Q, \text{Esterification}]} \\
\text{H}_3C-\text{C-O-CH}_2 & \quad \text{S} & \quad \text{S} & \quad \text{S} & \quad \text{C≡C-CH=CH}_2
\end{align*}
\]
3.4.8 5'-Hydroxymethyl-5-(3-butene-1-ynyl)-2,2'-bithiophene (92):

Pet-ether soluble part of the methanolic extract of *Blumea obliqua* was chromatographed on silica gel using different proportions of pet-ether and chloroform as eluent. Elution with 20% chloroform in pet-ether yielded 92 in semi-purified form and was purified by prep. TLC.

![Chemical Structure of 5'-Hydroxymethyl-5-(3-butene-1-ynyl)-2,2'-bithiophene (92)](image)

The electron impact mass spectrum (EIMS) exhibited a molecular ion peak \([M^+]\) at \(m/z\) 245.7 and the high resolution electron impact mass spectrum (HREIMS) gave an exact value of \(m/z\) 246.0155 which was in accordance with the molecular formula \(C_{13}H_{10}OS_2\). The molecular formula showed nine degrees of unsaturation in the compound.

The UV spectrum (\(Et_2O\)) displayed absorption maximum at 347.2 nm, characteristic of a 2,2'-bithiophene acetylenic chromophore conjugated with a double bond [25].

In the \(^1H\)-NMR spectrum a doublet of two protons appeared at \(\delta\) 4.77 (\(J=0.8\) Hz) which was assigned to H-6'. Three doublet of doublets at \(\delta\) 5.52 (\(J=2.0, 11.1\) Hz), 5.69 (\(J=2.0, 17.5\) Hz) and 6.00 (\(J=11.1, 17.5\) Hz) were characteristic of an unsubstituted terminal methylene group and were assigned to H-9 (cis),
H-9 (trans) and H-8 protons, respectively. The presence of four protons at δ 6.88 (dq, J=0.8, 3.6 Hz), 6.99 (d, J=3.8 Hz), 7.00 (d, J=3.6 Hz) and 7.08 (d, J=3.8 Hz) suggested the presence of a 5,5'-substituted 2,2'-bithiophene system in the molecule. These protons were assigned to H-4', H-3, H-3' and H-4, respectively. The value of the coupling constant of H-4' proton suggested its allylic attachment to H-6' proton which was also confirmed by the COSY-45° spectrum. The COSY-45° spectrum also exhibited strong correlations for H-4'/H-3', H-4/H-3, H-8/H-9 (cis), H-8/H-9 (trans) and H-9 (cis)/H-9 (trans) protons. The complete COSY-45° interactions are presented in 92 a.

The 13C-NMR (broad band) exhibited signals for all the thirteen carbons. The multiplicities of the various carbons were confirmed with the help of the DEPT experiments which showed that two methylenes, five methines and six quaternary carbons were present in the molecule. The chemical shifts of the various carbon atoms (Table 3.9) were assigned with the help of HMQC and HMBC spectra and were according to the values reported in the literature for
these type of compounds [25, 204]. The complete HMBC correlations are shown in 92 b. The compound has earlier been reported [208] from many species of the Asteraceae but isolated for the first time from B. obliqua.

\[ \text{Diagram} \]

92 b

3.4.8.1 Biological Activity:

Antifungal and antibacterial activity:

Photometric microtiter broth dilution method [205] was used to evaluate the antifungal and antibacterial activities of compound 92. It showed potent antifungal activity against Microsporum canis, Pleurotus ostreatus, Alternaria solani, Curvularia lunata, Epidermophyton floccosum, Trichophyton schoenleinii, T. longifusus, Pseudallescheria boydii, Candida albicans, Trichophyton mentagrophytes, T. simii, Fusarium solani, Macrophomina phaseolina and Rhizoctonia solani. The MIC values of 92 against various fungi are shown in Table 3.10. The table also shows the MIC values of compound 92 against various bacteria tested.
Antileishmanial Activity:

Compound 92 was tested for in vitro antileishmanial activity [206] against promastigotes of *Leishmania major* and was found to be highly leishmanicidal with IC\(_{100}\) value of 12.5 μg/ml in the dark. Under similar conditions the IC\(_{100}\) of the reference compound, pentamidine, was 100 μg/ml. When exposed to long wave UV light along with control its IC\(_{100}\) value was less than 0.1 μg/ml while that of pentamidine did not change.
Table 3.9: $^{13}$C-NMR data and $^1H/^{13}$C connectivities (HMOC) for compound 92.

<table>
<thead>
<tr>
<th>Position</th>
<th>Chemical Shift ($\delta$)</th>
<th>Multiplicity (DEPT)</th>
<th>$^1H/^{13}$C Connectivity ($J=Hz$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>138.5</td>
<td>C</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>123.4</td>
<td>CH</td>
<td>6.99 (d, $J=3.8$)</td>
</tr>
<tr>
<td>4</td>
<td>132.8</td>
<td>CH</td>
<td>7.07 (d, $J=3.8$)</td>
</tr>
<tr>
<td>5</td>
<td>121.9</td>
<td>C</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>83.1</td>
<td>C</td>
<td>--</td>
</tr>
<tr>
<td>7</td>
<td>93.0</td>
<td>C</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>116.8</td>
<td>CH</td>
<td>6.00 (dd, $J=11.1, 17.5$)</td>
</tr>
<tr>
<td>9</td>
<td>126.9</td>
<td>CH$_2$</td>
<td>5.52 (dd, $J=2.0, 11.1$) cis</td>
</tr>
<tr>
<td>9</td>
<td>126.9</td>
<td>CH$_2$</td>
<td>5.69 (dd, $J=2.0, 17.5$) trans</td>
</tr>
<tr>
<td>2'</td>
<td>138.9</td>
<td>C</td>
<td>--</td>
</tr>
<tr>
<td>3'</td>
<td>123.8</td>
<td>CH</td>
<td>7.00 (d, $J=3.6$)</td>
</tr>
<tr>
<td>4'</td>
<td>126.1</td>
<td>CH</td>
<td>6.88 (d, $J=3.6$)</td>
</tr>
<tr>
<td>5'</td>
<td>143.8</td>
<td>C</td>
<td>--</td>
</tr>
<tr>
<td>6'</td>
<td>60.1</td>
<td>CH$_2$</td>
<td>4.77 (d, $J=0.8$)</td>
</tr>
</tbody>
</table>
Table 3.10: MIC values of compound 92 against tested fungi and bacteria.

