SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF SOME SYNTHETIC CURCUMINOIDS AND THEIR METAL COMPLEXES

THESIS

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requirements for the Degree of
DOCTOR OF PHILOSOPHY

By

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Forwarded

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CERTIFICATE

This is to certify that the thesis entitled **SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF SOME SYNTHETIC CURCUMINOIDS AND THEIR METAL COMPLEXES** is an authentic record of the research work carried out by **Sri. Joseph John**, under my supervision in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Chemistry of the University of Calicut. This work or part thereof has not been presented before for the award of any other degree.

Dr. K. Krishnankutty
(Supervising Teacher)
Professor & Head
Department of Chemistry
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Calicut University,
23.07.2007.

DECLARATION

Certified that the thesis bound herewith is an authentic record of the research work on **SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF SOME SYNTHETIC CURCUMINOIDS AND THEIR METAL COMPLEXES** carried out by me under the supervision of Dr. K. Krishnankutty, Professor, Department of Chemistry, University of Calicut in partial fulfilment of the requirements for the award of the degree of the Doctor of Philosophy in Chemistry of the University of Calicut, and further that no part thereof has been presented before for any other degree.

Calicut University,
23.07.2007.

JOSEPH JOHN

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Finally, I bow before the Almighty for the blessings bestowed on me throughout the investigation.

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CHAPTER 3
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Preface

Studies on naturally occurring ligand systems and their synthetic analogues constitute an important area of modern coordination chemistry. Biological importance of many plant chemicals are known to be associated with their ability to form complexes with various inorganic species including metal ions. Active chemical constituents of several medicinal plants are good coordinating ligands, since they contain functional groups such as –OH, –COOH, –C=O, –SH, $–OCH_3$, –O– etc. However, structural and biological aspects of these types of complexes have not received as much attention as they deserve. The present investigation is an attempt in this direction.

Among the traditional Indian medicinal plants, turmeric (*Curcuma longa*) occupies a pride of place. The rhizomes of turmeric is an unavoidable constituent in numerous medicinal formulations, in Ayurveda, Unani, Siddha, etc. of the Indian subcontinent, from time immemorial. The medicinal properties of turmeric have been attributed mainly due to curcuminoids, the yellow constituent of the rhizomes. Structurally curcuminoids are 1,3-diketones in which the diketo function is attached directly to olefinic linkages. Numerous reports on the medicinal properties such as antitumour, antioxidant, antiinflammatory, antibacterial, etc. of turmeric and curcuminoids have been appeared recently. Several synthetic curcuminoid analogues and their metal complexes have been reported to exhibit enhanced biological properties compared to natural curcuminoids. Therefore studies on new synthetic analogues of curcuminoids have considerable importance. This is particularly true when such compounds containing biologically important heterocyclic ring systems. The present investigation is mainly on the

chemical and biochemical aspects of curcuminoid analogues containing indolyl ring, an important heterocycle in many biomolecules, and their typical metal complexes.

The thesis is divided into five chapters.

Chapter 1 is a general introduction highlighting the importance of various aspects of coordination chemistry in biological processes particularly in medicinal inorganic chemistry and chemotherapeutic application of food phytochemicals, with special emphasis on some heterocyclic analogues. Since compounds included in this study are typical 1,3-diketones, some of the salient structural features of 1,3-diketones and metal 1,3-diketonates are also discussed in this chapter. A brief account on the photochemical behaviour curcuminoids is also included. Importance of the present investigation has been interspersed at appropriate places.

Chapter 2 is a general description on various chemicals and methods employed, instruments used and techniques adopted for the study.

The present investigation focused on the study of various aspects of three different curcuminoids. They are 1,7-diindolyl-1,6-heptadiene-3,5-dione, 1-phenyl-5-indolyl-4-pentene-1,3-dione and 6-indolyl-5-hexene-2,4-dione. For convenience the results of the studies of these compounds are presented separately in three chapters.

Chapter 3 section 1 deals with the synthesis and characterisation of a new curcuminoid analogue, 1,7-diindolylheptanoid and its metal complexes. Spectral data clearly indicate the existence of the compound predominantly in the intramolecularly hydrogen bonded *cis* enol form. The behaviour of the compound as monobasic bidentate ligand in its

complexes are clearly indicated. The use of uv, ir, nmr, mass, esr and TG data in elucidating the structure of the compound and its metal complexes are well illuminated in this section. The spectral data unequivocally showed that only the diketo function is involved in bonding with metal ion. In **section 2** fluorescence characteristics of the 1,7-diindolylheptanoid are discussed. The effect of solvents, water, pH of the medium and metal ions on the fluorescence intensity of the compound are discussed. The quantum yield of fluorescence of the compound is higher than that reported for natural curcuminoids. Details of the biological studies of the compound and its metal chelates are given in **section 3**. Results obtained on superoxide scavenging activity, hydroxyl radical scavenging activity and inhibition of lipid peroxidation showed that the diindolylheptanoid and their metal complexes are effective antioxidants. The cytotoxic and antitmour behavour of the compound and its metal complexes are discussed separately. Result obtained on short *in vitro* cytotoxicity studies towards DLA cells are given. The effect of the compounds in reducing ascites tumours and tumour volume in mices are also discussed.

In **chapter 4, section 1** details on the synthesis and spectral characterisation of 1-phenyl-5-indolyl-4-pentene-1,3-dione and its metal chelates are discussed. The single crystal XRD structure of 1-phenyl-5-indolyl pentanoid is also included in this section. In **section 2** the experimental procedure employed for the study of the fluorescence behaviour of 1-phenyl-5-indolyl-1,3-dione are discussed. Effect of solvents, pH and metal ions on the fluorescence intensity of the compound are discussed. The fluorescence quantum yield is also given. In **section 3** cytotoxicity towards DLA cells and antioxidant activity towards superoxide radical, hydroxyl radical and inhibition lipid

peroxidation are discussed. The results showed that the compounds are less active than 1,7-diindolylheptanoid and its metal complexes.

Chapter 5 is on synthesis and characterissation of 6-indolyl-5-hexene-2,4-dione and its metal chelates. The results obtained in the various spectral and analytical techniques showed that the compound exists entirely in the intramolecularly hydrogen bonded *cis* enol form and the diketo function is involved in metal coordination.

References are given in serial order.

The work described in the **thesis** has partially been published/ accepted/communicated for publication.

1. Metal chelates of 1,7-diindolyl-1,6-heptadiene-3,5-dione. *J. Indian Chem. Soc.*, 2007, **84**, 478.

2. Synthesis, characterisation and antioxidant activity of 1,7-diindolyl-1,6-heptadiene-3,5-dione and its metal chelates. Proceedings of the National Seminar on FRONTIERS IN CHEMISTRY, March 24-25, 2006, organised by Dept. of Applied Chemistry, Cochin University of Science and Technology.

3. Crystal study of 1-phenyl-5-indolyl-4-pentene-1,3-dione, *Acta Cryst* (communi-cated).

4. Synthesis, characterisation and antitumour activity of 1,7-diindolyl-1,6-heptadiene-3,5-dione. *J. Exp. Clin. Cancer Res.* (communicated).

5. Synthesis, characterisation and antioxidant study of 1-phenyl-5-indolyl-4-pentene-1,3-dione and its metal chelates. *J. Ind. Chem. Soc.* (communicated).

6. Synthesis and characterissation of 6-indolyl-5-hexene-2,4-dione and its metal complexes (to be communicated).

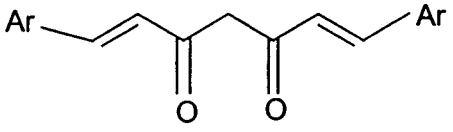
7. Fluorescence behaviour of 1,7-diindolyl-1,6-heptadiene-3,5-dione (to be communicated).

8. Fluorescence behaviour of 1-phenyl-5-indolyl-4-pentene-1,3-dione (to be communicated).

Nomenclature and Abbreviations

Nomenclature

In the present investigation both the systematic and trivial names are used wherever necessary. "Curcuminoids" is a general term used to designate unsaturated 1,3-diketones of structure **1**, because such compounds were first isolated from the yellow pigment of the rhizomes of the plant *Curcuma longa* (turmeric). In the present investigation the term curcuminoid is used in a broader sense to include related unsaturated 1,3-diketones also. The three types of unsaturated 1,3-diketones considered in the present study are systematically named as 1,7-diinidolyl-1,6-heptadiene-3,5-dione (**2**), 1-phenyl-5-indolyl-4-pentene-1,3-dione (**3**) and 6-indolyl-5-hexene-2,4-dione (**4**). For brevity and better readability these compounds are designated as 1,7-diindolylheptanoid (**Hdih**), 1-phenyl-5-indolylpentanoid and 6-indolylhexanoid, respectively. The name curcumin is used in the thesis for representing the major curcuminoid (curcumin I) present in turmeric.



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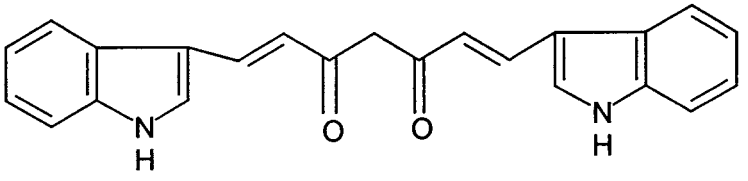

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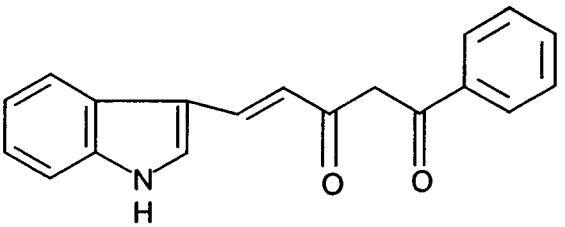

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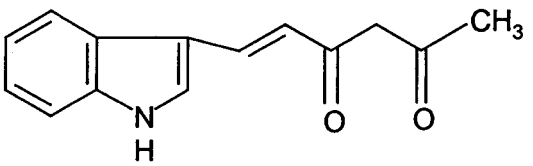

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Abbreviations

A	-	hyperfine splitting constant in ESR spectra
Ar	-	aryl group
BDE	-	Bond dissociation energy
BM	-	Bohr Magneton
DIM	-	3,3'-diindolylmethane
DLA	-	Daltons Lymphoma Ascites
DMF	-	Dimethyl formamide
DMSO	-	Dimethyl sulphoxide
EDTA	-	Ethylene Diamine Tetra Acetic acid disodium salt
FAB	-	Fast atom bombardment
g	-	gyromagnetic ratio
I3C	-	Indole-3-carbinol
ILS	-	Increase in life span
IP	-	Ionisation Potential
J	-	coupling constant
L	-	Deprotonated ligand

MAE	-	Microwave Assisted Extraction
MW	-	Microwave
NBT	-	Nitroblue tetrazolium
PBS	-	Phosphate buffer saline
Ph	-	Phenyl group
Q_F	-	Fluorescence quantum yield
ROS	-	Reactive Oxygen Species
S_1	-	Singlet State
SOS	-	Sodium dodecyl sulphate
TBA	-	Thio Barbituric acid
TBARS	-	Thiobarbituric acid reacting substances

CHAPTER 1
GENERAL INTRODUCTION

Metal complexes in biology and medicine

The growth of interest in coordination chemistry in recent years has been mainly due to the realization of the vital role of metal complexes in biology and medicine, where much progress has been made during the last few decades.[1,2] The ability of metal ions to influence many of the complex reactions upon which the vital processes of living organisms depends has been well established. This is also evident from the fact that coordination compounds of many varieties exist in nature. Several synthetic metal complexes which mimic the behaviour of complicated biomolecules are known and at present the study of such compounds are receiving much attention. Although the results obtained so far do not always parallel those in nature, a knowledge of the chemistry is being built up and the role of metal ions in natural biological systems is beginning to be better understood.

Various functions of metal complexes are dependent on several factors such as the nature of the metal ion and the ligands attached to it, the structure, conformation, etc. Metal ions available for complex formation are considerable, however, limited. But variation in ligands is

virtually limitless because of the ingeneousness of synthetic organic chemists. Literature reveals that ligand systems based on certain structural types such as azo, azomethine, hydrazones, diketones, etc. have proliferated much during recent years. This trend is evident from the reports of numerous ligand systems derived from β-dicarbonyl compounds and allied derivatives.

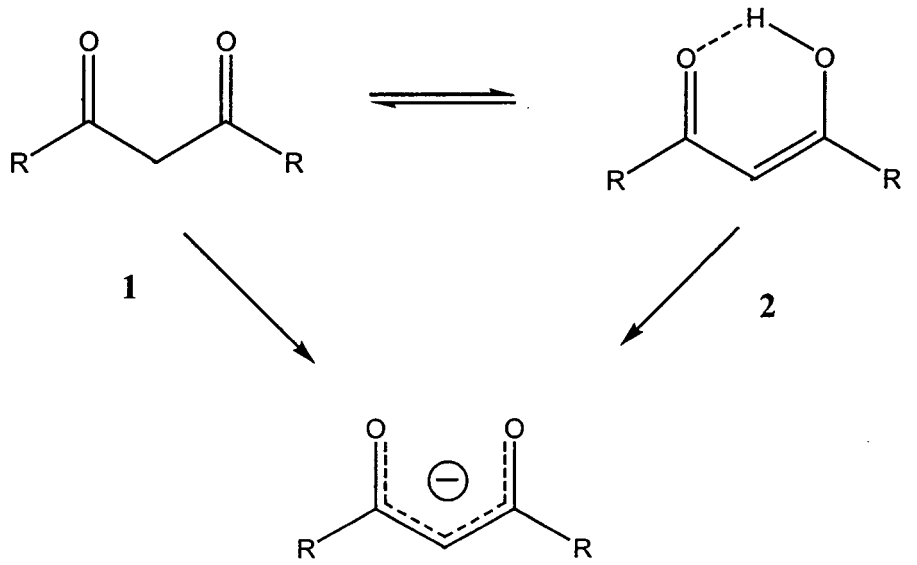
Carbonyl compounds in general serves as the starting material for the design and synthesis of large number of compounds having wide applications in many fields such as bioinorganic studies. This is particularly true in the case of 1,3-diketones. This is mainly because of the fact that proton transfer and hydrogen bonding are two important factors that governs the structure and functions of many molecules starting from water to DNA. The 1,3-diketones possess both these features. Therefore, studies on diverse types of 1,3-diketones and their metal complexes have considerable importance.

Most of the reported studies on 1,3-diketones and metal 1,3-diketonates are based on synthetic compounds. Only very few reports exist on naturally occurring β-dicarbonyl compounds and their synthetic analogues. The present study is mainly on chemical and biochemical aspects of new synthetic analogues of certain naturally occurring β-diketones and their metal complexes. Therefore it is very necessary to

mention some of the salient features of 1,3-diketones and their metal complexes which are quite relevant to the present investigation are briefly outlined below.

Tautomerism of 1,3-diketones

The structural features of 1,3-diketones and allied derivatives opened a new chapter in all branches of chemistry. The most striking structural aspects of this class of compounds is their ability to exhibit keto-enol tautomerism. In β diketones there is a methylene group interposed between acyl or aroyl groups. The hydrogen atom of this methylene group is activated by the adjacent C=O groups, and conjugated system can arise by a prototropic shift, hence they exist as a mixture of keto (**1**) and enol (**2**) forms.



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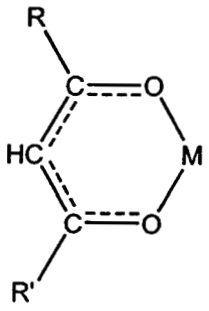
The existence of keto-enol tautomerism in β-diketones was confirmed by various analytical and spectroscopic techniques.[3-14] The relative amount of the enol form depends on various factors like nature of the solvent, temperature, substituent at 1, 2 and 3-positions, and the presence of other hydrogen bonding species. In general bulky alkyl substituents on the 2-carbon tend to produce a large decrease in the enol proportion. While, Cl^-, F^-, Br^-, $COOCH_3$ and SCH_3 groups lead to almost 100% enol form.[15-20] The methine proton in the enol form is acidic. The removal of this proton generate 1,3-diketonate anion **3** which gives the broad class of coordination compounds referred to as metal 1,3-diketonates.

Metal complexes of 1,3-diketones

The coordination chemistry of 1,3-diketones begins as early as in 1887, when Combes reported the synthesis of acetylacetonates of a large number of metals.[21-24] This was followed by the pioneering work of Werner,[25] Morgan[26] and Sidgwick,[27] who confirmed the bifunctional chelating character of this ligand. In 1945 Wilson and Calvin,[28] ascribed aromatic character to the six membered ring (**4**) on the basis of stability constant measurements.



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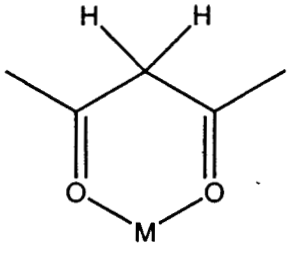
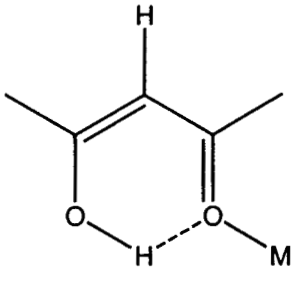
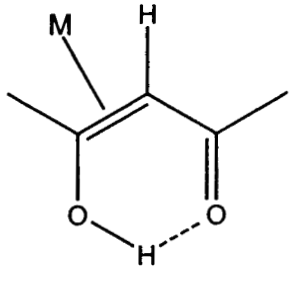
Thus the chemistry of metal β-diketonates in a sense, reflects the different eras in the development of inorganic chemistry as a whole, and an attempt to summarise all these works is purposefully avoided, since excellent reviews, books,[29] etc. are available.[30] However, since 1,3-diketonates exhibit a variety of coordination modes,[31-35] some of the important bonding modes are given in figure 1.

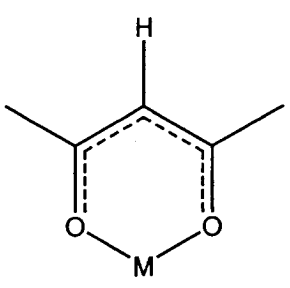
i) As neutral molecule

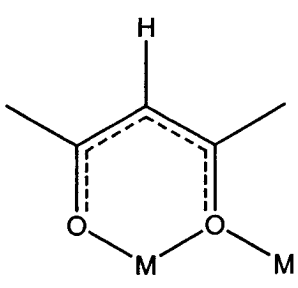


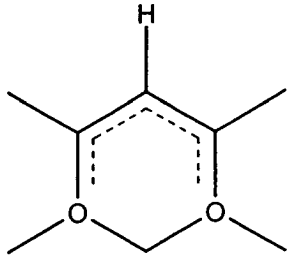


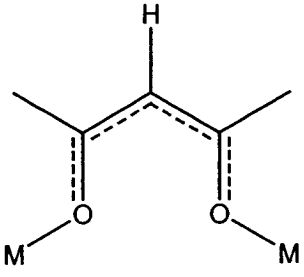


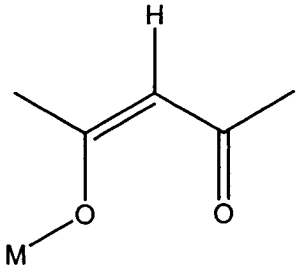
ii) As mono anion

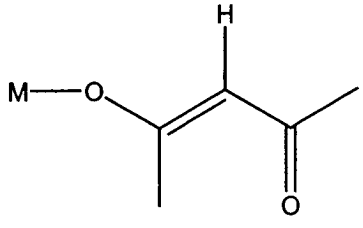


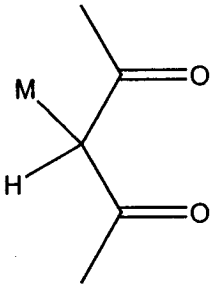


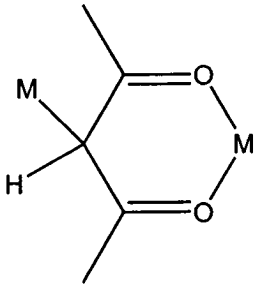


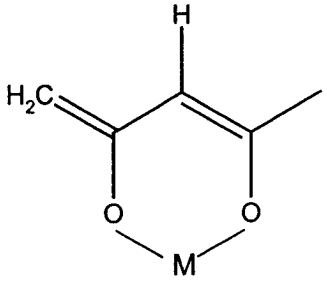


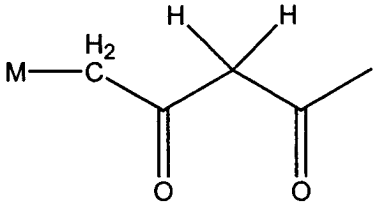












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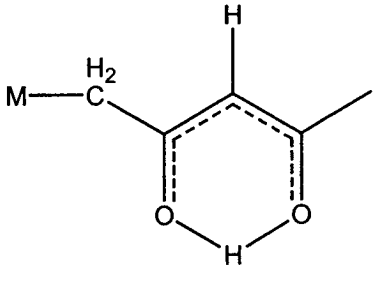

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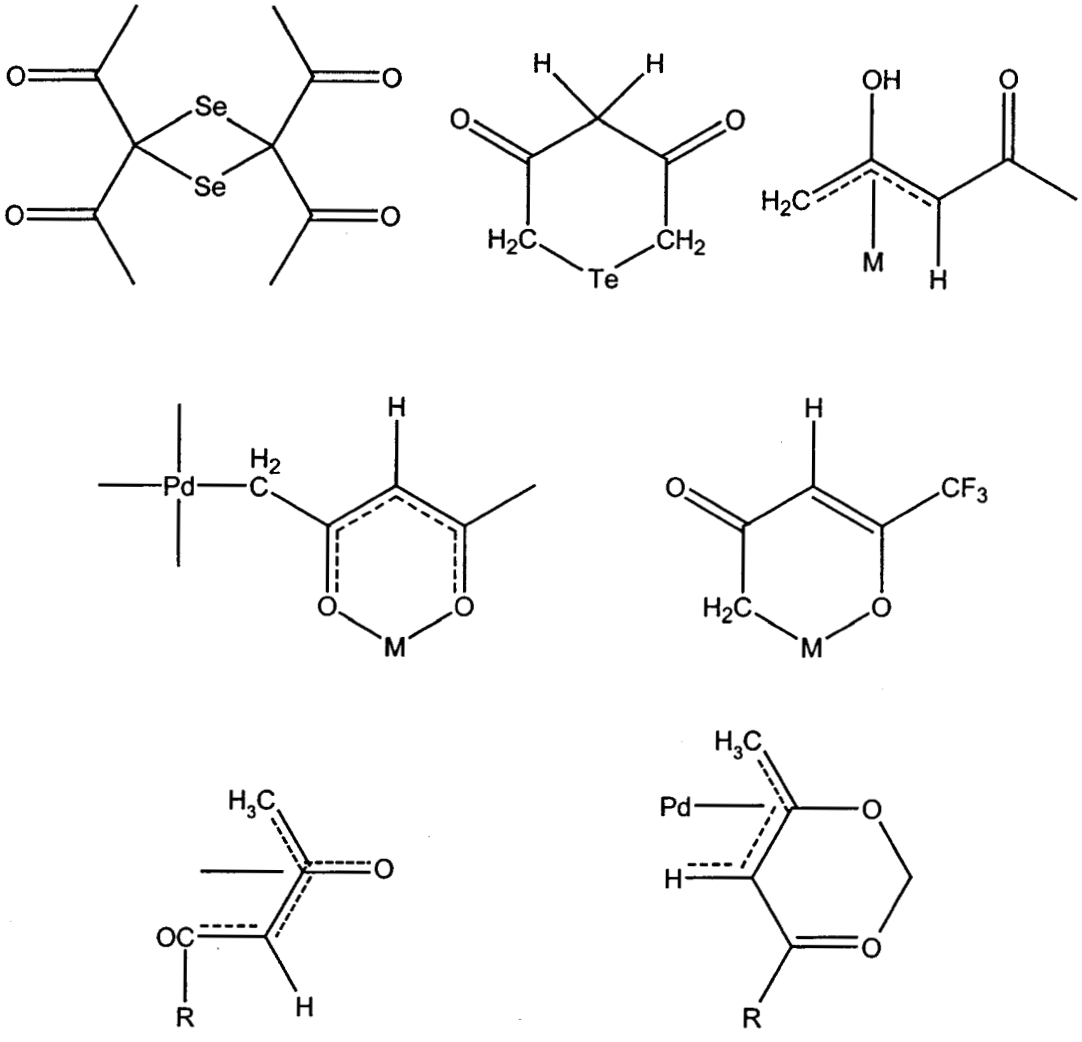


Fig. 1. Different bonding modes of β-diketones

Almost all structural characterization techniques such as spectral and diffraction data along with other physical and chemical methods have been extensively employed in establishing the structure and nature of bonding in these complexes.

Applications of metal 1,3-diketonates

Metal complexes of β-diketones have number of practical applications. Fundamentally, the various applications of metal chelates depend on their chemical properties coupled with attractive physical nature such as volatality, thermal and solvolytic stability.

Lanthanide chelates of β-diketonates are extremely useful as nmr shift reagent.[36-39] Metal β-diketonates like $Cr(acac)_3$ are extensively used in measurement of carbon-13 nmr spectra of metal carbonyls.[40-44] Many of the rare earth metal β-diketonates have got considerable importance because of their practical use as potential laser material.[45-49] A number of metal β-diketonates are suitable in vapour phase chromatographic separation of metals.[50-54] Since substitution of the hydrogen in β-diketone with fluorine increases the stability and volatility of the metal chelates, thereby reduces the retention time, they are widely used for this purpose. Fluorination also increases the acidity of the enol form and hence fluorinated β-diketones are used in the solvent extraction of metals.[55-59]

Although lot of work had been reported on the chemistry and applications of 1,3-diketones and their metal chelates, it can be seen that majority of these studies are on 1,3-diketones in which the diketo function is directly linked to alkyl/aryl groups. Only very few reports exist on

metal complexes of 1,3-diketones in which the diketo function directly attached to olefinic groups. The present investigation, therefore, designed in such a way as to provide an opportunity for the synthesis and structural characterisation of a series of 1,3-diketones in which the diketo function is attached to olefinic bond(s). Such unsaturated 1,3-diketones and related compounds constitute the active chemical species of several medicinal plants. Hence they are biologically important ligand systems.

Ligands in biological systems

Animals and plants contain numerous ligand systems that can effectively coordinate with various metal ions. Foodstuffs that originate in plants and animals are made mostly of proteins, carbohydrates, nucleic acids and lipids. They undergo chemical transformation in the body providing energy for the activities of the organisms for growth, maintenance and repair of its structural and functional constituents. Even, the most complex foodstuffs become a mixture of mostly amino acids, a few carbohydrates, fatty acids, glycerol, pyrimidines, purines, etc. and a few inorganic compounds when processed by digestive enzymes. Since all these chemical compounds of the biosphere have a number of electronegative functional groups, it is not surprising that they interact with metal ions. Several reviews, monographs, etc. are available on these aspects of biochemistry.[60-66]

Metal ions in biological systems

Metal ions in biological systems most frequently bind to donor ligands according to preferences dictated by HSAB principle.[67,68] The structure, reactivity, stability and other properties of transition metal complexes found in biological systems have been conveniently explained on the basis of the electronic configuration of the metal ion and by the ligand field-molecular orbital theories.[69]

The biochemical literature of the last 40 years led to understand that many of the biological activity of proteins and enzymes can be attributed to the metal centers and the role of the metal ion is crucial in effecting the requisite transformations.

Medicinal inorganic chemistry comprises the introduction of metal ions into a biological system either by fortuity or by intention. In the case of fortuitous introduction, the metal has to be removed from the biological system, because excess metal causes poisoning. This is usually done by using suitable chelating agents like EDTA.

The intentional introduction of metal ion into a biological system will be for either therapeutic or diagnostic purpose.[70] For instance γ-emitting radio pharmaceuticals, based on Technetium 99m (^{99m}Tc) complex, the magnetic imaging control agents containing complexes of

Gd and X-ray contrasting agents based on $BaSO_4$ are in routine clinical examination.[71]

Frust[72,73] has suggested the metal that penetrate living cells can either advance or retard the kinetics of metabolic or catabolic enzymes. He further suggested that viruses may aid cell penetration by these metals. Metals, either complexes or not, if present as unstable isotopes, emit ionising radiations that can cause cross mutations and eventually cancer. Sometime metal ions have the power of determining whether a carcinogen is active or not. Experiments in animals revealed that, sodium salt of cyclohexyl sulphuric acid under the most severe conditions, manages to produce a mild self-limiting lesion, whereas traces of calcium salt produce progressive lesions.[74]

Many diseases are also caused by the accumulation of free radicals which are generated directly in human body.[75] Cell damage caused by free radicals appears to be a major contributor to aging, and degenerating diseases of aging such as cancer, cardiovascular diseases, catracts, immune system decline and brain disfunction.[76] Reactive oxygen species (ROS) is a term which encompasses all highly reactive oxygen containing molecules including free radicals. Types of ROS include hydroxyl radical, superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical and various lipid peroxides.[75] These ROS induce all forms of DNA

damage and stimulate cell division, which is a critical factor in mutagenesis.[77,78] When a cell with damaged DNA strand divides, cell metabolism and duplication become deranged. Thus cell mutation arises which inturn is an important factor in carcinogenesis.

Another cause of the production of ROS is the absorption of short wavelength photons.[79] In many photochemical reactions in living systems, the product is singlet oxygen and several molecules are susceptible to attack by this O_2 to form lipid hydroperoxide that can cause damage and disfunction in cell.[80] Human have evolved a highly sophisticated and complex antioxidant protection system, which involves a variety of compounds, both endogenous and exogenous in origin. They include, nutrient derived antioxidant (vitamin C & E), enzymes[81] (catalase, peroxidase, etc.), metal binding proteins and numerous other phytonutrients, flavanoids, alkaloids, polyhydroxylated chalcones, β-carotene, etc.[82]

Treatment of cancer is mainly aimed to prevent the replication of DNA. There are many anticancer drugs that have increased anticancer activity when administered as metal complexes.[83-86] Hence, recently, metallotherapy has got great attraction for treating cancer. There are two classes of anticancer metallotherapeuticals; those acting outside the cell, and those acting inside the cell.[83] Livingstone has reported a series of

nickel, platinum and palladium dialkyl dithiophosphates which are capable of reducing mice tumor.[86] A range of platinum complexes like cis platin, [dichlorodiamine platinum(II), DDP], are in use as effective drug for the treatment of advanced cancer especially testicular and ovarian cancers.[87-91]

Role of food phytochemicals in chemoprevention

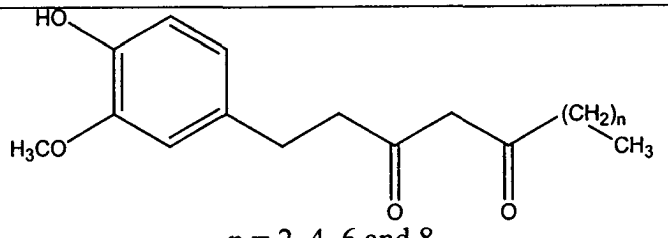
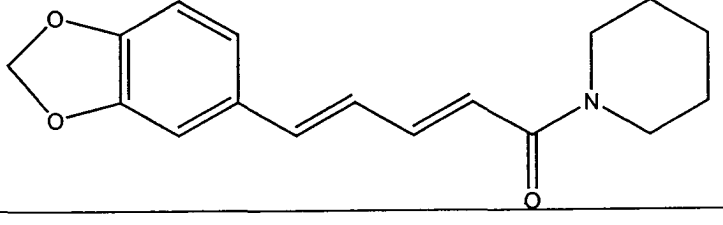
Though modern therapeutic drugs are mostly based on synthetic organic compounds, drug formulations derived from natural resources also occupies an important position.[92] This is clearly evident from the growing awareness in the clinical system of medicine such as Ayurveda, Siddha, Unani, Amachi, etc. which are largely based on herbal components. This is because of the well established facts that natural resources constitute greatest source of drugs and pharmaceuticals. Many plant derived drugs used in modern medicine are developed by ethnomedical concepts.

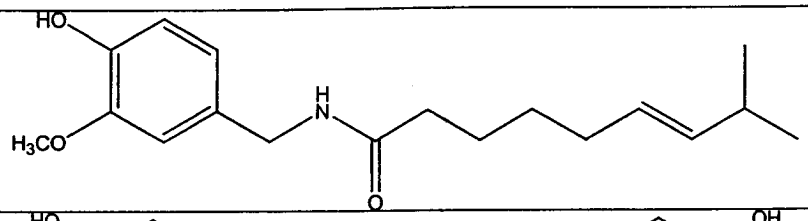
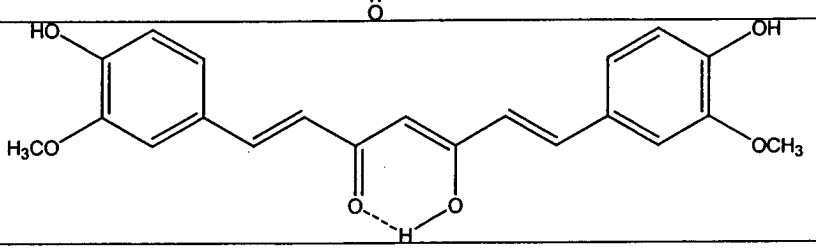
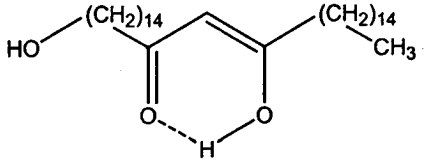
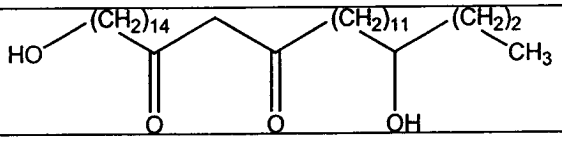
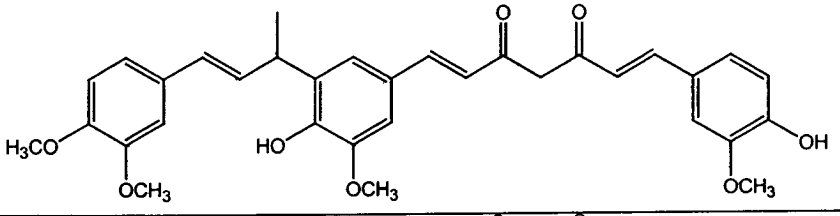
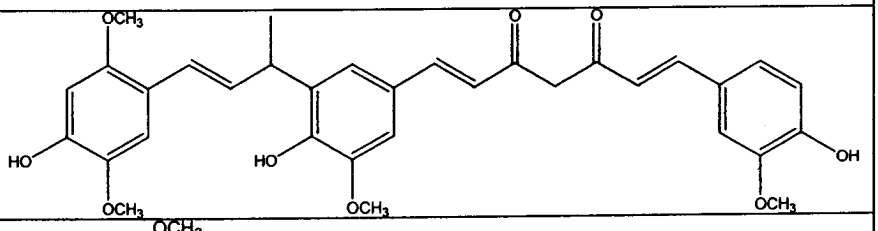
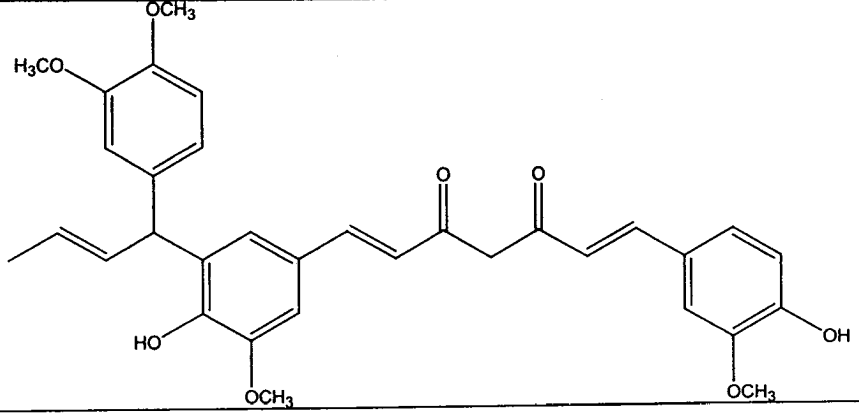
The Indian subcontinent is endowed with rich and diverse local health tradition which is matched with an equally rich and diverse plant genetic resources.[93] A variety of plants and plant products are widely used as food additives. Food additives are a group of substances added while processing in order to input certain desirable properties such as colour, flavour, and texture and also as preservatives. These food additives are

generally from rhizomes, barks, seeds, fruits, leaves and flowers of certain plants which are pungent or aromatic in nature and are known generally as spices. Spices in general increases salivary flow and secretion of neuramic acid and hexosamine which helps digestion. Traditionally these spices were used for the treatment of several ailments such as gastro intestinal disorders, biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis.[94-101] The distinct quality and associated physiological properties of many spices have been attributed to the presence of certain active chemical species with well defined structures. Some of the major spices, medicinal plants and their active chemical constituents are given in table 1.

TABLE 1

Active constituents of some common spices and medicinal plants

Spice (plant species) /Active Principle	Structure
Ginger (*Zingiber officinale*) Gingerols	 n = 2, 4, 6 and 8
Black pepper (*Piper nigrum*) Piperine	

Spice (plant species) /Active Principle	Structure
Red pepper (*Capsicum annum*) Capsaicin	
Turmeric (*Curcuma longa*) Curcumin-1	
Eucalyptus (*Eucalyptus globulus*) n-Tritriacontan-16,18-dione	
4-hydroxy-tritriacontan-16,18-dione	
Indonesian medicinel ginger (*Zingiber cassumunar*) Cassumunin A	
Cassumunin B	
Cassumunin C	

Indole derivatives in biological systems

It can be stated that the active chemical constituents of many medicinal plants possess one or more potential donor sites that can form stable complexes with metal ions, and many of them are acting as potential anticancer agents. Many of these compounds are simple organic molecules or complex heterocyclic compounds. Among the heterocyclic compounds of plant origin and possessing medicinal applications, indole and its derivatives such as indole-3-carbinol (I3C),[102-106] indole-3-acetic acid, and 3,3'-diindolylmethane (DIM) etc. occupies an important position.

Tryptophan, an amino acid which plays crucial role in the functions of many biomolecules is a derivative of indole. In plants, the growth factor, indole acetate is derived from tryptophan, by an oxidative pathway. Some of the intermediates in tryptophan catabolism are precursors for the biosynthesis of other important biomolecules. Tryptophan gives rise to the plant growth hormone, indole-3-acetic acid.

There is a growing public and scientific interest in the influence of hormonically active substances in the diet, on both the risk to develop breast cancer and to control breast cancer, with minimal side effects.[107] There are naturally occurring compounds in the diet that could either prevent or slow down the growth of breast cancer. Indole-3-carbinol (I3C)

is one among such compounds which can effectively reduce breast cancer growth.[107] Indole-3-carbinol is a naturally occurring hydrolysis product of glucobrassium found in many vegetables.

Among the various acid condensation products, the most important is 3,3'-diindolylmethane (DIM) which shows promising cancer chemopreventive properties *in vitro*[108] and *in vivo*.[109] I3C also has anti-istrogenic activities which should prevent cancer in cervical cells.[110] I3C is capable of acting as a scavenger of free radicals in *in vitro* system. Indole-3-acetic acid is also capable of scavenging free radicals, but I3C is more effective.[111]

Although several studies exist on the wide range of physiological and various chemical aspects of the active chemical components of numerous medicinal plants, only a very few reports are available on the synthesis, characterisation and biochemical aspects of their metal complexes.

The present investigation is mainly on the synthesis, characterisation and biochemical studies of certain new curcuminoid analogues containing indole rings and their metal complexes. Curcuminoids are the active chemical constituents present in the traditional medicinal plant, turmeric. Some of the reported studies on turmeric and curcuminoids are given below.

Curcuminoids, a group of structurally related compounds are the most important active chemical components present in the herbaceous medicinal plant *Curcuma longa* Linn. (turmeric). Curcuminoids are 1,3-diketones, in which the diketo function is directly attached to olefinic groups. The structural similarity of these compounds, with 1,3-diketones made their study more interesting. 1,3-diketones are known to form complexes with almost all metal and metalloid ions in the periodic table.[15,29,112-116] Curcuminoids and allied derivatives possess several interesting physico-chemical and biochemical properties.

Chemical and biochemical properties of turmeric and curcuminoids

Biodiversity of natural resources has not only served for the primary human needs, but also served for health care, since time immemorial. They are in different forms of phytochemicals, like alkaloids, terpenes, steroids and other chemical substances which are being used to cure variety of diseases.

Recently organic and biochemists were seriously engaged in the isolation, structural characterisation, chemical synthesis and biochemical investigation of the active chemical constituents of various medicinal plants. With the advancement of science and technology and the introduction of sophisticated equipments, it has become practically possible to isolate, and characterise almost all chemical compounds

present in medicinal plants.[117-119] The studies on the biochemical properties have undoubtedly proved that, to a large extent the medicinal uses of a particular plant is due to the presence of certain specific compounds and their synthetic analogues also exhibit similar medicinal properties.