<table>
<thead>
<tr>
<th>Name of Fungi</th>
<th>MIC (µg/100µl)</th>
<th>Name of Bacteria</th>
<th>MIC (µg/100µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichophyton schoenleinii</em></td>
<td>100</td>
<td><em>Bacillus cereus</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Trichophyton longifuses</em></td>
<td>150</td>
<td><em>Corynebacterium diptheriae</em></td>
<td>125</td>
</tr>
<tr>
<td><em>Pseudallescheria boydii</em></td>
<td>150</td>
<td><em>Escherichia coli</em></td>
<td>125</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>150</td>
<td><em>Shigella sonii</em></td>
<td>75</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>100</td>
<td><em>Proteus mirabilis</em></td>
<td>125</td>
</tr>
<tr>
<td><em>Trichophyton simii</em></td>
<td>100</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>75</td>
</tr>
<tr>
<td><em>Fusarium solani</em></td>
<td>100</td>
<td><em>Salmonella typhi</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Macrophomina phaseolina</em>.</td>
<td>100</td>
<td><em>Shigella boydii</em></td>
<td>150</td>
</tr>
<tr>
<td><em>Rhizoctonia solani</em>.</td>
<td>100</td>
<td><em>Staphylococcus aureus</em></td>
<td>125</td>
</tr>
<tr>
<td><em>Microsporum canis</em></td>
<td>150</td>
<td><em>Streptococcus pyogenes</em></td>
<td>200</td>
</tr>
</tbody>
</table>
Proposed biosynthesis of

5'-Hydroxymethyl-5-(3-butenyl-1-ynyl)-2,2'-bithiophene (92).

\[
\begin{align*}
\text{CH}_3 & \text{(CC)}_5 \text{CH=CH}_2 \\
\text{[H}_2\text{S]} & \\
\text{CH}_3 & \text{(CC)}_2 \text{S} \text{C=C-CH=CH}_2 \\
\text{[H}_2\text{S]} & \\
\text{CH}_3 & \text{S} \text{S} \text{C=C-CH=CH}_2 \\
\text{[O]} & \\
\text{HO-CH}_2 & \text{S} \text{S} \text{C=C-CH=CH}_2
\end{align*}
\]
3.4.9 5-(3-Butene-1-ynyl)[2,2'-bithiophene]-5'-carboxaldehyde (93):

Compound 93 was isolated from column chromatography and prep. TLC of the pet.-ether soluble part of the methanolic extract.

The molecular ion peak [M⁺] was observed in the electron impact mass spectrum (EI-MS) at m/z 243.7 with a strong [M+2]⁺ peak showing that this compound also contained sulfur. The high resolution electron impact mass spectrum (HREIMS) of the molecular ion peak corresponded with an accurate value of 243.9994 which agreed to an acceptable molecular formula, C₁₃H₉OS₂. This formula showed ten degrees of unsaturation in the compound.

The UV spectrum (Et₂O) displayed an absorption maximum at 370.0 nm. With the help of literature [25] it could be concluded that the absorption at a longer wave length was due to the extension of conjugation on 5-(3-butene-1-ynyl)-2,2'-bithiophenyl skeleton.

The ¹H-NMR spectrum also suggested the presence of an terminal vinyl group and two thiophene rings. Three doublet of doublets at δ 5.58 (J=1.9, 11.2 Hz), 5.73 (J=1.9, 17.5 Hz) and 6.01 (J=11.2, 17.5 Hz) were due to the presence of an end vinyl group and were assigned to H-9 (cis), H-9 (trans) and H-8 protons,
respectively. In the aromatic region of the spectrum four peaks, each for one proton, appeared as doublets at δ 7.13 (J=3.8 Hz), 7.21 (J=3.9 Hz), 7.23 (J=3.8 Hz) and 7.65 (J=3.9 Hz). These signals were assigned to the four thiopheneic protons. The literature shows [25] that the proton of the thiophene ring attached to a carbon to which an allylic carbonyl is present resonates at about δ 7.58. By this comparison, the doublet at δ 7.65 (J=3.9 Hz) could be assigned to H-4'. As the coupling constant for the doublet at δ 7.23 is also 3.9 Hz, so this value could be assigned to H-3' proton. Similarly, it is also confirmed from the literature [25] that H-4 always appears down field to H-3 when an acetylenic carbon is attached to C-5. By analogy to this fact, it could be decided that H-4 proton resonated at δ 7.21 (J=3.8 Hz) and H-3 at 7.13 (J=3.8 Hz). A singlet of one proton at δ 9.85 was assigned to the aldehydic proton. This confirmed the structure of compound 93. Earlier, Bohmman et al. has mentioned 93 from *Dyssodia acerosa* [158], but no structural data exist anywhere in the literature.
Proposed biosynthesis of

5-(3-Butene-1-ynyl)[2,2'-bithiophene]-5'-carboxaldehyde (93).

\[
\begin{align*}
\text{CH}_3-(\text{C} \equiv \text{C})_3-\text{CH} \equiv & \text{CH}_2 \\
\rightarrow & \ [\text{H}_2\text{S}] \\
\uparrow & \\
\text{CH}_3-(\text{C} \equiv \text{C})_2-\text{S} \uparrow & \text{C} \equiv \text{C} \rightarrow \text{CH} \equiv & \text{CH}_2 \\
\rightarrow & \ [\text{H}_2\text{S}] \\
\uparrow & \\
\text{S} \quad \text{S} & \quad \text{S} \quad \text{S} \quad \text{C} \equiv \text{C} \rightarrow \text{CH} \equiv & \text{CH}_2 \\
\rightarrow & \ [\text{O}] \\
\quad \text{H} & \quad \text{S} \quad \text{S} \quad \text{S} \quad \text{C} \equiv \text{C} \rightarrow \text{CH} \equiv & \text{CH}_2
\end{align*}
\]
3.4.10 *α*-Taraxasteryl acetate (94):-

The compound 94 was obtained from the fractions of silica gel column upon elution of the pet.-ether soluble part of the methanolic extract with pet.-ether.

![Chemical Structure of 94](image)

The electron impact mass spectrum (EI-MS) showed a molecular ion peak $[M^+]$ at m/z 468. The high resolution electron impact mass spectrum (HREI-MS) of the molecular ion peak gave an exact value of m/z 468.7660, corresponding to the molecular formula C$_{32}$H$_{52}$O$_2$. The IR spectrum revealed the presence of an acetate group (1725 and 1320 cm$^{-1}$) and an exocyclic double bond (1640 and 880 cm$^{-1}$).