India has a very rich cultural tradition in the use of indigenous plants and plant products for treatment of human ailments. Fortunately this subcontinent is highly rich in diverse types of medicinal plants. In this context, one of the well known example is turmeric. Turmeric occupies an inevitable position in the life of Indian people not only as a spice but also as a common remedy for many diseases.

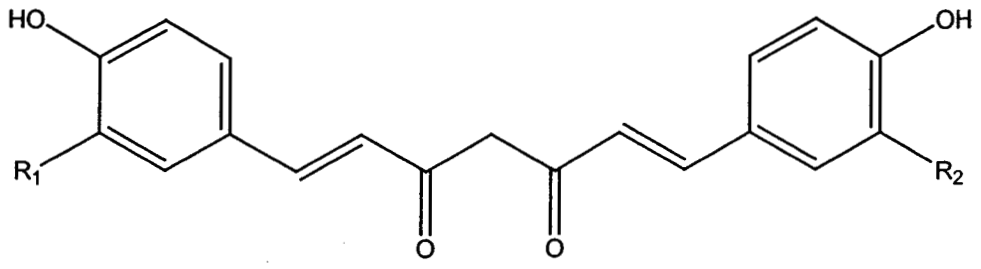
Turmeric products

The most important natural source of curcuminoids is turmeric. Turmeric (*Curcuma longa* L. Zingiberaceae family), a native of South and South East Asia, is a medicinal plant extensively used in Ayurveda, Unani and Siddha systems of medicine and as home remedy for various diseases.[120,121] Traditionally, addition of turmeric powder in food preparation preserved its freshness and nutritive value, improved the palatability, aesthetic appeal and shelf life of perishable food items. It is also considered as auspicious and is a part of religious rituals.

The composition of turmeric consists of various percentages of protein, fat, minerals, moisture and carbohydrate.[122] The important commercial products derived from raw turmeric are turmeric powder, turmeric oleoresin, turmeric oil and curcuminoids.[98,123] Aroma of turmeric, caused by the steam volatile oil fraction ranges from 2.5-7.2% of the spice. The major constituents of turmeric oil are, zingiberene, sesquiterpene and small amount of α-phellandrene, sabinene, cineole and borneol.[98,124,125] For extracting curcuminoids from powdered dried rhizomes of *Curcuma longa*, hot ethanol is the suitable extractant, composed to other solvents.

Constitution of curcuminoids

The crude pigment extracted from turmeric was found to contain three well defined compounds[126,127] and these compounds were identified as curcumin-I (diferuloylmethane) (1) or curcumin, curcumin II (feruloyl-p-hydroxycinnamoylmethane) (2) and curcumin-III (bis-4-hydroxy-cinnamoylmethane) (3).[126,127] Curcumin-I which eluted first from the column is the major component (~ 40%) followed by curcumin II (~ 16%) and curcumin III (~ 10%). The structure of the compounds (1, 2, 3) were later confirmed by chemical degradation studies.



5

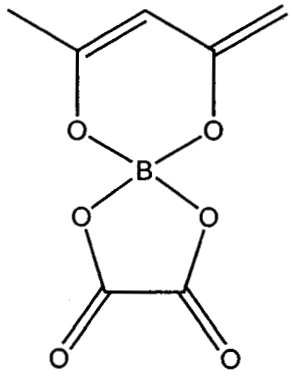
(1)	OCH_3	OCH_3	Curcumin I or curcumin
(2)	OCH_3	H	Demethoxycurcumin
(3)	H	H	bis-demethoxycurcumin

Metal chelates of curcuminoids

Since curcuminoids contains a diketo moiety, they are expected to form large number of metal chelates as 1,3-diketones do. But the number of compounds reported are very few.[128-130] When curcuminoids react with slaked lime, the natural yellow colour turns to deep red. This colour change may be attributed to the replacement of enolic/phenolic proton by the Ca^{2+} ion. A gold(I) complex of curucmin I, was reported to possess antiarthritic activity.[131] Cu(II) complexes of curcumin inhibit γ-radiation induced lipid peroxidation in liposomes and it is a good free radical scavenger.[132]

The ability of curcumin to form complexes with metal ions has got numerous application in analytical chemistry. It has been reported that curcumin form different complexes with boron under different reaction

conditions.[133-137] A red complex Rubrocurcumin was formed by the reaction between boric acid, curcumin and oxalic acid, in Cu ratio 1:1:1 in the complex **(6)** and another species rosocyanin formed by reaction between boric acid, and curcumin in presence of mineral acids (sulphuric acids) in combination with glacial acetic acid **(7)**. These reactions were used for the determination of microlevel of boron in soil. Complex formation similar to rubrocurcumin takes place with different α-hydroxy carboxylic acids and dicarboxylic acids instead of oxalic acid.



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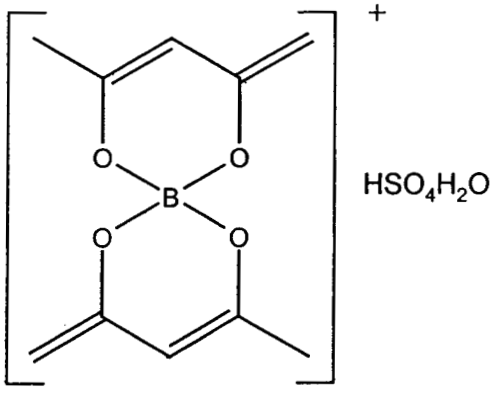


(7)

The change in colour of curcuminoids with pH change of the media has got applications in designing acid-base indicators. "Turmeric paper" available in the market is a commercialised curcumin indicator system.[138] Curcumin is also used as an effective spot reagent for identifying the

presence of boron. This has been utilized in agriculture, waste water and metallurgical anlaysis.[133,139]

Metabolism of curcuminoids

From the available reports on the uptake, distribution and excretion of curcuminoids in rats, it was revealed the 65-85% of the orally administered dose was excreted in the faeces, while negligible amounts were recovered in urine.[140-143] After intraperitoneal administration of curcumin in mice, the major part were found in the intestine, and the concentration in bile, liver, spleen, kidney and brain were very negligible.

Studies on the nature of metabolates of curcumin in plasma suggested that curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin (THC). Further studies on the pH dependent stability of these compounds suggest that curcumin gluocoronoside, dihydro curcumin-glucuronoside, THC-glucuronoside and THC are the major metabolites of curcumin in vivo.[144] Ireson *et al.* studied the bio transformation of curcumin by human and rat hepatocytes and identified hexahydrocurcumin and hexahydrocurcuminol as the major metabolites of curcumin.[145]

Combinatorial studies of curcumin with drugs also reveals synergistic actions. Curcumin enhances the antitumor effect of cis platin,

when used in combinatin against fibrosarcoma.[146] Therefore efficiency and synergistic effects of curcumin in combination with other dietary constituents warrants further study to exploit its full potential.

Medicinal applications of curcuminoids

All the three curcuminoids present in turmeric are pharmacologically important. As a result numerous studies exist in various biological activities of these curcuminoids both natural[147-151] and synthetic.[152-154] Besides, recently, a number of compounds structurally related to curcuminoid, have been synthesised.[155-157] These compounds exhibit a broad spectrum of medicinal activities. Most of these studies are on antimicrobial, antiinflammatory, antioxidant and anticarcinogenic activity.

The antidiabetic effect of curcumin was proved by its ability to prevent galactose-induced cataract formation at very low doses.[158] Both turmeric and curcumin decreases blood sugar level in alloxan-induced diabetes in rat.[159] Most importantly, curcuminoids show anti-HIV activity by inhibiting the HIV-1 integrase needed for viral replication.[160,161] It also inhibits UV light induced HIV gene expression.[162] Thus curcumin and its analogues may have the potential for novel drug development against HIV.

The phenolic group together with the β-diketone moiety present in curcuminoids are suggested to be responsible for their high biological activity and this leads to further studies using compounds having structural similarity to curcuminoids.[163,164]

Photophysical properties of curcuminoids

A striking photophysical property of these curcuminoid is, their fluorescent nature. Curcuminoids are insoluble in water, but freely soluble in alkali as well as in acids. But as the pH changes the colour of curcumin changes.[128,129] In organic solvents and aqueous media its colour is not constant due to degradation. These show that the fluorescence behaviour of curcuminoid greatly depends on the solvent.[165] The fluorescence spectra of these compounds revealed that, the various chromophore moieties such as β-diketone, diene, phenyl rings and substituents etc. interact strongly with each other, due to conjugation of their π electrons in the enolic configuration as well as the electronic properties of substituents.

The fluorescence characteristics of the curcuminoids considered in the present investigation were also studied.

CHAPTER 2

MATERIALS, INSTRUMENTS AND METHODS

Materials

Chemicals used for synthesis were of C.P. grade. For analytical purposes 'AnalaR' grade chemicals were employed. Commercial solvents were distilled and used for synthesis. Solvents purified by methods recommended by Weissberger[166] were employed for physico-chemical measurements.

The following metal salts were used for the synthesis of metal complexes. Iron(III) chloride hexahydrate, cobalt(II) acetate tetrahydrate, nickel(II) acetate tetrahydrate, copper(II) acetate monohydrate, Zn(II) acetate dihydrate and aluminium(III) sulphate hexahydrate.

Only compounds isolated analytically pure are reported in the thesis. The complexes reported here are stable and hence good keeping qualities. Compounds for recording spectra were recrystallised from proper solvents several times till chromatographically pure (tlc-silica gel) samples were obtained.

Instruments and Methods

Elemental analysis: The ligands and metal complexes were analysed by standard methods. Carbon, hydrogen and nitrogen percentages were determined by microanalysis using HERAEUS CHNO rapid analyser. The metal percentage were recorded using PERKIN ELMER 2380, atomic absorption spectrophotometer, after decomposing with concentrated sulphuric acid and nitric acid mixture.

UV-Visible spectra were recorded from JASCO V-550 Uv-visible spectrophotometer, using a 10^{-6} M solution of the compounds in methanol unless otherwise mentioned.

Infrared spectra were recorded using JASCO FT/IR 4100 instrument. The spectra were recorded from disc with KBr. Bands were calibrated using the nearest polystyrene bands.

^{1}H nmr spectra: The instrument employed was JEOL 400 nmr spectrometer (RSIC, IIT, Bombay). The spectra were recorded using $CDCl_3$ / DMSO-d_6 as solvents and TMS as internal reference.

FAB mass spectra were obtained from CDRI Lucknow. The spectra were recorded at room temperature using argon (6 KV, 10 mA) as the FAB gas and meta-nitrobenzyl alcohol (NBA) as the matrix. The probable matrix

peaks are located at m/z 136, 137, 154, 289 and 307. If metal ions such as Na^+ are present these peaks may be shifted accordingly.

EI mass spectra were obtained from CDRI Lucknow and they were recorded by imparting vapourised samples with a beam of electron at 7-0 eV.

ESR spectra (X-band) were obtained from RSIC, IIT Chennai. The spectra of copper complexes were recorded by using Varian E 112 ESR spectrometer, at 77 K, in glassy state between 8.5-9.5 GHz and calibrated with Diphenyl Picryl Hydrazil (DPPH) free radical for which g = 2.0036 in DMF. The microwave power employed was 5 mW and the field set up at 3000 G. Scan time was 4 hrs.

Magnetic susceptibility measurements were done by using SHERWOOD scientific magnetic susceptibility balance at room temperature (28 ± 1°C). The instrument was calibrated using the standard $Hg[Co(NCS)_4]$ as standard.[167] χ_g was calculated using the formula

$$\chi_g = \frac{C_{bal} \times \ell \times R\text{-}R^\circ}{10^9 \times m}$$

where C_{bal} is a constant with value 1.0192, ℓ the length of the sample taken, R observed value, R° value for empty tube (its value at 29°C is –31) m is the mass of the substance taken.

Molecular weights of the compounds reported were determined by Rast's method[168] using naphthalene / camphor as medium.

pH measurements were done by using Systronics pH meter at required concentration.

Thermal analysis data were obtained from Department of Chemistry, Kerala University. The thermogram were recorded by Mettler Toledo SR TG at a heating rate 10°C per minute.

Fluorescence spectra were recorded by using Elico spectrofluorometer SL 174. Solutions of concentrations (range $10^{-3} - 10^{-6}$ M) were employed for taking the fluorescence spectra.

To determine the quantum yield, quinine sulphate was taken as the standard. Various concentrations of quinine sulphate were prepared by dissolving calculated amount of the substance in 0.1 M sulphuric acid. To study the effects of metal ions on the fluorescence of various compounds, the solutions of metal salts were prepared in pure methanol.

Ground state absorption measurements were carried out with systronics UV-Vis double beam spectrophotometer. To record the fluorescence spectra of the compounds the fluorescence emitted in the direction perpendicular to that of the excitation was dispersed by a spectrograph and detected by a diode array based optical analyser.

Single crystal XRD data were obtained from a BRUKER AXS KAPPA APE X-II instrument using Mo (K_α) as the source of X-rays. Polarised light microscope was used to detect the crystalinity of the sample. Various informations regarding the cell parameter, space group, cell morphology, three dimensional molecular structure and molecular packing, etc. are obtained from the X-ray diffraction study. Details on the techniques employed for the determination of crystal structure are given in appropriate section.

Microwave oven of conventional type was used for microwave assisted synthesis of the compounds.

Biological studies: Materials, animals, chemicals, techniques and instruments employed for various biological studies are given in the respective section in chapter 3.

CHAPTER 3

SYNTHESIS, CHARACTERISATION, FLUORESCENCE CHARACTERISTICS AND BIOLOGICAL ACTIVITIES OF 1,7-DIINDOLYL-1,6-HEPTADIENE-3,5-DIONE AND ITS METAL COMPLEXES

Synthesis, characterisation and certain biological activities of 1,7-diaryl-1,6-heptadiene-3,5-diones, where the aryl groups are phenyl, naphthyl, substituted phenyl and naphthyl and some of their metal complexes were reported earlier.[155,169] However reports are scanty on 1,7-diarylheptanoids containing heteroaryl ring systems. It is to be expected that presence of heteroaryl rings will modify the chemical and biochemical properties of curcuminoids (1,7-diarylheptanoids). With this intention, a new curcuminoid analogue, in which indole rings are linked to the unsaturated diketo group, namely, 1,7-diindolyl-1,6-heptadiene-3,5-dione (1,7-diindolylheptanoid) and its metal complexes were synthesised and characterised in the present study. The fluorescence characteristics of the diindolylheptanoid was also studied. Biochemical properties such as antioxidant, antitumour and cytotoxicity of the 1,7-diindolylheptanoid and its metal complexes were also examined. For better readability the results are presented in three sections as below:

Section 1 : Synthesis and characterisation of 1,7-diindolyl-1,6-heptadiene-3,5-dione and its metal complexes.

Section 2 : Fluorescence characteristics of 1,7-diindolylheptanoid.

Section 3 : Biological studies of 1,7-diindolylheptanoid and its metal complexes.

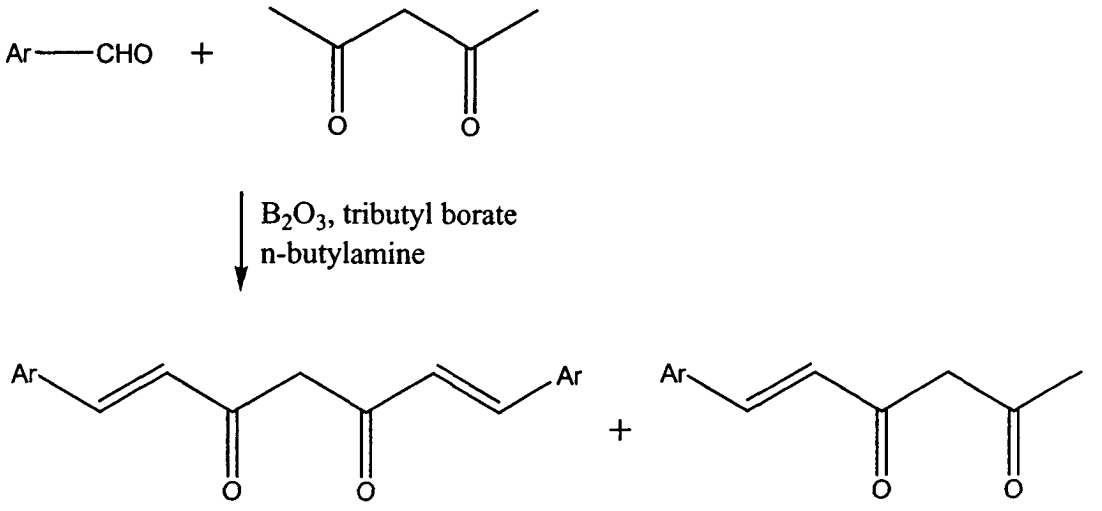
SECTION 1

SYNTHESIS AND CHARACTERISATION OF 1,7-DIINDOLYL-1,6-HEPTADIENE-3,5-DIONE AND ITS METAL COMPLEXES

Introduction

As early as in 1913 Lampe and Milobetzka reported the synthesis of diferuloylmethane (curcumin I of the natural curcuminoids) by the condensation of vaniline and acetylacetone.[170,171] This method was modified by H.J.J. Pabon in 1964 as a general route for the synthesis of curcuminoids (1,7-diarylheptanoids)[172] and has been successfully employed for the preparation of a number of 1,7-diarylheptnaoids and allied derivatives. The method involves the condensation of aromatic aldehydes with acetylacetone in presence of boric oxide, tri(sec-butyl)borate and n-butylamine in ethyl acetate medium. The use of B_2O_3 and butylborate is to prevent Knoevenagel type condensation and to facilitate only aldol type condensation at the methyl groups of acetylacetone. It has been shown that the condensation occur only at the methyl group due to the formation of a boron complex with the diketo function. The boron complex prevent the condensation of the aldehyde at the methylene group.

The synthetic route usually leads to the formation of both monocondensation and bis condensation products as in scheme 3.1.



Scheme 3.1

The yield of the 1,7-diarylheptanoids (biscondensation product) depends on the reaction temperature. Maximum yield was found to be obtained in the range 85-110°C. The same method was employed for the synthesis of the curcuminoid analogue considered in the present study.

Curcuminoids are typical 1,3-diketones and are expected to form complexes with various metal ions. Recently metal complexes of some synthetic and natural curcuminoids have been reported.[129,130-135,173-176] For characterisation of these curcuminoids and their metal complexes various spectral and physicochemical techniques have been reported. To avoid repetition these studies will be quoted at appropriate places while discussing the results of the present investigation.

Microwave assisted synthesis of curcumin and curcuminoids: Microwave (MW) activation as a non-conventional energy source has become a very popular and useful technology in synthetic chemistry. The acceleration of reactions by microwave exposure results from material-wave interactions leading to thermal effects and specific effects.[177,178] The thermal effects can result from dipolar polarization as a consequence of dipole-dipole interactions between polar molecules and the electric field. The origin of specific effect is two-fold, namely those which are not purely thermal and a thermal effect connected to the intervention of hot spots. Microwave effects also depends on the reaction medium.[179,180]

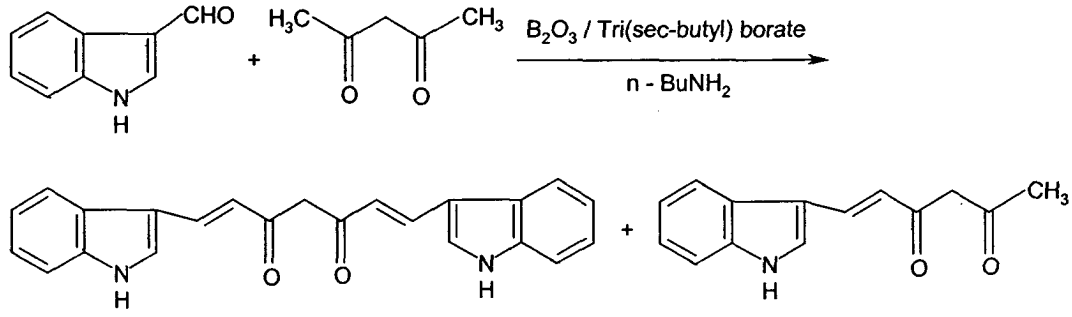
A novel microwave assisted extraction (MAE) technique was reported for selective and rapid extraction of curcuminoids from *Curcuma longa*, into organic solvents. The extraction process was optimised using acetone at 20% power level (PL) giving 60% extraction of curcuminoids with 75% purity.[181] The diindolylheptanoid compound in the present study was also synthesised by microwave method.

Experimental

Synthesis of 1,7-diindolyl-1,6-heptadiene-3,5-dione (1,7-diindolyl-heptanoid, Hdih)

The 1,7-diindolylheptanoid was synthesised by the condensation of indole-3-carbaldehyde with acetylacetone-boron complex in the presence

of tri (sec-butyl) borate and n-butylamine as the condensing agent as in the reaction **scheme 3.2** below. The reaction leads to the formation of 1,7-diindolylheptanoid as the major product (65%) along with small amounts of 6-indolylhexanoid (the mono condensation product). Pure 1,7-diindolyl heptanoid was obtained through column chromatographic separation of the products. A brief procedure for the synthesis and purification of 1,7-diindolylheptanoid is give below:



Scheme 3.2

Acetylacetone (0.5 mL, 0.005 mol) and boric oxide (0.25 g, 0.0035 mol) were mixed in a bottle and stirred for ~ 1 h. To this, a solution of indole-3 aldehyde (1.45 g, 0.01 mol) in 10 mL dry ethyl acetate and tri(sec-butyl) borate (4.6 mL, 0.02 mol) were added. The mixture was stirred continuously with the addition of n-butylamine (0.1 mL), dropwise in 40 minutes. The stirring was continued for an additional period of ~ 4 h while maintaining the temperature between 90-90°C and set aside

overnight. Hot (~ 60°C) hydrochloric acid (0.4 m, 7.5 mL) was then added and to mixture and stirred for ~ 1 h. Two layers were separated and the organic layer was extracted thrice with 5 mL ethyl acetate and the combined extracts were evaporated to a paste, which was stirred with HCl (50%, 10 mL) for 1 h. The solid product separated was collected, washed with water and dried in vacuum. The tlc of the product obtained revealed the presence of two compounds. The 1,7-diindolylheptanoid were quantitatively separated by column chromatography as illustrated below.

The crude product obtained was dissolved in minimum amount of dry ethyl acetate and placed over a column packed with silica gel (mesh 60-120) and eluted with 1:9:16 v/v acetone-benzene-chloroform mixture at a uniform flow rate of 2 mL per min. As the elution proceeds, two bands were developed in the column. The eluate from the pale yellow lower region and the junction between the two bands, were discarded. The elution was repeated by using 1:5 v/v mixture acetone-chloroform to recover the red band retained in the upper portion of the column. The eluates were collected in aliquots of 10 mL in separate test tubes, checked by tlc and the combined extracts were evaporated to remove the solvent to get the 1,7-diindolylheptanoid. The compound was recrystallised from ethanol-toluene (1:1) mixture to get spectroscopically pure sample.

Synthesis of the metal chelates

The general method adopted for the preparation of Al(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes is given below.

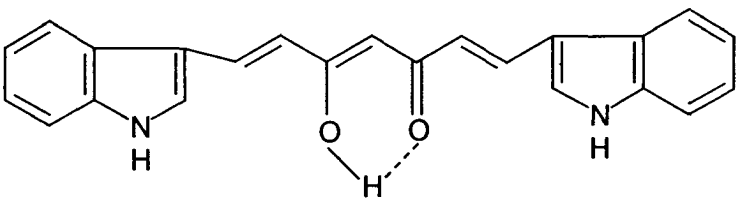
To a refluxing solution of the compound in ethanol (0.002 mol, 20 mL) a solution of the metal salt (0.001 mol, 20 mL) in 1:1 methanol-water mixture was added drop by drop with stirring. The pH of the solution was adjusted around 6 using sodium acetate and refluxing was continued for ~ 4 h. The solution was concentrated to half the volume and then cooled in ice. The precipitated complex was filtered washed several times with 1:1 mixture of methanol and water, recrystallised from acetonitrile and dried in vacuum.

Results and Discussion

Characterisation of 1,7-diindolylheptanoid, Hdih

Although the compound 1,7-diindolylheptanoid is a simple symmetrical β-diketone, its synthesis is not a straight forward di-aldol condensation on 2,4-pentanedione. The C_3 of 2,4-pentanedione bears more acidic protons than those on C_1/C_5 and therefore aldol condensations on terminal methyl groups (C_1 andC_5) must be carried out successively via the dienolate; this is hard to obtain and reaction at C_3 often leads to side products. Use of boron based protection of the 1,3-

diketone circumvents the Knoevenagel type condensation at C_3 and facilitates aldol type condensation at C_1 and C_5 of 2,4-pentanedione. Boron based reagent such as boric oxide complexes as Lewis acid with the β-diketone system and consequently reduces the nucleophilicity of the C_3 position and the reaction occurs at the terminal methyl groups resulting in the formation of diindolylheptanoid. This is evident from the fact that maximum yield of the compound was obtained when the reaction was carried in presence of tri(sec-butyl) borate and condensing agent n-butylamine.



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The compound is crystalline in nature shows sharp melting point and is freely soluble in common organic solvents. The observed C, H, N percentages and molecular weight (Table 3.1) together with mass spectral data of the compound clearly suggest the formation of the bis condensation product in which two equivalents of aldehyde has condensed with one equivalent of acetylacetone (scheme 3.2). The observed spectral data confirm the structure 3.1 of the compound.

TABLE 3.1

Physical, analytical and uv spectral data of the 1,7-diindolylheptanoid,Hdih

M.P. °C	Elemental analysis			Mo. Wt.	λ_{max} in Methanol	log ε
	C	H	N			
	Found / (Calcd.)					
165	77.04 (77.96)	4.61 (5.08)	7.20 (7.90)	352 (354)	449 276	4.87 4.05

Uv spectrum : The uv spectrum of the compound shows two absorption maxima (Table 3.1). Based on earlier reports,[15,182-184] the band at 449 nm corresponds to the n → π* transition and the one at 276 nm due to π→π* transition. The n→π* absorption values of simple diketones are much lower than that of the 1,7-diarylheptanoids.[182] The increase in the values for **Hdih** may be due to the α,β-unsaturation of the diketo group.

Infrared spectrum: Assigning of the correct tautomeric structure of these types of compounds can be done with the help of ir spectra. In these types of compound, the dicarbonyl group is the most useful function available for characterisation and structure elucidation. The position and intensity of the carbonyl stretching band is determined by molecular structure in its immediate vicinity and is, therefore, very valuable for characterising the type of carbonyl function.

Normal acetyl carbonyl gives stretching band at ~ 1720 cm^{-1}. The carbonyl stretching frequency of aroyl groups is at ~ 1650 cm^{-1}, which is much lower than that of an acyl group. Hydrogen bonding decreases carbonyl frequency. The carbonyl stretching further lowered when C=O is in conjugation with C=C, C=N, etc., Thus in the case of 1,3-diketones and allied derivatives, the nature and position of carbonyl stretching bands can provide valuable information regarding their structure.[15,134,185,186]

The enol tautomer does not show the normal carbonyl absorption. Instead a broad intense band appears in the region 1600-1640 cm^{-1}. This is because of the strong intramolecular O–H...O hydrogen bonding possible in the enol form. Another characteristic feature of the enol tautomer is the stretching of the OH bands at ~ 3600 cm^{-1}. However, the absorption is usually seen as a broad band at 2700-3800 cm^{-1}, presumably due to its involvement in the strong intramolecular hydrogen bonding.[173,185]

The infrared spectrum of the 1,7-diindolylheptanoid is dominated by the presence of two intense bands at 1613 and 1634 cm^{-1} in the double bond region, and in the X–H stretching region an intense broad band in the range 3100-3800 cm^{-1}. From a comparison of the reported spectral data of typical 1,3-diketones[15,134] and 1,7-diarylheptanoids,[155,183,184] the band at 1634 cm^{-1} can be assigned to the stretching of intramolecularly

hydrogen bonded carbonyl function. However the frequency value is significantly higher than that of 1,7-diarylheptanoids. This can be attributed to the influence of the heterocyclic ring system on the degree of enolisation.

The ethylenic double bond in unsaturated ketones usually shows two absorption band at 1600-1625 and ~ 990 cm^{-1}, the latter being characteristic of trans CH=CH– group. Thus the band at 1613 appears to be due to the olefinic functions of the compound. A prominent band observed at 1084 cm^{-1} can be assigned to the *trans* nature of the olefinic function since *cis* ethylene function usually show weak intensity bands at much lower region.[185,186] Several medium intensity bands observed in the region 1500-1600 cm^{-1} are due to various ν(C=C) vibrations.

The presence of intramolecular hydrogen bonding is clearly indicated from the appearance of the broad band in the X–H stretching region. The stretching band of indole N–H group also appears in this region. Other bands appeared in the region are due to various aromatic and aliphatic C–H stretching vibrations. Thus the ir spectrum fully support the intramolecularly hydrogen bonded enol form of the compound. The spectrum is reproduced in figure 3.1.

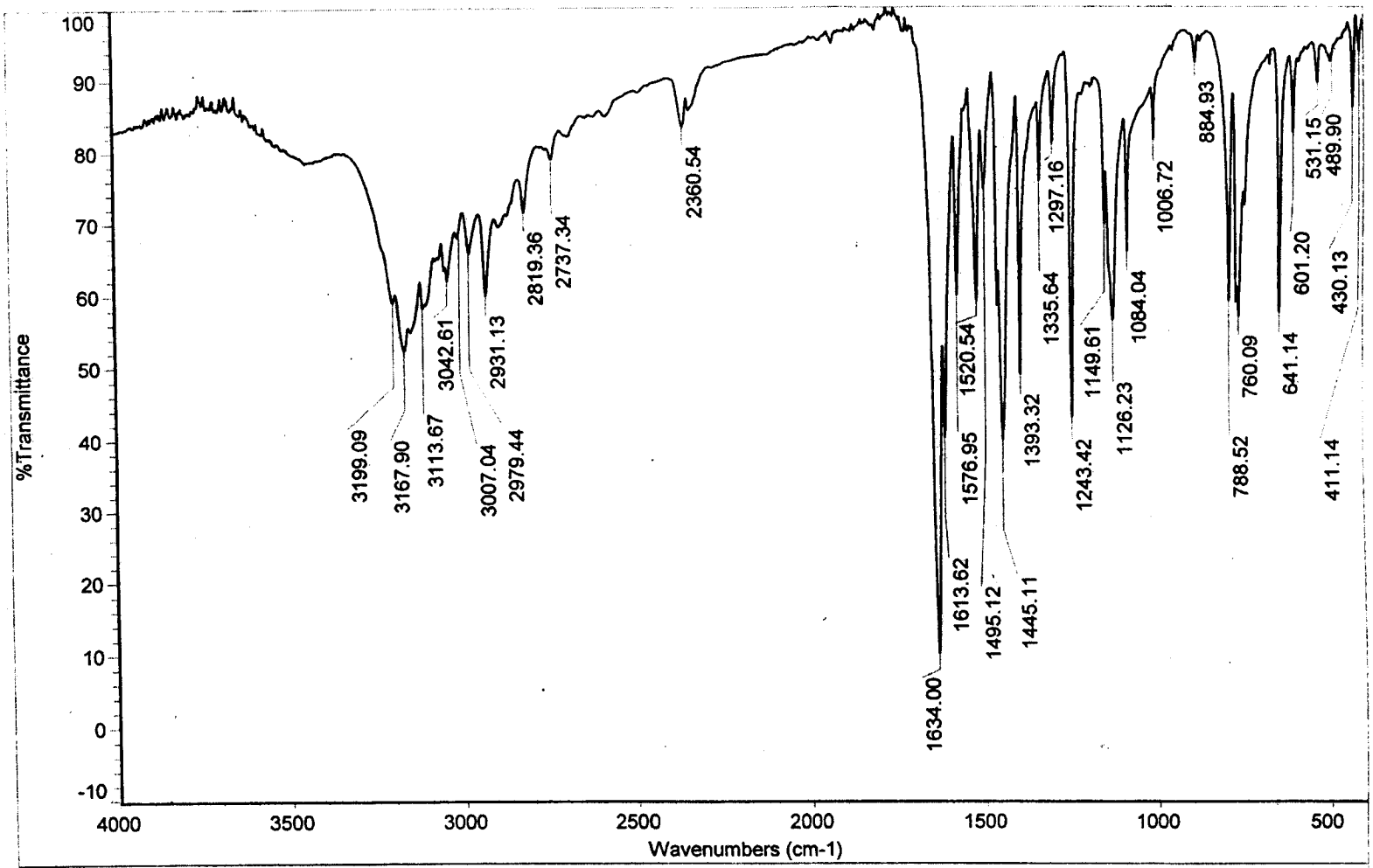


Fig. 3.1. Ir spectrum of 1,7-diindolyl-1,6-heptadiene-3,5-dione

^{1}H nmr spectrum: In the study of keto-enol tautomerism of 1,3-diketones ^{1}H nmr spectroscopy has perhaps been the most important technique.[5,15,187,188] The resonance signal of a proton participating in strong intramolecular hydrogen bonding generally appears in the downfield region of the nmr spectrum and is characteristically broad. However, such protons are prone to rapid exchange at room temperature between the different possible sites and in many cases it requires low temperature to quench such exchanges. The position of the methine proton signal, characteristic of the enol form, is also influenced by the electronic effects of the groups attached to the carbonyl function. Thus it has been shown that the chemical shift of the enolic proton in ^{1}H nmr spectra of 1,3-diketone is a measure of the strength of the intramolecular hydrogen bond.[187,188]

The percentage of enol tautomer present in a β-diketone, which can exist in the keto-enol tautomeric forms, can be roughly calculated from the integrated intensity of the downfield enolic proton signal. The chemical shift of the methine and enolic proton is also indicative of the tautomeric nature of the compound under consideration. The chemical shift values of methylene proton and enolic proton of some β-diketones given in table 3.3 show that these values are greatly influenced by the groups attached to the diketo function. It is due to the resonance between the aromatic and pseudo aromatic chelate ring which weakens the C=O

and strengthens the O–H bond by increasing the electron density on oxygen, and as a result, the enolic proton is deshielded. Phenyl substitution on 2,4-pentanedione also results in a downfield shift of the methine proton resonance. Each additional phenyl group shifts the signal 0.6 to 0.7 ppm downfield.[188] This shift is attributed to a number of phenomena such as inductive effects, long range anisotropic effects of the phenyl ring arising from its coplanarity with the chelate ring or the electron release of the phenyl ring by resonance.

The ^{1}H nmr spectrum of the 1,7-diindolylheptanoid shows a low field signal at ~ 15.00 ppm. This strongly support the existence of the compound in the intramolecularly hydrogen bonded enol form and also the influence of electronic and steric effects of the groups attached to the diketo function. This is also reflected in the position of the methine proton signal. In the case of 1,7-diindolylheptanoid the signal due to the methine proton appeared at 5.7 ppm, an upward shift compared to the acetylacetone and dibenzoylmethane. The shift in methine proton as well as enolic proton can be attributed to the influence of the heterocyclic ring on the degree of enolization and strength of the hydrogen bond. The indole NH proton signal appeared as a 2 proton singlet, at 10.15 ppm, the alkenyl at 8.25 (doublet) and the aryl protons in the range 6.9-7.9 ppm. The ^{1}H nmr spectrum of the compound is reproduced in the Fig. 3.2.

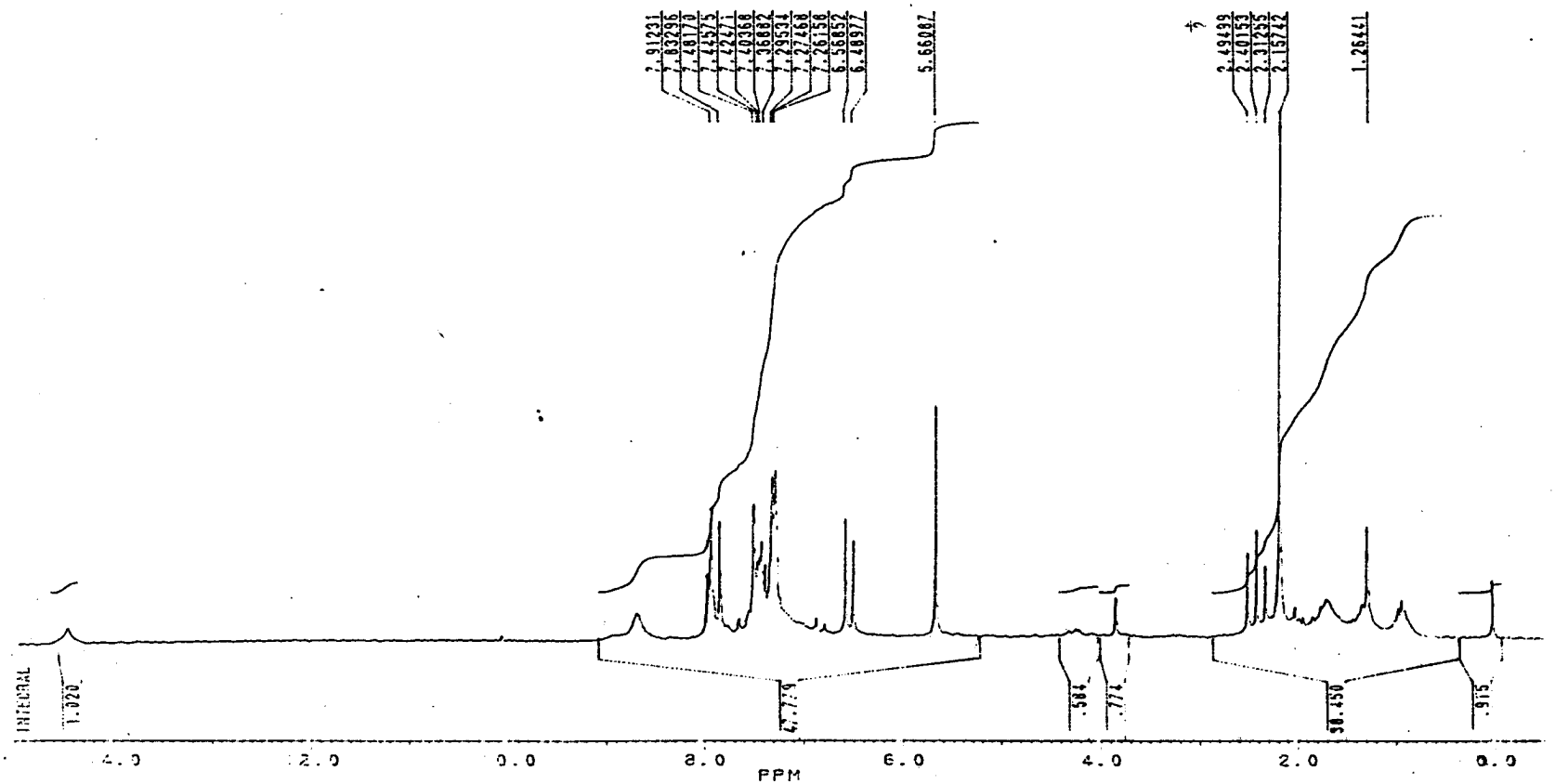
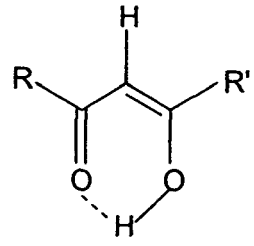


Fig. 3.2. ^{1}H NMR spectrum of 1,7-diindolyl-1,6-heptadiene-3,5-dione

TABLE 3.3

^{1}H nmr chemical shifts of methylene and enolic protons in typical β-diketones

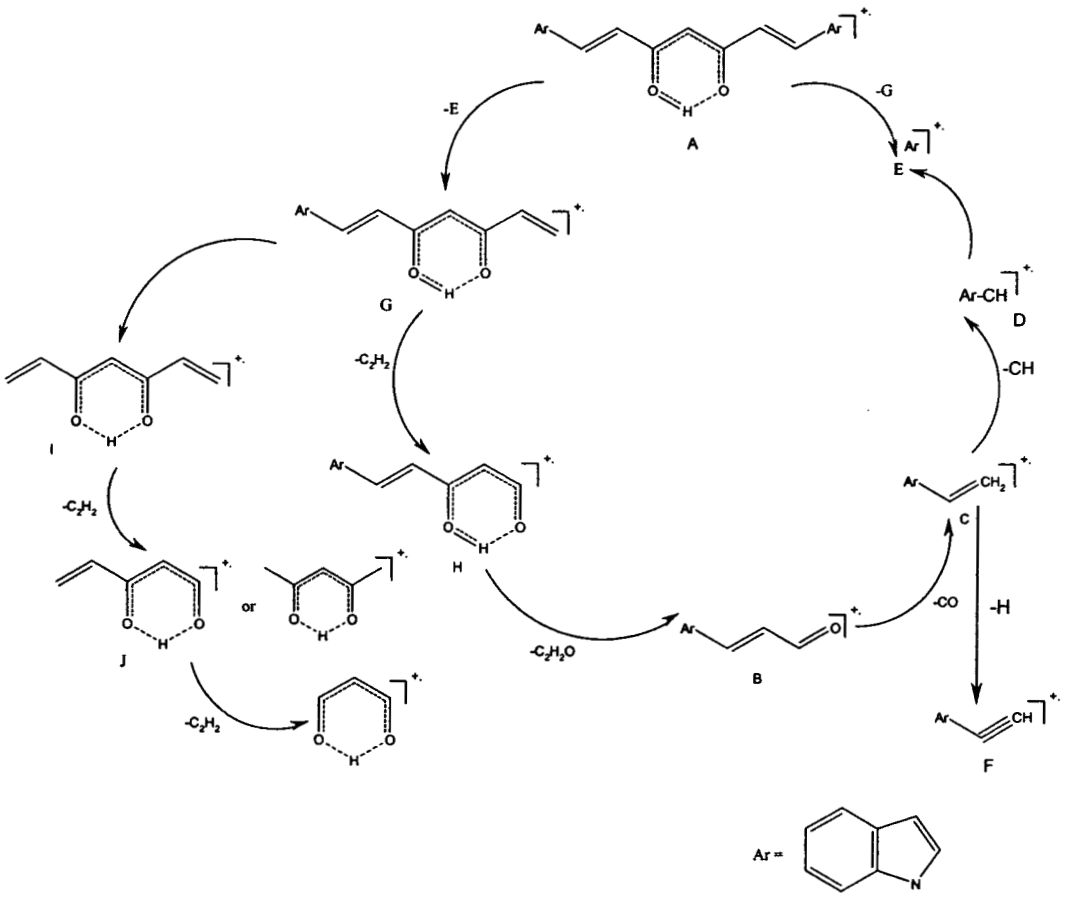


R	R'	Chemical shift (δ ppm)	
		Methylene proton	Enolic proton
CH_3	CH_3	5.44	15.40
CH_3	CF_3	5.80	14.24
CF_3	CF_3	6.43	13.00
C_6H_5	C_6H_5	6.80	17.13
CH_3	C_6H_5	6.08	16.24
CF_3	C_6H_5	6.56	15.23
2-C_4H_3S	CF_3	6.5	16.2
C_6H_5–CH=CH=	C_6H_5CH=CH	5.72	16.04

Mass spectrum: Several reports are available on mass spectral studies on diverse types of 1,3-dicarbonyl compounds.[189] The fragmentation patterns depend mainly on the nature of groups attached to the diketo function.[190] Elimination of CO, O, OH, $C_3HO_2^+$, CH_2=C=O (ketene), etc. are characteristic of acetylacetone and related 1,3-diketones.