The $^1$H-NMR spectrum exhibited signals for vinylic protons at δ 4.60 and 4.68 (br. s, 2H each), an equatorial acetoxy group (dd at δ 4.52, 1H, H-3β and a singlet at δ 2.08, 3H, OCOCH$_3$), one secondary methyl group at δ 0.9 and six tertiary methyl groups at δ 0.81 x 2 (Me-24, Me-28), 0.83 (Me-25), 1.01 x 2 (Me-23, Me-26) and 1.04 (Me-27). The mass spectrum of 94 showed
characteristic fragmentation pattern of pentacyclic taraxastane type triterpene with saturation in rings A, B, C and D [209-210]. The $^{13}$C-NMR exhibited thirty two carbon atoms. The DEPT experiments revealed the presence of seven methyl, eleven methylene and six methine carbon atoms.

The compound 94 corroborated to be taraxasteryl acetate by co-TLC with an authentic sample and through direct comparison of physical constants and spectral data to those already reported in literature [211-212].
3.4.11 \( \beta \)-Sitosterol (24-R Stigmast-5-ene-3-\( \beta \)-ol) (95):

\( \beta \)-Sitosterol was obtained from the column chromatography (silica gel) of the pet.-ether soluble part of the methanolic extract.

The melting point of 95 was found to be 135-136\(^\circ\)C, optical rotation \([\alpha]_D^{20}\) 25-35.5 (CHCl\(_3\)), UV (MeOH) absorption at 250 nm and maximum absorption in the IR spectrum (CHCl\(_3\)) were at 3450 (OH), 3050 (C-H), 2900 (C-H) and 1680,1460 (C=C) cm\(^{-1}\). The ElMS displayed a molecular ion peak \([M^+]\) at \(m/z\) 414. The HREiMS showed an exact value of 414.4091 which corresponded to the molecular formula \(C_{29}H_{50}O\). The other prominent fragments appeared at \(m/z\) 399 ([M-CH\(_3\)]\(^+\)), 396 ([M-H\(_2\)O]\(^+\)), 381 ([M-CH\(_3\)-H\(_2\)O]\(^+\)), 329 ([M-C\(_5\)H\(_7\)-H\(_2\)O]\(^+\)), 303 ([M-C\(_5\)H\(_7\)-H\(_2\)O]\(^+\)), 273 ([M-side chain]\(^+\)) and 255 ([M-side chain-H\(_2\)O]\(^+\)) which were characteristic of steroe with double bond at C-5 [213]

The \(^1\)H-NMR of the compound exhibited five methyl signals at \(\delta\) 0.76 (H-18) and 0.83-0.99 (H-21, 26, 27 and 29). The signals for 14-3\( \beta \) and 16-6 were found
at δ 3.20 and 5.34. The $^{13}$C-NMR spectrum showed signals for all the twenty nine carbons. The mass fragmentation pattern and $^1$H and $^{13}$C-NMR data showed close identity of 95 to that reported for β-sitosterol [214]. It was further confirmed by co-TLC and mix melting point with an authentic sample of β-sitosterol.
3.4.12 *Lupeol* (96)

Lupeol 96 was isolated by repeated column chromatography of the fractions which yielded 95.

![Chemical Structure of Lupeol](image)

It showed a molecular ion peak in its electron impact mass spectrum (EIMS) at *m/z* 426 which showed an exact value of 426.6998 in high resolution mass spectrum, corresponding to the molecular formula C\textsubscript{30}H\textsubscript{50}O. The IR spectrum displayed a strong absorption band for a hydroxyl group at 3440 cm\textsuperscript{-1} and for a C=CH\textsubscript{2} group at 3070, 1650 and 880 cm\textsuperscript{-1}. The \textsuperscript{1}H-NMR spectrum revealed the presence of seven tertiary methyl groups (singlets at δ 0.76, 0.85, 0.90, 0.96 x 2, 1.05 and 1.59), along with a multiplet at δ 4.63 which was assigned to the olefinic protons. The signal at δ 3.65 (dd, *J*=4.2, 10.6 Hz) was attributed to a proton geminal to alcoholic group. Further information about the structure of the compound was provided by its mass spectrum which exhibited typical fragmentation pattern for pentacyclic triterpene of the lupane series [215-216].

The \textsuperscript{13}C-NMR spectrum of the compound showed all the thirty carbon atoms, the multiplicity assignments of each of these was determined by performing the
DEPT experiments. The spectral data of 96 was in complete agreement to those previously reported for lupeol [217-218].
3.4.13 3-O-[β-D-Glucopyranosyl]-β-sitosterol (97):

3-O-[β-D-Glucopyranosyl]-β-sitosterol was obtained from the elution of the main column with chloroform methanol (9:1).

The IR spectrum of 97 revealed the presence of hydroxyl group (3400 cm⁻¹). The electron impact mass spectrum (EIMS) showed the absence of molecular ion peak and the highest peak appeared at m/z 396 which was due to the loss of glucose moiety from the molecular ion peak. The negative ion fast atom bombardment mass spectrum (FABMS) exhibited [M-H]⁻ peak at m/z 575. The fragment which appeared at m/z 413 confirmed the loss of sugar moiety.
The $^1$H-NMR spectrum showed two singlets due to tertiary methyls at $\delta$ 0.64 and 0.95 which were assigned to Me-18 and Me-19, respectively. Other methyl signals appeared as doublets at $\delta$ 0.78 (Me-27), 0.80 (Me-26) and 0.89 (Me-21). A 3H triplet at $\delta$ 0.82 was due to Me-29. The anomeric proton of the sugar [H-1'] resonated as a doublet at $\delta$ 4.21 due to diaxial coupling, thus showing that the sugar moiety was $\beta$-linked to sitosterol.

The acid hydrolysis of 97 with 2M HCl in aqueous methanol yielded aglycone 97a which was identified as $\beta$-sitosterol by comparing with spectral data reported in the literature [214]. The sugar obtained from the hydrolysis was identified as glucose on silica gel TLC as well as by paper chromatography, by comparing with an authentic sample of glucose.

The $^{13}$C-NMR data deduced the $\beta$-D-pyranosyl configuration for glucose [219-220].
4.0 EXPERIMENTAL
4.1 General Experimental

All chemical and instrumental analyses were performed at the International Center for Chemical Sciences, H. E. J. Research Institute of Chemistry, University of Karachi, Karachi. All solvents used for TLC were purchased from E. Merck. For other chromatographic techniques commercially available solvents were used after distillation at their respective boiling points. Distilled pet.-ether was collected between 64 °C and 68 °C.