The mass spectrum of the compound shows the parent ion peak (P^+) at m/z 354 as expected from its formulation. Although peaks due to the elimination of O, OH are very weak, a relatively intense peak

corresponds to the elimination of C_3HO_2 from P^+ at m/z 285 is present. The mass spectrum of the compound is reproduced in the fig. 3.3. The important peaks appeared in the spectrum can be conveniently accounted by the fragmentation patterns given in **scheme 3.3**. Thus all the available evidences support that the compound exists in the intramolecularly H-bonded enol form as in structure 3.1.



Scheme 3.3

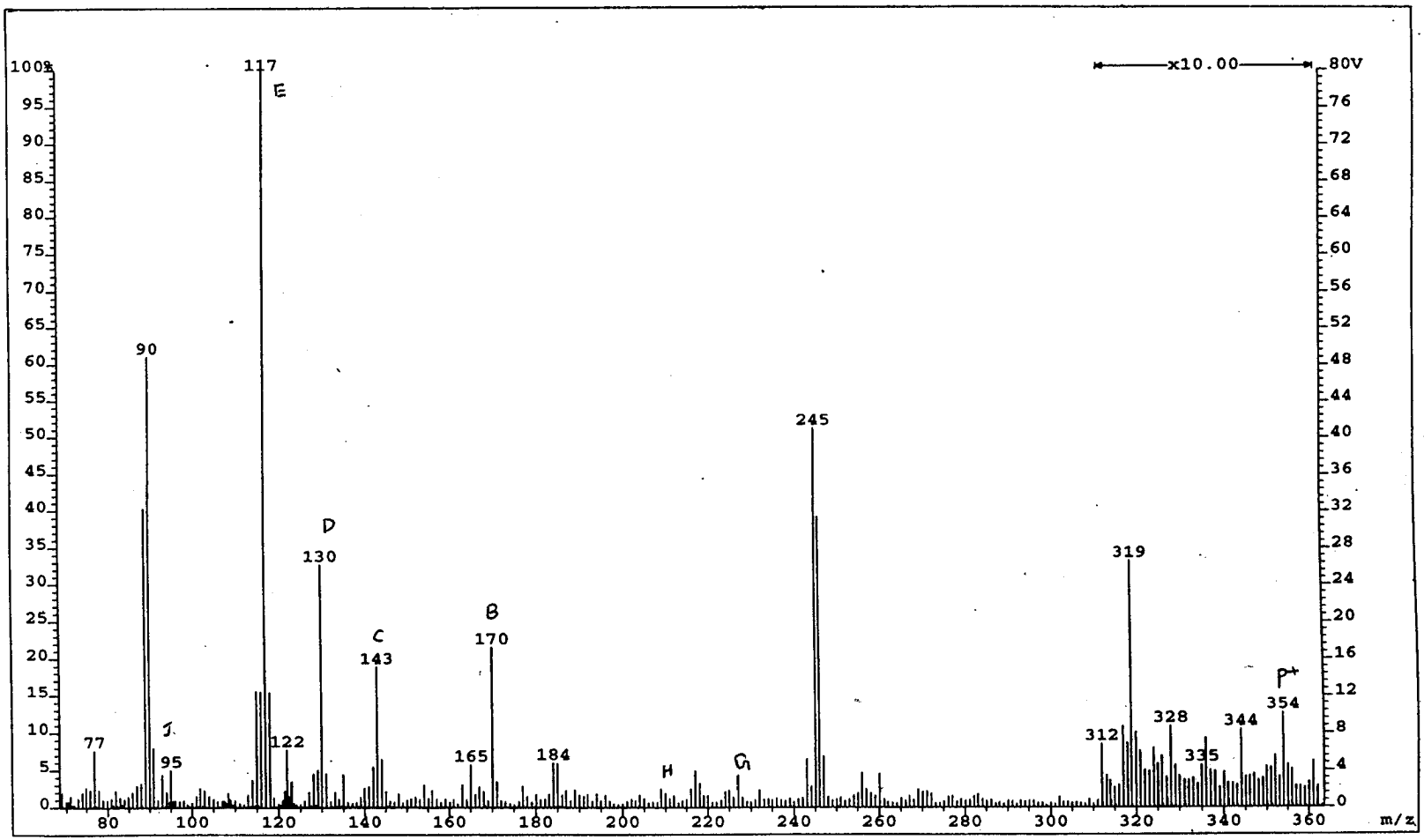
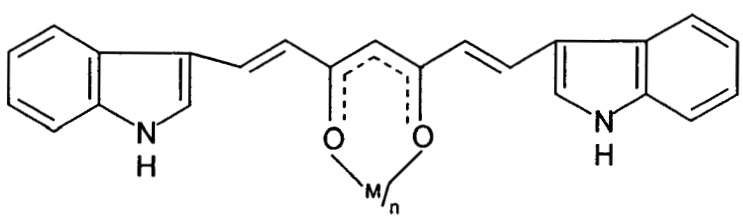


Fig. 3.3. Mass spectrum of 1,7-diindolyl-1,6-heptadiene-3,5-dione

Chracterisation of metal chelates

The 1,7-diindolylheptanoid formed well defined crystalline complexes of Al(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II). Elemental analysis (C, H, N and metal percentages) and physical data of the metal complexes are given in table 3.3. The analytical data suggests a 1:2 metal ligand stoichiometry for Ni(II), Co(II), Cu(II) and Zn(II) complexes and 1:3 for Al(III) and Fe(III) complexes. Conductometric studies showed that all the complexes behave as non electrolytes in DMF and do not contain the anion of the metal salts used for their preparation. Magnetic moment measurements showed that except aluminium and zinc complexes all other complexes are paramagnetic. The observed uv, ir, nmr, mass, esr, spectral data and thermal data are in agreement with the structure **3.2** of the complex.



Structure 3.2

n = 2 for Co(II), Ni(II), Cu(II) and Zn(II)
n = 3 for Al(III) and Fe(III)

TABLE 3.3

Physical and analytical data of Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Al(III) chelates of 1,7-diindolyl heptanoid, Hdih

Compound	M.P. °C	μ_{eff} B.M.	Elemental analysis (%) Found / (Calcd.)			
			C	H	N	M
$[Fe(dih)_3]$	> 260	5.313	7.92 (74.30)	4.08 (4.57)	7.14 (7.54)	4.83 (4.97)
$[Co(dih)_2(H_2O)_2]$	156	3.610	69.50 (69.60)	5.12 (4.71)	6.52 (6.90)	7.86 (7.36)
$[Ni(dih)_2(H_2O)_2]$	141	2.724	69.84 (69.01)	5.03 (4.80)	6.45 (7.02)	7.30 (7.33)
$[Cu(dih)_2(H_2O)_2]$	> 260	1.705	69.32 (68.57)	4.54 (4.72)	6.02 (6.95)	7.65 (7.88)
$[Zn(dih)_2(H_2O)_2]$	132	--	69.54 (69.92)	4.54 (4.45)	6.83 (7.02)	8.49 (8.10)
$[Al(dih)_3]$	242	--	75.94 (76.24)	5.08 (4.6)	6.98 (7.70)	2.81 (2.48)

Uv spectra: The uv spectra of the complexes bear close resemblance to that of the free ligand, indicating that no structural alteration of the ligand took place during complexation. Compared to the free ligand the two absorption maxima are shifted to longer wavelength in complexes. This indicate the involvement of the carbonyl oxygens in bonding with metal ion.[29,191,192]

Infrared spectra: The use of vibrational spectra in establishing the structure and nature of bonding in various metal 1,3-diketonates has been well illuminated.[29,113-116,193] This is because in metal 1,3-diketonates, the most important functions available for structural studies are the carbonyl groups, O–H, C=C and M–O bonds and all these groups show characteristic ir absorption, and from their position and nature, various structural informations can be derived.

Although the ligand 1,7-diindolylheptanoid is a complex molecule, the infrared spectra of its metal complexes are very simple and easy to interpret. In general, upon complexation, the carbonyl stretching frequency of 1,3-diketones show a shift to lower values[155,169] and an additional band due to ν(M–O) vibration appear in the region 400-500 cm^{-1}. A comparison of the spectra of metal chelates with that of the free ligand spectrum revealed that the band due to the intramolecularly hydrogen bonded carbonyl group of the enol form at 1634 cm^{-1} totally disappeared in the spectra of complexes. The only band present in the region 1600-2000 cm^{-1} in the spectra of the complexes is the band at ~ 1610 cm^{-1}. In the spectrum of the free ligand this band has been assigned to the stretching of the C=C groups.

A striking feature in the spectra of the complexes is the presence of an intense band at ~ 1565 cm^{-1}, not found in the ligand spectrum. From

its position and based on earlier reports[29,155,169,191] on spectra of 1,3-diketonates, the band can confidently be assigned to the stretching of metal bonded dicarbonyl function. Another characteristic feature of the spectra of the complexes is the disappearance of the broad free ligand band in the 3100-3800cm^{-1}. Instead medium intensity bands at ~ 3410, 3100, 2950 cm^{-1} appeared in spectra of all the complexes. The band at ~ 3410 cm^{-1} can safely be assigned to the stretching of the indolyl NH group and other bands to the stretching of various C–H groups. Thus it can be presumed that the heterocyclic NH remained as such in complexes also. Therefore in metal coordination the indole nitrogens are not involved and only the diketo oxygens are engaged is clearly evident from the ir spectra of complexes.

That the dicarbonyl group is involved in bonding with the metal ion is evident from the appearance of two new bands in the range 420-480 cm^{-1} in spectra of all the complexes assignable to the stretching of metal bonded carbonyl (M–O). A band at ~ 990 cm^{-1} due to trans –CH=CH– remain unaffected in the metal complexes also. The characteristic ir bands are given in **table 3.4**. Presence of weak bands in the region 3200-3400 cm^{-1} suggests the coordination of water molecules to the metal ion.

TABLE 3.4

IR spectral data of various metal chelates 1,7-diindolyl heptanoid

Compound	Characteristic ir bands (cm^{-1})		
	ν(C=O)	ν(C=C) olefinic	ν(M–O)
Hdih	1634	1613	
$[Fe(dih)_3]$	1558	1610	482 424
$[Co(dih)_2(H_2O)_2]$	1566	1614	458 446
$[Ni(dih)_2(H_2O)_2]$	1560	1613	466 426
$[Cu(dih)_2(H_2O)_2]$	1566	1610	479 434
$Zn(dih)_2(H_2O)_2]$	1564	1612	458 427
$[Al(dih)_3]$	1562	1610	483 426

^{1}H nmr spectra: In the ^{1}H nmr spectrum of the diamagnetic Zn(II) complex of **Hdih**, the low field signal of the free ligand due to the hydrogen bonded enol has disappeared which indicates that the metal ion replaced the enolic proton during complexation. The methine proton singlet and aromatic proton signals shifted slightly to downfield.[155] Further the signal due to the NH proton remained almost unaffected suggests that the heterocyclic nitrogens are not involved in coordination. The ^{1}H nmr spectrum of Zn(II) chelate is reproduced in the **fig. 3.4.** Thus

the nmr spectrum strongly support the bonding mode of the ligands as in structure **3.2**. The integrated intensities of all the protons agree well with the formulation of the complex.

Mass spectra: The use of mass spectrometry for the establishment of the stoichiometry and structural elucidation of metal 1,3-diketonates has been well rooted.[29,194-196] It has been shown from the mass spectral analysis of a series of copper(II) chelates of 1,3-diketones, that, stepwise removal of alkyl/aryl group(s) is a characteristic feature of all the complexes.[155,169] Electronic and steric effect of the group(s) attached to the diketo function strongly influence the stability of various fragments formed under mass spectral condition. The FAB mass spectra of Cu(II) and Al(III) complexes of Hdih are shown in **figs. 3.5, 3.6**. The spectrum of Cu(II) shows the step wise removal of aryl groups. The molecular ion peak at 767 and 769 in agreement with the $[CuL_2]$ stoichiometry. Peaks due to $[ML]^+$, L^+ and fragment of L^+ are also detected in the spectrum. The mass spectrum of the Al(III) complex clearly indicates $[AIL_3]$ stoichiometry of the complex. From the observed peaks in the spectrum of the Cu(II) complex it can be seen that certain fragments rearrange to form stable cyclic species as given in the fragmentation **scheme 3.4**.

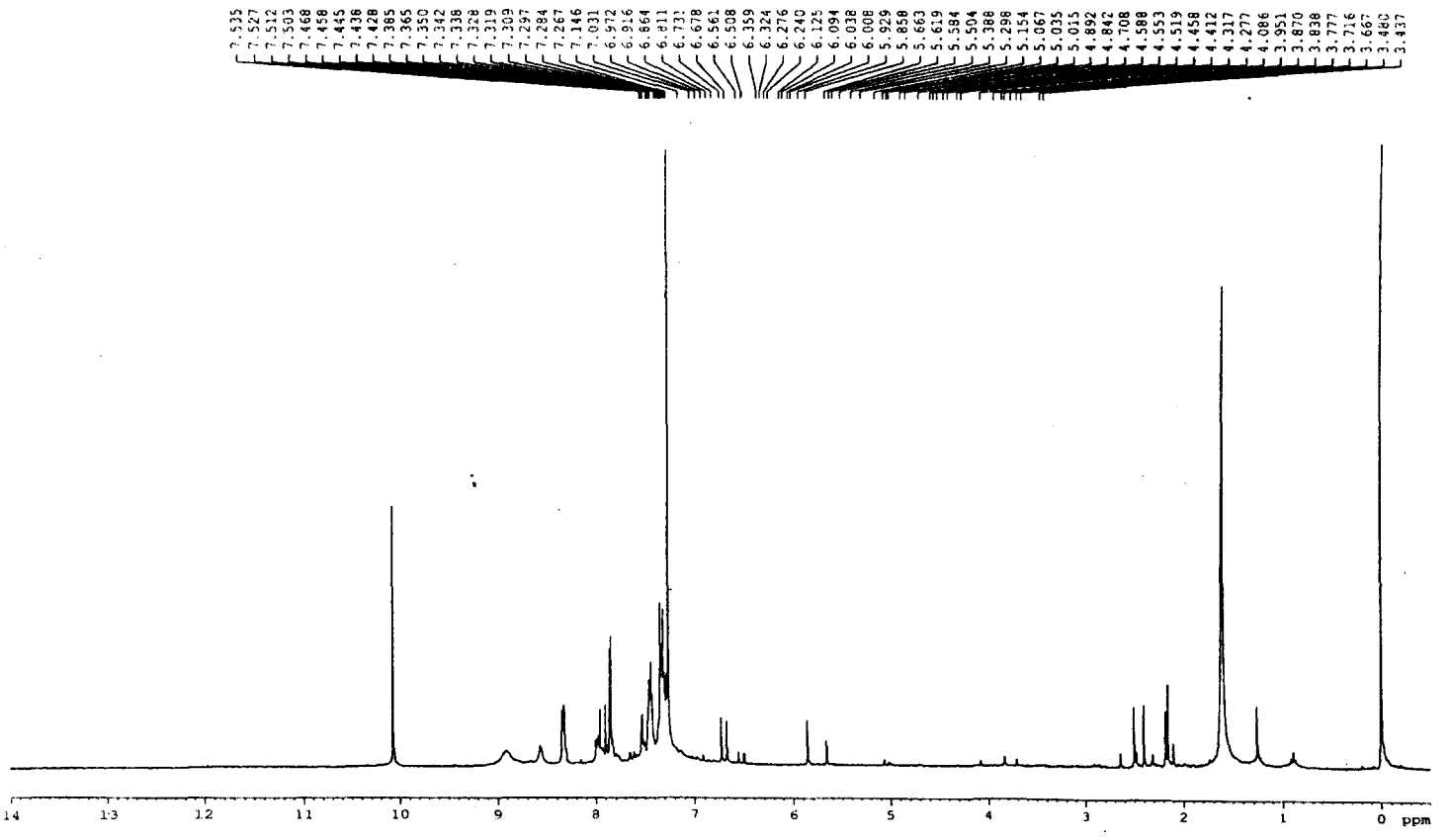


Fig. 3.4. Nmr spectrum of zinc(II) complexes of 1,7-diindolyl heptanoid

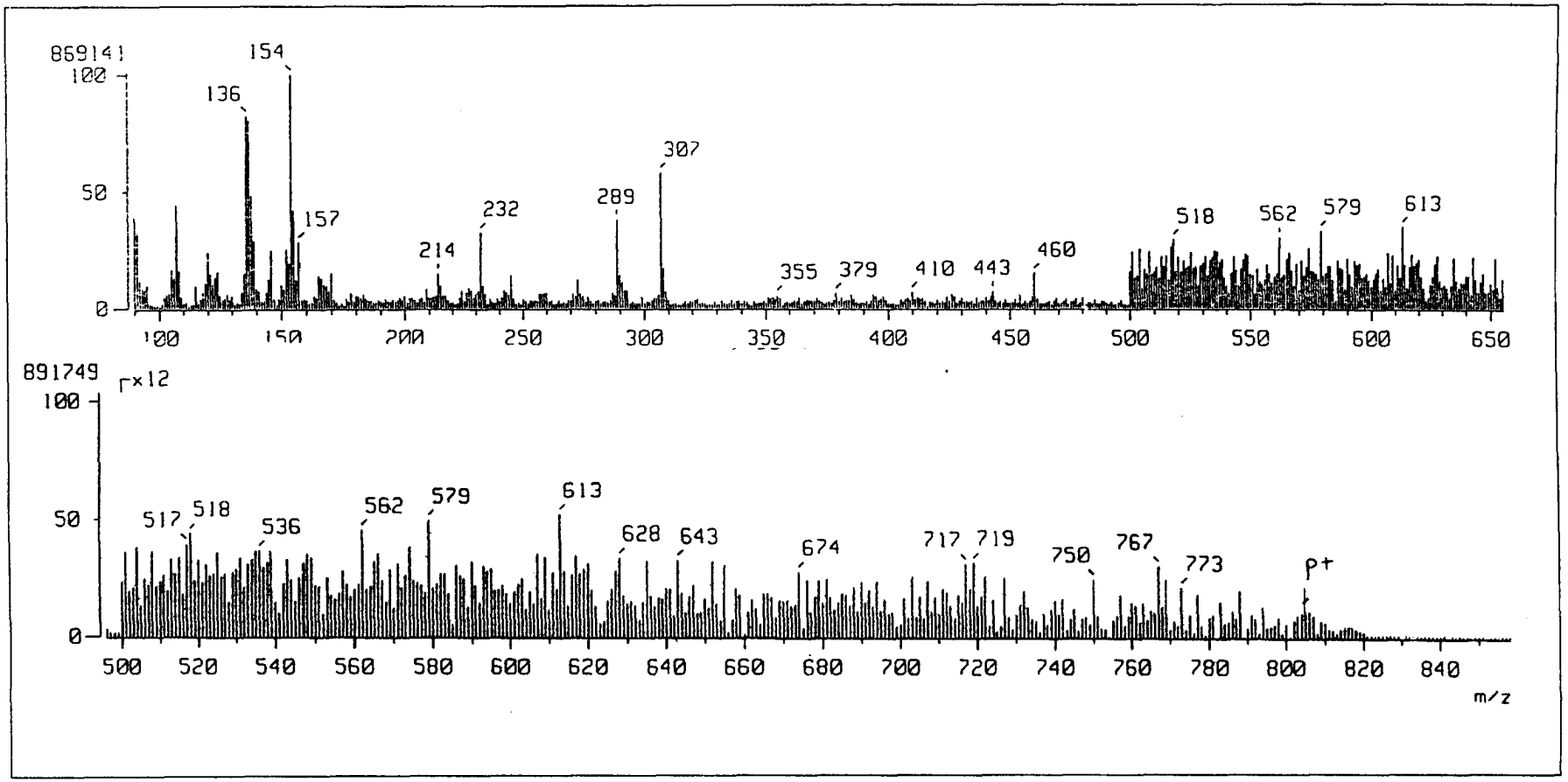


Fig. 3.5. Mass spectrum of Cu(II) complex of 1,7-diindolylheptanoid

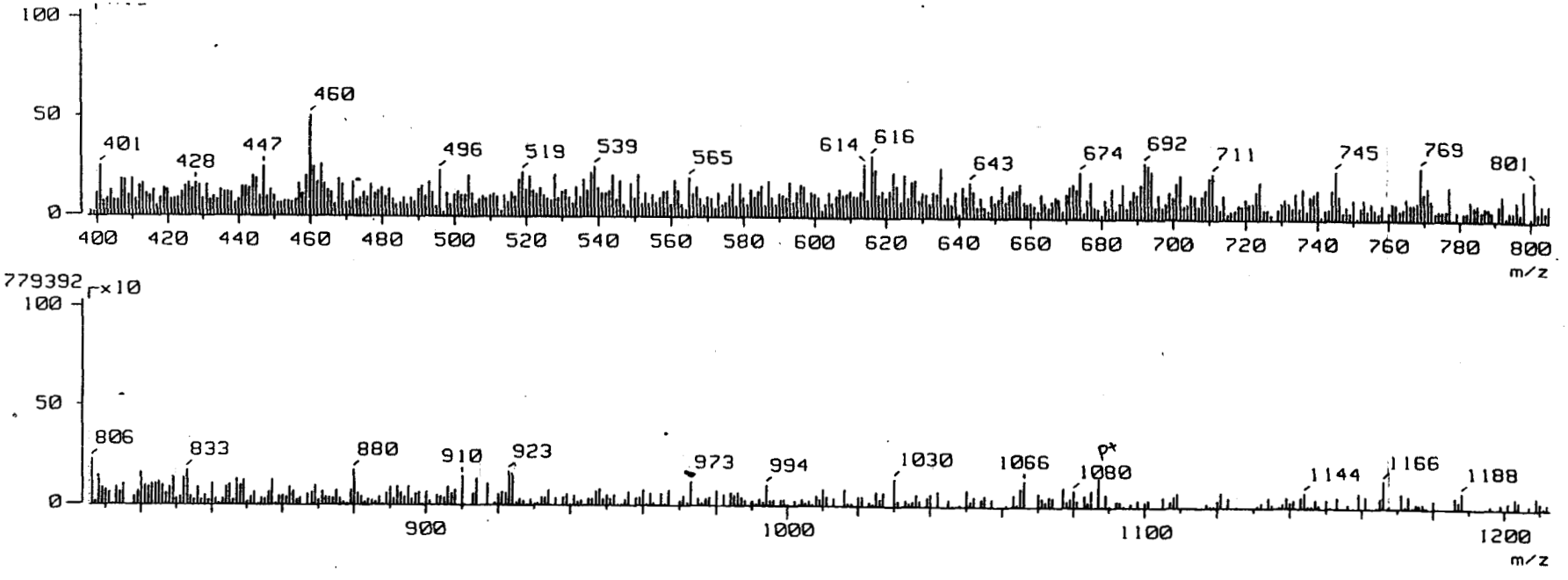
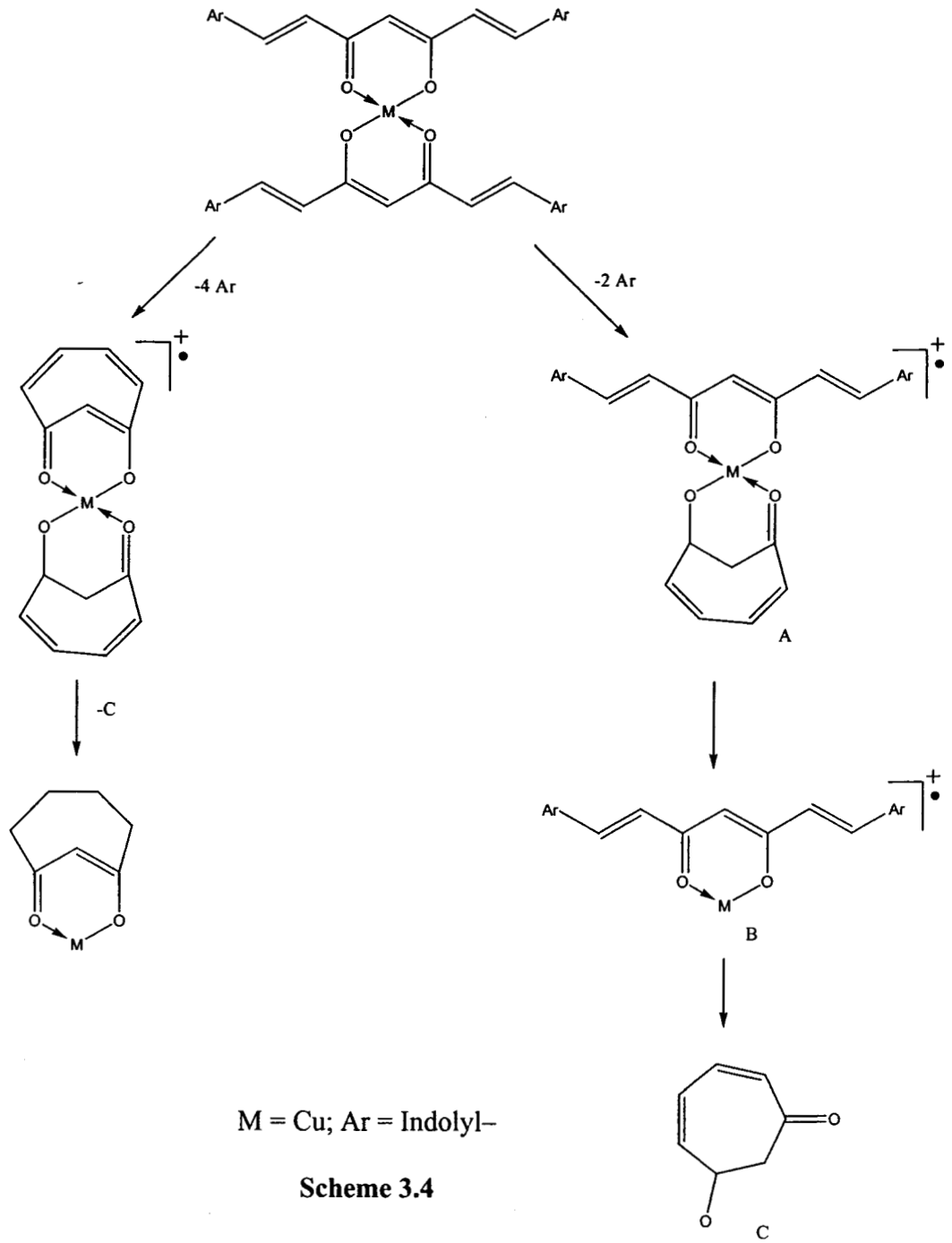


Fig. 3.6. Mass spectrum of Al(III) complex of 1,7-diindolylheptanoid



M = Cu; Ar = Indolyl–

Scheme 3.4

Geometry of the metal complexes: The stoichiometry and the magnetic moment data together with the observed electronic spectra of the Fe(III), Co(II), Ni(II) and Cu(II) complexes suggest their octahedral geometry.

The visible spectrum of Co^{2+} complex is dominated by a broad band with maxima at ~ 19,400 cm^{-1}. This value together with the observed magnetic moment 3.610 B.M. suggests the octahedral geometry of the complex.

The observed paramagnetism and presence of three absorption bands in the visible spectrum of Ni(II) complex clearly indicated the octahedral geometry due to the water coordination. The three well-separated absorption bands at λ_{max} ~ 7,938, ~ 14,280 and ~ 24,565 cm^{-1} correspond to transitions $^3A_{2g} \rightarrow {}^3T_{2g}$, $^3A_{2g} \rightarrow {}^3T_{1g}(F)$, $^3A_{2g} \rightarrow {}^3T_{1g}(P)$ respectively.

ESR spectral study of copper(II) complex : ESR spectroscopy is a valuable tool in the studies of paramagnetic transition metal complexes, which gives information regarding the magnetic properties of the unpaired electrons and thereby some understanding about the nature of the bonding between the metal ion and the ligands.[197] It has been demonstrated that the effective spin-orbit coupling constant and nuclear hyperfine structure of an ion will vary with the covalent character of metal-ligand bond.

The esr spectrum of copper(II) complex of 1,7-diindolylheptanoid in DMF solution at 77 K is reproduced in **fig.3.7**. In general complexed Cu^{2+} ion in solution exhibits four hyperfine lines in esr spectrum with varying line width. Spectrum in the glassy state is very useful for further studies of ligand interactions. It has been reported that the g values are

very sensitive to the covalent nature of the metal-ligand bond.[197-199] The g and A values are given in **table 3.5**. For comparison the values reported for copper acetylacetonates[199] and $CuCl_2$ are also included in this table.

TABLE 3.5

Esr spectral data of Cu(II) complex of 1,7-diindolyl heptanoid

Compound	$g_{\|\|}$	$g_{\perp}$	$A_{\|\|}$ (x 10^4 cm^{-1})	$A_{\perp}$ (x 10^4 cm^{-1})	Solvent
$Cu(dih)_2$	2.2750	2.0725	168.4	478.8	DMF
$Cu(acac)_2$	2.264	2.036	14.55	29	60% Toluene 40% $CHCl_3$
$CuCl_2$	2.340	2.05	112	--	60% Toluene 40% $CHCl_3$

DMF is strongly coordinating solvent. So it is likely that the geometry of the complex is tetragonally elongated octahedron belonging to D_{4h} point group. The observed g values suggest the presence of appreciable delocalisation of π electron cloud of the chelate ring C_3O_2Cu and the metal-ligand bond has considerable covalent character.[199]

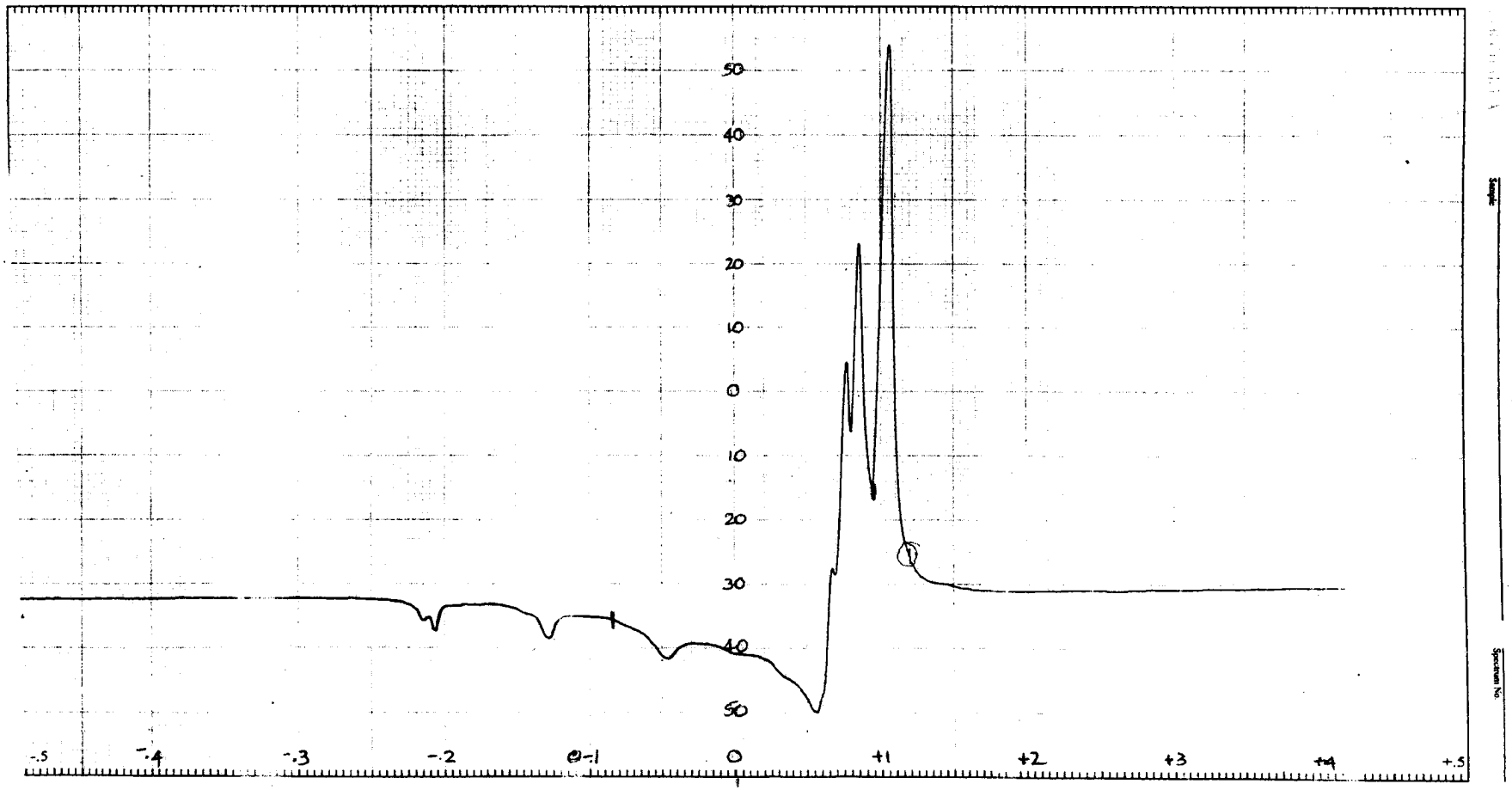


Fig. 3.7. ESR spectrum of Cu(II) complex of 1,7-diindolylheptanoid

Thermogravimetric studies of the copper complex of 1,7-diindolyl heptanoid

Thermogravimetry has been extensively used for structural elucidation and for comparing the relative volatilities of metal chelates of 1,3-diketonates.[29] The thermogram of Cu(II) complex of 1,7-diindolylheptanoid in air agree well with the $[Cu(dih)_2(H_2O)_2]$ stoichiometry. Elimination of the two coordinated water molecules (found 4.4%, cald 4.27%) occurred in the 150-250°C range. The percentage of metal oxide residue obtained (found 9.69,cald 9.86) also agree with formulation of the complex. The thermogram of Cu(II) complex is reproduced in **fig. 3.8**.

Microwave-assisted synthesis of 1,7-diindolylheptanoid

For the microwave-assisted synthesis of 1,7-diindolylheptanoid, acetylacetone (5 mmol) and boric oxide (3.5 mmol) were mixed in a 50 mL conical flask. It was then stoppered and the mixture was irradiated with microwave radiation at medium power for 1 minute. The flask was cooled and the product was mixed with indole-3-aldehyde (10 mmol), tri (sec-butyl) borate (20 mmol) and n-butylamine (0.1 mL). The mixture was made into a paste with 3 mL ethyl acetate and again irradiated with microwave radiation at medium power for 3 minutes. The flask was cooled for 2 minutes, and ethyl acetate (10 mL) was added.

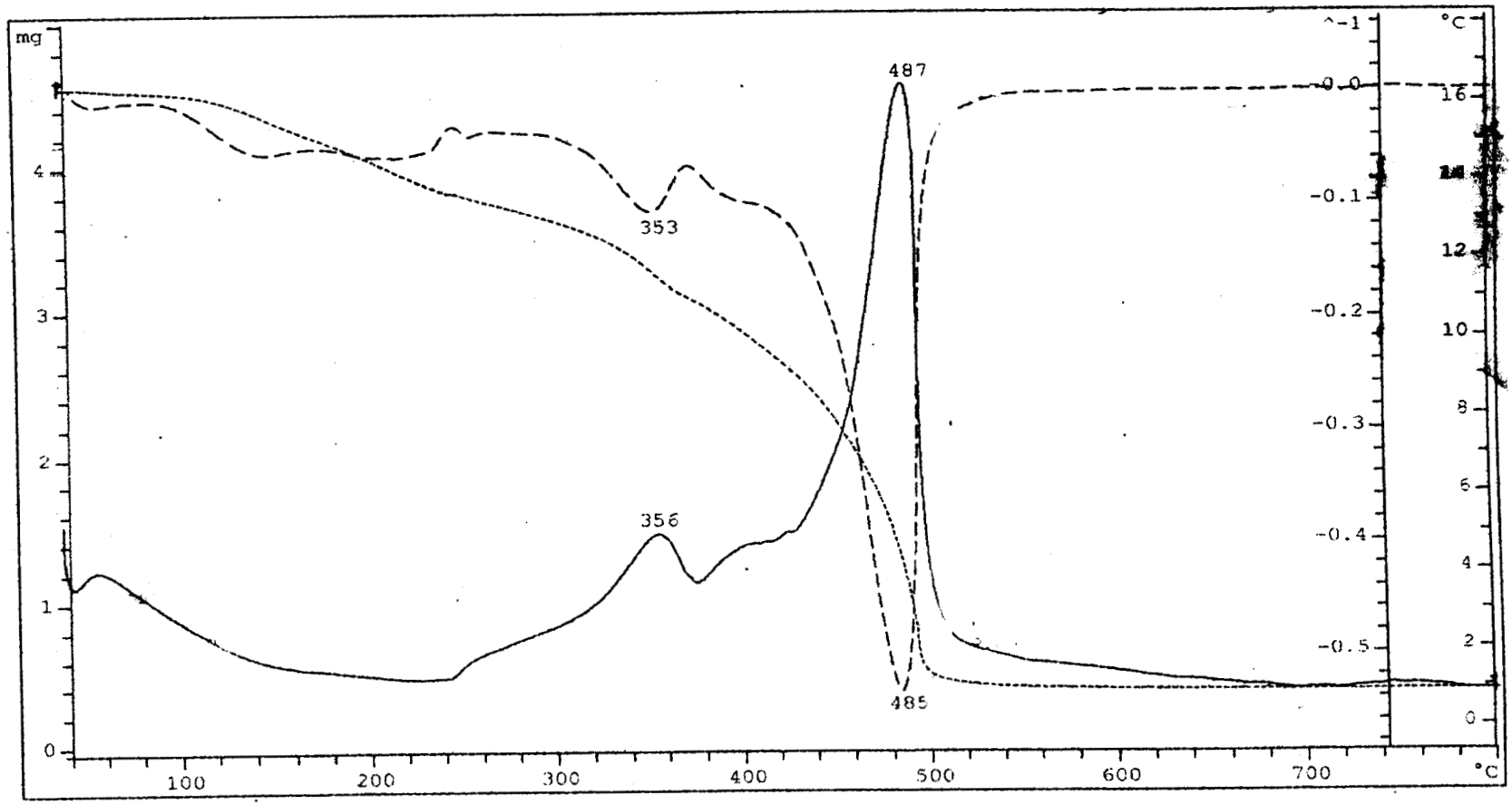


Fig. 3.8. Thermogram of Cu(II) complex of 1,7-diindolylheptanoids

The solution was then stirred with 0.4 M HCl for 30 minutes at 60°C. The organic layer was extracted with ethyl acetate and then evaporated to a paste, which was again stirred with 50% HCl. The solid separated was filtered, washed with water and dried in vacuum.

The product obtained was 1,7-diindolylheptanoid, with very small amount of monocondensation product as revealed from tlc. Further purification was done by column chromatography as described under the synthesis of 1,7-diindolylheptanoid

Numerous microwave-assisted aldol condensation have been reported, but the use of microwave energy in carrying out boron-assisted regioselective aldol condensation are very few.[200,201] In the case of 1,7-diindolylheptanoid moderate to excellent yields of the compound was obtained when the reaction mixture was irradiated for 3 minutes by microwave. It should be noted that the reaction was carried out in a conventional microwave oven at medium power, where the control of the reaction course is not ideal, however the reaction was found to be reproducible.