Instrumental Details

All melting points were recorded in glass capillary tubes using Büchi melting point apparatus. Optical rotations were measured on JASCO DIP-360 (Japan Spectroscopic Co. Ltd., Tokyo, Japan) digital polarimeter.

Spectroscopy

The UV Spectra were recorded on Shimadzu UV-240 (Shimadzu Corporation, Kyoto, Japan) spectrophotometer.

The IR spectra were recorded on Jasco IRA-1 (Japan Spectroscopic Co. Ltd., Tokyo, Japan) and Shimadzu IR-460 (Shimadzu Corporation, Kyoto, Japan) instrument.

The Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker AM 300 FT NMR, AM 400 FT NMR and AM 500 FT NMR spectrometers using TMS as internal standard.

The $^{13}$C-NMR spectra were recorded at 75, 100 and 125 MHz on Bruker AM 300 FT NMR, AM 400 FT NMR and AM 500 FT NMR spectrometers respectively.

Mass spectra were recorded on Varian-MAT 112s and Finnigan MAT-112 and 312A double focusing mass spectrometers connected to DEC PDP 11/34 and
IBM-AT compatible PC based system, respectively. Electron impact, peak matching, field desorption (FD) and fast atom bombardment (Neg. FAB) experiments were performed on MAT-312A or Jeol-JMS HX-110 mass spectrometers. FABMS were recorded in a glycerol-water (1:1) matrix in the presence of KI. High resolution electron impact mass spectra were recorded on a Jeol-JMS H x 110 mass spectrometer.

Chromatography

Column chromatography was performed with silica gel (E. Merck, 60 and 70-230 mesh). TLC and preparative TLC was performed on silica (GF254) precoated TLC plates (E. Merck and Riedel De Haen). UV lamp (250 nm) and ceric sulphate spray were used for visualization.

4.2 Antimicrobial Assays:

Micro-organism and Media:

Media employed in the tests were Sabouraud Dextrose Broth and Nutrient Broth, except for compound 86, where Sabouraud Dextrose Agar was used. All pathogenic microbes were clinical isolates and kindly provided by Microbiology Department, University of Karachi, Karachi.

Bacteria and Yeast: were grown over night in NB and SDB respectively at 37 °C. Cell suspensions in sterile media were adjusted to give a final concentration of 10^2-10^6 viable cells/ml.

Dermatophytes: In case of dermatophytes spore suspensions were obtained by flooding the tubes with 0.85% saline and diluted to make inocula containing 2.5-3.0 x 10^6 cells or spores with colony forming ability per milliliter [221].
Antimicrobial assays:

Antimicrobial assays were performed by photometric microtiter broth dilution protocol (by tube dilution method in case of compound 86). The test samples were first dissolved in hexane then serial two-fold dilutions were made in media in 96-well microtiter plates to give different concentrations ranging from 1 mg/ml to 0.067 mg/ml. Using a multi-channel micropipette, an inoculum of 5μL of the cell/spore suspension was added to their respective wells. The plates were incubated for 24, 36 hours to 5 days [205] according to the control microbes growth at 28-30 and 37°C depending on optimal growth conditions.

Microbial growth in the sample containing media were determined measuring their turbidity or O.D. values at 540 [222] and 600 nm [223] for fungi and bacteria respectively using 96 well-microtiter plate reader (Spectramax 340, Molecular Devices, USA) and growth inhibition was calculated with reference to the negative control.

In case of compound 86, the antifungal activity was performed by tube dilution method [207]. Compound was serially diluted in DMSO and added to molten SDA in order to make slants. The slants were inoculated with the fungus and incubated at 29°C for seven days after which they were observed for the inhibition of growth and MIC values were determined.

In Vitro Antileishmanial Activity:

The parasites were obtained from open ulcerated lesion of infected patients. The pus containing parasites was inoculated aseptically into liquid phase (medium 199 + 10% heat inactivated fetal calf serum (FCS)) of solid biphasic medium (NNN) composed of 0.9% NaCl, 1.1% plain agar, 0.35% brain heart infusion (BHI) and 25% v/v heat inactivated (56°C for 30 min.) rabbit blood. The tubes were kept at 21-23°C in dark for 3-5 days and presence of leishmania promastigotes was confirmed microscopically.
The isolated parasites were immediately sub-cultured in liquid medium RPMI-1640 + 10% heat inactivated fetal calf serum (FCS) + 2% human urine buffered with 20 M TES buffer pH 7.4. The culture was kept at 21-23 °C in dark. The parasite strain was confirmed by isoenzyme analysis [224] and found to be *Leishmania major* (MHOM/PK/88/DESTO).

The compound was dissolved in 5% MeOH/phosphate buffer (PBS, pH 7.4) so that the final concentration was 1.0 mg/ml. The toxicity of MeOH was checked by adding different amounts of solvents (0.1 to 5%) to suspension of *Leishmania* culture. After three days the value of non-toxic dilution was found to be 0.62% and a % inhibition of 10% was observed between 0.62 and 1.25% MeOH.

To study the effect, 4x10^6 promastigotes/ml were taken in 96-well microtiter plate and the compounds were added and diluted serially so that the minimum concentration of the compound was less than 0.1µg/ml where as the maximum concentration was 100 µg/ml. Only methanol/PBS was added in control. The culture was left for 3-5 days in dark and growth was assessed by counting the parasites microscopically on an improved neubauer chamber. Pentamidine, a known antileishmanial drug, was tested simultaneously for comparison [206].

**UV exposure:**

The 96-well microtiter plate was half foiled. The well under the foil were used for dark control and photoactivated leishmanicidal activity of 92 was assessed in the exposed wells (along with control) under a UV light source [6Watt Black Light FL6BL, Toshiba-Japan (350-400 nm)] from a distance of 20 cm for three hours at 23 °C. After this the plate was kept in dark at 21-23 °C for three days and the growth was assessed.
4.3 Plant Material

The fresh plant (aerial parts of *Blumea obliqua*, 20 Kg) was collected from Mangopir near Karachi in May 1991. A voucher specimen was deposited in the herbarium of the Department of Botany, University of Karachi (herbarium No. 63484 KUH).

Aerial parts of *B. obliqua* (60 Kg fresh wt.) were recollected in July 1995 to work on some minor components.

4.4 Extraction and Isolation

The fresh plant (20 Kg) was chopped and macerated twice for 10 days with methanol. The combined extract was filtered and evaporated under reduced pressure to a viscous mass (480 g) which was partitioned between methanol-water (9:1) and pet.-ether. The pet.-ether soluble portion was evaporated under reduced pressure to an oily paste (57 g) which was chromatographed on a silica gel column and eluted with pet.-ether, pet.-ether/chloroform and chloroform. Repeated column chromatography and prep. TLC of the various fractions yielded compounds 85, 86, 87, 89, 90, 91, 94, 95 and 96.

The second collection of *B. obliqua* (60 Kg fresh weight) was air dried, chopped and macerated twice for 7 days in methanol. The extract was filtered and evaporated to a viscous mass (1132 g) which was extracted with methanol-water (9:1) and pet.-ether. The pet.-ether soluble part was evaporated under reduced pressure to an oily paste (51 g) and was loaded onto a silica gel column and eluted with pet.-ether, pet.-ether/chloroform and chloroform. Repeated column chromatography and prep. TLC of the various fractions yielded compounds 88, 92, 93 and 97 along with the previously isolated compounds.
4.4.1 5'-Methyl-5-[4-(3-methyl-1-oxohutoxy)-1-butylnyl]-2,2'-bithiophene (85):

Compound 85 was isolated from the pet.-ether soluble part of the methanolic extract of *Blumea obliqua*. It was eluted from the silica gel column with 10% chloroform in pet.-ether and was further purified by repeated column chromatography (silica gel). Finally, preparative TLC developed in chloroform-pet.-ether (1:1, Rf=0.5) afforded 85 (50 mg, yellowish oil).

EIMS m/z (rel. int. %): 332.0903 [C₁₈H₂₀O₂S₂, M⁺] (19), 230.0186 [M-C₅H₁₀O₂] (100), 217 (9), 197 (5), 115 (7), 102 (4).

UV λ_{MAX} (Et₂O): 339 nm (ε 26,726).

IR ν_{MAX} (CCl₄): 2850 (C—H stretching), 2300(C≡C), 1717 (ester carbonyl), 1600 (C=C) cm⁻¹.

¹H-NMR (CDCl₃, 300 MHz): δ 0.97 (6H, d, J=6.5 Hz, H-4a), 2.12 (1H, m, H-3a), 2.22 (2H, d, J=6.5 Hz, H-2a), 2.48 (3H, d, J=1.0 Hz, H-6'), 2.78 (2H, t, J=6.7 Hz, H-3''), 4.24 (2H, t, J=6.7 Hz, H-4''), 6.64 (1H, dq, J=1.0, 3.5 Hz, H-4'), 6.88 (1H, d, J=3.7 Hz, H-3), 6.93 (1H, d, J=3.5 Hz, H-3'), 6.99 (1H, d, J=3.7 Hz, H-4).

¹³C-NMR (CDCl₃, 75 MHz): δ 15.3 (C-6'), 20.4 (C-3''), 22.4 (2xC-4a), 25.8 (C-3a), 43.4 (C-2a), 60.6 (C-4''), 75.3 (C-1''), 90.5 (C-2''), 121.6 (C-5) 122.5 (C-3), 124.3 (C-3'), 126.0 (C-4'), 132.5 (C-4), 134.5 (C-2'), 138.6 (C-2), 139.8 (C-5'), 172.8 (C-1a).
4.4.2 5'-Hydroxymethyl-5-(butyl-3-ene-1-yn)-2,2'-bithiophene (86):

Compound 86 was isolated from the pet.-ether soluble part of the methanolic extract of *Blumea obliqua*. It was eluted from the silica gel column with 10% chloroform in pet.-ether along with compound 86 as a semi-purified fraction which was purified by repeated column chromatography (silica gel) and finally by preparative TLC (chloroform:pet.-ether 1:1, Rf=0.6) as a yellow labile oil (80 mg).

ElMS m/z (rel. int %): 330.0750 [C_{15}H_{18}O_{2}S_{2}, M^+] (38), 229.0107 [M-C_{5}H_{9}O_{2}] (100).

UV λ_{MAX} (Et_{2}O): 344 nm (ε 27,620).

IR ν_{MAX} (CCl_{4}): 2840 (C—H), 2180 (C≡C), 1725 (ester carbonyl), 1600 (C=C) cm\(^{-1}\).

\(^{1}\)H-NMR (CDCl\(_{3}\), 500 MHz): δ 0.94 (6H, d, J=7.0 Hz, H-4a), 2.12 (1H, m, H-3a), 2.22 (2H, d, J=7.0 Hz, H-2a), 5.2 (2H, s, H-6'), 5.54 (1H, dd, J=1.5, 11.0 Hz, H-4''(cis)), 5.72 (1H, dd, J=1.5, 17.5 Hz, H-4''(trans)), 6.01 (1H, dd, J=11.0, 17.5 Hz, H-3''), 6.96 (1H, d, J=3.5 Hz, H-4'), 7.00 (1H, d, J=3.5 Hz, H-3''), 7.01 (1H, d, J=3.5 Hz, H-3'), 7.07 (1H, d, J=3.5 Hz, H-4).

* Assignments are interchangeable.

\(^{13}\)C-NMR (CDCl\(_{3}\), 125 MHz): δ 22.4 (2xC-4a), 24.7 (C-3a), 43.2 (C-2a), 60.2 (C-6'), 83.1 (C-1''), 93.1 (C-2''), 116.8 (C-3''), 122.2 (C-5) 123.7 (C-3), 123.7 (C-3'), 127.0 (C-4''), 128.8 (C-4'), 132.8 (C-4), 137.9 (C-2'), 138.0 (C-5'), 138.6 (C-2), 172.2 (C-1a).
4.4.3 5'-Methyl-5-(3-hydroxy-4-isovaleroxy-1-butynyl)-2,2'-bithiophene (87):

The column chromatography of the pet-ether soluble part of the methanolic extract of Blumea obliqua was performed with pet-ether and increasing proportions of chloroform. The fractions obtained with 10% chloroform in pet-ether were combined. Repeated column chromatography of this fraction gave 87 in semi-purified form which yielded 87 as yellow oil (4mg) on preparative TLC (pet-ether:chloroform, 1:1, Rf=0.4).

FDMS m/z: 348 [M⁺].
Peak Matching m/z: 348.08519 [C₁₈H₂₀O₃S₂].
EIMS m/z (rel. int. %): 348.0 [M⁺] (66), 246.0 [M-C₅H₁₀O₂] (100), 233 (17), 190 (32), 171 (29).

UV λₘₐₓ (Et₂O): 338.0 nm.