This method has several advantages over the conventional method. Isolation and purification of the product have become simple. The yield of the product is considerably higher (82.31%) than those obtained under

conventional conditions (65%). Moreover, this method was found to be more product specific, i.e., the major product is 1,7-diindolylheptanoid.

The compound was characterised by ir spectral analysis. The ir spectrum of the sample and that obtained by microwave-assisted irradiation is reproduced in the **fig. 3.9.** The very similarity observed between these two spectra confirmed that the both samples are identical. Thus it can be concluded that the microwave-assisted synthesis developed, is an efficient, expeditious,[202] and simple method for the synthesis of 1,7-diindolylheptanoid and similar compounds.

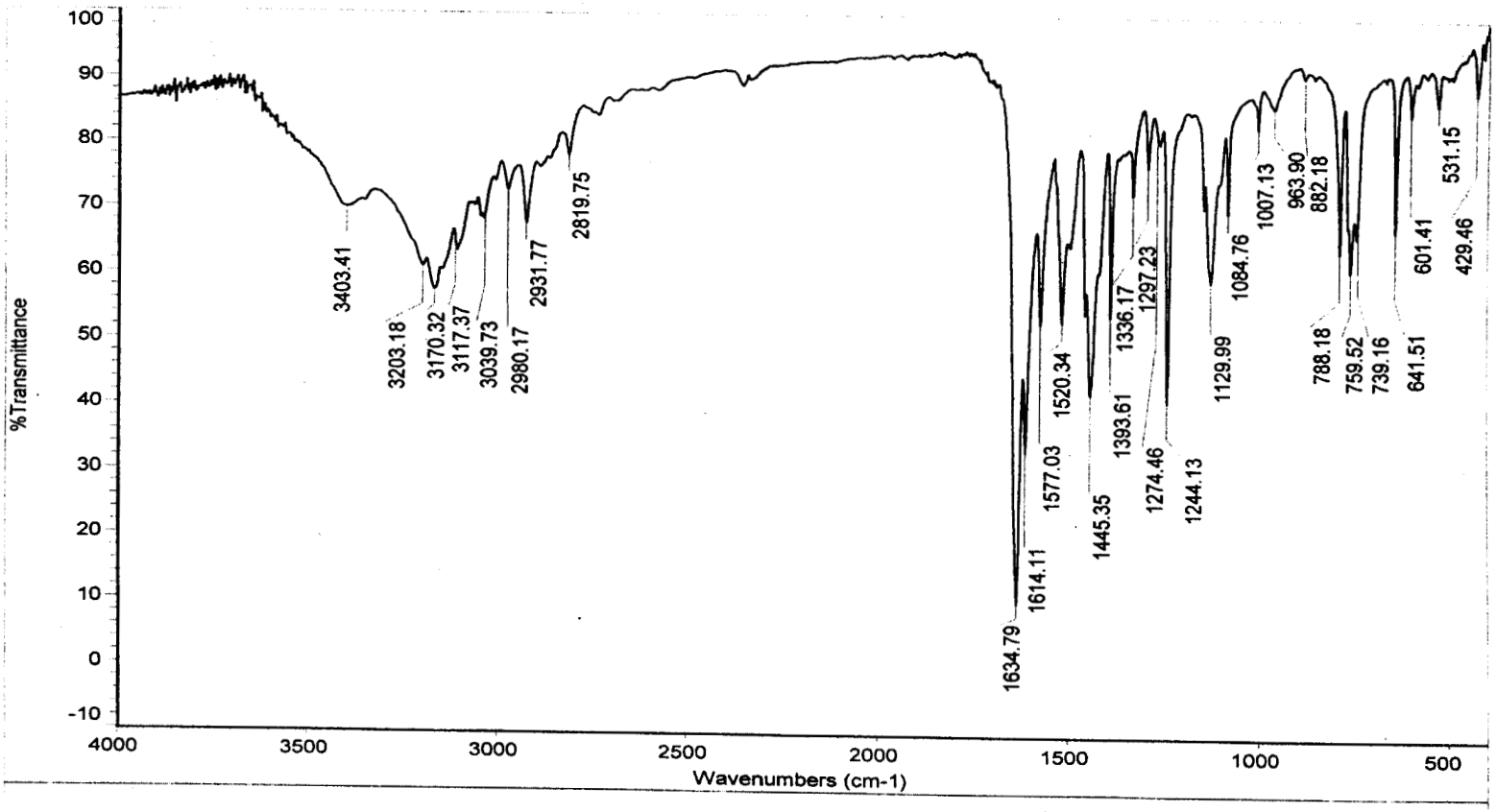


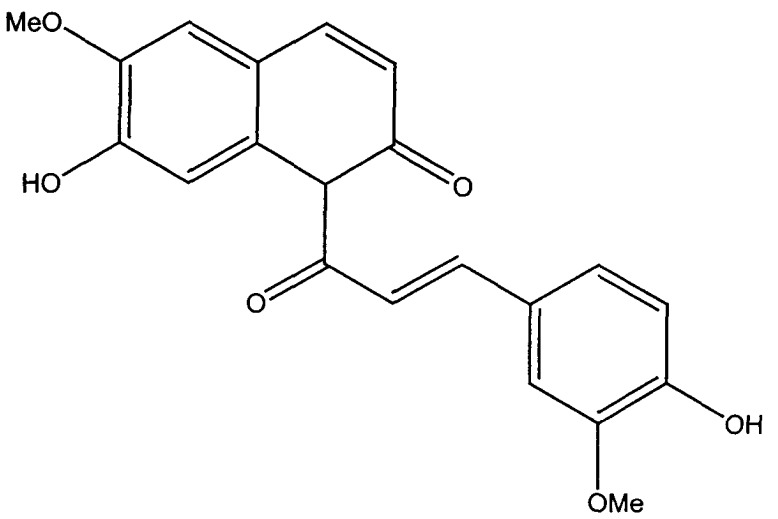
Fig. 3.9. IR spectrum of 1,7-diindolylheptanoid

SECTION 2

STUDIES ON FLUORESCENCE CHARACTERISTICS OF 1,7-DIINDOLYLHEPTANOID

Introduction

Photochemical properties of various natural and synthetic curcuminoids were well studied by many research groups, and explained the behaviour of these molecules in the excited states. Curcuminoids are generally less stable to uv radiation, and hence the yellow colour of turmeric products fades considerably when exposed to light.[203,204] Formation of cyclisation product (II) on exposure to light have been reported.



Structure 3.3

The reaction mechanism and kinetics of the overall photochemical degradation of curcuminoids suggest that it acts as a photosensitiser of singlet oxygen. Fading of colour also involves various other mechanisms independent of oxygen. The possibility that curcumin itself can act as a photosensitising agent is an interesting aspect in many drug formulations. Tonneson and coworkers have shown that the antibacterial activity of curcumin is generally enhanced by visible radiation.[205] Photophysical properties of curcumin have been studied recently by Pill Hoon Bong using time resolved fluorescence spectra.[206] Studies on the steady-state absorption and fluorescence of curcumin showed that absorption/ emission maxima are solvent dependent.[165]

Curcumin in solution exists as an equilibrium mixture of diketo and enol form, and the interconversion is very slow in ground state. Nmr study indicated that the amount of the enol form in equilibrium is more than 95%. Absorption and emission spectral characteristics indicate that the two tautomeric forms have similar absorption and emission spectra, and hence when excited at 443 nm, both forms are probably excited with the same equilibrium percentage as in the ground state. Fluorescence emission can take place before any new equilibrium between the excited state is established. Hence the components having shorter life time and higher percentage has been assigned to the enol form and the other long

lived but smaller percentage to the diketo form. In curcumin the two ends of the chromophore can communicate via resonance structures in the enol form, conjugation of the π electrons may results in the coupling of the two feruloyl chromophores into one excited state π electron system.[207]

The life time of the enol form can be very short, due to either strong intramolecular or intermolecular hydrogen bonding with the solvent, which provide a very efficient radiationless pathway for the excited state energy relaxation mechanism. The intramolecular hydrogen bonding in solvents like cyclohexane explains the low solubility and low quantum yield and solvents of strong hydrogen bond donors or acceptors, the life time of the enol form is relatively large and its contribution is reduced compared to the diketo form. Thus the major photophysical properties of curcumin are indicated by those of the enol form, since it is the predominant form which is present in solution. Life time of the singlet state is very short and the fluorescence and triplet quantum yield are very low in all the solvents due to strong intra or intermolecular hydrogen bonding. The internal conversion process is the major relaxation pathway for the excited singlet state.

It has been observed that water quenches the fluorescence of curcumin in solvents such as acetone, ethanol, etc. Jasmin and Alli[208,209] attributed to reaction between H_2O (electron donor, D) and the

fluorescent curcumin (C*) resulting in the formation of a non fluorescent and more stable form (C^{*-} D^{+}) with lower vibrational energy levels.

Fluorescence of curcumin depends on the pH of the medium. Curcumin is slightly soluble in water, but, readily soluble in alkali or in glacial acetic acid. Its colour is not constant in aqueous media or in an organic solvent due to its degradation or dissociation. It is exposed to hydrolytic degradative reaction in aqueous solution. From the above observations it is possible to conclude that pH effect on the fluorescence of curcumin is either due to its degradation or due to the change in its properties of first excited singlet state.[206]

Heavy metal ions will quench the fluorescence of curcumin. They often quench the triplet state.[210-212] Largest effects are noted for paramagnetic metal ions, suggest that paramagnetic species increases the rate constants for intersystem crossing in the organic molecule. Metal ion quenching proceeds by formation of an excited state, charge transfer complex between fluorescent molecule and metal ions, with latter acting as electron donor. But this type of quenching is not observed in light metals. The fluorescence quenching of curcumin by metal ions is not well established, and more studies in this direction are required for more information about the fluorescence behaviour of curcumin.

A detailed study on the fluorescence of the 1,7-diindolylheptanoid was carried out. The effect of various solvents, pH of the medium and various metal ions on the fluorescence were investigated. The quenching of fluorescence of the compound by water was also studied. A brief out line of the determination of fluorescence quantum yield was also included in this section.

Experimental

Effect of solvents on the absorption and fluorescence maxima: Solutions (10^{-6} M) of the compound in different solvents (methanol, ethanol, acetone, acetonitrile and DMSO) were prepared. The absorption and the fluorescence spectra were recorded. The fluorescence emission was measured at 350 volts.

Effect of water on the fluorescence: The effect of various concentrations of water on the fluorescence was studied by measuring the fluorescence intensity of the compound in different ratio of methanol and water. For this different percentage by volume of water to a methanol solution ($2x10^{-5}$M) of the compound was prepared and in each case fluorescence intensity was measured at 400 volts. The intensity of fluorescence of the compound in pure anhydrous methanol was also determined.

Effect of pH of the medium on the fluorescence: The effect of pH on the fluorescence of 1,7-diindolylheptanoid was studied in methanol - water solvent (2/8, v/v). For this 2 x 10^{-5} M solution of the compound in methanol-water mixture at different pH were prepared and the fluorescence intensity of each solution was measured.

Effect of metal ions on the fluorescence: Fluorescence intensity of organic molecules are greatly influenced by metal ions. Paramagnetic metal ions are effective quencher of fluorescence. The effect of metal ions was studied by measuring the fluorescence intensity after adding the metal ions. Different concentrations of a methanolic solution of metal salts were added to 1mL of 10^{-5}M solution of 1,7-diindolylheptanoid in methanol and the total volume was then made up to 10 mL. The fluorescence intensities of these solutions were measured.

Determination of quantum yield: The fluorescence quantum yield (Q_F) is the ratio of photons absorbed to photons emitted through fluorescence. It gives the probability of the excited state being deactivated by fluorescence rather than by other, non-radiactive mechanisms. The most reliable method for recording Q_F, is the comparative method of Williams *et al.*[213] which involves the use of well characterised standard samples with known Q_F values. Essentially solutions of the standard and test samples with identical absorbance at the same excitation wave length can be

assumed to be absorbing the same number of photons. Hence, a simple ratio of the integrated fluorescence intensities of the two solutions, recorded under identical conditions, will yield the ratio of the quantum yield values. Since Q_F, of the standard sample is known, it is trivial to calculate Q_F of the test sample.

Selection of standard : The standard should be chosen to ensure that they absorb and emit in a same region as the test sample. A solution of quinine sulphate in 0.1 M H_2SO_4 was taken as the standard, whose quantum yield is 0.54.

Cuvettes: Standard 10 mm path length fluorescence cuvettes were used for fluorescence measurements.

Sample preparation: A 10 ppm solution of quinine sulphate, was prepared in 0.1M H_2SO_4. Solutions of various concentrations were prepared by diluting the above solution. Absorbance of these solutions were measured by using spectrophotometer. The fluorescence spectra of these solutions were recorded, and the integrated fluorescence intensity ie, the area under the fluorescence spectra were calculated.

Solution of 1,7-diindolylheptanoid (10^{-6} M) at different concentrations were prepared in pure methanol. As described above the

absorbance and the fluorescence intensities of solutions of different concentrations were measured.

Results and Discussion

Effect of solvents: The 1,7-diindolylhptanoid is insoluble in water but shows differential solubility in several organic solvents. Its solubility is very high in polar organic solvents, but is only slightly soluble in aliphatic or alicyclic organic solvents, like hexane and cyclohexane. In alkaline water it shows marginal solubility, but it is negligibly soluble in neutral and acidic water. The absorption spectra of the compound showed a strong and intense band in the 400-500 nm wave length region (**Table 3.6**).

TABLE 3.6

Absorption and emission maxima of 1,7-diindolylheptanoid in different organic solvents
(Emission measured at 350 Volts)

Solvent	$^{*}\lambda_{a(nm)}$	$^{+}\lambda_{fl(nm)}$
Methanol	450	555
Ethanol	448	550
Acetone	440	500
Acetonitrile	443	513
DMSO	465	545

* Absorption maxima; $^{+}$ Fluorescence maxima.

Absorption maxima show a large red shift in more polar solvents. eg. the maxima in DMSO. The absorption spectrum in each solvent is very broad and shows the presence of shoulder. All these features probably indicate the presence of more than one isomeric form in the ground state.

Fluorescence spectra also show significant solvent dependent shifts in maxima (Table 3.6). A large red shift from 500 nm in acetone to 545 nm in DMSO, the most polar among the solvents considering the dielectric constant values. However, maximum red shit appears at 555 nm in methanol, a strong hydrogen bond-donating as well as a hydrogen bond accepting solvent. The fluorescence spectrum of the compound in methanol is reproduced in **fig. 3.10.** The widths of the fluorescence spectra increases with increase in the solvent polarity and hydrogen bond-donating or accepting ability of the solvents. These steady-state absorption and fluorescence characteristics of the molecule suggest that the excited singlet (S_1) state has a large intramolecular charge transfer character.[136,212] In the S_1 state, reasonable amount of charge is transferred from the aromatic subtituent moiety to the dicarbonyl moiety. The large solvochromic red shift observed in polar protic solvents like methanol may be due to the combination of both the polarity and hydrogen bonding effect of the solvent on the excited state of the compound.

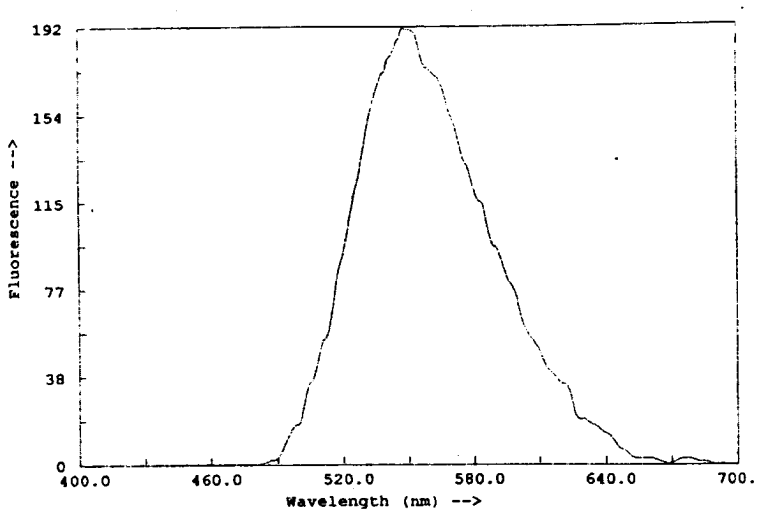


Fig. 3.10. Fluorescence spectrum of 1,7-diindolylheptanoid in methanol

For most polar aromatic molecules whose lowest-singlet states are (π, π^*), the excited state is more polar than the ground state.[165,206,212] Therefore, any increase in the polarity of the solvent will produce a relatively greater stabilization of the excited state than the ground state. Thus, as the solvent polarity increases, both the absorption and fluorescence spectra will shift to lower energies.

Effect of water: Earlier reports showed that water quenches the fluorescence intensity of curcuminoids.[206] The results obtained in the case of 1,7-diindolylheptanoid (**Table 3.7**) also agree with the above observation. Water quenches the fluorescence of the diindolyl curcuminoid. Jasmin and Alli[209] reported that fluorescence quenching of

curcumin can be attributed to a reaction between H_2O (electron donor, D) and the fluorescence curcumin C^* resulting in the formation of a nonfluorescent and a stable complex of the type (C^{*-} D^+). A similar process may occur in the case of diindolylheptanoid also.

TABLE 3.7

Effect of various concentrations of water (% v/v) on the fluorescence of 1,7-diindolyl heptanoid in methanol[a]

$[H_2O]$ (% v/v)	Excit. wave length (nm)	E_{max} (nm)	Fluorescence Intensity	[b] η^o/η	[c] I^o_{fl}/I_{fl}	η^o/η x I^o_{fl}/I_{fl}
0	470	552	376	1.00	1.00	1.000
3	470	552	325	0.99	1.1616	1.150
5	470	553	283	0.96	1.3375	1.284
7	470	551	271	0.95	1.3694	1.301
10	470	552	243	0.92	1.5597	1.435
20	470	551	174	0.85	2.1764	1.850
30	470	552	127	0.79	2.959	2.338

a. Concentration of the compound is 2×10^{-5}
b. from ref. 207.
c. I^o_{fl} is the fluorescence intensity in anhydrous methanol.

The Stern-Volmer relationship: The effect of water on fluorescence can be studied with the help of modified Stern-Volmer equation:

$$\frac{I^0_{fl}}{I_{fl}} = 1 + Kq.\tau.[H_2O] / \eta^0/\eta$$

where K_q is the rate constant for quenching, τ is the life time of the excited singlet state, and I°_{fl} is the fluorescence intensity in the absence of water. η° and η are the viscosities of pure methanol and methanol-water mixture. Thus from a plot of $\frac{I_{fl^{\circ}}}{I_{fl}}\eta^{\circ}/\eta$ Vs $[H_2O]$, the effect of water on the fluorescence quenching can be studied. Such a graph in the case of Hdih is shown in the **Fig. 3.11**. The slope of the plot is 4.6 x $10^{-2}M^{-1}$, but reported value for curcumin is 1.38 x 10^{-2} M^{-1}, which indicates that the effect of water quenching on the fluorescence of 1,7-diindolylheptanoid is slightly higher than that of curcumin. This may be due to the fact that, the fluorescent life time of the compound is different from that of curcumin, and the non-fluorescent complex $[C^{*-}\ D^{+}]$ of the diindolyl derivative is more stable than that formed with curcumin.

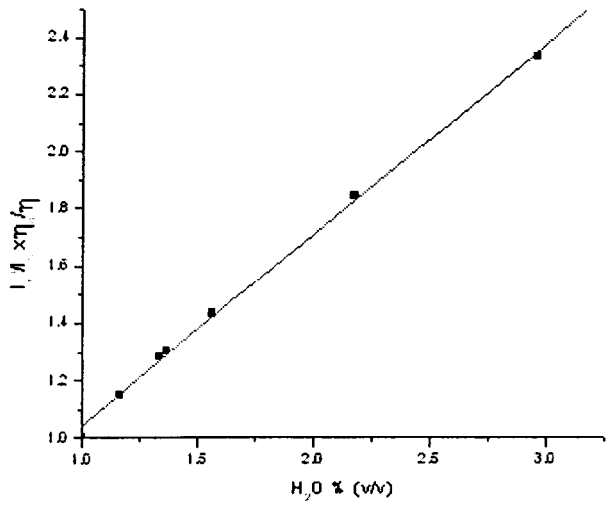


Fig. 3.11. Effect of water on the fluorescence emission of 1,7-diindolylheptanoid

Effect of pH of the medium: The potential use of curcumin and other curcuminoids as colouring agents, in pharmaceutical prescriptions or drugs is closely related to the stability of these compounds. Their colour is not constant in aqueous media or in an organic solvent due to degradation or conversion to other species. The intensity fluorescence of 1,7-diindolylheptanoid in methanol at different pH are given in the table 3.8. No regular trend was observed with variation in pH. The intensity decreases from pH 1.2-2.1. At pH 3 the intensity considerably increases and then gradually decreases upto pH 8.7 and again goes to pH 13.6.

If proton forms a complex with diketo oxygens and hydroxide ion forms an enolate anion of β-diketone,[206] the proton and hydroxide ion will raise the energy of the lowest $^1(n, \pi^*)$ state. Because the proton is not expected to affect the energy of $^1(\pi, \pi^*)$ state, the intensity of fluorescence originating from the $^1(\pi, \pi^*)$ state decreases as the pH increases above the pKa value. Thus it appears that pH effect on the fluorescence of 1,7-diindolyheptanoid is either due to its degradation or due to the change in its properties of first excited singlet state.

TABLE 3.8

Effect of pH on the fluorescence of 1,7-diindolylheptanoid fluorescence intensity (measured at 400 volt)

pH	Emission intensity	I^{o}_{fl}/I_{fl}	pH	Emission intensity	I^{o}_{fl}/I_{fl}
1.2	735	1.08	5.9	784	1.01
1.6	770	1.03	7	794	1.00
2.0	810	0.98	7.6	796	0.99
2.1	8.53	0.93	8.4	804	0.98
3	676	1.174	8.7	863	0.92
3.4	744	1.06	9.3	806	0.98
3.6	759	1.05	9.8	606	1.31
4.1	779	1.02	10.7	498	1.59
			11.6	367	2.16

Concentration of the compound is 2×10^{-5} M.
I^{o}_{fl} is the fluorescence intensity at pH 7.

Effect of metal ions: Presence of metal ions greately affect the fluorescence of many organic molecules. The effects of metal ions such as Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Al(III) on the fluorescence of 1,7- diindolylheptanoid are summarized in **tables 3.9 to 3.14**. The results revealed that Cu(II) is the effective quencher of the fluorescence and to certain extent Fe(III), Co(II) and Ni(II) also suppress the fluorescence, while Zn(II) has no effect on the fluorescence intensity. But interestingly Al(III) ions increase the fluorescence intensity of the compound. From these observations it is clear that whether or not metal ions actually complex the ground state of the organic molecule, they have influence on its fluorescence. Generally heavy metal ions will quench the fluorescence

of organic molecules. The largest effects of Cu(II), Fe(III) and Co(II) are expected due to the paramagnetic nature of these metal ions. It is known that paramagnetic species increases the rate constants for intersystem crossing in organic molecules.[210-212] The decreased effect of the Ni(II) shows that some species in solution exists as diamagnetic species due to complexation. Most paramagnetic ions are effective quenchers. In general, metal ion quenching proceeds by formation of an excited state charge transfer complex between the fluorescent molecule and metal ion, with the latter acting as electron donor.[212] The rate of quenching also depends on the rate of formation of the complex. The actual mechanism of quenching also involve paramagnetic enhancement of spin-orbit coupling.

TABLE 3.9

Effect of Fe(III) ions on the intensity of fluorescence of 1,7-diindolylheptanoid

Concentration of Fe(III) salt solution	Fluorescence maxima	Fluorescence intensity
1×10^{-5}M	557	250
3×10^{-5} M	557	231
5×10^{-5} M	557	194
7×10^{-5} M	557	156
1×10^{-4} M	557	72
3×10^{-4} M	556	52
5×10^{-4} M	557	34
7×10^{-4} M	--	--

Concentration of 1,7-diindolylheptanoid is 10^{-5} M.
The fluorescence measurements were done at 450 volts.

TABLE 3.10

Effect of Co(II) ions on the intensity of fluorescence of 1,7-diindolylheptanoid

Concentration of Co(II) solution	Fluorescence maxima	Fluorescence intensity
1×10^{-5}M	550	290
3×10^{-5} M	550	254
5×10^{-5} M	550	202
7×10^{-5} M	550	178
1×10^{-4} M	550	79
3×10^{-4} M	551	57
5×10^{-4} M	550	41
7×10^{-4} M	--	--

Concentration of 1,7-diindolylheptanoid is 10^{-5} M.
The fluorescence measurements were done at 450 volts.

TABLE 3.11

Effect of Ni(II) ions on the intensity of fluorescence of 1,7-diindolylheptanoid

Concentration of Ni(II) solution	Fluorescence intensity	Fluorescence maxima
1×10^{-5}M	415	550
3×10^{-5} M	383	550
5×10^{-5} M	334	551
7×10^{-5} M	297	552
1×10^{-4} M	276	550
3×10^{-4} M	228	550
5×10^{-4} M	173	550
7×10^{-4} M	131	551
1×10^{-3} M	75	550

Concentration of 1,7-diindolylheptanoid is 10^{-5} M.
The fluorescence measurements were done at 450 volts.

TABLE 3.12

Effect of Cu(II) ions on the intensity of fluorescence of 1,7-diindolyl heptanoid

Concentration of Cu(II) solution	Fluorescence maxima	Fluorescence intensity
1×10^{-5}M	550 nm	270
3×10^{-5} M	550	195
5×10^{-5} M	550	114
7×10^{-5} M	551	83
1×10^{-4} M	550	54
3×10^{-4} M	550	21
5×10^{-4} M	--	--

Concentration of 1,7-diindolylheptanoid is 10^{-5} M.
The fluorescence measurements were done at 450 volts.

TABLE 3.13

Effect of Zn(II) ions on the intensity of fluorescence of 1,7-diindolylheptanoid.

Concentration of Fe(III) salt solution	Fluorescence maxima	Fluorescence intesntiy
1×10^{-5}M	537	298
3×10^{-5} M	537	294
5×10^{-5} M	536	284
7×10^{-5} M	537	283
1×10^{-4} M	537	281
3×10^{-4} M	538	281
5×10^{-4} M	538	279
7×10^{-4} M	537	281
1×10^{-3} M	537	282

Concentration of 1,7-diindolylheptanoid is 10^{-5} M.
The fluorescence measurements were done at 450 volts.

TABLE 3.14

Effect of Al(III) ions on the fluorescence of 1,7-diindolylheptanoid.

Concentration of Al(III) solution	Fluorescence maxima	Fluorescence intensity
1×10^{-5}M	537	557
3×10^{-5} M	537	585
5×10^{-5} M	537	624
7×10^{-5} M	536	651
1×10^{-4} M	537	667
3×10^{-4} M	535	742
5×10^{-4} M	538	846
7×10^{-4} M	537	897
1×10^{-3} M	537	973

Concentration of 1,7-diindolylheptanoid is 10^{-5} M.
The fluorescence measurements were done at 450 volts.

The increase in fluorescent intensity observed in the case of Al(III) ion may be due to the formation of a highly fluorescent chelate. Here the complex itself is fluorescent, and addition of increasing amount of Al(III) will increase the fluorescent intensity due to the formation of the complex. This method may be employed for the quantitative analysis of aluminium.

Fluorescent intensity is not affected by Zn(II) ion. This may be due to the diamagnetic nature of the Zn(II) ion, and also the complex formed is nonfluorescent. Thus the general conclusion is that paramagnetic metal ions can quench the fluorescent intensity of the 1,7-diindolyheptanoid, but

the effect of diamagnetic ion depends on the fluorescent nature of the adduct formed.

Quantum yield : The absorbance and integrated fluorescence of the standard solution of quinine sulphate in 0.1 M H_2SO_4 and that of 1,7 diindolylheptanoid in methanol are given in the **table 3.15.**

TABLE 3.15

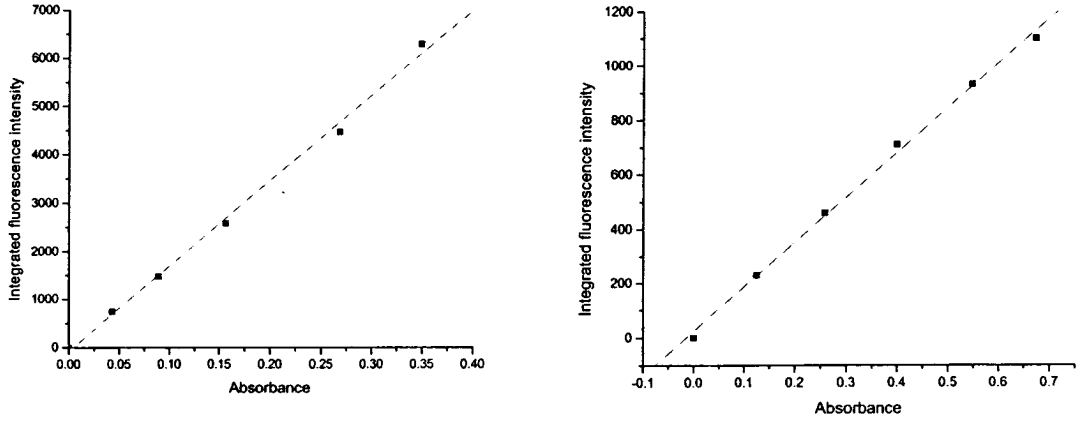
Absorbance and fluorescence intensity of quine sulphate and 1,7-diindolylheptanoid at various concentration at voltage 350

Substance	Concentration	Fluorescence max	Fluorescence intensity	λ_{max}	Absorbance	Integrated fluorescence (area of the peak)
Quinine sulphate in 0.1M. H_2SO_4	1 ppm	438	160	344	0.043	737
	3 ppm	438	442	344	0.089	1474
	5 ppm	437	708	344	0.156	2582
	8 ppm	438	1034	344	0.268	4470
	10 ppm	438	1268	344	0.349	6301
1,7 diindolyl heptanoid in methanol	1×10^{-6}M	552	114	452	0.037	223
	2×10^{-6} M	552	192	452	0.066	419
	3×10^{-6} M	552	255	452	0.104	558
	4×10^{-6} M	552	320	452	0.140	773
	5×10^{-6} M	552	475	452	0.183	978

The plots of absorbance Vs integrated fluorescence are given in the **Fig. 3.12.** From the slopes of the two plots the quantum yield of 1,7-diindolylheptanoid was calculated using the equation.[213]

$$Q_X = Q_{ST}\left[\frac{Grad_X}{Grad_{ST}}\right]\left[\frac{n_X^2}{n_{ST}^2}\right]$$

where the subscripts $_{ST}$ and $_X$ denote standard and test sample respectively, Q is the fluorescence quantum yield, Grad, the gradient from the plot of integrated fluorescence intensity Vs absorbance and n is the refractive index of the solvent.



(a) (b)

Fig. 3.12. Plot of integrated fluorescence intensity Vs. absorbance (a) Standard (b) 1,7-diindolylheptanoid

The quantum yield obtained for 1,7-diindolylheptanoid is 0.1581. This shows that, process other than fluorescence play an important role in the deactivation process of the fluorophore. If fluorescence was the only process for deactivation, the quantum efficiency should be one. Thus the non radiative process, intersystem crossing, etc. are exist. Compared to curcumin (0.017) the quantum yield of 1,7-diindolylheptanoid is much higher and this may be due to the presence of the heterocyclic ring.

SECTION 3

BIOLOGICAL STUDIES OF 1,7-DIINDOLYL-HEPTANOID AND ITS METAL COMPLEXES

Introduction

Numerous reports exist on the various biological and medicinal applications of curcuminoids and their synthetic analogues. Most of these properties including antitumour and cytotoxicity are attributed to the antioxidant and free radical scavenging nature of curcuminoids in particular due to the presence of phenolic, olefinic and diketo functions in these compounds.[150] The indolyl groups may definitely alter the various biological properties of curcuminoids and their metal complexes.

In the present study the synthetic curcuminoid **Hidh** was investigated for its *in vitro* cytotoxicity and anti oxidant activity and also its *in vivo* antitumour activity. All the metal complexes discussed in section 1 were also examined for their cytotoxicity, antioxidant and antitumour activities. Experimental details and results obtained of these studies are discussed separately below.

SECTION 3 A. Antioxidant activity of 1,7-diindolylheptanoid and its metal complexes

Free radical intermediates are produced in living system from a number of sources such as the ionisation of water by high energy radiation, through metabolism, by triggered inflammatory phagocytes to reactive oxidants, partial reduction of oxygen during oxidative phosphorylation,[214-216] etc. The resulting free radicals, such as, superoxide anion and hydroxyl radicals, as well as the non radical hydrogen peroxide can damage macromolecules including DNA.[217]

Curcuminoids are effective antioxidants and can scavenge superoxide radicals, hydrogen peroxide, hydroxyl radicals, nitric oxide from activated microphages, inhibits *in vitro* lipid peroxidation and the inducible nitric oxide[122,153,218] and synthase activity in macrophages,[219] which play an important role in inflammation. Curcuminoids exerts Keralinocytes,[220,221] also enhances the activities of other antioxidants such as superoxide dismutase, catalase, glutathione peroxide and prevent the damage of skin due to the exposure to sunlight.

In view of the importance of dietary antioxidants such as curcuminoids, in chemo prevention of degenerative diseases, such as, cancer, Alzheimers, Parkinsons and cardiovascular diseases, antioxidant

and chemopreventive mechanism of curcumin have been reported by several investigators.[222,224]

The antioxidant mechanism of curcuminoids have been attributable to its unique conjugated structure, aryl substituent, etc.[225] The structure shows typical radical traping ability as a chain-breaking antioxidant.[225] The antioxidant process of phenolic materials are believed to be mediated through the following steps:

$$S\text{–}OO^{\cdot} + AH \rightarrow SOOH + A^{\cdot}$$

$$A^{\cdot} + X^{\cdot} \rightarrow \text{non radical material}$$

S is the substance oxidised, AH is the phenolic antioxidant, $A^{\cdot}$ is the antioxidant radical, and $X^{\cdot}$ is another radical species or the same as $A^{\cdot}$. Masuda *et al.*[226] studied the antioxidant mechanism of curcumin using linoleate as an oxidisable poly unsaturated lipid and proposed that the mechanism involves oxidative coupling reaction at 3 position of curcumin with the lipid and a subsequent intramolecular Diels-Alder reaction.

According to Tonnesen *et al.*[227] the enolised diketone system of curcumin appears to be the part of the molecule involved in scavenging of oxygen free radicals. Later it was proved that the phenolic group is essential for the free radical scavenging and the presence of methoxy group further increased the activity.[228] It was also reported that curcumin

analogues lacking both phenolic and methoxy groups was just as active as curcumin in scavenging hydroxyl radical. Another mechanism of antioxidant activity, involving H-atom transfer from a methylene CH bond has also been proposed.[229] Theoretical prediction of the antioxidant activity of curcuminoids was also reported.[230] The studies showed that curcumin react with methyl peroxyl radical through the enolic OH bond.

In the present investigation the antioxidant activities of the diindolyheptanoid and its typical metal complexes towards superoxide radicals, hydroxyl radicals and ferrous ion induced lipid peroxidation were studied. The techniques employed for the studies are briefly mentioned below.

Experimental

Materials and Methods

Ascorbic acid, Deoxy ribose, Nitroblue tetrazolium (NBT) and Riboflavin were purchased from Sisco Research Laboratories Pvt. Ltd. Bombay. All other chemicals and reagents used were of analytical grade. The tissue homogenizer from yorco Delhi.

Ferric chloride solution (1 mM) was prepared by dissolving 16.22 mg $FeCl_3$ in 100 mL water. 1 mL of this solution was diluted to 10 mL to get 0.1 mM.

Ascorbic acid solution (1 mM) was prepared by dissolving 17.61 mg of ascorbic acid in 100 mL water and 1 mL of this solution was diluted to 10 mL to get 0.1 mM solution.

Tris Buffer (0.2 mM; pH 7) Prepared by dissolving 2.42 g tris buffer in 100 mL water and pH was adjusted with HCl.

Ammonium ferrous sulphate solution (0.8 mM) was prepared by dissolving 31.37 mg in 100 mL water.

KCl solution (150 mM) was prepared by dissolving 1.12 g in 100 mL water.

TBA solution (0.8%) prepared by dissolving 400 mg TBA in 50 mL water.

Sodium dodecayl sulphate solution (8%) was prepared by dissolving 8 g SDS in 100 mL water.

Nitroblue tetrazolium (NBT) solution (1.5 mM). It was prepared by dissolving 12.3 mg in 10mL phosphate buffer.

Riboflavin solution (0.12 mM) was prepared by dissolving 4.5 mg in 100 mL distilled water.

NaCN (0.0015%) in 0.1M EDTA was prepared by dissolving 1.5 mg NaCN in 100 mL of 0.1M EDTA (3.724 g EDTA is 100 mL water).

Phosphate buffer: pH 7.8 (0.06M) was prepared by mixing 8.5 mL 0.06M NaH_2PO_4. $2H_2O$ (0.936 g/100 mL) and 0.15 mL 0.06 M. Na_2HPO_4 (1.068 g/100 mL) solutions in water.

Deoxy Ribose (2.8 mM) was prepared by dissolving 9.38 mg in 2.5 mL phosphate buffer of pH 7.4 and diluted to ten times.

Tissue Homogenate prepared by the following method.

Liver tissue from healthy mice was cut into small pieces and 25% homogenate was prepared in tris-HCl buffer. The homogenate was centrifuged at 3000 rotation per minute (rpm) for 5 minutes and the supernatant was used for the assay.

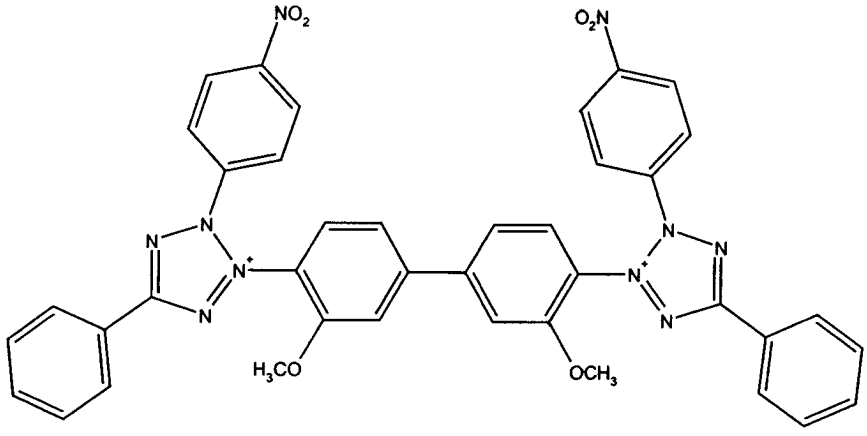
Inhibition of superoxide radicals

Superoxide scavenging was determined by the Nitro Blue Tetrazolium (NBT) reduction method of McCord and Frikdovich.[231] Superoxide anion (O_2^-) acts as a one electron reducing agent in several reactions including reduction of NBT. The reduction of NBT to blue formazan has been widely used as a probe of $O^{\cdot}{}_2^-$ generation in chemical and biological systems. The method is based on the following reactions.

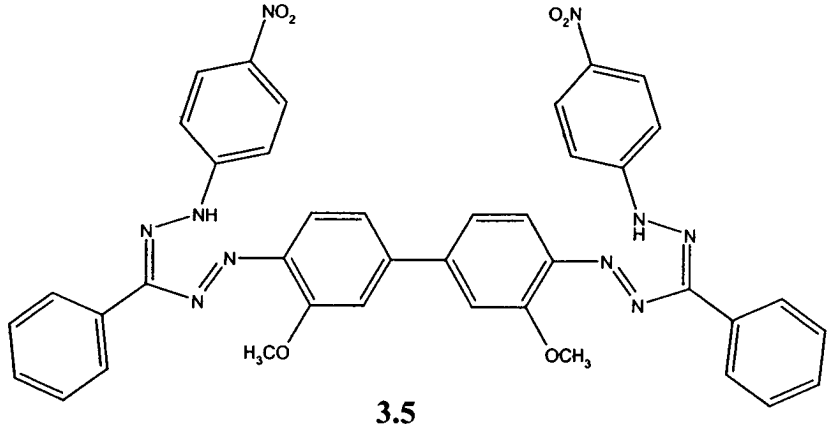
Reduction of NBT to Formazan: NBT is an electrophilic dicationic compound (structure 3.4) which can easily be reduced. The complete

reduction to diformazan (structure 3.5) requires four electrons and four protons according to the equation

$$NBT^{++}: 2Cl^{--} + 4\,e^{-} + 2H^{+} \rightarrow \text{Diformazan} + 2\,HCl$$



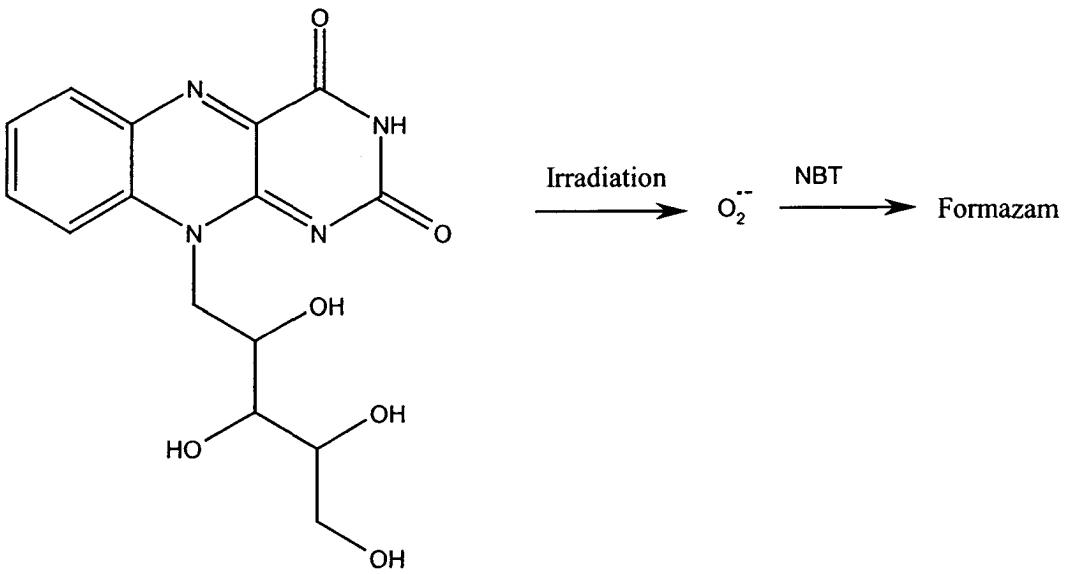
3.4



3.5

In order to determine the superoxide scavenging activity of the compound $O^{\cdot}_2{}^{-}$ species are to be produced. In the NBT method free superoxide radicals ($O^{\cdot}_2{}^{-}$) are generated by the irradiation of riboflavin. The $O^{\cdot}_2{}^{-}$ on reaction with NBT gives blue formazan as in **scheme 3.5**. From the absorbance of the solution the amount of $O_2{}^{\cdot -}$ formed can be

determined. If superoxide scavengers are present in the solution the formazan formation will be prevented and the intensity of the blue colour decreases, hence the absorbance of the solution also decreases. Thus the free radical scavenging activity of a compound can be studied spectrophotometrically. The procedural details are given below.



Scheme 3.5

Different concentrations of the test sample were taken in a test tube. To this solution, EDTA (0.1 M) containing 0.0015% NaCN, riboflavin (0.12 mM), NBT (1.5 mM) were added, and the total volume was adjusted to 3 mL by adding phosphate buffer (0.06 mM, pH 7.8). The tubes were uniformily illuminated under an incandescent lamp for 15 minutes, and thereafter the optical density was measured at 530 nm. The percentage inhibition of superoxide production was evaluated by

comparing the optical density of the control and samples. The percentage inhibition was calculated by using the formula $\frac{C-T}{C} x100$ where C and T are the optical densities of the control and test sample.

Assay protocol

Control		**Sample**	
NBT	- 100 µL	NBT	- 100 µL
NaCN/EDTA	- 200 µL	NaCN/EDTA	- 200 µL
Riboflavin	- 50 µL	Riboflavin	- 50 µL
Buffer	- 2650 µL	Buffer	- 2650 µL (Adjusted accordingly)
Sample	- 0 µL	Sample	- Different concentration

Hydroxyl radical scavenging activity: The presence of ·OH radicals are very harmful to living systems, as they can initiate oxidation of organic substrates, through addition or hydrogen atom abstraction.

$$\cdot OH + R \rightarrow \cdot ROH \rightarrow \text{hydroxylated product}$$

$$\cdot OH + RH_2 \rightarrow \cdot RH + H_2O \rightarrow \text{oxidized product.}$$

In order to study *in vitro* hydroxyl scavenging activity of a compound hydroxyl radicals are to be generated. An important method for this is by Fenton reaction given below.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^-$$

In the absence of added free radical scavengers, the hydroxyl radical will oxidise another ferrous ion: $Fe^{2+} + OH^{\cdot} \rightarrow Fe^{3+} + OH^{-}$. In presence of protons the overall reaction is: $2Fe^{2+} + H_2O_2 + 2H^{+} \rightarrow 2Fe^{3+} + 2H_2O$.

In cyclic Fenton-type reactions, a reducing agent such as ascorbic acid is added to reduce Fe^{3+} salt. Thus Fe^{3+} will be reduced to Fe^{2+} and the ascorbic acid gets oxidised to dehydroascorbic acid. In presence of H_2O_2 the Fe^{2+} will be oxidised to Fe^{3+} with the formation of $\cdot OH$ radical and ascorbic acid is regenerated as given below.

$$Fe^{3+} + AH_2 \text{ (ascorbic acid)} \rightarrow Fe^{2+} + \cdot AH + H^{+}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^{-}$$

$$\cdot AH + \cdot AH \rightarrow AH_2 + A$$

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the test compounds for hydroxyl radicals. In the absence of any scavenger, $\cdot OH$ will attack the deoxyribose, that eventually results in the formation of thiobarbituric acid reacting substances (TBARS).[232] Presence of a radical scavenger prevents its formation and this can be studied by spectrophotometric method.

To different concentrations (in the range 25-50 μg/ml) of the test sample, deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1.0 mM), ascorbate (0.1 mM) were added and the total volume was

adjusted to 1 ml with KH_2PO_4-KOH buffer (20 mM, pH 7.4) and incubated for 1 h, at 37°C. After incubation 0.4 mL of the reaction mixture was mixed with 200 µL sodium dodecyl sulphate (SDS) (8.1%), 1.5 mL acetic acid (pH 3.5), 1.5 ml TBA and volume was made up to 4 mL with distilled water. The mixture was heated at 100°C in a water bath for 1 h, cooled and then 1 mL of water was added. To this 5 mL of 15:1 butanol pyridine mixture was added, stirred well, centrifuged at 3000 rpm for 15 minutes and the optical density was measured at 560 nm. The percentage inhibition was calculated using the formula C-T/C x 100 where C and T are the optical density of the control and test samples respectively.

Assay Protocol

Control		**Sample**	
Deoxy ribose	- 100 µL	Deoxy ribose	- 100 µL
$FeCl_3$	- 100 µL	$FeCl_3$	- 100 µL
Ascorbic acid	- 100 µL	Ascorbic acid	- 100 µL
EDTA	- 100 µL	EDTA	- 100 µL
Buffer	- 100 µL	Buffer	- Adjusted to 1mL
H_2O_2	- 100 µL	H_2O_2	- 100 µL
Sample	- 0 µL	Sample	- Different concentrations

Inhibition of lipid peroxidation

Hydroxyl radical can initiate lipid peroxidation readily by abstracting a hydrogens from methylene groups. $R–CH_2– + OH \rightarrow R–CH^{\cdot} + H_2O$. The $R–CH^{\cdot}$ radical, under aerobic conditions combine with O_2 gives peroxyl radical $RCOO^{\cdot}$. Low concentration of O_2 might favour self-reaction of carbon centered radicals, or reacts with other membrane components such as –SH group or protons. Peroxy radicals are capable of abstracting H from another lipid molecule: $ROO^{\cdot} + RH \rightarrow ROOH+R^{\cdot}$. The carbon radical can react with O_2 to form another peroxy radical and the chain reaction of lipid peroxidation continue.

Fe^{2+} induced lipid peroxidation: The ability of a test compound to inhibit lipid peroxidation is usually studied by the following method. Hydroxyl radicals are generated by the Fenton reactions (reaction 3). H_2O_2 required for the above reaction is produced in the system from the dismutation of superoxide (reaction 2) which is formed by the reaction of Fe^{2+} with O_2 (reaction 1).

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{-} \qquad (1)$$

$$2O_2^{\cdot -} + 2H^{+} \rightarrow H_2O_2 + O_2 \qquad (2)$$

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH + OH \qquad (3)$$

Thus the addition of Fe^{2+} salt (or Fe^{3+} salt + reducing agent such as absorbate) to a peroxide-free unsaturated lipid in presence of O_2 will initiate lipid peroxidation (H abstraction by OH). The peroxidation can be inhibited by H_2O_2 removing enzymes (catalase), scavengers of OH and chelating agents that bind iron.

In the present study lipid peroxide inhibition was measured by the method of Ohkawa *et al.*[232] after induction by Fe^{2+}/ascorbate system. The reaction mixture contained normal rat liver homogenate (0.1 mL, 25% w/v) in Tris-HCl buffer (40 mM, pH 7.0), KCl (30 mM), ferrous ion (0.16 mM) ascorbic acid (0.06 mM) and various concentrations (range 2.5 - 50 μg/mL) of the test sample in a final volume of 0.5 mL was incubated for 1 h at 37°C. From this reaction mixture 0.4 mL was mixed with SDS (0.2 mL, 8.1%) TBA (1.5 mL 0.8%) and acetic acid (1.5 ml, 20% pH 3.5). The total volume was then made upto 4 mL by adding distilled water, and kept in a water bath at 90°C for 1h. After cooling, 5 mL of a mixture of n-butanol and pyridine (15:1 v/v) were added and shaken vigourously. After centrifugation, the organic layer was taken and its optical density was measured at 530 nm. The percentage inhibition of lipid peroxidation was determined by comparing the results of the test sample with those of the control using the formula C-T/C x 100 where C and T are the optical density of the control and test sample respectively.

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Assay protocol

Control		Test Sample	
Tissue Homogenate	- 100 µL	Tissue Homogenate	- 100 µL
Tris buffer	- 100 µL	Tris buffer	- 100 µL (Adjusted accordingly)
Ascorbic acid	- 100 µL	Ascorbic acid	- 100 µL
Ferrous Ammonium-Sulphate	- 100 µL	Ferrous Ammonium Sulphate	- 100 µL
KCl	- 100 µL	KCl	- 100 µL
Sample	- 0 µL	Sample	- Different concentration

Results and Discussion

Inhibition of superoxide radical generation

The effects of diindolyl curcuminoid analogues and its metal complexes on superoxide inhibition are given in the **Fig. 3.13**. All the seven compounds produced superoxide radical inhibition. Generally the metal complexes are more active than the free ligand. Cu(II) complex showed 50% inhibition at 42 µg/ml concentration, and the free ligand produced same effect at 48 µg/ml. All other compounds required more than 50 µg/ml to produce 50% inhibition.

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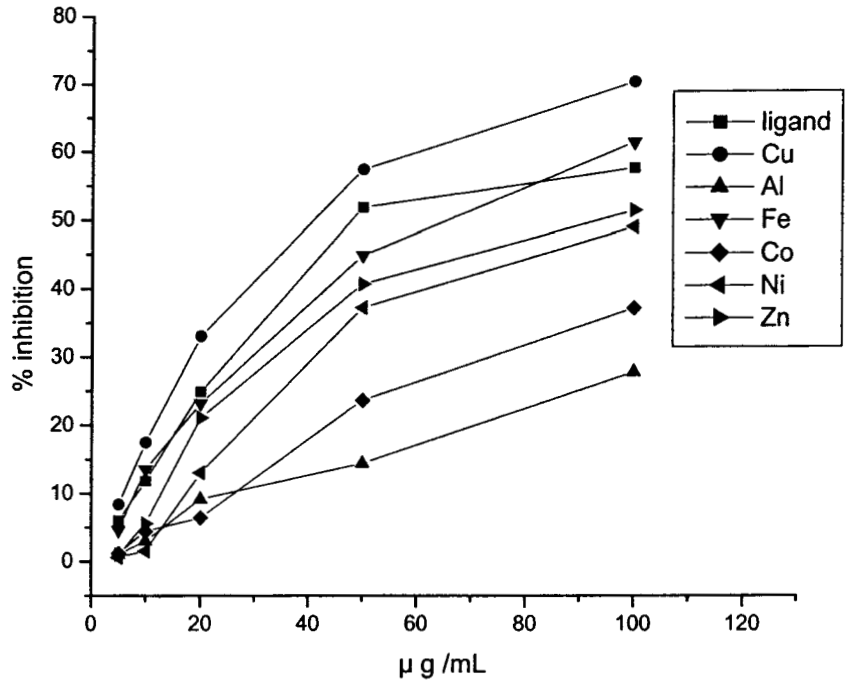


Fig.3.13. Percentage inhibition of superoxide radical generation by Hdih and its metal complexes

Hydroxyl radical scavenging activity

The effect of the ligand and the metal complexes on scavenging hydroxyl radicals is given in **Fig. 3.14**. Hydroxyl radicals produced by Fenton' reaction were effectively savenged by all compounds. The metal complexes showed higher activity than the ligand. At lower concentration Fe(III) complex possess maximum activity, but at higher concentration its activity decreases. The same is observed in the case of Ni(II), Co(II) and Zn(II) complexes. At higher concentration Cu(II)

complex is the best scavenger of hydroxyl radicals. Fe(III) complex produced 50% inhibition at 2.6 μg/ml while Cu(II) complex produced the same effect at 5.5 μg/ml concentration. The decreased activity of the Fe(III), Co(II), Ni(II) and Zn(II) complexes at higher concentrations may be due to the dissociation of the complex under the reaction condition, which have little effect upon the overall auto oxidation rate of the Fenton's reaction.

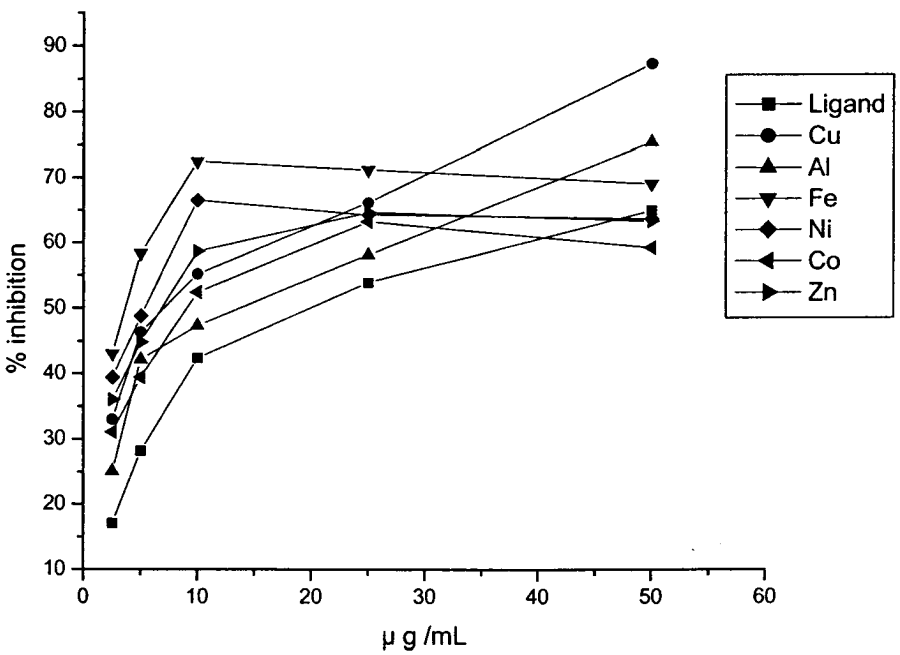


Fig.3.14. Percentage inhibition of hydroxyl radical by Hdih and its metal complexes

Inhibition of lipid peroxidation

All the compounds were investigated for their ability to inhibit the ferrous ion induced peroxidation of rat liver homogenate. Al(III) and Cu(II) complexes produced 50% inhibition of lipid peroxidation at concentrations 10.5 and 12.μg/mL and the free ligand Hidh at concentration 37 μg/mL. The complexes of Co(II), Ni(II) and Zn(II) did not have any effect on inhibition of lipid peroxidation **Fig. 3.15**.

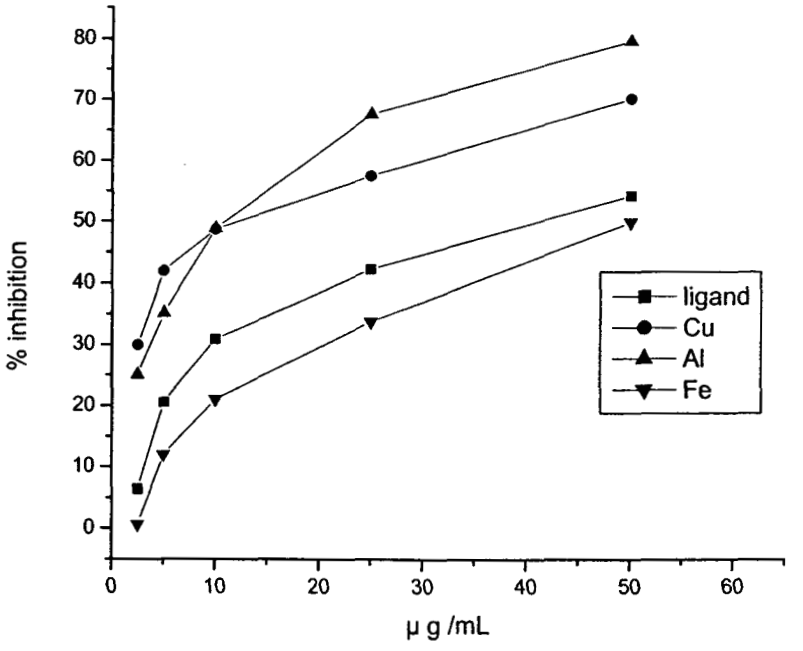


Fig. 3.15. Percentage inhibition of lipid peroxidation by Hdih and its metal complexes

Section 3 B: Cytotoxicity and Anticancer activity

The short term *in vitro* cytotoxicity of many synthetic curcuminoids and their metal complexes were studied. All these studies showed that many metal chelates are biologically active than the free curcuminoids.[75,233,234]

Anticarcinogenic activity of curcuminoids and their complexes correlate with their ability to protect biomolecules against singlet oxygen and other prooxidants. It has been shown that compounds with phenolic group or which can yield phenolic structure upon metabolism, together with presence of conjugated double bond can exhibit considerable anticancer activity.[235] Reports are available supporting this fact that first raw transition metal complexes especially Cu(II) complexes play a vital role as anticancer and antiartharitic agents.[139,236]

Experimental

Materials and Methods

Cells: Daltons Lymphoma Ascites (DLA) cells, were obtained from the cancer institute, Adayar, India. The cells were maintained as ascites tumour in Swiss albino mice.

Animals: Female Swiss albino mices 6-8 weeks old (25 $\pm$ 3g) were obtained from veterinary college, Thrissur, Kerala. They were kept in

polypropylene cage under controlled temperature (25-27°C) and humidity (60%) and fed with pellet diet (Lipton, India, Ltd.) and water and libitum.

Normal saline: Normal saline was prepared by dissolving A.R. NaCl (0.85 g) in 100 mL of distilled water. Normal saline was essential for preparing cell suspension, as the osmotic pressure due to it is isotonic with the fluid inside the cells and this will not cause death of the cell.

Phosphate buffer saline (PBS): It is used for maintaining the pH and isotonicity of the cells, failing, which the cells may rupture during experiments. It is prepared by dissolving NaCl (8 g). KCl (0.2g), $Na_2H\ PO_4.2H_2O$ (1.15 g) and KH_2PO_4 (0.2g) in one litre distilled water.

Trypan Blue: Cell viability was determined using the dye which penetrates into dead cells and makes the identification of dead cells easier. The live cells can be counted under a microscope using a haemocytometer. It is prepared by dissolving 1.00g of trypan blue in 100mL of distilled water.

Maintaining cell lines: DLA cells lines were maintained as ascites tumour in Swiss albino mice. The cells were aspirated, washed thrice with cold PBS to make it free from RBC, etc. and counted using a haemocytometer under a microscope. The cells were suspended in saline or PBS so as to get a cell suspension of 1 million cells/mL. One mL of

the suspension was injected to the peritonial cavity of fresh albino mices. The test animals were given normal diet and within 10-14 days ascites fluid that contain cancer cells were accumulated in the abdomen. The animals with this tumour die within 18-25 days. These cells are propagated regularly by transferring it, as mentioned above to other normal mice and thus the cell lines were maintained.

Determination of *in vitro* cytotoxicity

The short term *in vitro* cytotoxic activity of the compounds were analysed using DLA cells. The dinidolylheptanoid and its Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Al(III) complexes were dissolved in minimum quantity DMSO. Different concentrations (1-100 μg/mL) of these compounds were prepared by diluting this solution. The cell suspension (0.1 mL stock solution which contain ~ 1 million cells) was added to tubes containing different concentrations of these compounds and volume was finally made up to 1 mL using PBS, and incubated for ~ 3 h at 37°C. After incubation 0.1 mL trypan blue was added to each tube and kept for 2 minutes. The number of dead cells were counted using a haemocytometer.

Assay Protocol

Control			Sample		
Cell lines	-	100 μL	Cell lines	-	100 mL
PBS	-	800 μL	PBS	-	800 μL (adjust accordingly)
Dye	-	100 μL	Dye	-	100 μL
Sample	-	0 μL	Sample	-	Different concentration.

Determination of reduction in solid tumour development

Solid tumours were induced in groups of Swiss albino mice (6 nos./group) by subcutaneous injection of DLA cells (1 x 10^6 cells/animal) on the right hind limbs. One group was kept as control and other groups were simultaneously injected (ip) with the test compounds (200 μg/kg body weight) and continued for 10 days. Tumour diameter was measured every fourth day for one month and tumour value was calculated using the formula $V=4/3\ \pi r_1 r_2$ where r_1 and r_2 are the major and minor radii respectively.[237-239]

Determination of reduction of ascites tumour development

Five groups of Swiss albino mice (6 nos./group) were injected ip with DLA cells (1 x 10^6 cells/animal). One group was kept as control and the remaining groups were simultaneously injected ip with test compounds (160 mg/kg body weight) dissolved in DMSO, and injection

of the compound continued for 10 days. The animals were observed for survival for one month and their increase in life span (ILS) was calculated using the formula % ILS = T-C/C x 100, where T and C are the mean number of days survived by the treated and control animals respectively.

Results and Discussion

Short term in vitro cytotoxicity studies

Short term *in vitro* cytotoxicity of the compound **Hdih** and its Cu(II), Al(III), Co(II), Ni(II), Zn(II) and Fe(III) complexes are shown in table 3.17. The experiments were carried out with five different concentrations of the compounds. Cu(II) complex was found to be more cytotoxic than free ligand, **Hdih**. The ligand Hdih produced 50% cell death at a concentration 22 μg/ml and Cu(II) complex produced the same result at a concentration 17 μg/ml. All other complexes produced 50% cell death at concentrations above 30 μg/ml. The insolubility of **Hdih** and the metal complexes in PBS may be the reason for the low activity. The increased activity of the Cu(II) complex than the ligand Hdih may be due to the presence of increased reaction site or the special geometry of the complex which will make easy for the DLA cells to interact. The percentage of cell death at various concentrations of different compounds are given in **fig. 3.16.**

TABLE 3.16

Short term *in vitro* cytotoxicity of ligand Hdih and its metal complexes towards DLA cells

Compounds	Percentage cell-death at different concentrations (μg/ml)				
	5	10	20	50	100
Hdih	14	27	48	71	84
$[Cu(dih)_2 (H_2O)_2]$	19	32	58	83	100
$[Al(dih)_3]$	9	12	18	36	54
$[Co(dih)_2(H_2O)_2]$	--	3	12	20	27
$[Ni(dih)_2(H_2O)_2]$	4	7	16	28	34
$[Zn(dih)_2(H_2O)_2]$	8	17	23	48	56
$[Fe(dih)_3]$	7	13	15	32	41

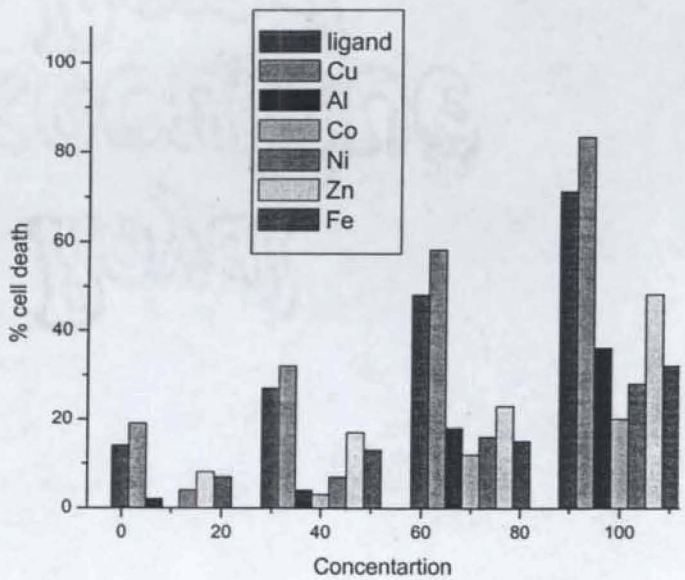


Fig.3.16. Percentage cell-death at different concentrations of Hdih and its metal complexes

Effect of the compounds on solid tumour development

The 1,7-diindolylheptanoid and its Cu(II), All(III) and Fe(III) complexes were studied for their antitumour activity. All compounds were showed significant reduction of solid tumour volume in mices when injected intraperitoneally (**Fig.3.17**). The tumour volume on 36th day was found to be 2.42 cm^3 in the case of control and 1.420, 1.183, 1.021; and 0.816 and 3 for the indolyl curcuminoid and its Fe(III), Al(III) and Cu(II) complexes respectively. However the administration of these curcuminoids did not prevent solid tumour death in these animals.

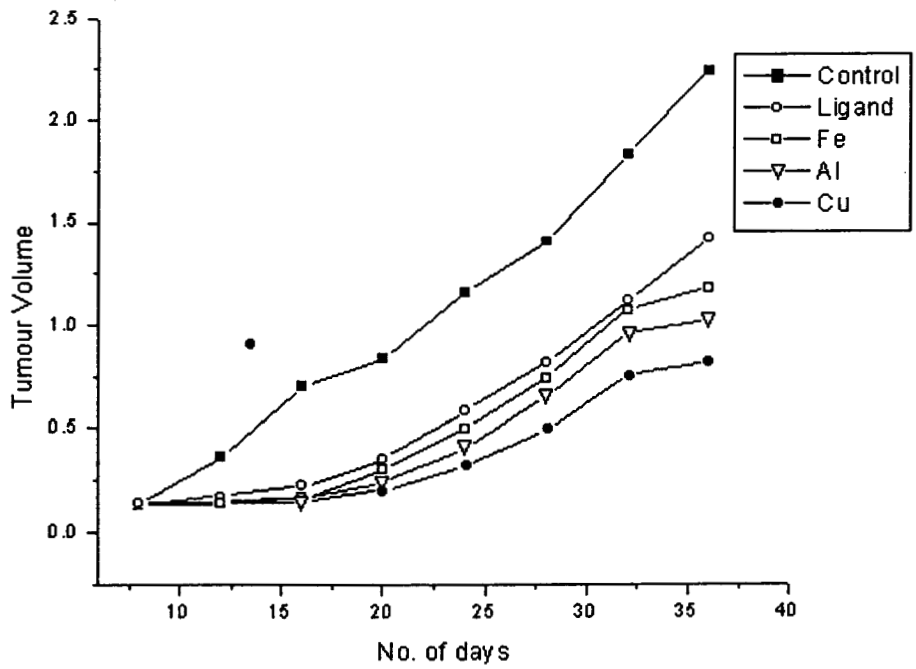


Fig.3.17.Effect on tumour reduction by Hdih and its metal complexes

Effect of the compounds on ascites tumour reduction

The curcuminoid (Hdih) and its metal complexes were found to be less active towards reduction of ascites tumour compared to their activity towards solid tumour development. This may be due to the difference in the mode of action of the metabolites. However, among the various compounds the Al(III) complex was found to possess slightly greater activity (table 3.17).

TABLE 3.17

Effect of the compounds on ascites tumour reduction

Compounds	% increase in life span(in days)
Control	16.25 ± 3.02
Hdih	18.25 ± 1.96
Cu $(dih)_2$	20.58 ± 3.58
Al $(dih)_2$	21.58 ± 2.39
Fe $(dih)_2$	19.25 ± 2.99

Earlier studies on natural and synthetic curcuminoids showed considerable reduction in animal tumour volume, and they are found to be good antioxidants. The above results revealed that Hdih and its metal complexes are cytotoxic towards DLA cells. Cu(II) complex is more active than the ligand. The ligand and the metal complexes are

significant antioxidants. Among the various compounds the Cu(II), complex is the most active. The antioxidant activities of the natural and synthetic curcuminoids are mainly attributed to the presence of phenolic OH and the conjugated double bonds. But diindolyl curcuminoid does not have such -OH group, the activity may be due to the conjugated double bond. Jovanovic *et al.* proposed a hydrogen atom transfer mechanism for explaining the antioxidant activity of curcuminoids.[240] According to this concept the methylene C-H of the diketo group is responsible for the antioxidant activity of the compounds. The increased activity of certain metal complexes reveal the involvement of the metal ion in the hydrogen atom transfer mechanism. But the actual mechanism of the process is not known.

SUMMARY

1,7-diindolyl-1,6-heptadiene-3,5-dione was synthesised by the condensation of indole-3-carboxaldehyde and acetylacetone. The metal chelates of Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Al(III) were also synthesised. The diindolylheptanoid and its metal chelates were characterised by various spectral and analytical techniques. The results obtained are fully supporting the intramolecularly hydrogen bonded enol form of the compound. The metal chelates of Al(III) and Fe(III) have 1:3 stoichiometry while a 1:2 stoichiometry for the remaining chelates.

The study of the fluorescence characteristics showed that the fluorescence maxima of the diketone depends on the nature of the solvent. The fluorescence intensity is influenced by water, pH of the medium and metal ions. The fluorescence quantum yield of the compound was also determined. The value obtained was higher than that of natural curcumin.

Study of antioxidant activity of these compounds, such as superoxide scavenging activity, hydroxyl radical scavenging activity and inhibition of lipid peroxidation revealed that 1,7-diindolyl curcuminoid and its metal chelates are good antioxidants, the Cu(II) chelate possess maximum activity.

The short term *in vitro* cytotoxic activity of these compounds were analysed using DLA cells. 1,7-diindolylheptanoid and its Cu^{2+} complexes were found to be more active than other compounds.

All the compounds were investigated for their antitumour activity. It was found that Cu(II) chelates are remarkably active in reducing solid tumour volume induced by DLA cells.

CHAPTER 4

SYNTHESIS, CHARACTERISATION, FLUORESCENCE CHARACTERISTICS AND BIOLOGICAL ACTIVITY OF 1-PHENYL-5-INDOLYL-4-PENTENE-1,3-DIONE AND ITS METAL COMPLEXES

Introduction

The 1,7-diinidolylheptanoid (chapter 3) was readily formed by the condensation of indole-3-aldehyde at the γ-methyl groups of acetylacetone. Therefore, it is logical to extend the reaction to other 1,3-diketones having atleast one such methyl group. When the condensation reaction was carried out using benzoylacetone and indole-3-aldehyde a new 1,3-diketone was formed in good yield. The reaction can be represented as in scheme 4.1. The unsaturated 1,3-diketone forms well defined complexes with various metal ions. Details on the synthesis and characterisation of these compounds are presented in **section 1**. The fluorescence properties of the 1,3-diketones are discussed in **section 2**. The antioxidant activities, cytotoxicity and antitumor properties of the compound and its metal complexes are discussed in **section 3**.

SECTION 1

SYNTHESIS AND CHARACTERISATION OF 1-PHENYL 5-INDOLYL-4-PENTENE-1,3-DIONE AND ITS METAL COMPLEXES

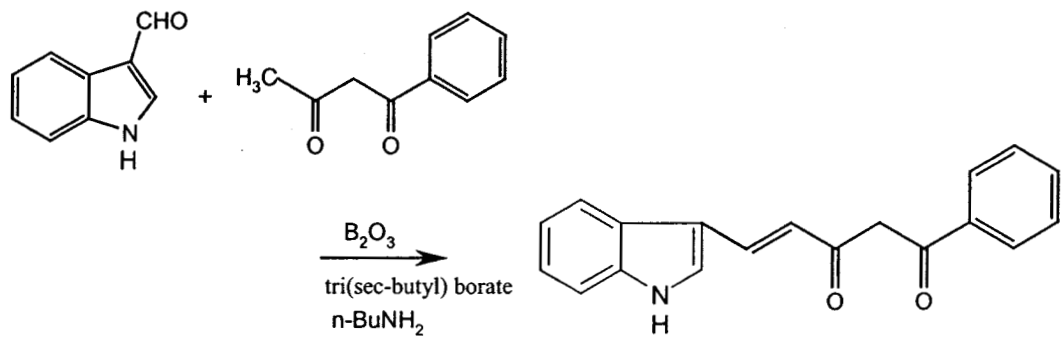
Experimental

Synthesis of 1-phenyl-5-indolyl-4-pentene-1,3-dione

The 1-phenyl-5-indolylpentanoid was synthesised by the condensation of indole-3-aldehyde with benzoylacetone. Compared to the 1,7-diindolylheptanoid, the yield was high. The condensation was performed in the presence of tri(sec-butyl) borate, boric oxide, and n-butylamine. Procedural details are given below.

A mixture of benzoylacetone (0.005 mol) and boric oxide (0.0035 mol) was stirred well in 5 mL dry ethyl acetate for ~ 1 h. The stirring was continued for ~ 1 h with slow addition of a solution of indole-3-aldehyde (0.005 mol) in 10 mL dry ethyl acetate followed by tri (sec-butyl) borate (0.01 mol) and 0.05 mL n-butylamine. After stirring an additional period of ~ 3 h, at 80°C, the solution was set aside overnight. Hot (~60°C) hydrochloric acid (0.4 M. 7.5 mL) was then added to the reaction mixture and stirred again for ~ 1h. The product was then extracted with ethyl acetate. The combined extract was concentrated by evaporation and the

residual paste obtained was again stirred with hydrochloric acid (2M, 10 mL). The solid separated was collected, washed with water, and dried under reduced pressure. The compound was recrystallised from ethanol-toluene mixture to get chromatographically (tlc) pure sample.



Scheme 4.1

Microwave assisted synthesis of 1-phenyl-5-indolyl-4-pentene-1,3-dione: The microwave-assisted synthesis of 1,7-diindolylhpetanoid (chapter 3) revealed that the method is rapid and gave good yield compared to the conventional method.[151] Therefore, the method was extended for the synthesis of the indolylpentanoid considered in this chapter. Details are illustrated below.

Benzoylacetone (5 m mol) was mixed with boric oxide (3.5 mM) in a 50 mL conical flask. It was made in to a paste with few drops of dry ethyl acetate and then stoppered the flask. The mixture was irradiated with microwave radiation at medium power for 1 minute in a conventional microwave oven. The flask was then cooled and the

product was mixed with indole-3-aldehyde (5 mM), tri (see-butyl) borate (10 mM) and 0.05 mL n-butylamine and made a paste with 3ml dry ethyl acetate and irradiated again with microwave radiation for 3 minutes. Then the flask was cooled and ethyl acetate was added. The solution was stirred with 0.4 M. HCl for 30 minutes at 60°C. The organic layer was separated and evaporated to a paste, which was stirred with hydrochloric acid (2 M). The solid product separated was filtered, washed with water and dried under vacuum. The compound was recrystallised from ethanol-toluene mixture to get spectroscopically pure (tlc) sample.

Synthesis of metal chelates

The general method synthesis of complexes of Al(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) is given below.

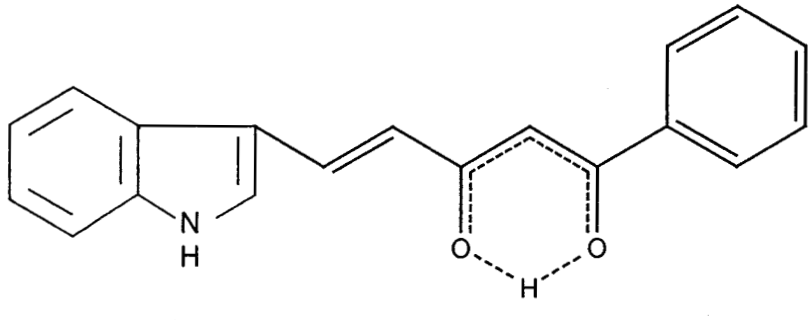
A solution (25 m) of the metal salt in a 1:1 mixture of methanol and water was added slowly with stirring to a boiling solution (30 mL) of the ligand (0.001 mol) in ethanol. The reaction mixture was gently refluxed for ~2 h and the volume was reduced to half. On cooling to room temperature the complex get precipitated. The pH of the solution was kept ~ 6 by the addition of sodium acetate. The precipitated complex was filtered, washed with ethanol-water mixture and recrystallised from hot methanol.

Results and Discussion

Characterisation of 1-phenyl-5-indolylpentanoid

The compound is reddish brown plate like crystalline solid with sharp melting point and freely soluble in common organic solvents like acetone, ethanol, chloroform, etc. The yield was 72%. But MW assisted synthesis gave a better yield (91%), which revealed that this technique is superior to the conventional method.

The C, H, N analysis and molecular weight determination (**Table 4.1**) together with mass spectral data of the compound clearly suggest that one equivalent of aldehyde has condensed with one equivalent of benzoylacetone as in scheme 4.1. The spectral data are in agreement with the intramolecularly hydrogen bonded structure (**4.1**) of the compound. The spectral data are discussed below.



4.1

TABLE 4.1

Physical Analytical and uv spectral data of 1-phenyl –5-indolyl pentanoid

m.p. °C	Elemental analysis (Found/ Calcd.)			Mole. wt.	λmax in methanol	log ε
	C	H	N			
199	77.97 (78.89)	6.02 (5.19)	4.08 (4.84)	289	420 232	4.26 4.01

Uv spectrum: The uv spectrum of the compound (10^{-3} M, in methanol) shows two strong broad absorptions (**Table 4.1**). The band at 420 nm corresponds to the n→π* transition. As expected these bands shifted to higher wave length compared to benzoylacetone due to conjugation of the double bond to the indole ring. But the delocalisation is much less compared to 1,7-diindolylheptanoid and hence the absorption maxima are observed at lower wave length.

Infrared spectra: The 1-phenyl-5-indolylpentanoid is an unsymmetrical 1,3-diketone. As a result, the carbonyl frequencies should differ, ie, two types of carbonyls, the C_6H_5-CO and Ind-CH=CH-CO. Benzoyl carbonyls usually absorb in the region 1670-1690 cm^{-1} and conjugation decreases the value to low frequencies. The cinnamoyl carbonyl frequencies are usually in the range 1640-1660cm^{-1}. However, the heterocyclic group indole and the phenyl group may lower the carbonyl

frequency. Thus the net effect is to lower the stretching frequency of both the carbonyl groups. Therefore in such compounds it is very difficult to assign precisely even the carbonyl stretching frequencies.

The infrared spectrum of the compound is characterised by the presence of two strong bands at 1639 cm^{-1} and another peak at 1615 cm^{-1}. In the enol form of the compound, the benzoyl and the indolyl carbonyls are expected to stretch in the region 1620-1650 cm^{-1}. Thus the broad band at 1639 cm^{-1} is presumably due to both these carbonyl groups. The presence of a very weak band at 1719 cm^{-1} assignable to free acetyl carbonyl suggests that the compound contains a small percentage of the diketo form also. But the intensity of the band is very low suggest that the compound exists predominantly in the hydrogen bonded enol from. The band at 1615 cm^{-1} is due to the stretching of νC=C olefinic.

The stretching of the NH group appeared at 3238 cm^{-1}. Presence of a comparatively broad band in the range of 3100-3300 cm^{-1} confirm the intramolecularly hydrogen bonded enol form of the compound. There are several medium intensity bands in the rage of 2300-3100 cm^{-1} due to various aliphatic and aromatic C–H stretches. The spectrum is reproduced in **figure 4.1**.