¹H-NMR (CDCl₃, 300 MHz): δ 0.96 (6H, d, J=6.5 Hz, H-4a), 2.15 (1H, m, H-3a), 2.26 (2H, d, J=6.5 Hz, H-2a), 2.46 (3H, d, J=1.0 Hz, H-6'), 4.29 (2H, d, J=2.3 Hz, H-4''), 4.82 (1H, br.t, H-3''), 6.66 (1H, dq, J=1.0, 3.5 Hz, H-4'), 6.91 (1H, d, J=3.8 Hz, H-3), 6.97 (1H, d, J=3.5 Hz, H-3'), 7.08 (1H, d, J=3.8 Hz, H-4).

¹³C-NMR (CDCl₃, 75 MHz): δ 14.0 (C-6'), 22.3 (2xC-4a), 24.8 (C-3a), 43.2 (C-2a), 61.8 (C-4''), 65.0 (C-3''), 79.7 (C-1''), 90.4 (C-2''), 121.1 (C-5) 122.6 (C-3), 124.4 (C-3'), 126.2 (C-4'), 133.6 (C-4), 139.4 (C-2), 140.0 (C-2'), 140.2 (C-5'), 173.0 (C-1a).

* assignments may be interchanged.
4.4.4 *Methylene-bis-[5-(3-butene-1-ynyl)-2,2'-bithiophene* (88):

Compound 88 was isolated from the pet.-ether soluble part of the methanolic extract of *Blumea obliqua*. The pet.-ether soluble portion was loaded onto a silica gel column and eluted with different proportions of pet.-ether and chloroform. The fractions obtained with 10% chloroform in pet.-ether were combined and rechromatographed repeatedly on different sizes of silica gel columns. Finally, elution with 3% chloroform in pet.-ether yielded 4 as colorless needle-like crystals (10 mg).

EIMS m/z (rel. int. %): 444.2 [M⁺] (78.1), 229.1 (19.5), 57 (100).

HR EIMS m/z: 444.0146 [C<sub>25</sub>H<sub>16</sub>S<sub>4</sub>].

UV λ<sub>MAX</sub> (Et<sub>2</sub>O): 347.2 nm.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): δ 4.27 (2H, s, H-6'), 5.52 (1H, dd, J=1.9, 11.2 Hz, H-9 (cis)), 5.72 (1H, dd, J=1.9, 17.5 Hz, H-9 (trans)), 6.01 (1H, dd, J=11.2, 17.5 Hz, H-8), 6.80 (1H, d, J=3.6 Hz, H-4'), 6.95 (1H, d, J=3.8 Hz, H-3), 7.00 (1H, d, J=3.6 Hz, H-3'), 7.06 (1H, d, J=3.8 Hz, H-4).

<sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz): δ 30.7 (C-6'), 83.2 (C-6), 92.9 (C-7), 116.9 (C-8), 121.6 (C-5), 123.2 (C-3'), 124.0 (C-3), 126.3 (C-4'), 126.8 (C-9), 132.7 (C-4), 135.8 (C-2'), 139.1 (C-2), 142.5 (C-5').
4.4.5 5'-Methyl-5-(4-acetoxy-1-butynyl)-2,2'-bithiophene (89):

The elution of the column, loaded with the pet.-ether soluble part of the methanolic extract of *Blumia obliqua*, with 10% chloroform in pet.-ether gave a fraction which was further chromatographed on silica gel column. Finally preparative TLC of this fraction, using pet.-ether and diethyl ether as solvent (9:1, *R* < sub > f </ sub > = 0.3) resulted in the purification of compound 89 as yellow oil (4 mg).

EIMS *m/z* (rel. int. %): 290.0409 [C<sub>13</sub>H<sub>14</sub>O<sub>2</sub>S<sub>2</sub>, M<sup>+</sup>] (32), 230.0205 [C<sub>13</sub>H<sub>10</sub>O<sub>2</sub>, M-acetate] (100), 217 (20), 197 (14), 150 (40)

UV λ<sub>MAX</sub> (Et<sub>2</sub>O): 339.2 nm.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz): δ, 2.08 (3H, s, H-2a), 2.48 (3H, d, J=0.8 Hz, H-6'), 2.76 (2H, t, J=6.9 Hz, H-3''), 4.22 (2H, t, J=6.9 Hz, H-4''), 6.64 (1H, dq, J=1.0, 3.5 Hz, H-4'), 6.88 (1H, d, J=3.7 Hz, H-3), 6.93 (1H, d, J=3.5 Hz, H-3''), 6.99 (1H, d, J=3.9 Hz, H-4).

<sup>13</sup>C-NMR (CDCl<sub>3</sub>, 125 MHz): δ 14.1 (C-6'), 20.9 (C-2a), 22.7 (C-3''), 62.1 (C-4''), 77.5 (C-1''), 90.5 (C-2''), 121.1 (C-5) 122.5 (C-3), 124.0 (C-3'), 126.0 (C-4'), 132.5 (C-4), 140.0 (C-5')
4.4.6 5'-Methyl-5-(4-hydroxy-1-butynyl)-2,2'-bithiophene (90):

Repeated column chromatography of a fraction obtained from the elution of a column loaded with the pet.-ether soluble part of the methanolic extract of Blumea obliqua yielded 90 in the semi purified form. It was further purified by preparative TLC with pet.-ether and chloroform as solvent system (1:1, $R_f=0.5$) in the form of yellow oil (3 mg).

FDMS $m/z$: 247.8 [$M^+$]
Peak Matching $m/z$: 248.0324 [C$_{13}$H$_{12}$OS$_2$]
UV $\lambda_{MAX}$ (Et$_2$O): 322.0 nm.
IR $\nu_{MAX}$ (CHCl$_3$): 3630 (OH), 2860 (C-H) cm$^{-1}$
$^1$H-NMR (CDCl$_3$, 500 MHz): 2.24 (3H, d, J=1.1 Hz, H-6'), 2.70 (2H, t, J=6.2 Hz, H-8), 3.80 (2H, t, J=6.2 Hz, H-9), 6.64 (1H, dq, J=1.1, 3.8 Hz, H-4'), 6.89 (1H, d, J=3.5 Hz, H-3'), 6.93 (1H, d, J=3.8 Hz, H-3'), 7.01 (1H, d, J=3.5 Hz, H-4).
4.4.7 5'-Acetomethyl-5-(3-butene-1-ynyl)-2,2'-bithiophene (91):

Compound 91 was isolated from the pet.-ether soluble part of the methanolic extract of *Blumea obliqua*. It was eluted from the silica gel column with 10% chloroform in pet.-ether along with compound 90 as a semi purified fraction which was purified by repeated column chromatography (silica gel) and finally by preparative TLC (pet.-ether:diethyl ether 9:1, Rf=0.5) as amorphous solid (6 mg).

ElMS m/z (rel. int. %): 287.8 [M⁺] (62), 228.9 (100), 217 (4), 149 (7)

HREIMS m/z: 288.0277 [C₁₃H₁₁₂O₂S₂], 229.0122 [C₁₃H₉S₂, M-acetate]

UV λₓₜₐₓ (Et₂O): 347.2 nm.

¹H-NMR (CDCl₃, 500 MHz): δ 2.08 (3H, s, H-2a), 5.19 (2H, s, H-6'), 5.54 (1H, dd, J=1.9, 11.3 Hz, H-9 (cis)), 5.72 (1H, dd, J=1.9, 17.5 Hz, H-9 (trans)), 6.01 (1H, dd, J=11.3, 17.5 Hz, H-8), 6.97 (1H, d, J=3.6 Hz, H-4'), 7.00 (1H, d, J=3.8 Hz, H-3), 7.02 (1H, d, J=3.6 Hz, H-3'), 7.07 (1H, d, J=3.8 Hz, H-4).

¹³C-NMR (CDCl₃, 125 MHz): δ 20.9 (H-2a), 60.5 (C-6'), 83.0 (C-6), 93.1 (C-7), 116.8 (C-8), 122.2 (C-5) 123.7 (C-3), 123.7 (C-3'), 127.1 (C-9), 129.0 (C-4'), 132.8 (C-4), 137.6 (C-5'), 138.1 (C-2), 138.6 (C-2'), 172.0 (C-1a).
4.4.8 5'-Hydroxymethyl-5-(3-butene-1-ynyl)-2,2'-bithiophene (92):

Pet.-ether soluble part of the methanolic extract of Blumea obliqua was chromatographed on silica gel using different proportions of pet.-ether and chloroform as eluent. Elution with 20% chloroform in pet.-ether yielded a fraction which on preparative TLC, developed in pet.-ether and diethyl ether (4:1, RF=0.4) gave compound 92 as yellow needle-like crystals (6 mg).

EI-MS m/z (rel. int. %):  245.7 [M+] (94.6), 228.8 (70), 216.8 (100).
HREI-MS m/z :  246.0155 [C₁₃H₁₀O₂S₂].
UV λ_{max} (Et₂O):  347.2 nm.

^1H-NMR (CDCl₃, 500 MHz): δ 4.77 (2H, d, J=0.8 Hz H-6'), 5.52 (1H, dd, J=2.0, 11.1 Hz, H-9 (cis)), 5.69 (1H, dd, J=11.1, 17.5 Hz, H-9 (trans)), 6.00 (1H, dd, J=11.1, 17.5 Hz, H-8), 6.88 (1H, dq, J=0.8, 3.6 Hz, H-4'), 6.99 (1H, d, J=3.8 Hz, H-3'), 7.00 (1H, d, J=3.6 Hz, H-3'), 7.08 (1H, d, J=3.8 Hz, H-4).

^13C-NMR (CDCl₃, 125 MHz): δ 60.1 (C-6'), 83.1 (C-6), 93.0 (C-7), 116.8 (C-8), 121.9 (C-5) 123.4 (C-3), 123.8 (C-3'), 126.1 (C-4'), 126.9 (C-9), 132.8 (C-4), 138.5 (C-2), 138.9 (C-2'), 143.8 (C-5').
4.4.9 5-(3-Butene-1-ynyl)[2,2'-bithiophene]-5'-carboxaldehyde (93):

Compound 93 was isolated from column chromatography of the pet.-ether soluble part of the methanolic extract. Preparative TLC (pet.-ether:diethyl ether, 4:1) of a fraction eluted with 20% chloroform in pet.-ether yielded 93 as yellow amorphous solid (4 mg).

EIMS m/z (rel. int. %): 243.7 [M+](100), 214.7 (5.3).
Peak Matching m/z: 243.9994 [C_{13}H_{8}OS_{2}].
UV λ_{max} (Et_{2}O): 370.2 nm.

^{1}H-NMR (CDCl_{3}, 500 MHz): δ 5.58 (1H, dd, J=1.9, 11.2 Hz, H-9 (cis)), 5.72 (1H, dd, J=1.9, 17.5 Hz, H-9 (trans)), 6.04 (1H, dd, J=11.2, 17.5 Hz, H-8) 7.13 (1H, d, J=3.8 Hz, H-4)^{a}, 7.21 (1H, d, J=3.8 Hz, H-3)^{a}, 7.24 (1H, d, J=3.8 Hz, H-3)^{a}, 7.65 (1H, d, J=3.8 Hz, H-4)^{a}, and 9.85 (1H, s, H-6').

(a=assignments may be interchanged).
4.4.10  *α*-Taraxasteryl acetate (94):

The compound 94 was obtained from the fractions of silica gel column upon elution of the pet.-ether soluble part of the extract with pet.-ether. On evaporation, the fractions yielded the rosette shaped crystals. Repeated recrystallization from ethyl acetate gave shining needles.

Melting Point:  255-256° C.
Optical rotation $[\alpha]_D^{18}$:  +100° (CHCl₃, c=0.21).
EIMS m/z (rel. int. %):  468 [M⁺] (16), 453 (20), 408 (14), 393 (18), 386 (12), 356 (24), 249 (50), 189 (100), 100 (43).
HREIMS m/z:  468.7677 [C₃₂H₃₂O₂].
IR ν$_{MAX}$ (CHCl₃):  1725, 1640, 1320, 880 cm$^{-1}$.

$^1$H-NMR (CHCl₃, 300 MHz): δ 4.68-4.60 (3H, br.s, H-30), 4.52 (1H, dd, J=4.7, 9.8 Hz, H-3), 2.08 (3H, s, OCOCH₃), 1.04 (6H, s, H-27), 1.01 (3H, s, H-23 and H-26), 0.90 (3H, d, J=6.2 Hz, H-29), 0.83 (3H, s, H-25), 0.81 (6H, s, H-24 and H-28).