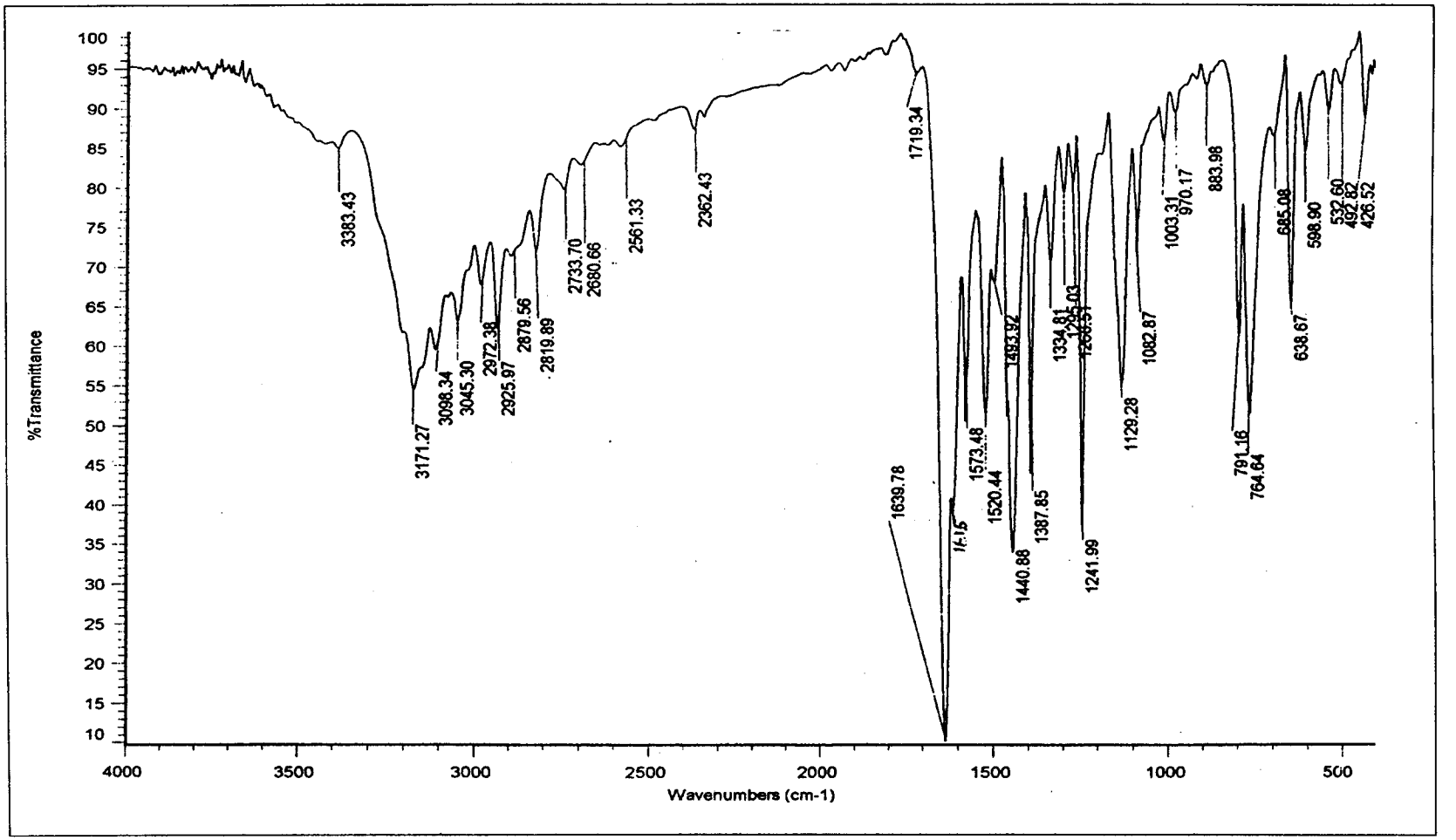


Fig. 4.1. IR spectrum of 1-phenyl-5-indolyl-4-pentene-1,3-dione

^{1}H nmr spectrum: The ^{1}H nmr spectrum of the compound is reproduced in **Fig. 4.2.** The spectrum shows a downfield one proton signal at δ16.1 ppm assignable to the intramolecularly hydrogen bonded enol proton. Another one proton signal at δ 10.1 ppm is due to the NH of the indolyl group. The signal at δ 6.61 is due to the methine proton. The presence of a weak signal at δ 4.05 ppm is probably due to the methylene proton of the small amount of keto form present in the compound. The alkenyl signals (8 ppm) with their observed J value (~ 16 Hz) suggests a trans configuration about the olefinic function in the compound.

Mass spectrum: The mass spectrum of the compound showed intense molecular ion peak at m/z 289. However the most intense peak are at m/z 183 and 170. The formation of the fragment at m/z 183 is interesting since its origin can only be explained by the elimination of the fragment C_6H_5C-OH. This clearly demonstrate that the compound exists predominantly in the enol form and the enolisation is more towards the benzoyl carbonyl. The formation of the fragment of m/z 170 and other fragments appeared in the spectrum can be conveniently explained by considering the fragmentation pattern given in the **scheme 4.2.** The spectrum is reproduced in **Fig. 4.3.** Thus all the available evidence strongly support the structure (**4.1**) of the compound.

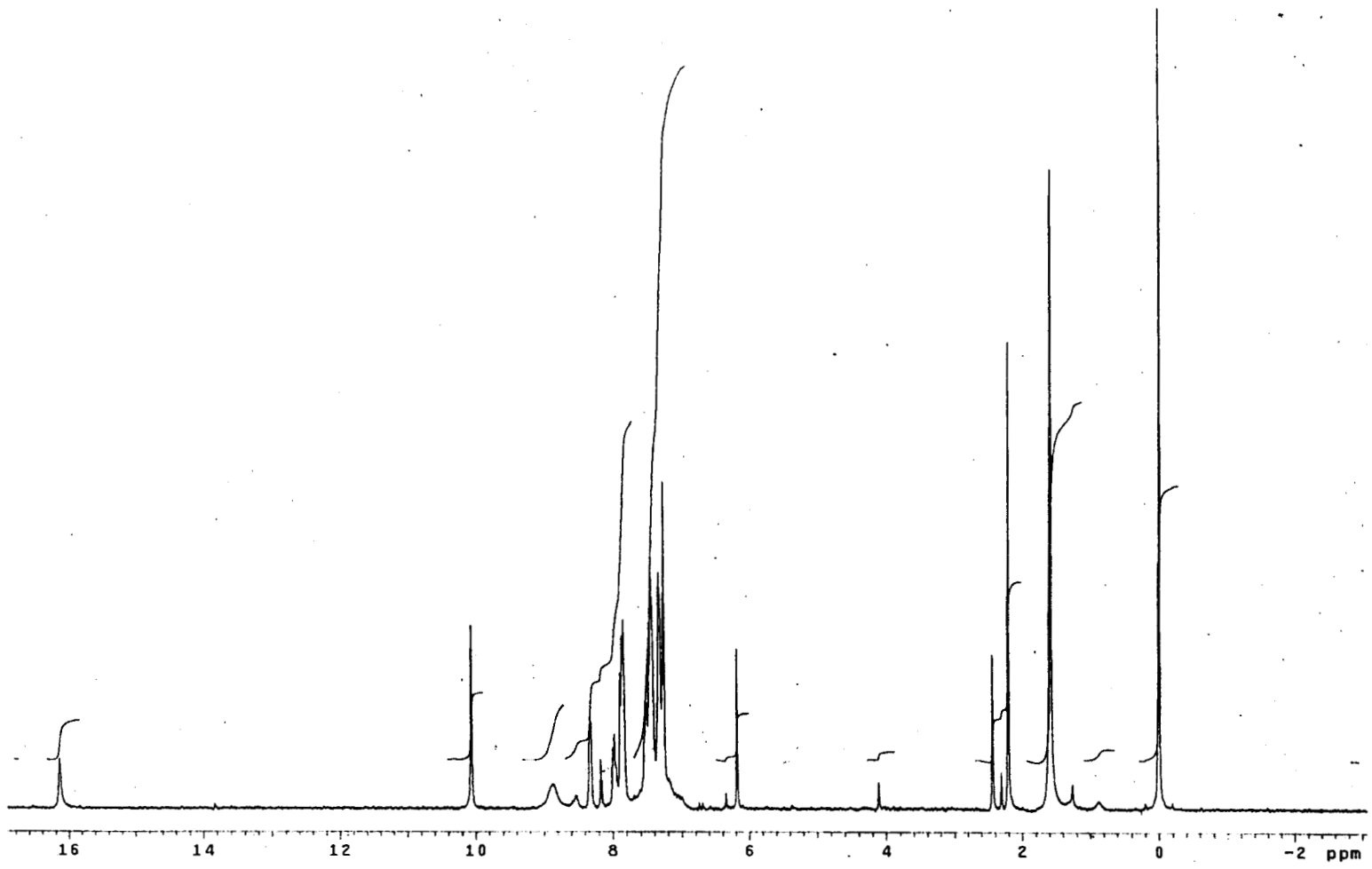


Fig. 4.2. NMR spectrum of 1-phenyl-5-indolyl-4-pentene-1,3-dione

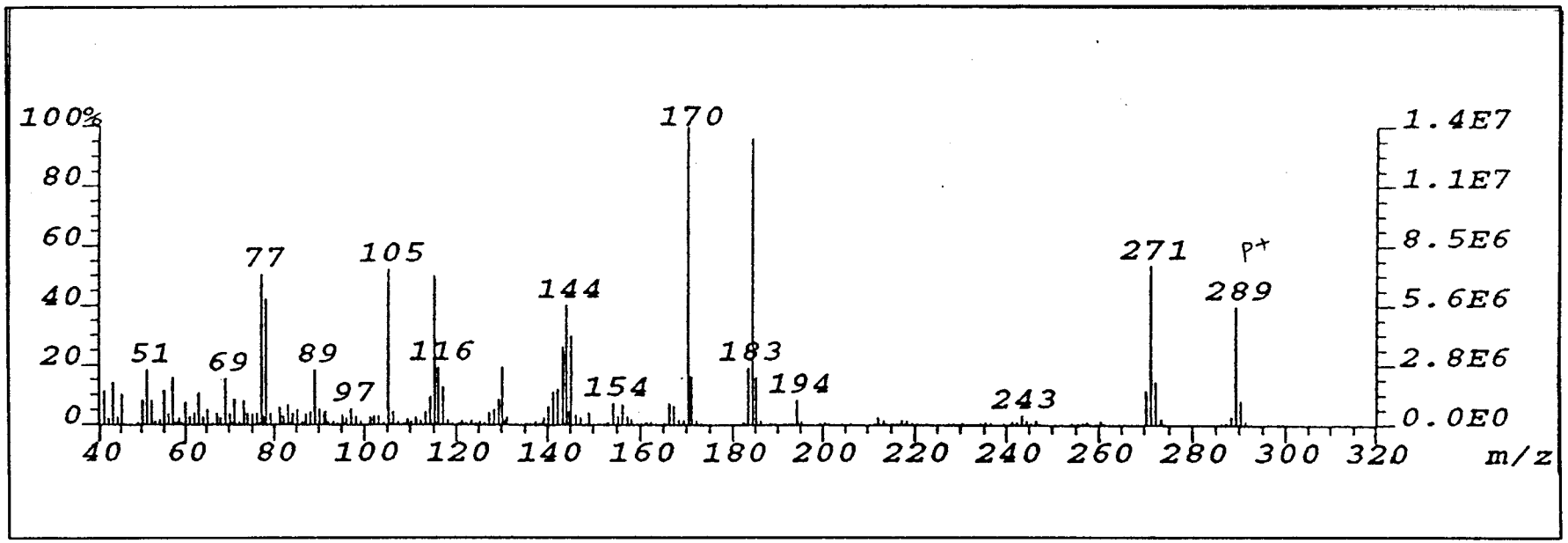
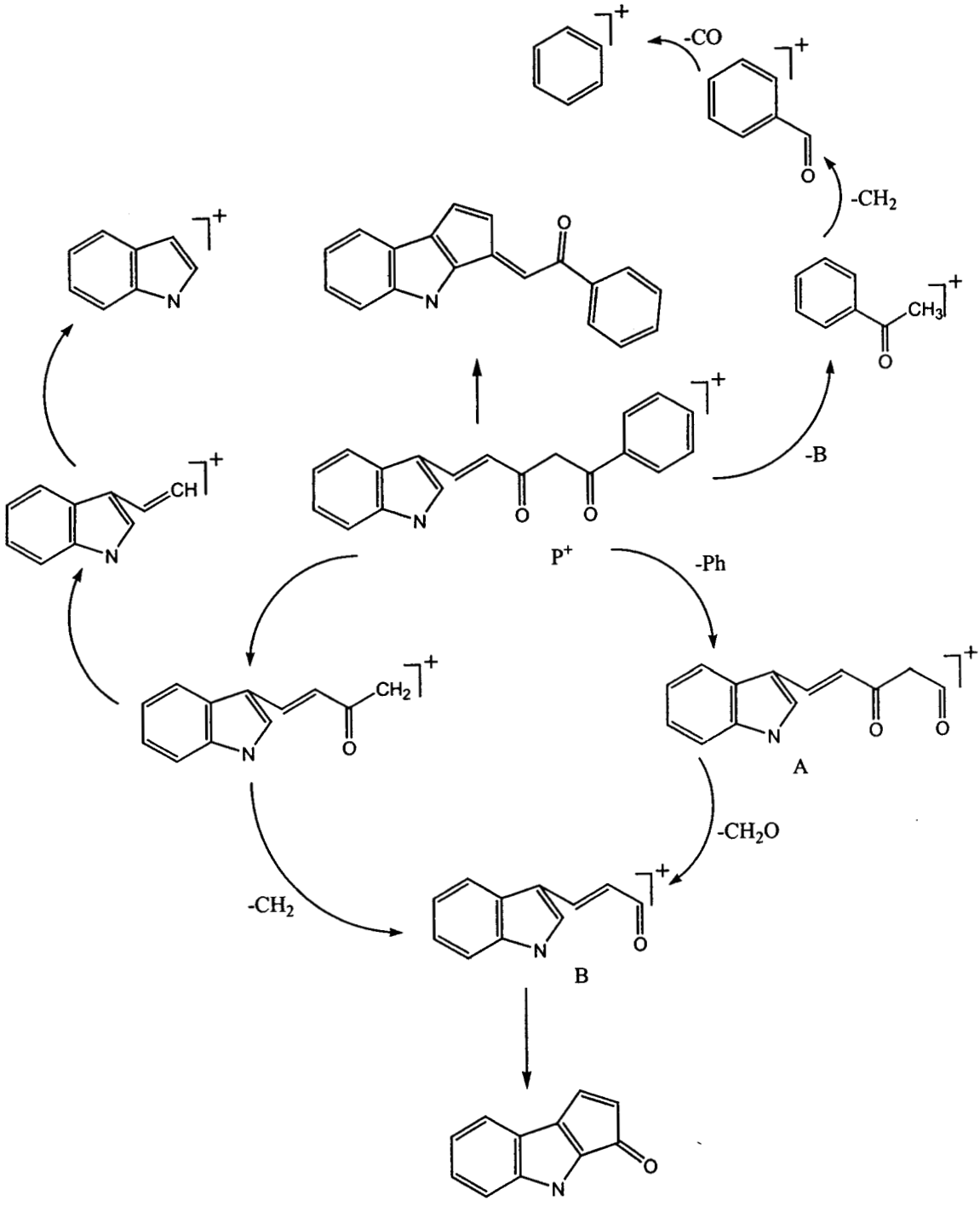


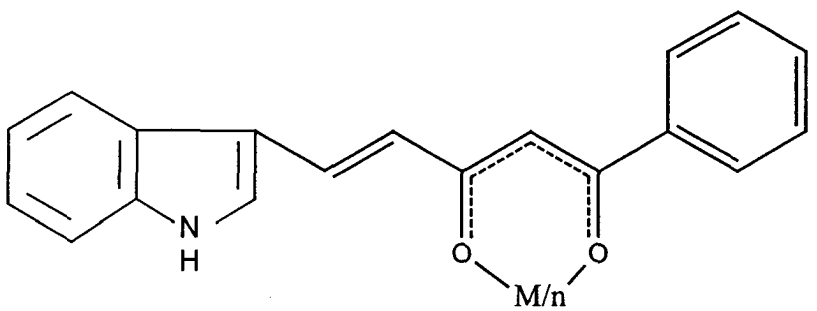
Fig. 4.3. Mass spectrum of 1-phenyl-5-indolyl-4-pentene-1,3-dione



Scheme 4.2

Characterisation of metal chelates

The 1-phenyl-5-indolyl-4-pentene-1,3-dione formed stable complexes with Al(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) with sharp melting points. Solution of all these metal complexes in DMSO behaved as non electrolytes. The complexes of Fe(III), Co(II), Ni(II) and Cu(II) complexes showed normal magnetic moment. Elemental analysis and other physical data of the complexes are given in table 4.2. The metal percentage agree well with the $[ML_2]$ stoichiometry for divalent metal ions and $[ML_3]$ stoichiometry for trivalent metal ions. The observed uv, ir, nmr, and mass spectral data of the metal complexes conform the replacement of enolic proton of the ligand by the metal ion with the formation of a stable six membered metal chelate ring in which both oxygens of the dicarbonyl function are involved in bonding with the metal ion as in structure 4.3.



n = 2 or 3

4.3

Uv spectra: The observed uv absorption maxima of the complexes are given in **table 4.2**. The data suggests that no structural alteration of the ligand has occurred during complexation. However, the minor bathochromic shift of the two major bands of the complexes compared to the free ligands suggest that both the carbonyl oxygens are involved in bonding with the metal ion.

TABLE 4.2

Physical and analytical data of metal chelates of 1-phenyl-5-indolyl- pentanoid (HL)

Metal Complex	M.P. °C	$\delta\mu_{eff.}$ B.M	Elemental analysis % Found/ (Calcd)				$\lambda_{max(nm)}$
			C	H	N	M	
$[Al\ L_3]$	216	--	75.28 (76.76)	5.78 (4.71)	3.81 (4.71)	4.14 (3.02)	421, 232
$[Fe\ L_3]$	254	5.32	73.21 (74.35)	5.71 (4.56)	3.98 (4.56)	6.81 (6.07)	425, 237
$[CoL_2(H_2O)_2]$	158	3.83	67.75 (68.70)	5.82 (5.61)	3.9 (4.1)	8.16 (8.77)	420, 234
$[NiL_2(H_2O)_2]$	146	3.00	65.41 (67.90)	6.01 (4.78)	3.85 (4.01)	7.98 (8.75)	428, 237
$[CuL_2(H_2O)_2]$	> 260	1.62	67.61 (67.50)	5.11 (4.73)	4.05 (4.10)	9.72 (9.40)	420, 233
$[ZnL_2\ (H_2O)_2]$	154	--	69.31 (71.01)	5.32 (4.72)	4.20 (4.36)	8.84 (9.65)	421, 241

Infrared spectra: The most striking feature in the ir spectra of complexes is the absence of any band in the region 1630 - 2000 cm^{-1} due to free or hydrogen bonded carbonyl groups. However in the spectra of all the

complexes, a new strong band appeared at ~ 1585 cm^1 assignable to metal bonded carbonyl group. Two medium intensity bands, appeared at ~ 468 and ~ 420 cm^{-1} are due to the νM-O vibrations.

The breadth of the free ligand band in the region 3100-3300 cm^{-1} decreased considerably indicating the replacement of the enolic proton by metal. That the –NH group is not engaged in bonding with the metal ion is evident from the presence of a band assignable to the ν_{NH} in this region of the spectra of the metal complexes. Thus the observed ir data are in agreement with the formation of stable chelates through the 1,3-dicarbonyl function of the ligand. The characteristic ir spectral data of the metal chelates are tabulated in **table 4.3**.

TABLE 4.3

Characteristic ir data (cm^{-1}) of metal chelates of 1-phenyl 5-indolyl heptanoid

Probable Assignment	Complexes of					
	A(III)	Fe(III)	Co(II)	Ni(II)	Cu(II)	Zn(II)
νC=O chelated	1582	1580	1575	1578	1580	1585
νC=C olefinic	1610	1612	1608	1610	1605	1612
νC=C	1590	1588	1580	1586	1583	1569
νas C-C-C chelate ring	1442	1450	1468	1440	1454	1415
νS C-C-C chelate ring	1384	1366	1386	1387	1394	1382
β_{C-H} chelate ring	1116	1101	1120	1102	1096	1103
νCH=CH (trans)	967	1000	984	963	976	968
νC-H chelate ring	742	744	740	724	738	745
νM-O chelate ring	472	462	458	473	466	446
	436	421	420	424	426	428

^{1}H NMR spectra: The most characteristic feature of the ^{1}H nmr spectra of the diamagnetic metal chelates is the absence of the one proton signal of the free ligand at ~ 16 ppm. This strongly suggest the replacement of enolic proton by metal ions. The NH proton shifted slightly to the upfield. The ^{1}Hnmr spectrum of Zn(II) chelate is shown in the **Fig. 4.4**. The integrated intensities of the aryl and alkenyl signals are in agreement with the 1:2 metal-ligand stoichiometry of the chelates.

Mass Spectra: The FAB mass spectrum of the copper(II) chelate is given in the **Fig. 4.5**. The spectrum shows the stepwise removal of aryl groups. The molecular ion peak at 639 and 641 in agreement with $[CuL_2]$ formulation of the compound. Peaks due to $[ML]^+$, L^+ and fragments of L^{++} are also detected in the spectrum. The fragmentation pattern of the Cu(II) complex is illustrated in **scheme 4.3**.

ESR spectral study of Cu(II) complex: The esr spectrum of copper(II) complex of 1-phenyl-5-indolylpentanoid in DMF at 77 K is reproduced in **Fig. 4.6**. The $g_{||}$ (2.1428), $g_{\perp}$ (2.028) and $A_{||}$ (82 x 10^4 cm^{-1}), $A_{\perp}$ (28 x 10^4 cm^{-1}) values calculated for the complex indicate appreciable delocalisation in the chelate ring.

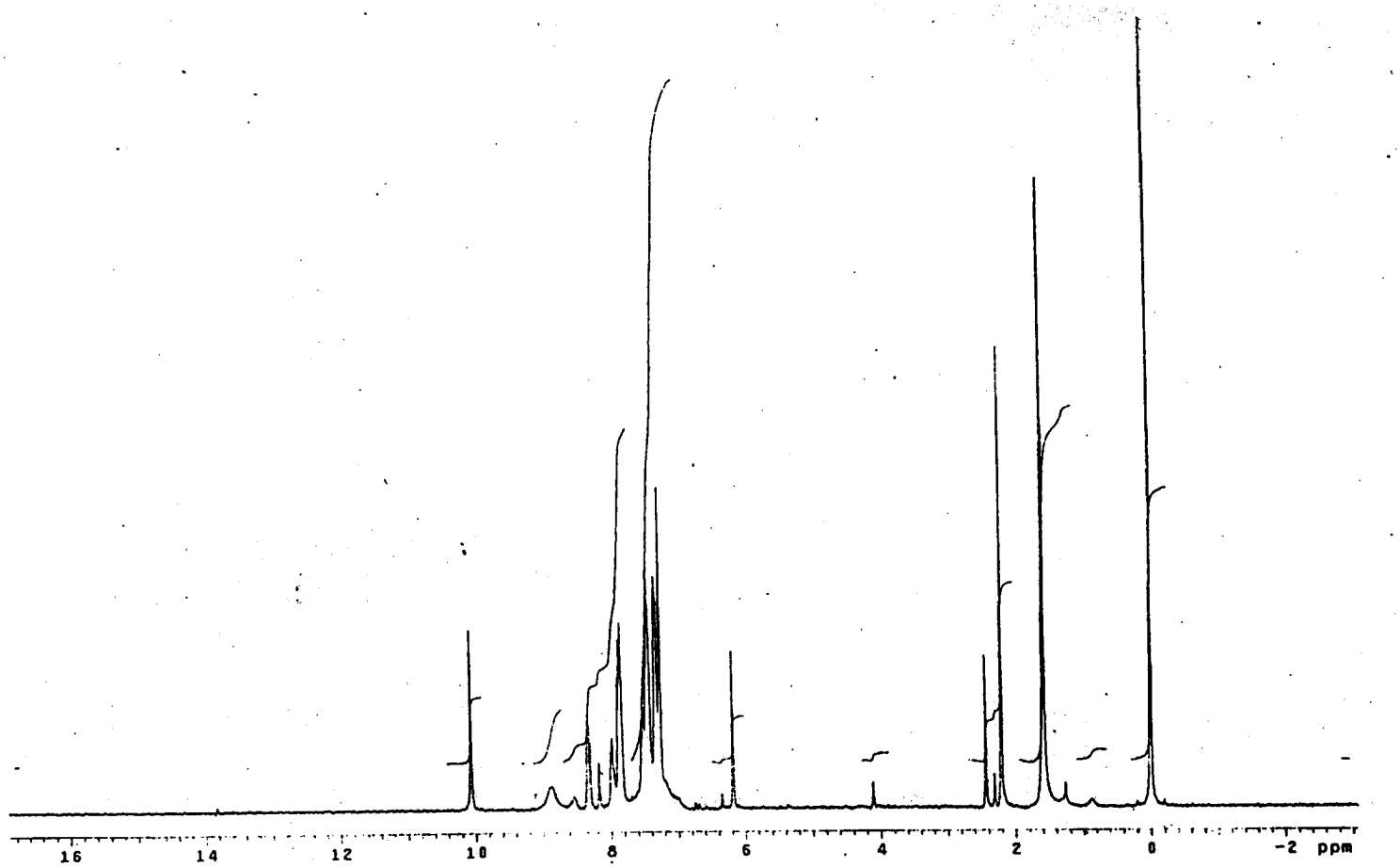


Fig. 4.4. NMR spectrum of Zn(II) complex of 1-phenyl-5-indolylpentanoid

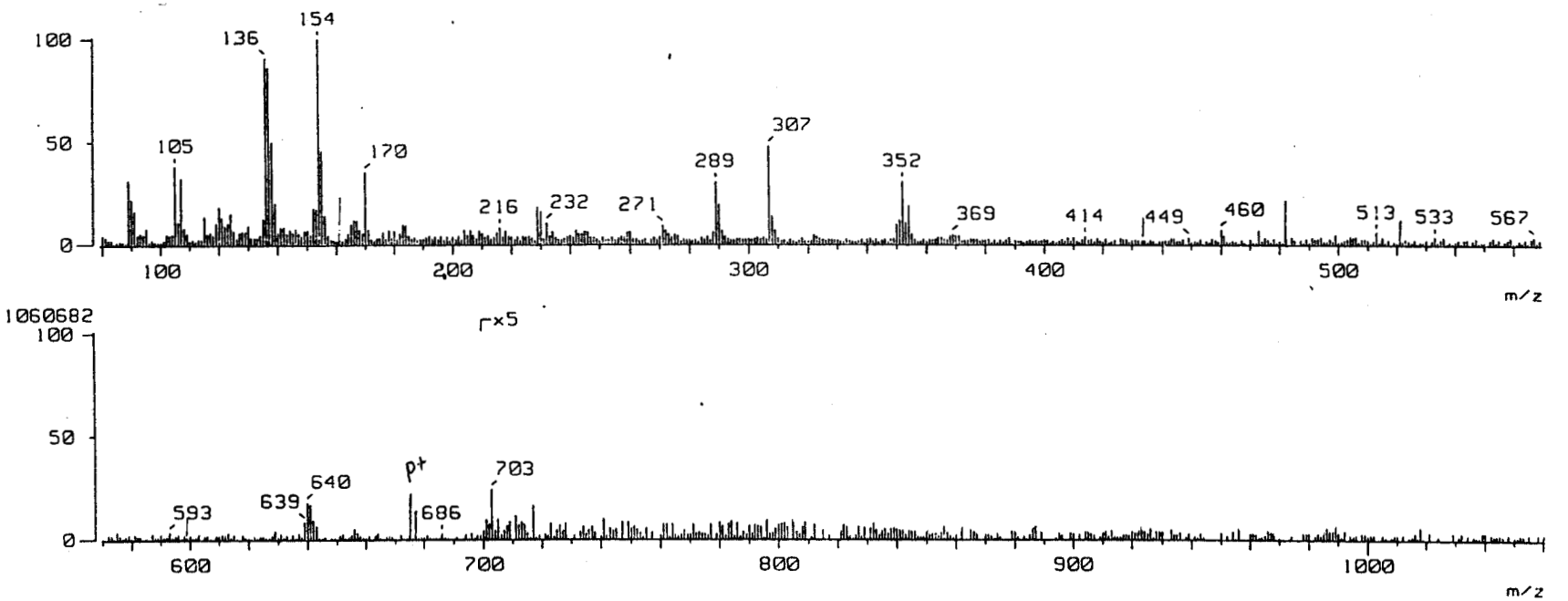
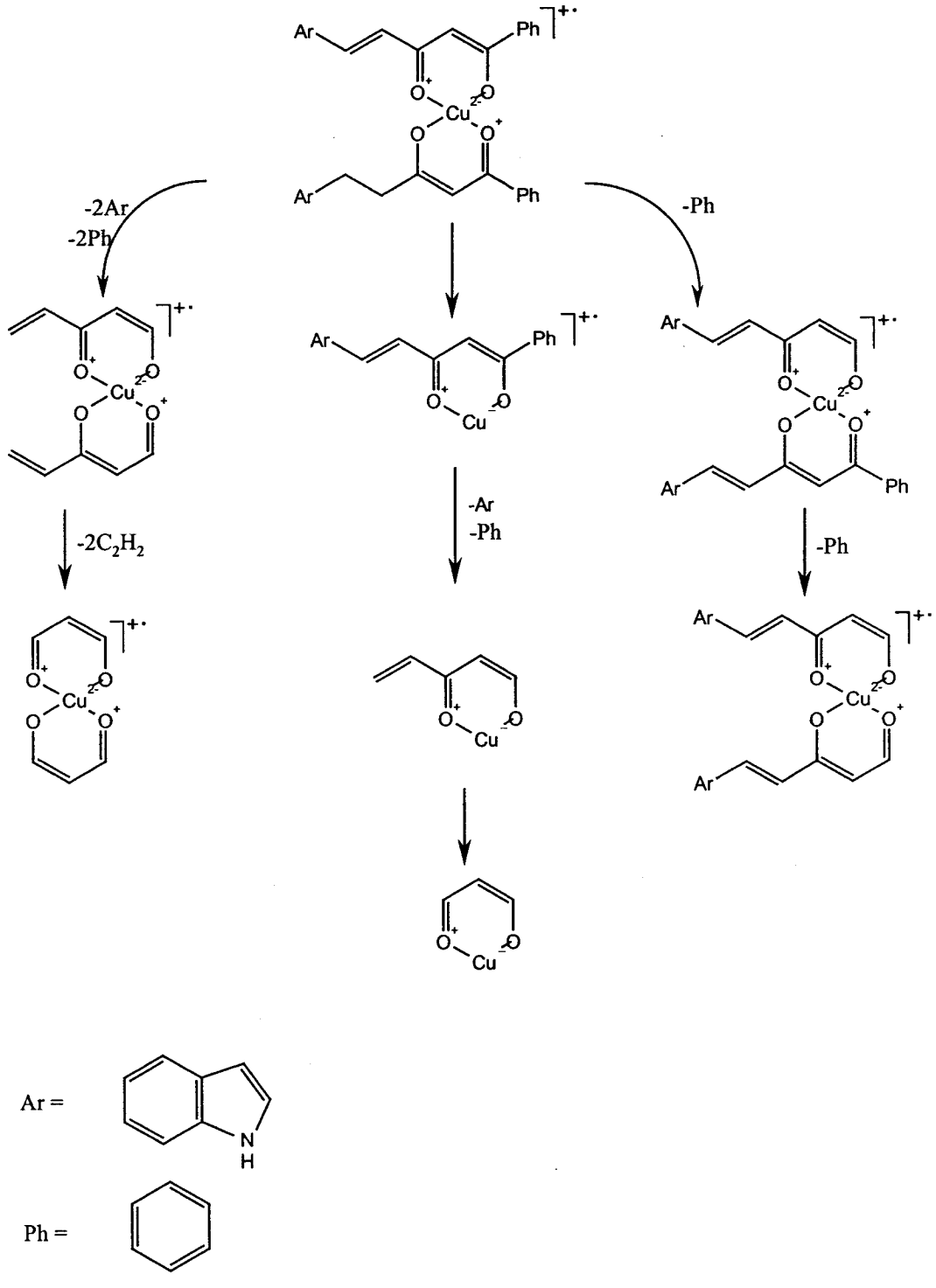


Fig. 4.5. Mass spectrum of Cu(II) complex of 1-phenyl-5-indolylpentanoid



Ar =

Ph =

Scheme 4.3

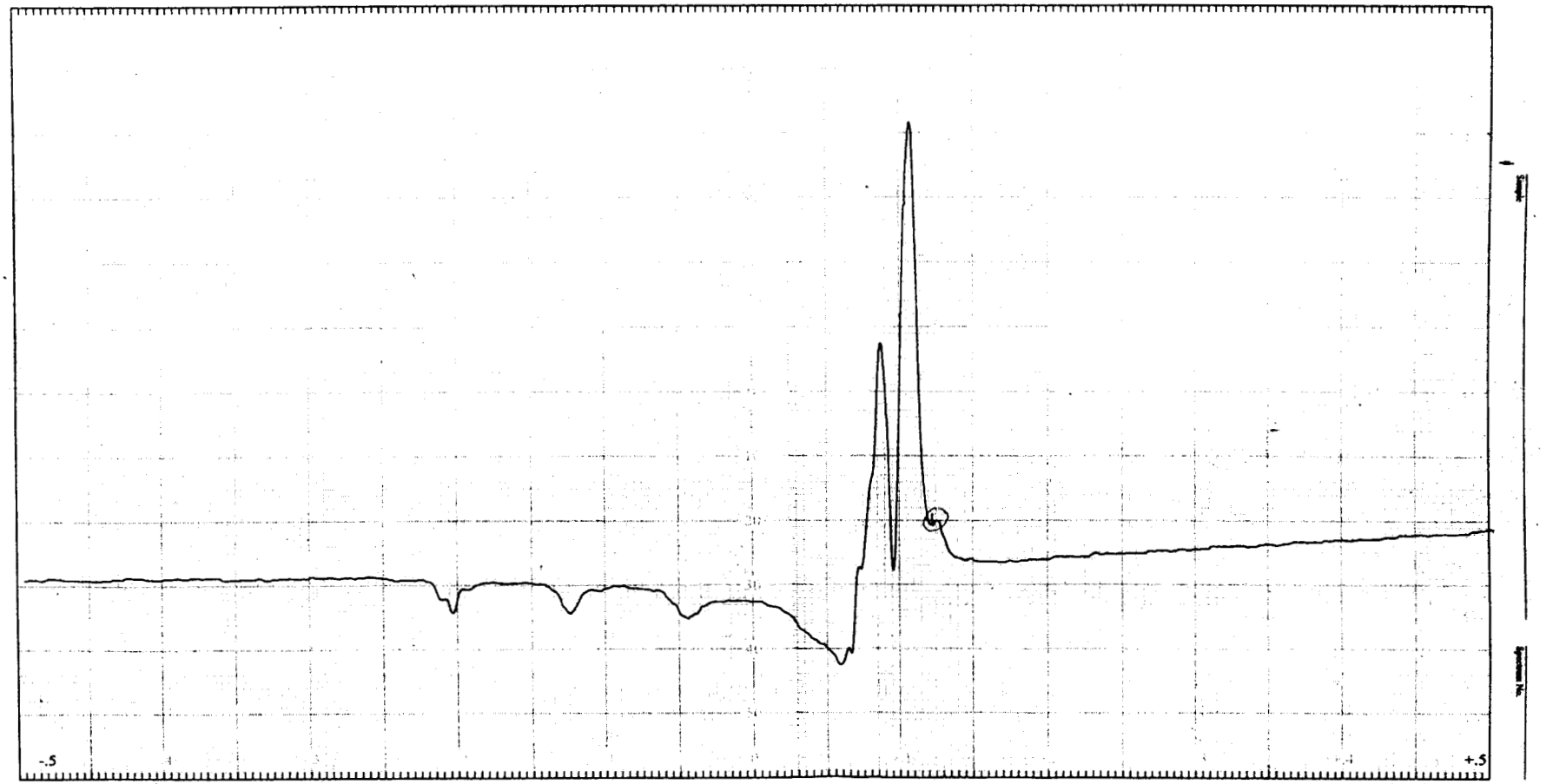


Fig. 4.6. ESR spectrum of Cu(II) complex of 1-phenyl-5-indolylpentanoid

Thermogravimetric study of Cu(II) complex: The thermogram of the Cu(II) complex in air shows a two stage decomposition pattern, and agree well with the formulation $[CuL_2(H_2O)_2]$. The first stage occurred in the temperature range 200-250°C. At this temperature range the percentage weight loss corresponds to the loss of two H_2O molecules (found 6.0, calculated 5.3). The loss of the ligand occurred in two steps (at 386°C and 450°C). The percentage of metal oxide residue obtained (found 12.4% calculated 11.78) also agree with the formulation of the complex. The thermogram of Cu(II) complex is reproduced in **Fig. 4.7.**

X-ray diffraction study of 1-phenyl-5-indolylpentanoid

Single crystal XRD analysis is a unique technique to determine the structure of various compounds.[241] It gives complete information regarding the type of space group, until cell dimensions, the way in which the molecules are packed in the unit cell and structural parameters such as bond length, bond angle, torsion angle and the possibility for inter or intra molecular hydrogen boding, etc.

The crystal and molecular structure of curcumin I has been reported by single crystal X-ray diffraction method.[152] The crystals are monoclinic and the space group P_2/n with unit cell dimensions a = 20.028 A°, b = 7.073 A°, c = 12.609 A° β= 94.94.

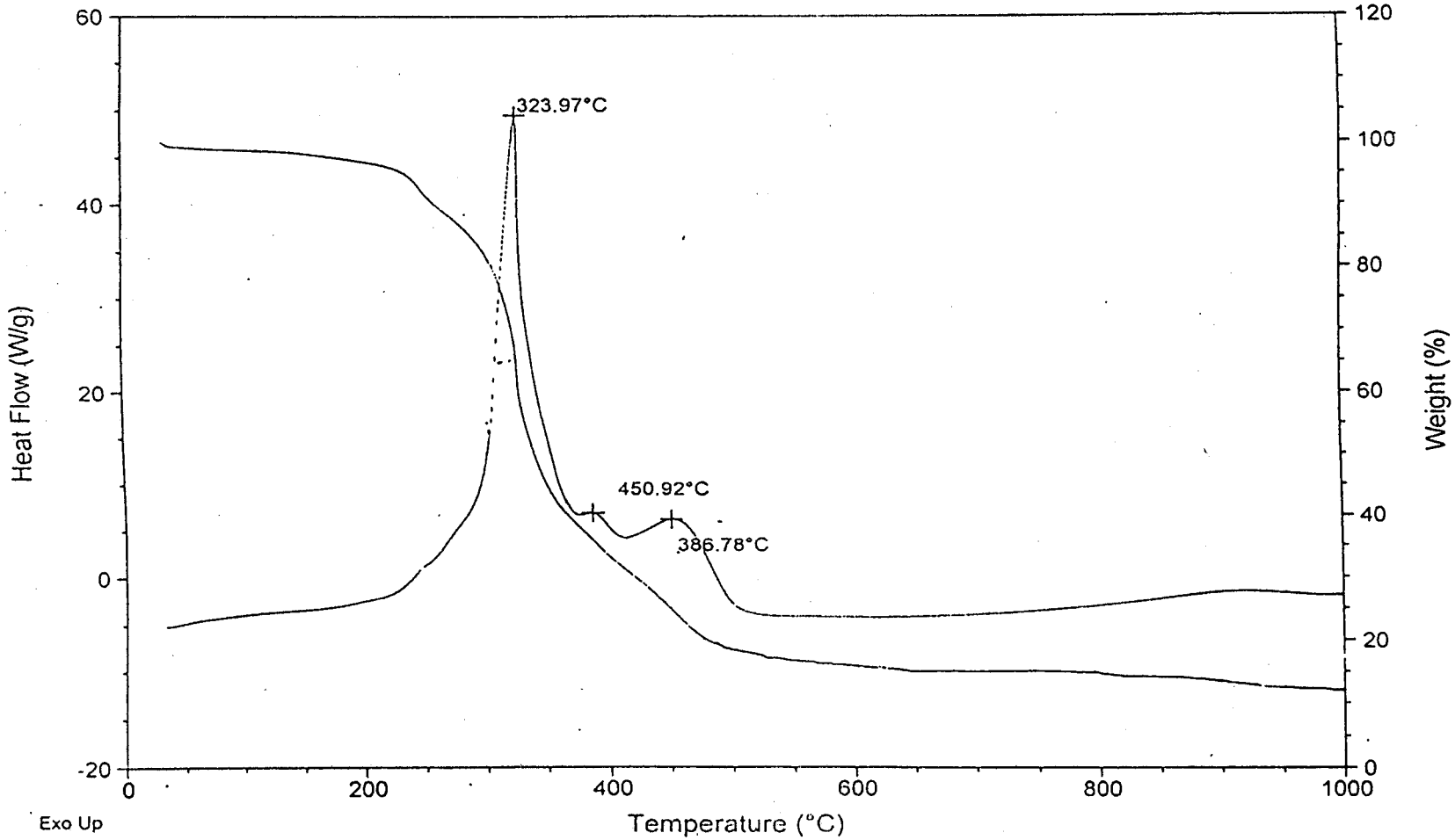
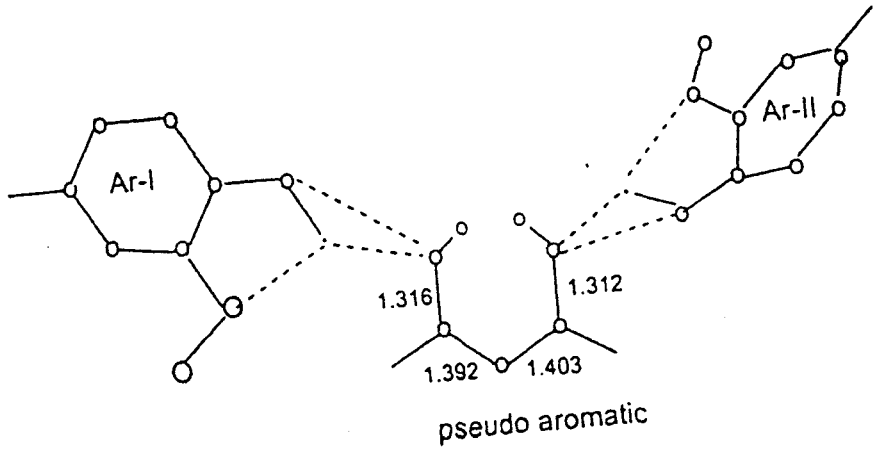


Fig. 4.7. Thermogram of Cu(II) complex of 1-phenyl-5-indolylpentanoid

The molecule may be described as consisting of three substituted planar rings interconnected through the two double bonds C_{17}–C_{18} and C_{12}–C_{13}.The two terminal groups are identical but the inherent symmetry of molecule is distorted in the crystal by rotation of 162° about the C_{11}–C_{12} bond. Molecular structure of curcumin is shown in **structure 4.4.**



4.4

The enolic hydrogen found to be equally associated with two oxygens. There is no significant difference in the C–C or C–O bonds in the enol ring giving a pseudoaromatic character to the chelate ring system. Conjugation between the ring 1 and the pseudoaromatic ring seems to be indicated by the distance between the two ring, which are essentially coplanar. The angle between the two ring planes being only 3°. The interaction between the π electron system in pseudoaromatic ring is

probably somewhat less as the angle between the two ring plains is about 45°.