$^{13}$C-NMR (CDCl₃, 75 MHz): δ 171.0 (OCORMe), 150.9 (C-20), 107.0 (C-30), 80.8 (C-3), 55.2 (C-5), 50.7 (C-9), 48.6 (C-18), 42.1(C-14), 41.0 (C-8), 39.2 (C-22), 39.1 (C-13), 38.4 (C-1), 38.2 (C-19), 36.9 (C-4), 36.9 (C-10), 36.2 (C-16), 34.9 (C-17), 34.5 (C-7), 27.9 (C-23), 27.5 (C-15), 27.0 (C-12), 26.1 (C-28), 25.4 (C-21), 23.6 (C-2), 21.7 (C-11), 21.3 (OCORCH₃); 19.3 (C-29), 18.1 (C-6), 16.1 (C-26), 15.8 (C-25), 15.2 (C-24), 14.6 (C-27).
4.4.11  

β-Sitosterol (95):

Compound 95 was obtained from the column chromatography (silica gel) of the pet.-ether soluble part of the methanolic extract. It got crystallized upon evaporation of the fractions obtained from elution of the silica gel column with 5% chloroform in pet.-ether. It was further purified upon recrystallization from chloroform.

Melting point:  
135° C.

Optical rotation [α]D\(^{25}\):  
-35.5° (CHCl₃, c=0.1429).

ElMS m/z (rel. int. %):  
414 [M⁺] (15), 399 (10), 396 (12), 381 (72), 329 (25), 303 (23), 275 (12), 255 (30).

HREIMS m/z:  
414.4091 [C$_{29}$H$_{50}$O].

IR ν\(_{\text{MAX}}\) (CHCl₃):  
3450, 3050, 1650, 815 cm\(^{-1}\).

$^1$H-NMR (CDCl₃, 300 MHz):  
δ 5.23 (1H, m, H-6), 3.32 (1H, m, H-3), 1.01 (3H, s, H-19), 0.92 (3H, d, J=6.2 Hz, H-21), 0.84 (3H, t, J=7.0 Hz, H-29), 0.83 (3H, d, J=6.5 Hz, H-26), 0.81 (3H, d, J=6.5 Hz, H-27), 0.68 (3H, s, H-18).

$^{13}$C-NMR (CDCl₃, 75 MHz):  
8.140.9 (C-5), 121.8 (C-6), 71.9 (C-3), 56.7 (C-14), 56.2 (C-17), 50.8 (C-9), 50.3 (C-24), 42.6 (C-13), 42.4 (C-4), 40.0 (C-12), 37.3 (C-1), 36.6 (C-10), 36.2 (C-20), 34.0 (C-22), 32.0 (C-7), 32.0 (C-8), 31.8 (C-2), 29.3 (C-23), 28.2 (C-16), 26.2 (C-25), 24.3 (C-15), 23.1 (C-28), 21.1 (C-11), 19.8 (C-27), 19.4 (C-19), 19.1 (C-21), 18.8 (C-26), 11.9 (C-18), 11.9 (C-29).
4.4.12  *Lupeol* (96):

Lupeol 96 was isolated by repeated column chromatography from the same fractions which yielded 95. It was further purified on repeated crystallization from a mixture of acetone-methanol as shining needles.

Melting point:  215-216°C.

Optical rotation $[\alpha]_D^{20}$:  $+26.40$ (CHCl$_3$, $c=0.21$).

EI-MS $m/z$ (rel. int. %):  426 [M$^+$] (14), 411 (30), 207 (10), 204 (7), 189 (20), 133 (11).

IR $\nu_{\text{MAX}}$ (KBr):  3350, 3070, 1650, 880 cm$^{-1}$.

$^1$H-NMR (CDCl$_3$, 300 MHz):  $\delta$ 4.63 (2H, m, H-29), 3.65 (1H, dd, J=4.27, 10.68, H-3), 1.65 (3H, br. s, H-30), 1.05 (3H, s, H-26), 0.96 (6H, s, H-25, H-27), 0.90 (3H, s, H-24), 0.85 (3H, s, H-28), 0.76 (3H, s, H-23).

$^{13}$C-NMR (CDCl$_3$, 75 MHz):  $\delta$ 150.6 (C-20), 109.2 (C-29), 78.8 (C-3), 55.2 (C-5), 50.4 (C-4), 48.2 (C-18), 47.9 (C-19), 42.9 (C-17), 42.8 (C-14), 40.8 (C-8), 39.9 (C-22), 38.8 (C-4), 38.7 (C-1), 38.0 (C-13), 37.1 (C-10), 35.5 (C-16), 34.2 (C-7), 29.8 (C-21), 28.0 (C-23), 27.4 (C-15), 27.4 (C-2), 25.1 (C-12), 20.9 (C-11), 19.3 (C-30), 18.3 (C-6), 18.0 (C-28), 16.1 (C-25), 15.9 (C-26), 15.4 (C-24), 14.5 (C-27).
4.4.13 3-O-[β-D-Glucopyranosyl]-β-sitosterol (97):

The fractions obtained from the elution of the column with chloroform methanol (9:1) showed similar TLC profiles and were combined and washed with methanol to purify compound 97.

FABMS (negative ion) m/z: 575 [M⁺-H]⁻ and 413 [M-H-glucose]⁻.

IR ν max (KBr): 3400, 2920, 2840, 1635 cm⁻¹.

¹H-NMR (DMSO-d₆, 300 MHz): 8 5.32 (1H, distorted t, H-6), 4.21 (1H, d, J=7.7 Hz, H-1'), 3.4 (1H, m, H-3), 0.94 (3H, s, H-19), 0.89 (3H, d, J=6.3 Hz, H-21), 0.82 (3H, t, J=7.3 Hz, H-29), 0.80 (3H, d, J=7.1 Hz, H-26), 0.78 (3H, d, J=7.2 Hz, H-27), 0.64 (3H, s, H-18).

¹³C-NMR (DMSO-d₆, 75 MHz): 8 140.5 (C-5), 121.1 (C-6), 100.8 (C-1'), 77.0 (C-3), 76.7 (C-3'), 76.6 (C-5'), 73.4 (C-2'), 70.1 (C-4'), 61.1 (C-6'), 56.1 (C-14), 55.4 (C-17), 49.6 (C-9), 45.1 (C-24), 41.8 (C-13), 39.2 (C-4), 38.3 (C-12), 36.8 (C-1), 36.2 (C-10), 35.4 (C-20), 33.3 (C-22), 31.4 (C-8), 31.3 (C-7), 29.2 (C-2), 28.7 (C-25), 27.7 (C-16), 25.5 (C-23), 23.8 (C-15), 22.6 (C-28), 20.5 (C-11), 19.6 (C-27), 19.0 (C-26), 18.9 (C-19), 18.6 (C-21), 11.7 (C-29), 11.6 (C-18).
5.0 REFERENCES
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