In the present study attempts were made to get single crystals of 1,7-diindolylheptanoid and 1-phenyl-5-indolylheptanoid and their metal complexes. However crystals suitable for XRD studies were obtained only in the case of 1-phenyl-5-indolylheptanoid. Details are given below.

Experimental

Deep red plate like crystals of 1-phenyl-5-indolylpentanoid were prepared by crystallization of the compound from a mixture of ethanol-toluene-ethyl acetate mixture. The compound was dissolved in the solvent mixture at room temperature in a 50 mL conical flask and the mouth was closed with cotton to allow slow evaporation of the solvent and kept aside for about 2 weeks when a plate like crystal was separated. A crystal with dimensions 0.30 x 0.30 x 0.10 mm was used for obtaining X-ray diffraction data. The experimental conditions employed for generating the XRD data, crystal data of the compound and methods employed for structure determination and the exact structure and crystal packing of molecules are discussed below.

Experimental conditions

Temperature	:	293 (2) K
Crystal dimensions	:	0.30 x 0.30 x 0.10 mm
Range (θ) for scanning	:	1.24 to 22.05 deg
Limiting indices	:	-8<=h<=8,-5<=k<=5, -34<=l<=34
Reflections collected/unique	:	4664/1659
Rint	:	0.0361
Completeness to theta = 22.5	:	95.6%
Absorption correlation	:	Semi-empirical from equivalents
Max. and min. transmission	:	0.9914 and 0.9744
Refinement method	:	Full matrix least squares on F^2
Data/restraints/parameters	:	1659 /1 / 209
Goodness of fit on F^2	:	1.211
Final R indices [I > 2σI]	:	RI = 0.0602, WR2 = 0.1375
R indices all data	:	RI = 0.0949, WR2 = 0.1987
Absolute structure parameter	:	-1 (4)
Extinction coefficient	:	0.76 (10)
Largest diff. peak and hole	:	0.328 and –0.409 e A^{-3}

Crystal Data

Compound	:	1-phenyl-5-indolylpentanoid
Empirical formula	:	$C_{19}H_{15}NO_2$

Formula weight	:	289.32
Crystal system	:	Orthorhombic
Space group	:	PCA 21
Unit cell dimensions	:	a=7.7847 (8) A°; b=5.6426 (7) A° ;
		c=32.781 (3) A° ; α=90°; β = 90°; γ= 90°
Volume	:	1439.9 (3) $A^{o\,3}$
Calculated density	:	1.335 mg/m^3
Z	:	4
Absorption coefficient	:	0.087 mm^{-1}
$F_{(000)}$	:	608

Structure determination

The structure was solved by direct methods using the programme assembly SIR97 WINGX. To refine structure, the programme SHEL XL97 was used. The program used for molecular graphics was ORTEP-32 WINGX and for molecular packing MERCURY program was used.

Fourier synthesis indicated the positions of all the non hydrogen atoms. The position of all the 14 hydrogen atoms were readily found from a difference synthesis. A final difference suggested two possible positions for the last hydrogen atom supposed to be attached to the O_1 or O_2 atom. The best result was obtained by placing a half hydrogen atom in each of the two positions. All positional parameters, anisotropic temperature

factors for the ion hydrogen atoms and isotropic temperature factors for the hydrogen atoms were refined in the final least-squares calculations giving a final R factor of 0.0602 and goodness of fit S on F^2 = 1.21. The final parameters are given in **Tables 4.4 to 4.9**.

TABLE 4.4

Anisotropic displacement parameters (A^2 x 10^3)

The anisotropic displacement factor exponent takes the form
$-2\pi^2 (U_{11}a^*h^1 + ... + 2U_{12}a^*b^*hk)$

	U11	U22	U33	U23	U13	U12
C(1)	48(3)	45(5)	60(5)	-10(4)	1(3)	4(4)
C(2)	38(3)	41(5)	44(3)	1(3)	-7(3)	-8(3)
C(3)	34(3)	62(5)	32(3)	8(3)	-5(3)	-13(3)
C(4)	45(3)	50(5)	53(4)	-1(3)	-2(3)	-7(3)
C(5)	45(3)	88(7)	49(4)	9(4)	8(3)	-9(4)
C(6)	55(4)	81(7)	43(3)	0(4)	2(3)	-15(4)
C(7)	51(3)	69(6)	56(4)	18(4)	-10(3)	-15(4)
C(8)	42(3)	61(6)	43(4)	5(4)	-1(3)	-13(3)
C(9)	40(3)	53(5)	48(4)	3(3)	-5(3)	2(3)
C(10)	51(4)	56(6)	57(5)	4(4)	8(3)	5(4)
C(11)	66(4)	61(6)	46(4)	0(4)	-5(3)	1(4)
C(12)	68(4)	62(6)	45(4)	2(4)	6(3)	6(4)
C(13)	64(4)	55(6)	46(4)	0(4)	1(3)	8(4)
C(14)	44(3)	48(5)	49(4)	2(4)	-2(3)	3(3)
C(15)	52(3)	54(5)	56(5)	3(4)	-1(3)	1(3)
C(16)	65(4)	65(6)	48(4)	8(4)	2(3)	6(4)
C(17)	60(4)	85(7)	46(4)	2(5)	8(3)	-1(4)
C(18)	69(5)	61(6)	53(5)	-7(4)	3(3)	12(4)
C(19)	63(4)	72(6)	48(4)	10(4)	-4(3)	9(4)
N(1)	55(3)	50(4)	55(4)	6(3)	-4(3)	7(3)
O(1)	134(5)	64(4)	56(3)	7(3)	15(3)	29(4)
O(2)	98(3)	61(4)	54(3)	2(3)	10(2)	13(3)

TABLE 4.5

Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3)

$U_{(eq)}$ is defined as one third of the trace of the orthogonalised U_{ij} tensor

	x	y	z	U(eq)
C(1)	2602(7)	4005(12)	1780(2)	51(2)
C(2)	3494(7)	1939(12)	1750(2)	41(2)
C(3)	4039(6)	1775(13)	1334(2)	43(2)
C(4)	4978(7)	142(12)	1103(2)	49(2)
C(5)	5292(8)	553(16)	699(2)	60(2)
C(6)	4691(8)	2571(16)	507(2)	60(2)
C(7)	3750(8)	4240(15)	724(2)	59(2)
C(8)	3439(7)	3813(13)	1133(2)	48(2)
C(9)	3797(7)	165(13)	2061(2)	47(2)
C(10)	3280(8)	292(14)	2450(2)	55(2)
C(11)	3543(8)	-1522(15)	2750(2)	58(2)
C(12)	2959(8)	-1299(15)	3147(2)	58(2)
C(13)	3175(8)	-3013(14)	3437(2)	55(2)
C(14)	2596(7)	-2775(12)	3865(2)	47(2)
C(15)	3036(8)	-4487(13)	4148(2)	54(2)
C(16)	2543(9)	-4263(15)	4550(2)	59(2)
C(17)	1630(9)	-2302(15)	4675(2)	64(2)
C(18)	1186(9)	-569(15)	4397(2)	61(2)
C(19)	1662(8)	-821(14)	3996(2)	61(2)
N(1)	2579(6)	5121(9)	1417(2)	53(2)
O(1)	3897(8)	-5018(10)	3343(2)	85(2)
O(2)	4357(6)	-3499(10)	2637(1)	71(2)

TABLE 4.6

Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3)

	x	y	z	U(eq)
H(1)	2084	4565	2017	61
H(4)	5391	-1233	1225	59
H(5)	5923	-547	550	72
H(6)	4919	2811	232	72
H(7)	3341	5604	598	71
H(12)	2391	88	3220	70
H(15)	3670	-5801	4067	65
H(16)	2828	-5436	4738	71
H(17)	1311	-2146	4947	77
H(18)	570	756	4481	73
H(19)	1354	341	3809	73
H(1A)	2093	6463	1371	64
H(2O)	4466	-4375	2835	350(130)
H(9)	4440(70)	-1470(100)	1985(14)	35(14)
H(10)	2590(70)	1820(120)	2559(15)	40(14)

TABLE 4.7

Bond length [A] and Angles [deg]

C(1)-N(1)	1.348(8)
C(1)-C(2)	1.361(8)
C(1)-H(1)	0.9300
C(2)-C(3)	1.429(8)
C(2)-C(9)	1.450(9)
C(3)-C(4)	1.400(9)
C(3)-C(8)	1.405(9)
C(4)-C(5)	1.365(9)
C(4)-H(4)	0.9300
C(5)-C(6)	1.382(10)
C(5)-H(5)	0.9300
C(6)-C(7)	1.388(10)
C(6)-H(6)	0.9300
C(7)-C(8)	1.385(9)

contd....

Table 4.7 contd....

C(7)-H(7)	0.9300
C(8)-N(1)	1.363(8)
C(9)-C(10)	1.338(9)
C(9)-H(9)	1.08(6)
C(10)-C(11)	1.435(10)
C(10)-H(10)	1.08(6)
C(11)-O(2)	1.335(9)
C(11)-C(12)	1.383(8)
C(12)-C(13)	1.368(10)
C(12)-H(12)	0.9300
C(13)-O(1)	1.301(8)
C(13)-C(14)	1.477(8)
C(14)-C(15)	1.384(8)
C(14)-C(19)	1.389(9)
C(15)-C(16)	1.379(9)
C(15)-H(15)	0.9300
C(16)-C(17)	1.377(10)
C(16)-H(16)	0.9300
C(17)-C(18)	1.379(10)
C(17)-H(17)	0.9300
C(18)-C(19)	1.375(10)
C(18)-H(18)	0.9300
C(19)-H(19)	0.9300
N(1)-H(1A)	0.8600
O(2)-H(2O)	0.8200
N(1)-C(1)-C(2)	110.0(6)
N(1)-C(1)-H(1)	125.0
C(2)-C(1)-H(1)	125.0
C(1)-C(2)-C(3)	106.1(6)
C(1)-C(2)-C(9)	128.5(6)
C(3)-C(2)-C(9)	125.3(6)
C(4)-C(3)-C(8)	117.2(5)
C(4)-C(3)-C(2)	135.6(6)
C(8)-C(3)-C(2)	107.2(6)
C(5)-C(4)-C(3)	120.5(7)
C(5)-C(4)-H(4)	119.8
C(3)-C(4)-H(4)	119.8
C(4)-C(5)-C(6)	121.4(7)
C(4)-C(5)-H(5)	119.3
C(6)-C(5)-H(5)	119.3
C(5)-C(6)-C(7)	120.4(7)
C(5)-C(6)-H(6)	119.8
C(7)-C(6)-H(6)	119.8
C(8)-C(7)-C(6)	118.0(7)

contd....

Table 4.7 contd...

C(8)-C(7)-H(7)	121.0
C(6)-C(7)-H(7)	121.0
N(1)-C(8)-C(7)	130.7(7)
N(1)-C(8)-C(3)	106.6(5)
C(7)-C(8)-C(3)	122.6(6)
C(10)-C(9)-C(2)	125.8(7)
C(10)-C(9)-H(9)	114(3)
C(2)-C(9)-H(9)	120(3)
C(9)-C(10)-C(11)	124.9(7)
C(9)-C(10)-H(10)	121(3)
C(11)-C(10)-H(10)	114(3)
O(2)-C(11)-C(12)	119.5(6)
O(2)-C(11)-C(10)	118.2(6)
C(12)-C(11)-C(10)	122.2(7)
C(13)-C(12)-C(11)	123.4(7)
C(13)-C(12)-H(12)	118.3
C(11)-C(12)-H(12)	118.3
O(1)-C(13)-C(12)	120.2(6)
O(1)-C(13)-C(14)	115.9(6)
C(12)-C(13)-C(14)	124.0(7)
C(15)-C(14)-C(19)	118.4(6)
C(15)-C(14)-C(13)	119.9(6)
C(19)-C(14)-C(13)	121.7(6)
C(16)-C(15)-C(14)	120.7(7)
C(16)-C(15)-H(15)	119.7
C(14)-C(15)-H(15)	119.7
C(17)-C(16)-C(15)	120.0(7)
C(17)-C(16)-H(16)	120.0
C(15)-C(16)-H(16)	120.0
C(16)-C(17)-C(18)	120.2(7)
C(16)-C(17)-H(17)	119.9
C(18)-C(17)-H(17)	119.9
C(19)-C(18)-C(17)	119.4(7)
C(19)-C(18)-H(18)	120.3
C(17)-C(18)-H(18)	120.3
C(18)-C(19)-C(14)	121.3(7)
C(18)-C(19)-H(19)	119.3
C(14)-C(19)-H(19)	119.3
C(1)-N(1)-C(8)	110.1(5)
C(1)-N(1)-H(1A)	124.9
C(8)-N(1)-H(1A)	124.9
C(11)-O(2)-H(2O)	109.5

Symmetry transformations used to generate equivalent atoms:

TABLE 4.8
Torsion Angles [deg]

N(1)-C(1)-C(2)-C(3)	-0.7(6)
N(1)-C(1)-C(2)-C(9)	-178.2(5)
C(1)-C(2)-C(3)-C(4)	-179.8(6)
C(9)-C(2)-C(3)-C(4)	-2.2(10)
C(1)-C(2)-C(3)-C(8)	0.4(6)
C(9)-C(2)-C(3)-C(8)	178.0(5)
C(8)-C(3)-C(4)-C(5)	0.4(8)
C(2)-C(3)-C(4)-C(5)	-179.4(6)
C(3)-C(4)-C(5)-C(6)	-0.3(9)
C(4)-C(5)-C(6)-C(7)	0.1(9)
C(5)-C(6)-C(7)-C(8)	0.0(9)
C(6)-C(7)-C(8)-N(1)	179.5(6)
C(6)-C(7)-C(8)-C(3)	0.1(9)
C(4)-C(3)-C(8)-N(1)	-179.8(5)
C(2)-C(3)-C(8)-N(1)	0.0(6)
C(4)-C(3)-C(8)-C(7)	-0.3(8)
C(2)-C(3)-C(8)-C(7)	179.6(5)
C(1)-C(2)-C(9)-C(10)	-2.3(10)
C(3)-C(2)-C(9)-C(10)	-179.4(6)
C(2)-C(9)-C(10)-C(11)	177.8(6)
C(9)-C(10)-C(11)-O(2)	-0.3(10)
C(9)-C(10)-C(11)-C(12)	-179.2(6)
O(2)-C(11)-C(12)-C(13)	0.3(10)
C(10)-C(11)-C(12)-C(13)	179.1(6)
C(11)-C(12)-C(13)-O(1)	-2.9(11)
C(11)-C(12)-C(13)-C(14)	178.4(6)
O(1)-C(13)-C(14)-C(15)	8.5(9)
C(12)-C(13)-C(14)-C(15)	-172.8(6)
O(1)-C(13)-C(14)-C(19)	-173.9(6)
C(12)-C(13)-C(14)-C(19)	4.8(10)
C(19)-C(14)-C(15)-C(16)	0.6(9)
C(13)-C(14)-C(15)-C(16)	178.4(6)
C(14)-C(15)-C(16)-C(17)	-1.1(10)
C(15)-C(16)-C(17)-C(18)	0.8(11)
C(16)-C(17)-C(18)-C(19)	0.1(10)
C(17)-C(18)-C(19)-C(14)	-0.6(10)
C(15)-C(14)-C(19)-C(18)	0.2(10)
C(13)-C(14)-C(19)-C(18)	-177.4(6)
C(2)-C(1)-N(1)-C(8)	0.7(6)
C(7)-C(8)-N(1)-C(1)	-179.9(6)
C(3)-C(8)-N(1)-C(1)	-0.4(6)

Symmetry transformations used to generate equivalent atoms:

TABLE 4.9

Hydrogen bonds [A and deg.]

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(2)-H(2O)...O(1)	0.82	1.76	2.493(7)	147.8

Symmetry transformations used to generate equivalent atoms:

Results and Discussion

The labelling of the atoms are indicated in **Fig. 4.8**. The bond length and bond angles are given in Table 4.7 and some torsion angles in Table 4.8. Figure 4.8 illustrates the molecule as it appears in the crystal and **fig. 4.9** gives the molecular packing in the unit cell. The unit cell dimensions reveals that the crystal system belongs to orthorhombic and with space group PCA 21.

The molecule may be described as consisting of three planar groups, out of these one is connected to the central ring through a double bond and other one is connected directly to the central ring. The terminal groups are different and hence the structure is not symmetric.

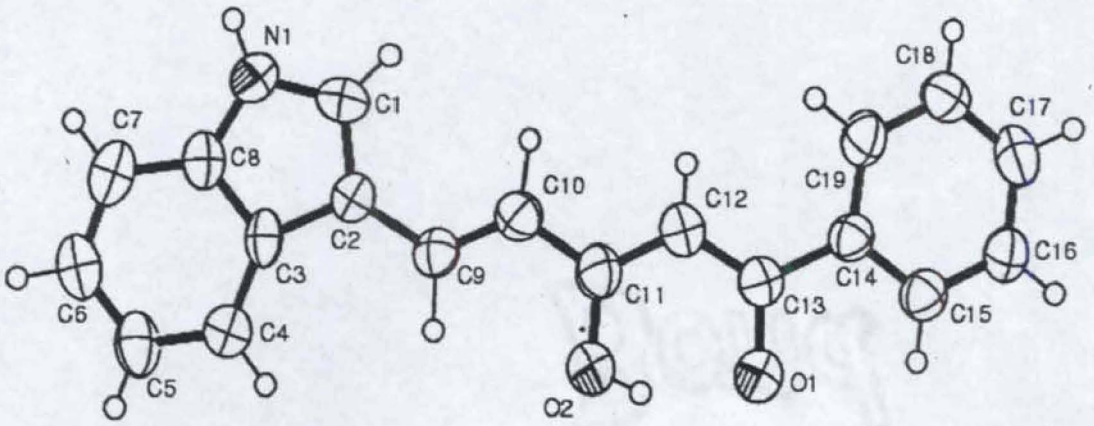


Fig. 4.8. Molecular structure of 1-phenyl-5-indolylpentanoid

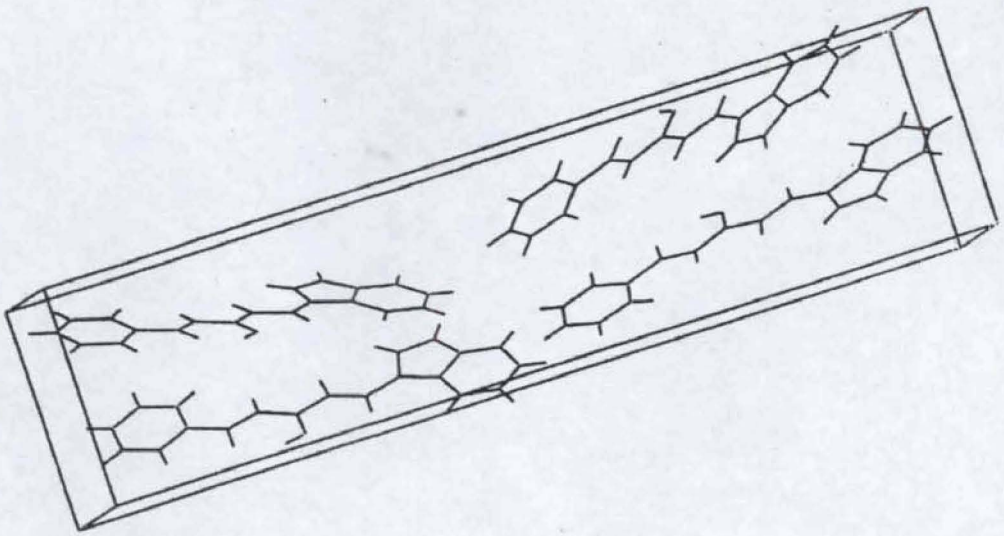


Fig. 4.9. Molecular packing of 1-phenyl-5-indolylpentanoid

Electron delocalization and intramolecular hydrogen bonding in the fragment –CO–CH=COH has been studied in a number of molecules. Of the possible tautomeric forms it appears that in the crystal phase, the β-diketones prefer the cis enol configuration stabilized by a strong intramolecular H-bond. This hydrogen bond appears moreover invariably to be asymmetrical, the hydrogen atom always found to be bonded to one unique oxygen atom.

In the present structure the oxygen atoms of the enol-ring are engaged in intramolecular hydrogen bond. It may be seen from the fig. 4.8 that there are no significant differences in the C–C and C–O bonds in the enol ring. Furthermore, the best model to fit the data is the one with the hydrogen atom statistically distributed between the two oxygen atoms.

A certain conjugation between the aromatic ring (indole) and the pseudoaroamtic ring (enol-ring) seems to be indicated by the distances between the atoms connecting the two rings, which essentially coplanar, the angle between the two ring planes being nearly zero. The difference in the bond length C_2–C_9 and C_{13}–C_{14} (1.477) shows that there is appreciable conjugation between indole ring and the enol ring (pseudoaromatic ring).

SECTION 2

FLUORESCENCE CHARACTERISTICS OF 1-PHENYL-5-INDOLYLPENTANOID

In this section a details on the fluorescent properties of 1-phenyl-5-indolyl-4-pentene-1,3-dione are presented. The effects of solvents, pH of the medium and various metal ions on the fluorescence were investigated. The fluorescence quenching by water and the fluorescence quantum yield of the compound were also studied.

Experimental

Various reagents such as quinine sulphate solution, metal salt solutions were prepared as given in section 2 of chapter 3.

Effect of solvents on the absorption and fluorescence maxima: A 10^{-6} M solution of the compound was prepared in different solvents. The absorption and fluorescence spectra were recorded and the λ_{max} for both absorption and emission were measured.

Effect of water on the fluorescence: The effect of water on the fluorescence nature was studied by measuring the fluorescence intensity of the compound in different mixtures of methanol and water, which were prepared by mixing different amounts of water and methanol (% of

volume). A $2x10^{-5}$M solution of the compound was prepared in these solvent mixtures. The intensity of fluorescence in pure anhydrous methanol was also determined. The effect of water on the fluorescence was studied from a plot of I°_{fl} | I_{fl} x η°/η Vs. the percentage of water as described in section 2 of chapter 3. Where I°_{fl} and I_{fl} are the intensities of fluorescence emission in pure anhydrous methanol and in methanol water mixture and η° and η are the viscosities of pure methanol and methanol-water mixture respectively.

Effect of pH of the medium on the fluorescence: The effect of pH on the fluorescence emission of 1-phenyl-5-indolylpentanoid was studied in methanol-water mixture (2/8, v/v). Solutions of the compound (2×10^{-4} M) in a mixture of methanol and water at different pH values were prepared. The fluorescence intensity of each solution was measured.

Effect of metal ions on the fluorescence: The effect of metal ions on intensity of fluorescence was studied by measuring the fluorescence intensity after introducing the metal ions. Various amounts of metal ions in methanol were added to a 10^{-4} M solution of 1-phenyl-5-indolyl pentanoid in methanol and in each case total volume of the solution was made up to 10 mL. The fluorescence intensities of these solutions were measured.

Determination of quantum yield: A 10 ppm solution of quinine sulphate was prepared in 0.1 M H_2SO_4. Solutions of required concentrations were prepared by diluting it. Absorbance of these solutions were measured by using a spectrophotometer. The fluorescence spectra of these solutions were recorded, and the integrated fluorescence intensities were calculated and were plotted against their corresponding absorbance. A straight line graph was obtained, and its slope determined. By similar method the integrated fluorescence intensity of the compound 1-phenyl-5-indolylpentanoid is also worked out.

Results and Discussion

Effect of solvent: The absorption spectra of the compound shows an intense broad absorption band in the region 400-500 with a shoulder. Absorption maxima in different solvents are given in **table 4.10.** The λ_{max} values shows a solvent dependence and increase with increase in solvent polarity. The appearance of the shoulder indicates the presence of more than one isomeric forms in the ground state.

TABLE 4.10

The absorption and emission maxima of 1-phenyl-5-indolyl pentanoid in different solvents. Emission was measured at 350 volts.

Solvent	[a.] $\lambda_{a\ (n.m)}$	[b.] $\lambda_{fl\ (n.m)}$
Methanol	416	528
Ethanol	426	520
Acetone	417	500
Acetonitrile	408	504
DMSO	434	520

a - Absorption maxima, b - Fluorescence maxima

The fluorescence spectra also show significant solvent dependent shifts in maxima, red shift from 500 nm in acetone to 520 in DMSO, the most polar among the solvents. However, more red shift is observed in methanol (527 nm), due to its strong hydrogen bond-donating as well as a hydrogen bond accepting nature.

The solvochromic red shift observed in polar protic solvents like methanol may be due to the combination of both polarity and hydrogen bonding effect of the solvent on the excited state of the compound. However, the red shift observed in 1-phenylindolylpentanoid is less than that observed in 1,7-diindolylheptanoid. This may be due to the replacement of one indole group by a phenyl substituent which will retard the extent of intramolecular charge transfer and to a certain extent influence the transition dipole moment.

Effect of water: The effect of water on the fluorescence intensity of the compound is given in the **table 4.11.**

TABLE 4.11

Effect of various concentrations of water (% v/v$_1$) on the fluorescence of 1-phenyl-5-indolyl pentanoid in methanol. Excitation wave length 470 nm Volt. 400

[H_2O] (% v/v)	E_{max} (nm)	Intensity	$(\eta^\circ/\eta)^b$	I°_{fl}/I_{fl}	$I^\circ_{fl}/I_{fl} \times \eta^\circ/\eta$
0	552	98	1.00	1.00	1.00
3	552	93	0.99	1.050	1.04
5	553	88	0.96	1.104	1.06
7	551	86	0.95	1.136	1.08
10	552	80	0.92	1.217	1.12
20	551	67	0.85	1.447	1.23
30	552	57	0.79	1.708	1.35

a. Concentration of the compound is 2 x 10^{-5}M
b. from ref. 207.
c. I°_{fl} - florescence intensity in anhydrous methanol.

The fluorescence intensity decreases with the increase of water content. Water quenching can be attributed to a reaction between H_2O (electron donor, D) and the fluorescence compound (C*) resulting in the formation of a non fluorescent and stable complex (C^{*-} D^+) with lower (S_o) vibrational energy levels.

$$I^\circ_{fl}/I_{fl} = (1+kq.\ \tau\ [H_2O]\ ) / \eta^\circ/\eta$$

The modified Stem-Volmer plots of I_{fl}°/I_{fl} x η°/η Vs $[H_2O]$ on the fluorescence quenching is shown in the **Fig. 4.10**. From the plot it is possible to arrive of the conclusion that fluorescence quenching by water is dependent on the viscosity of solvent. The slope of the plot is 1.2 x 10^{-2} M^{-1} but for curcuminoid (from earlier reports) is 1.38 x 10^{-2} M^{-1}, and that of 1,7-diindolyheptanoid (Chapter 3, section 2) 4.6 x 10^{-2} M^{-1}. These indicate that the effect of water on fluorescence quenching of 1-phenyl-5-indolylpentanoid is slightly lower than that of 1,7-diindolylheptanoid and almost equal to that of curcumin. Hence it can be concluded that the fluorescent life time of 1-phenyl-5-indolypentanoid is almost same as that of curcumin, and the non fluorescent complex (C^{*-} D^{+}) is less stable than that formed by 1,7-diindolyheptanoid.

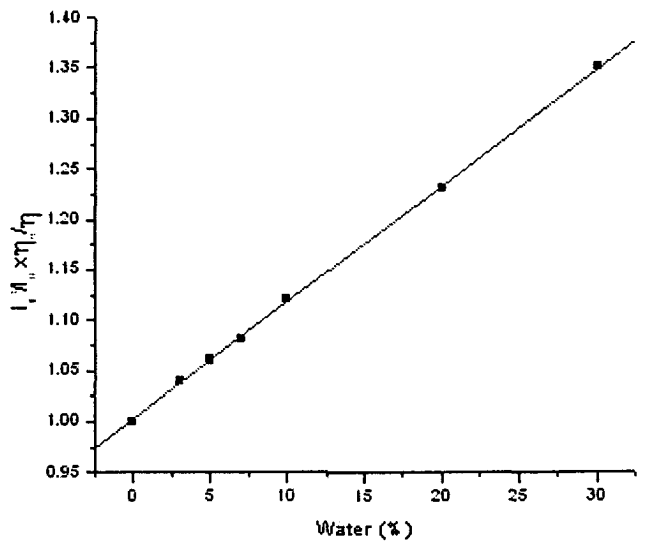


Fig. 4.10.

Effect of pH of the medium: The colour of curcuminoid is not constant in aqueous media or in organic solvent due to their degradation or conversion to their dissociation form. The fluorescent behaviour of the compound under investigation is also similar to the that of curcumin and other curcuminoids. The effect of pH on the fluorescence of 1-phenyl-5-indolyl pentanoid is summarized in the **table 4.12**. The fluorescence intensity varies with. Thus pH effect on the fluorescence of 1-phenyl-5-indolyl pentanoid is either due to its degradation or due to the change in its properties of first excited singlet state, as in the case of 1,7-indolylhpetanoid.

TABLE 4.12

Effect of pH on the fluorescence of 1-phenyl-5-indoly pentanoid. Fluorescence intensity was measured at 400 volt.
Emission maxima Emax - 530 nm

pH	Fluorescence intensity	$\frac{I_{fl^o}}{I_{fl}}$	pH	Fluorescence intensity	$\frac{I_{fl^o}}{I_{fl}}$
.79	50	1.76	7.30	98	0.89
.87	64	1.7	8.70	94	0.93
1.10	81	1.08	9.30	89	0.98
1.58	91	0.96	10.56	56	1.57
2.10	94	0.93	10.56	0	
3.20	96	0.91			
3.53	114	0.77			
4.00	106	0.83			
5.55	79	1.11			
5.64	84	1.04			
7.00	88	1			

Effect of metal ions

The effect of various metal ions on the fluorescence of 1-phenyl-5-indolyl pentanoid is given in the **tables 4.13 to 4.17**. The results revealed that Fe(III) is the most effective ion in quenching the fluorescence and next comes Cu(II). Metal ions Co(II) and Ni(II) suppresses the fluorescence, but A1(III) increases the fluorescence intensity. From these studies it can be stated that whether or not metal ions actually complex the ground state of the molecule, they will influence its fluorescence. Generally heavy metal ions will quench the fluorescence of organic molecules.

TABLE 4.13

Effect of Cu(II) ions on the intensity of fluorescence of 1-phenyl-5-indolyl pentanoid

Conc. of Cu(II) solution	Fluorescence maxima Emax.	Fluorescence intensity (I_{lf})
0 ml	520	314
1 x 10^{-4}M	520	141
4 x 10^{-4}M	520	47
5 x 10^{-4}M	520	23
6 x 10^{-4}M	520	--

TABLE 4.14

Effect of Al(III) ions on the intensity of fluorescence

Conc. of Al(III) in solution	Fluorescence maxima	Fluorescence intensity
0 ml	520	326
1×10^{-5}M	523	284
4×10^{-5}M	523	289
5×10^{-5}M	521	292
8×10^{-5}M	520	305
1×10^{-4} M	523	312

TABLE 4.15

Effect of Fe(III) ions on the intensity of fluorescence

Conc. of Fe(III) solution	E_{max}	Fluorescence intensity
0 ml	524	732
0.5×10^{-4}M	526	521
1×10^{-4}M	527	294
3×10^{-4}M	527	95
5×10^{-4}M	526	55
8×10^{-4}M	526	--

TABLE 4.16

Effect of Co(II) ions on the intensity of fluorescence

Conc. of Co(II) solution	E_{max}	Fluorescence intensity
0 ml	522	230
1 x 10^{-5}M	522	225
5 x 10^{-5}M	520	144
8 x 10^{-5}M	520	97
1 x 10^{-4}M	520	25

TABLE 4.17

Effect of Ni(II) ions on the intensity fluorescence

Conc. of Ni(II) solution	E_{max}	Fluorescence intensity
0 ml	523	225
1 x 10^{-5}M	520	216
5 x 10^{-5}M	521	205
1 x 10^{-4}M	520	183
5 x 10^{-4}M	520	154
1 x 10^{-3} M	521	94
5 x 10^{-3} M	520	23

Thus it can be concluded that paramagnetic metal ions can quench the fluorescence intensity of the compound 1-phenyl-5-indolylpentanoid, but the effect of diamagnetic ion depends on the fluorescent nature of the adduct formed.

Quantum yield of 1-phenyl-5-indoly pentanoid

Quantum yield of 1-phenyl-5-indolyl pentanoid was determined by the comparitive method of Williams *et al.* The absorbance and integrated fluorescence of the standard solution of quinine sulphate in 0.1 M H_2SO_4 and that of 1-phenyl-5-indolyl pentanoid in methanol are given in the **table 4.18.** The plots of absorbance Vs integrated fluorescence are given in **Fig. 4.11.** From the slop of the two plots, the quantum yield of the compound was calculated using the equation.

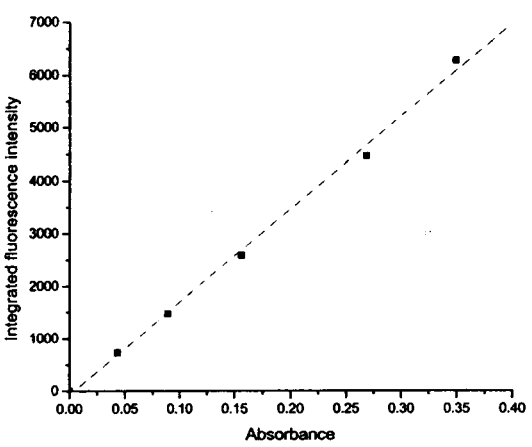
$$Q_X = Q_{ST}\left[\frac{Grad_X}{Grad_{ST}}\right]\left[\frac{n_x}{n_{ST}}\right]^2$$

TABLE 4.18

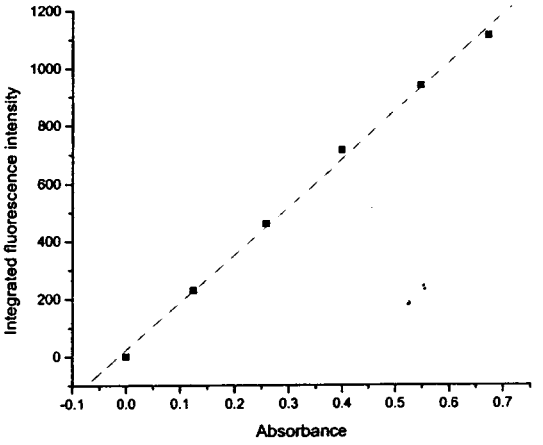
Absorbance and fluorescence intensity of quinine sulphate and the compound 1-phenyl-5-indolyl pentanoid at various concentration

Compound	Concentration	Fluorescence maxima	Fluorescence intensity	λ_{max}	Absorbance	Integrated fluorescence (area of the peak)
Quinine sulphate in 0.1 M H_2SO_4	1 ppm	438	160	344	0.043	737
	3 pm	438	442	344	0.089	1474
	5 pm	437	708	344	0.156	2582
	8 ppm	438	1034	344	0.268	4470
	10 ppm	438	1268	344	0.349	6301
1-phenyl-5-indolyl-pentanoid in methanol	1×10^{-5}M	525	71	416	0.124	230
	2×10^{-5} M	526	150	416	0.258	460
	3×10^{-5} M	525	195	416	0.399	710
	4×10^{5} M	525	242	416	0.547	930
	5×10^{-5} M	528	270	416	0.673	1100

Fluorescence measurements were done at 350 Volts.



Standard



1-phenyl-5-indolylpentanoid

Fig. 4.11

The quantum yield calculated for the compound using the above formula is 0.0157 in methanol. This value is very close to that of curcuminoids but it is less than the quantum yield of 1,7-diindolyl heptanoid (0.1581). This shows that, there are process other than fluorescence, play an important role in the deactivation of the fluorophore. If fluorescence was the only process for deactivation, the quantum efficiency should be one. Thus the non radiative process, intersystem crossing, etc. are also important for the emission, path way.

SECTION 3

BIOLOGICAL STUDIES OF 1-PHENYL-5-INDOLYL PENTANOID AND ITS METAL COMPLEXES

The compound 1-phenyl-5-indolylpentanoid and its metal complexes were investigated for their *in vitro* cytotoxicity and antioxidant activities.

Experimental

Determination of *in vitro* cytotoxicity

The short term *in vitro* cytotoxicity of the compounds were analysed by using DLA cells. The 1-phenyl-5-indolyl curcuminoid and its Al(III), Fe(III), Co(II), Ni(II) Cu(II) and (Zn(II) complexes were dissolved in DMSO and required concentrations of these solution were prepared by dilution. The cell suspension (1 million cells/mL) was added to tubes containing different concentrations (5-100 μg/mL) of these compounds and volume was finally adjusted to 1mL using PBS. The mixture was incubated for ~ 3h at 37°C. After incubation 0.1 mL trypan blue was added to each tube and kept for two minute. The number of dead cells were counted using a haemocytometer.

Assay of antioxidant activity

Inhibition of superoxide, scavenging of hydroxyl radicals and inhibition of lipid peroxidation by the ligand and the metal complexes were examined. The ligand and the metal complexes were dissolved in DMSO and various test concentrations were prepared by dilution and anti oxidant activity of each concentrations were studied.

Inhibition of superoxide radicals: Superoxide scavenging was determined by the NBT reduction method of McCord and Fridovich as detailed in chapter 3, section 3. The reaction mixture contained EDTA (0.1M) containing 0.0015% NaCN, riboflavin (0.12 mM) NBT (1.5mM) and various concentrations of test samples and total volume was adjusted by adding phosphate buffer (67 mM, pH 7.8) to 3 mL in a test tube. The tubes were uniformly illuminated under an incandesent lamp for 10 minutes and thereafter the optical density was measured at 530 nm, and the percentage inhibition of superoxide production was evaluated as described in chapter 3, section 3.

Hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity was measured by studying the competition between deoxy ribose and the test sample for hydroxyl radicals, generated from Fe^{3+}- ascorbate EDTA-H_2O_2 system. The hydroxy radicals attack deoxy ribose that evantually results in TBARs. The reaction mixture containing deoxy

ribose (2.8 mM) $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1.0 mM) ascorbate (0.1 mM) KH_2PO_4 - KOH buffer (20 mM, pH 7.4) and various concentrations (2.5 - 50 μg/mL) of the test sample in a final volume of 1 mL was incubated for 1h, at 37°C and deoxy ribose degradation was measured as TBARS as illustrated in chapter 3, section 3. The percentage inhibition was calculated.

Inhibition of lipid peroxidation: The inhibition of lipid peroxidation was measured by the method of Ohkawa *et al.* after induction by Fe^{2+}/ascorbate system. The reaction mixture contained normal rat liver homogenate 0.1 mL. 25% (w/v) in tris -HCl buffer (40 mM, pH 7.0), KCl (30 mM), Ferrous iron (0.16 mM) ascorbic acid (0.06 mM) and various concentrations (range 2.5 - 50 μg/mL) of the test sample in a final value of 0.5 mL was incubated for 1 h at 37°C. After incubation the inhibition of lipid peroxidation was determined by thio barbituric acid method as illustrated chapter 3, section 3.

Results and Discussion

Cytotoxicity of the compounds

Results obtained from the short term *in vitro* cytotoxic studies of the compounds are shown in the **table 4.19**. Cu(II) complex was found to be more cytotoxic than the free ligand. Compared to the 1,7-

diindolylhexanoid and its complexes, the compound showed less cytotoxicity. Complexes of Ni(II) and Co(II) did not show any cell death. But they were found to be active in a sense that they could change the cell morphology to a notable extent, which is considered to be a sign of the cytotoxic activity of the compounds. Among the various compounds only the ligand and the Cu(II) complex showed 50% cell death at a concentration above 100 μg/mL.

TABLE 4.19

Short term *in vitro* cytotoxicity of compounds towards DLA cells

Compound	Percentage Cell-death at different concentrations (μg/mL)				
	5	10	20	50	100
L	12	23	34	48	56
CuL_2	15	28	42	59	68
AlL_3	9	17	29	34	39
FeL_3	2	4	9	12	19
ZuL_2	3	8	14	19	21
NiL_2	Cell morphology changed				
CoL_2	Cell morphology changed				

L = 1-phenyl-5-indolyl pentanoid.

Inhibition of superoxide radical generation

The percentage inhibition of superoxide by the 5-indolylpentanoid and its metal complexes are given in **table 4.20**. The data suggest that the compounds are less active than the 1,7-diindolylhpetanoid and its

complexes. Among the compounds the Cu(II), Al(III) and Fe(III) complexes showed more superoxide inhibition.

TABLE 4.20

Percentage inhibition of superoxide

Compound	Percentage inhibition at various concentration (μg/mL)				
	5	10	20	50	100
L	2.62	8.71	12.45	23.89	38.72
CuL_2	8.93	12.86	27.46	41.35	50.36
AlL_3	6.38	14.35	32.76	44.61	56.45
FeL_3	5.74	11.75	29.64	42.58	46.84
CoL_2	--	4.57	6.08	9.41	11.45
NiL_2	3.64	9.42	19.36	29.64	40.05
ZnL_2	5.70	11.04	16.87	26.71	37.95

L = is the ligand 1-phenyl-5-indolyl pentanoid.

Hydroxyl radical scavenging activity

The effect of 1-phenyl-5-indolylpentanoid and the metal complexes in scavenging hydroxyl radicals is shown in the **table 4.21**. Cu(II) and Al(III) complexes showed higher activity than the ligand. Fe(III) showed slight increased activity at lower concentrations. Complexes of Ni(II), Co(II) and Zn(II) are less active towards scavenging hydroxyl radicals. The low activity of these complexes compared to complexes of 1,7-dindolylheptanoid, revealed the role of the ligand in the hydroxyl radical scavenging. Further, high concentrations of the metal ions such as

Fe(III), Co(III), Ni(II) and Zn(II) produced a reduction in the rate, may be due to some specific catalysed heterolysis of the hydroperoxide.

TABLE 4.21

Percentage inhibition of hydroxyl radicals

Compound	Percentage inhibition at various concentrations (μg/mL)				
	2.5	5	10	25	50
L	12.92	23.4	29.65	32.81	41.44
CuL_2	19.46	28.55	39.88	48.26	59.05
AlL_3	14.48	22.86	29.89	39.26	51.04
FeL_3	20.04	28.42	32.74	39.65	36.58
CoL_2	4.54	10.62	16.35	19.48	18.94
NiL_2	3.63	9.14	14.89	16.17	15.96
ZnL_2	9.08	14.25	18.34	21.44	19.37

L = 1-phenyl-5-indolylpentanoid.

Inhibition of lipid peroxidation

All compounds were investigated for their ability to inhibit the ferrous-ion induced peroxidation of rat liver homogenate. The ligand and the complexes of Al(III), Fe(III) and Cu(II) were found to exhibit activity. The complexes of Co(II), Ni(II) and Zn(II) did not have any effect on inhibition of lipid peroxidation. The percentage inhibition of the ligand 1-phenyl-5-indodyl pentanoid and the Cu(II), Al(III) and Fe(III) complexes are given in the **table 4.22**.

TABLE 4.22

Percentage inhibition of Ferrous ion induced lipid peroxidation

Compound	Percentage inhibition at various concentrations (μg/mL)				
	2.5	5	10	25	50
L	9.50	12.25	19.91	24.43	30.19
CuL_2	10.05	13.17	21.38	28.41	36.56
AlL_3	11.15	14.67	23.98	32.84	39.13
FeL_3	19.21	23.65	29.14	35.40	31.64

L = 1-phenyl-5-indolyl pentanoid.

SUMMARY

1-Phenyl-5-indolyl-4-pentene-1,3-dione was synthesised by the condensation of indole-3-carbaldehyde and benzoylacetone. The metal chelates of Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Al(III) were also synthesised. The intramolecular hydrogen bonded enol form for the 1-phenyl-5-indolyl curcuminoid was confirmed by various spectral data. Characterisation of the metal chelates of the compound revealed that Al(III) and Fe(III) chelates have a ML_3 stoichiometry where all other complexes have ML_2 structure.

Study of fluorescence characteristics of 1-phenyl-5-indolylpentanoid showed that the emission maxima are solvent dependent and the intensity of fluorescence is influenced by factors like pressure of water, pH of the medium and pressure of metal ions. Determination of quantum yield

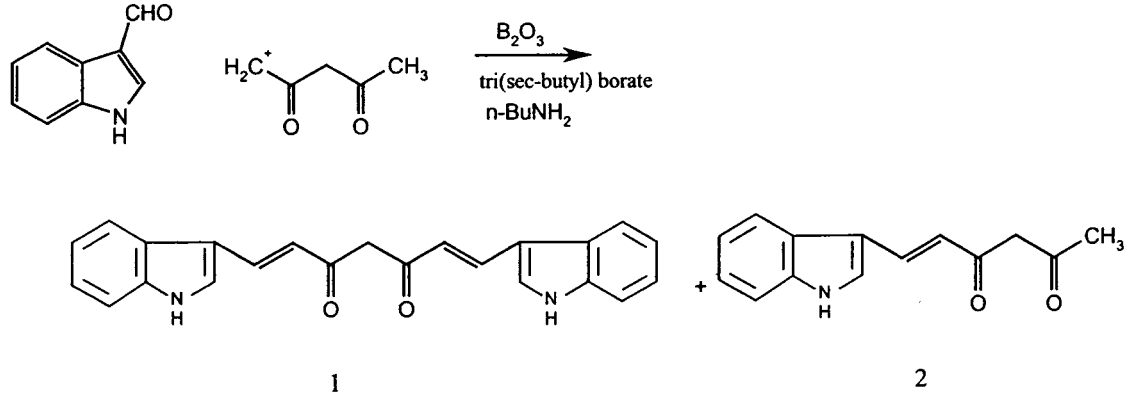
showed that the value is very close to that of curcumin but it is less than 1,7-diindolylheptanoid.

The studies of biological activities revealed that 1-phenyl-5-indolylpentanoid and its various metal chelates are not good antioxidant and cytotoxic as 1,7-diindolylheptanoid.

CHAPTER 5
SYNTHESIS AND CHARACTERISATION OF 6-INDOLYL-5-HEXENE-2,4-DIONE AND ITS METAL COMPLEXES

Introduction

The 1,7-diindolylheptanoids considered in chapter 3 was synthesised by the condensation of indole-3-aldehyde with acetylacetone under specified conditions. The reaction yielded two products as given below in **scheme 5.1.**



1 2

Scheme 5.1

The relative yield of **1** and **2** depends to a large extend on the temperature of the condensation reaction. At low temperature (< 10°C) the monocondensation is favoured. Thus by carrying out the reaction at ~ 0°C about 68% of monocondensation product was obtained. The

compound formed stable complexes with various metal ions. Unlike the 1,7-diarylheptanoids (chapter 3) and pentanoids (chapter 4) the 6-arylhexanoids did not exhibited appreciable fluorescent and biological properties. Therefore, in this chapter only the details on the synthesis and characterisation of 6-indolylhexanoid and its metal complexes are included.

Experimental

Synthesis of 6-indolyl-5-hexene-2,4-dione (6-indolylhexanoid)

Acetylacetone (0.5 mL, 0.005 mol) and boric oxide (0.25 g, 0.0035 mol) were mixed in a bottle and stirred for ~ 1 h. To the product, kept at ~ 0°C, a solution of indole-3-carbaldehyde (0.75 g, 0.005 mol) in 10 mL of ethyl acetate and tri (sec-butyl) borate (2.3 mL, 0.01 mol) were added. The mixture was stirred continuously with the addition of n-butylamine (0.05 mL) dropwise in 40 minutes. The stirring was continued for an additional period of ~ 2 h and the solution was set aside overnight. The reaction mixture was then stirred for ~ 1 h with hot (~ 50°C) hydrochloric acid (0.4 M, 20 mL) and extracted repeatedly with ethyl acetate. The combined extracts were concentrated in vacuum and subjected to chromatographic separation to get 6-indolylhexanoid as outlined below.

The concentrated extract was uniformly applied on the top of a silica gel (mesh 60-120) column (4 x 45 cm) and then eluted with a 1:3:9 v/v mixture of acetone-benzene chloroform at a flow rate of 2 mL per min. As the elution proceed, two bands were developed in the column an yellow lower band and an orange red upper band. The yellow band developed in the lower region was removed by successive elution and combined eluates on evaporation yield the 6-indolylhexanoid.

The elution was then repeated by using a 1:5 v/v mixture of acetone and chloroform to recover the orange red band retained at the upper portion of the column and the combined extracts on removing the solvent in vacuum yield 1,7-diindolylheptanoid (15-20%).

The isolated 6-indolylhexanoid was recrystallised from ethyl acetate toluene mixture to get chromatographically (tlc) pure material.

Synthesis of metal chelates

The Al(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes of the ligand were prepared by the following general procedure. An aqueous solution of the metal salt (0.001 mol) was added to a solution of the ligand (0.002 mol) in methanol (15 mL). The mixture was then refluxed for ~ 3 h and cooled to room temperature. The precipitated product was filtered, washed with water, ethanol and dried in vacuum.

Results and Discussion

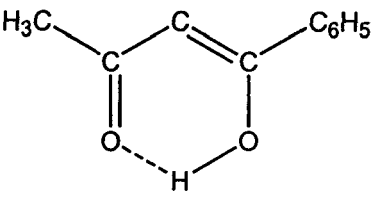
Characterisation of the 6-indolylhexanoid

The 6-indolylhexanoid is crystalline, showed sharp melting point and is soluble in common organic solvents. The elemental analytical data and observed molecular mass (Table 5.1) of the compounds suggest that only one equivalent of the aldehyde has condensed with one equivalent of acetylacetone. Like curcuminoids, 6-indolylhexanoid is soluble in both polar and non polar organic solvents but practically insoluble in water at neutral and acidic pH values. In strongly alkaline solutions the compound was less stable and underwent degradation. Spectral data of the compound are discussed below.

Uv spectrum: The uv spectra of the compound in different solvents (methanol, ethanol, acetone DMF and DMSO) show two bands with λ_{max} at ~ 380 nm and ~ 276 nm. From a comparison of the reported spectra of simple 1,3-diketones such as acetylacetone and the observed spectra of 1,7-diindolylheptanoid (chapter 3) the band at ~ 380 nm can be assigned to the $n \rightarrow \pi^*$ transition of the carbonyl chromophore and the band at ~ 280 nm to the conjugated C=C $\pi \rightarrow \pi^*$ transition. The position of the $n \rightarrow \pi^*$ band is considerably lowered in 6-indolylhexanoid compared to those observed for 1,7-diindolylheptanoid and this decrease in the λ_{max} is due to the weak external conjugation of the diketo function.

Absorption maxima show a large red shift in more polar solvents. The absorption band is very broad and shows the presence of more than one shoulder indicate the presence of more than one isomeric form in the ground state. Compared to 1,7-diindolylheptanoid, in 6-indolylhexanoid, since, the aryl group is present only at one end of the chromophore the extent of conjugation is limited and absorption maxima are shifted to lower wavelength. With the same reason, the compound is only weakly fluorescent.

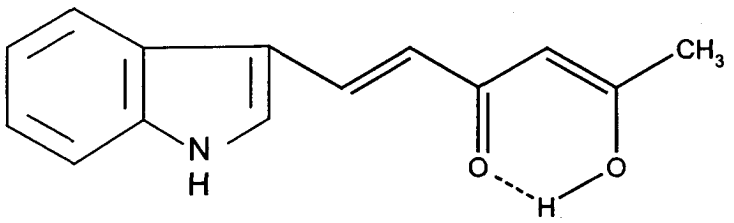
Infrared spectrum: The keto-enol tautomeric equilibria of unsymmetrical 1,3-diketones such as arolalkanoyl methanes, Ar–CO–CH_2–CO–R, has been the subject of numerous studies [1,2-4]. Electronic, infrared and ^1H nmr spectroscopy has been extensively employed in determining the exact percentage of the various tautomeric forms present in these types of compounds.[5,15,29] For instance, it has been shown that Ph–$COCH_2CO$–CH_3, exist mostly in the (> 90%) enol form in non polar solvents and are enolised towards the aryl group. Thus the infrared spectrum of benzoylacetone is characterised by the presence of a broad weak band in the region 2500-2700 cm^{-1} for the chelated carbonyl function of structure 5.1. It is to be noted that no free benzoyl carbonyl and acetyl carbonyl stretching bands were observed in the spectrum of benzoylacetone.[29,155]



5.1

The 6-arylhexanoid considered in this section is also an unsymmetrical 1,3-diketone similar to aroylalkanoyl methanes, where the aroyl group is indole–CH=CH–. Thus unlike in benzoylacetone both the carbonyl groups of 6-arylhexanoid are attached to aliphatic carbons as in structure 5.2 and therefore the tautomeric equlibria of these compounds may appear complex and spectral interpretation may become difficult. Contrary to these expectations, the ir spectrum of the compound appears to be very simple and afford easy assignments of the bands particularly in the double bond region.

The ir spectrum of the compound shows two prominent bands at 1634 and at 1613 cm^{-1} assignable to the stretching vibrations of the chelated acetyl C=O group and the C=O group connected to the Indole–CH=CH– group. The observed positions and intensity of these bands indicate that the compound exists predominantly in the enol form and are enolised towards the indolyl function. This is expected because of the possible extended delocalisation of the π electrons, which stabilises the whole molecule.



5.2

The intense and broad band observed in the region 2800-3200 cm^{-1} indicate the presence of strong intramolecular O–H....O hydrogen bonding in these compounds which is also evident from the lowering of carbonyl stretching frequency. Two prominent bands observed in the region 1500-1600 cm^{-1} are presumably due to various aryl νC=C vibrations. A medium intensity band observed at ~ 1006 cm^{-1} is possibly arising from the trans CH=CH double bond absorption. The spectrum of the compound is given in **figure 5.1**.

^{1}H nmr spectrum: From a comparison of the observed ^{1}H nmr signals of the 6-indolyl hexanoid with the reported spectral data of related 1,3-diketones unequivocally suggest that the compound exists predominantly in the enol tautomer. Thus the one proton singlet in the spectrum of the compound at 15.73 ppm confirm the existence of the compound in the intramolecularly H-bonded enol form.

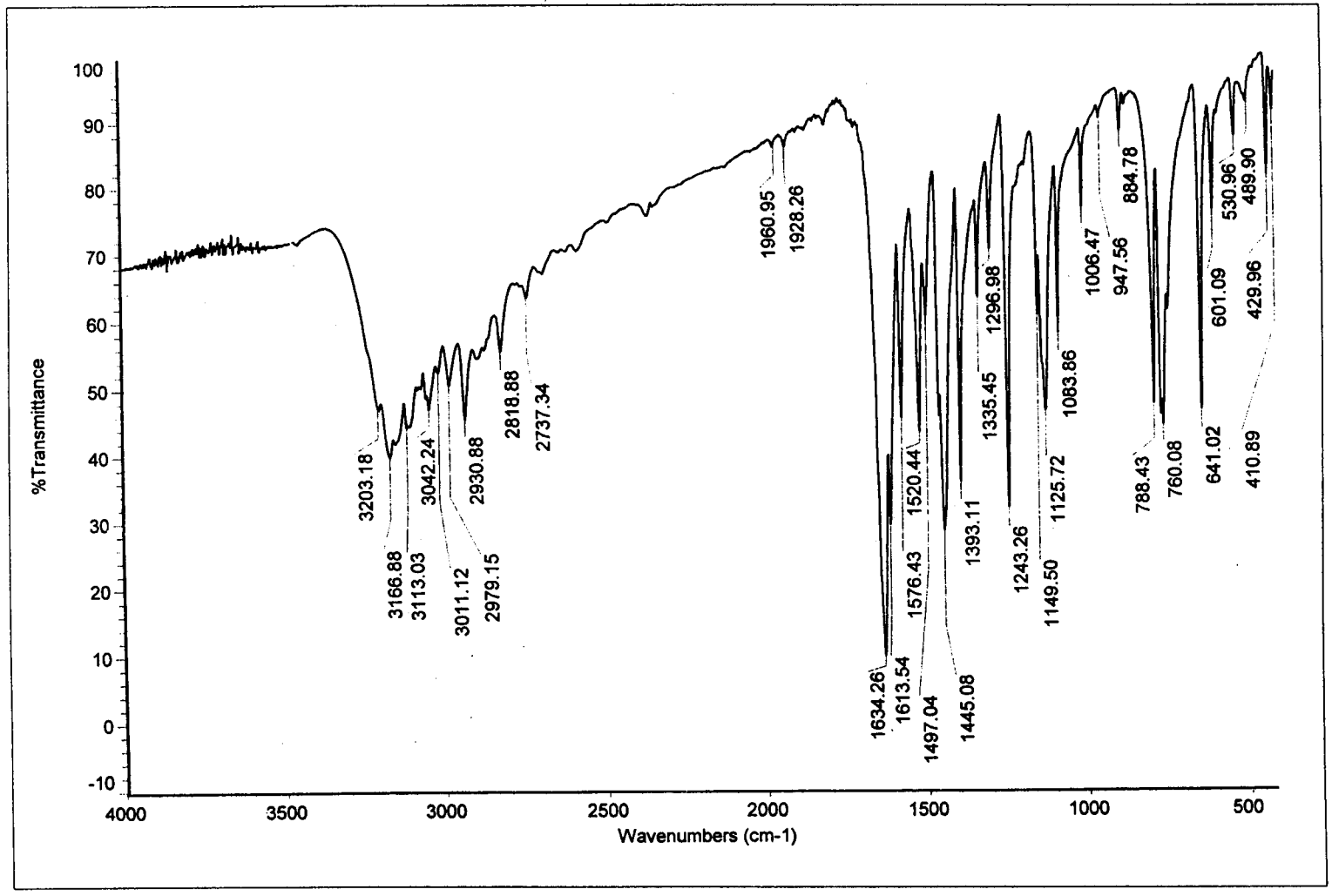


Fig. 5.1. IR spectrum of 6-indolyl-5-hexene-2,4-dione

Influence of steric and electronic effects of the aryl/alkyl group is also reflected in the position of the methine proton signal. In benzoylacetone the methane proton signal was appeared at 6.10 ppm which is 0.7 ppm downfield compared to that of acetylacetone (5.40 ppm). The same proton signal in the 6-indolylhexanoid appeared at about 7.1 which is 1.7 ppm downfield compared to that of acetylacetone. Another one proton signal at 10.1 ppm is due to the NH of the indolyl group. The alkenyl proton at 8.25 (doublet) and aryl protons in the range 6.7-7.9 ppm were also observed in the spectrum, which is reproduced in **figure 5.2**.

Mass spectrum: The 6-indolylhexanoid shows intense molecular ion peak at m/z. The spectra is reproduced in **figure 5.3**. Other prominent peaks appeared in the spectrum are due to Ind–CH=$CH^{]+}$, $(Ind)^{+}$, $(P–CH_3CO)^{]}$ etc. The formation of the important peaks can be conveniently explained by the fragmentation pattern given in **scheme 5.2**.

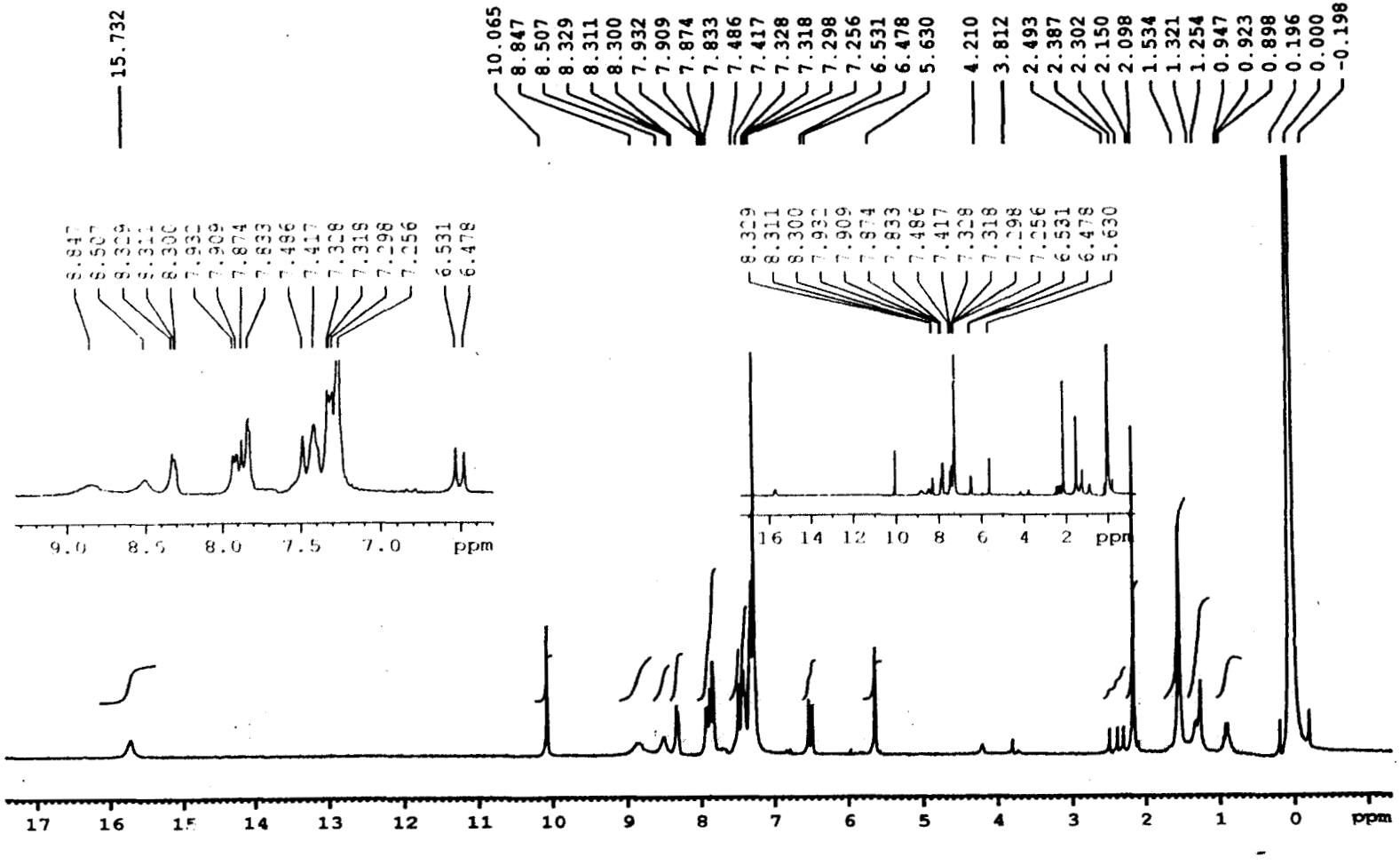


Fig. 5.2. NMR spectrum of 6-indolyl-5-hexene-2,4-dione

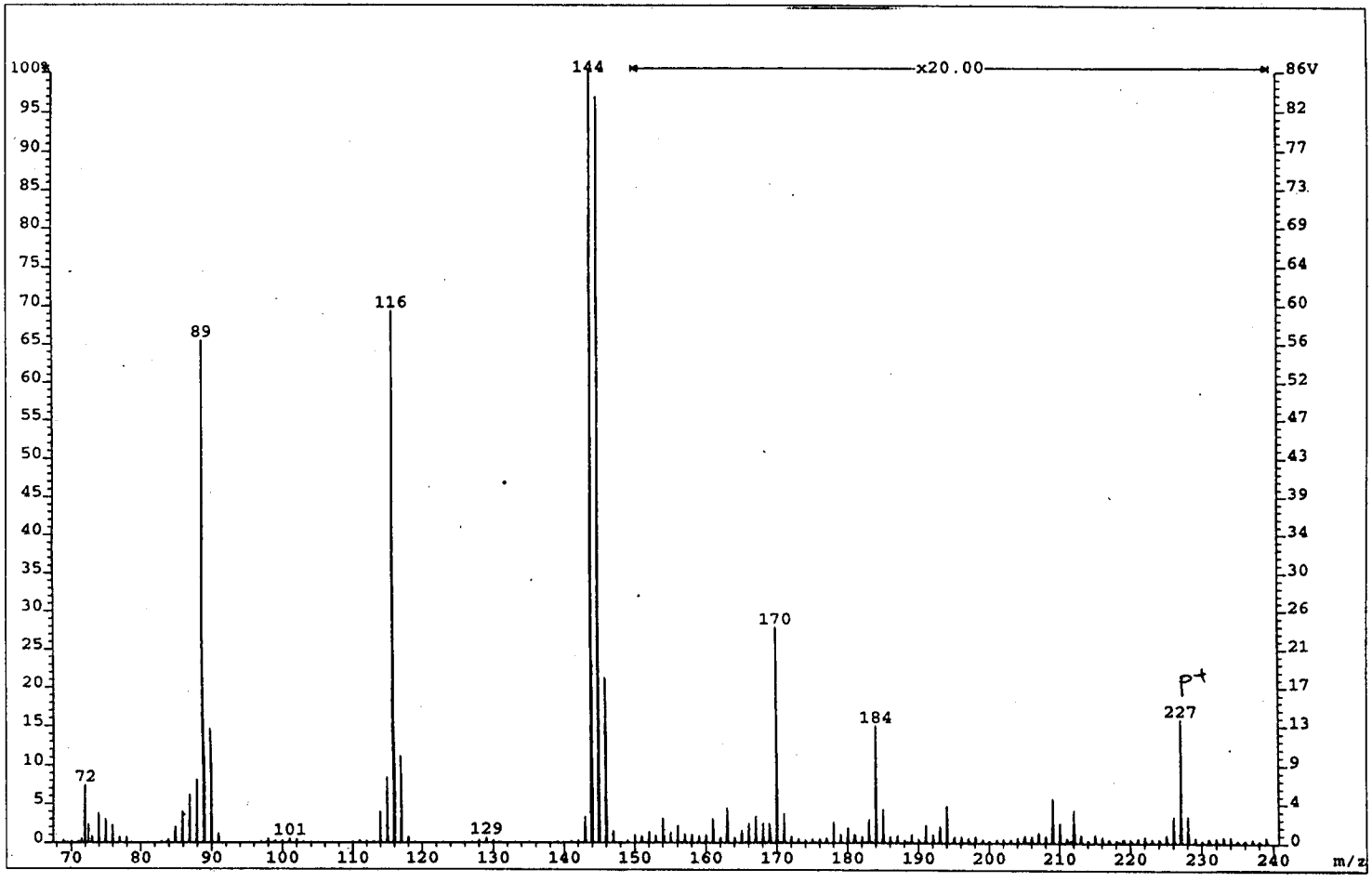
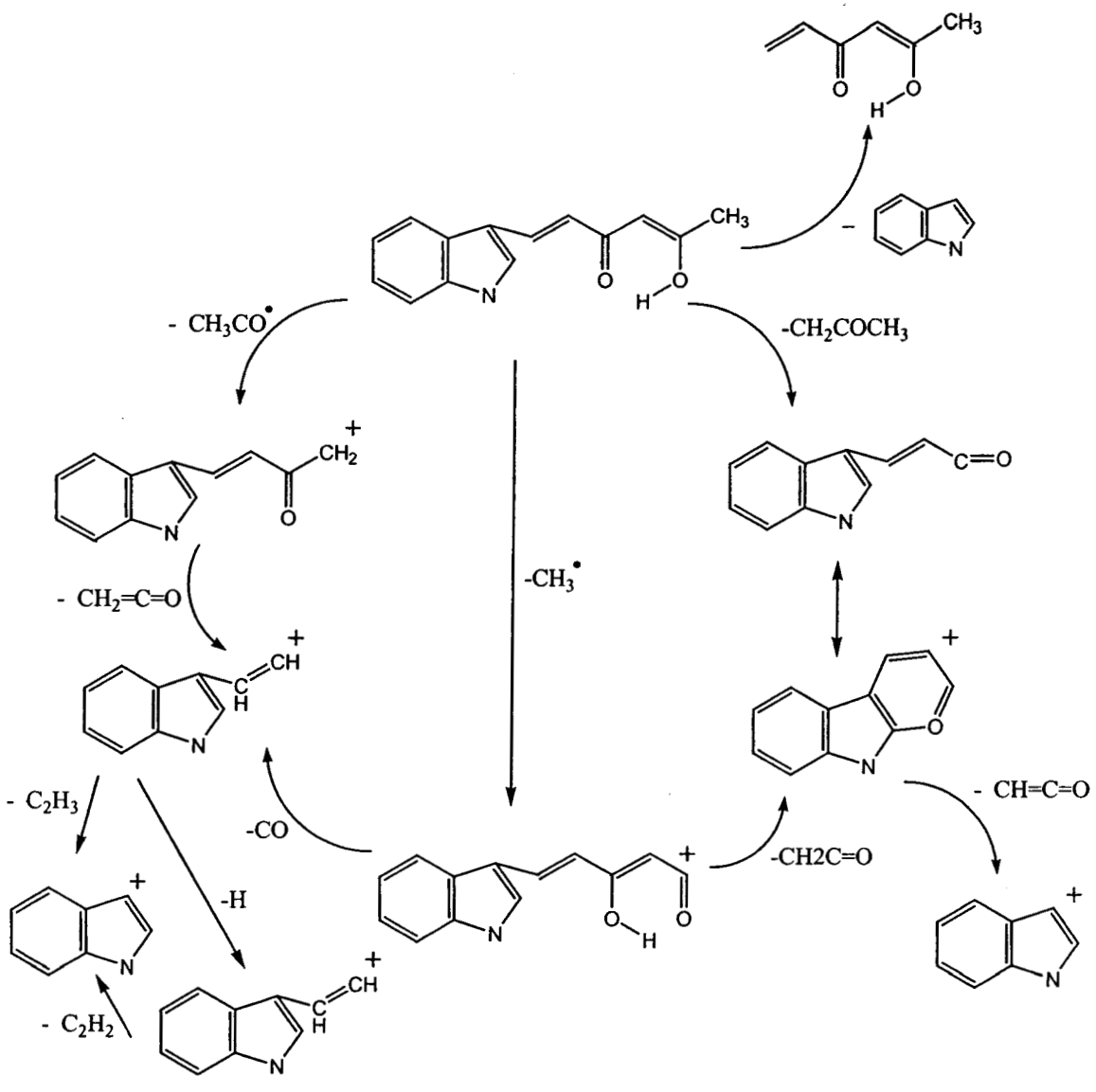


Fig. 5.3. Mass spectrum of 6-indolyl-5-hexene-2,4-dione



Scheme 5.2

Characterisation of metal chelates of 6-indolylhexanoid

Analytical and physical data of the metal complexes given in table 5.2 suggest a 1:2 metal ligand stoichiometry for Co(II), Ni(II), Cu(II) and Zn(II) while 1:3 stoichiometry was found in the case of Fe(III) and Al(III) complexes. The conductivity measurements from 10^{-3}M solution in

DMSO indicated their non ionic nature. Fe(III), Co(II), Ni(II) and Cu(II) complexes showed normal magnetic moment. The structure and nature of bonding in metal complexes were established on the basis of their electronic, ir, nmr, and mass spectral data, as discussed below.

TABLE 5.2

Analytical and physical data of metal complexes of 6-indolyl hexanoid, HL

Compound	M.P. (°C)	μ_{eff} (B.M.)	Elemental analysis (%) Found / (calcd.)				λ_{max} nm
			C	H	N	M	
HL	158	--	73.74 (74.00)	5.05 (5.72)	6.46 (6.16)	--	276, 372
$[AlL_3]$	195	--	72.51 (71.48)	5.01 (5.52)	6.32 (5.93)	4.25 (3.81)	278, 384
$[FeL_3]$	224	5.72	69.24 (68.38)	5.86 (5.29)	6.34 (5.69)	7.96 (7.61)	276, 388
$[CoL_2(H_2O)_2]$	175	4.20	61.65 (61.43)	5.40 (5.14)	6.52 (5.14)	9.83 (10.76)	276, 391
$[NiL_2(H_2O)_2]$	186	2.99	61.34 (61.43)	5.28 (5.10)	5.00 (5.12)	11.04 (10.72)	277, 394
$[ZnL_2(H_2O)_2]$	170	--	62.36 (61.18)	5.58 (5.05)	6.51 (5.77)	10.97 (11.82)	278, 387

Uv spectra: The uv absorption bands of the ligand almost unaffected by complexation with metal ions. The n $\rightarrow$ π^* transition of the dicarbonyl chromophore of the free ligand showed slight bathochromic shift indicating the involvement of the dicarbonyl moiety in the chelate formation.

Infrared spectra: A comparison of the spectra of the complexes with that of the ligand spectrum revealed that the bands due to the intramolecularly hydrogen bonded carbonyl group of the enol form at 1634 cm^{-1} is totally disappeared in the spectra of complexes. The only band present in the region 1600-2000 cm^{-1} in the spectra of the complexes is the intense band at ~ 1610 cm^{-1}. In the spectra of the free ligand this band has been assigned to the stretching of the C=C groups. Therefore this band in the complex can safely assigned to the stretching of the olefinic carbon bond.

Another characteristic feature of the spectra of the complexes is the presence of a very intense band at ~ 1570 cm^{-1} which is not found in the ligand spectrum. From its position, intensity and based on earlier reports on spectra of 1,3-diketonates, the band can be confidently be assigned to the stretching of metal bonded dicarbonyl function.

A striking feature in the spectra of the complexes is the presence of an intense broad band at ~ 3200 cm^{-1}. The broadening is very little in comparison with the ligand spectrum, where it has been assigned to the NH and the intramolecularly hydrogen bonded O–H...O group of the enol tautomer. The appearance of the band in complexes with comparatively less broadening clearly indicates that it is due to the NH stretching of the indolyl group. Thus it can be confirmed that the heterocyclic NH remained as such in complexes also. Thus the indole nitrogen in the

ligand are not involved in metal coordination and only the diketo oxygens are engaged, is clearly evident from the ir spectra of complexes.

TABLE 5.3

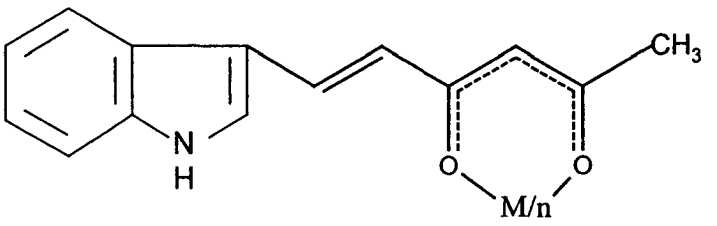
Characteristic ir data (cm^{-1}) of 6-indolyl hexanoid and its metal chelates

HL	Al(III)	Fe(III)	Co(II)	Ni(II)	Cu(II)	Zn(II)	Probable Assignments
1613	1610	1612	1608	1612	1614	1614	νC–C olefin
1634	1555	1558	1552	1560	1557	1560	νC–O
1613	1576	1578	1574	1576	15786	1572	νC–C aryl
1520	1509	1505	1504	1505	1509	1506	νC=C olefinic
1497	1483	1482	1485	1480	1483	1484	νC–C phenyl
1445	1424	1420	1428	1426	1430	1427	νC–C alkenyl
1393	1392	1390	1387	1386	1387	1386	ν_{as}C–C–C (chelate ring)
1149	1132	1135	1134	1132	1136	1135	ν_{s}C–C–C (chelate ring)
1083	1070	1072	1071	1073	1070	1070	BCH=CH chelate ring
947	940	942	945	944	951	940	νCH_2 CH trans
760	740	742	748	746	748	746	νC–H (chelate ring)
--	450	452	450	453	458	456	νM–O (chelate ring)
--	421	420	418	418	416	418	

L = 6-indolyl hexanoid

That the involvement of the carbonyl group in metal coordination is further evident from the appearance of a two medium intensity bands at ~ 450 and 420 cm^{-1} in the spectra of the complexes. This band can be

assigned to the stretching of metal bonded carbonyl (νM–O). Important bands and their assignments are given in **table 5.3**. Thus infrared spectra clearly showed that metal ions coordinated only through the dicarbonyl oxygens as in **structure 5.3**.



n = 2 or 3

5.3

^{1}H nmr spectra: In conformity with the structure 5.3 the enolic proton signal (δ ~ 16 ppm) of the free ligand disappeared in the ^{1}H nmr spectrum of the diamagnetic Al(III) chelate (**Fig. 5.4**). This indicates that the enolic proton has been replaced by the metal ion during complexation. The methine signals shifted towards the downfield of the spectrum indicating the decreased electron density around the central carbon atom of the pseudo-aromatic metal chelate ring system. The integrated intensities of the aryl and alkenyl signals are in agreement with their formulation. Further the signal due to the NH proton remained almost unaffected suggest that the heterocyclic nitrogen is not involved in coordination. Thus the nmr spectrum strongly support the bonding mode of the ligand as in structure 5.3.

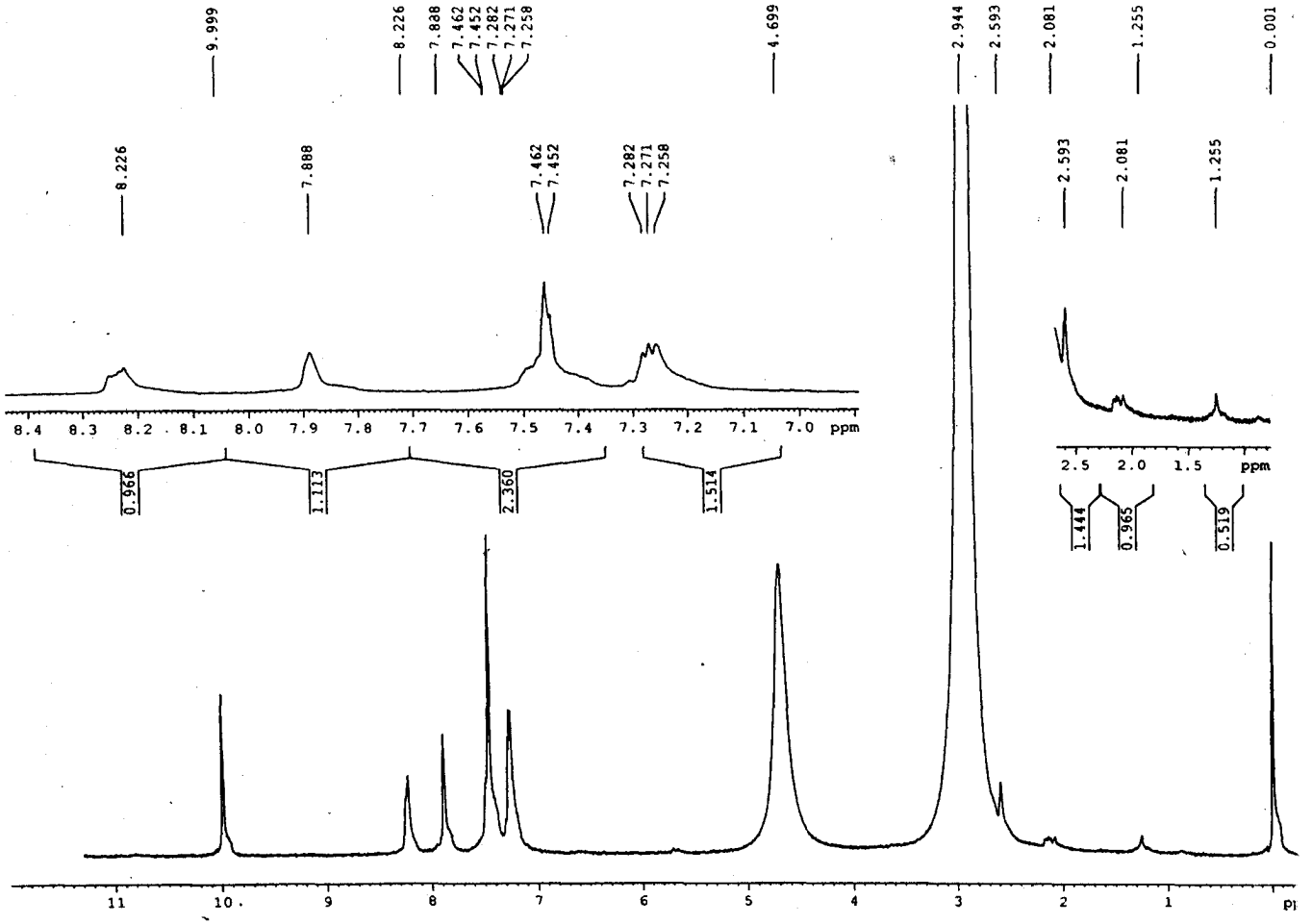


Fig. 5.4. NMR spectrum of Al(III) complex of 6-indolylhexanoid

Mass spectra: The copper(II) chelate of 6-indolylhexanoid show relatively intense peak at m/z 551 corresponding to the $[ML_2(H_2O)_2]$ formulation. A characteristic feature of the spectrum is the appearance of intense peaks due to the elimination of CH_3–, CH_3CO–, Ind, Ind–CH=CH– group from the molecular ion. The spectra of Cu(II) complex is reproduced in the **fig. 5.5**. The fragmentation pattern is shown in the **scheme 3.3**.

Thermal analysis of Cu(II) complex of 6-indolylhexanoid

Thermogram of Cu(II) complex of 6-indolylhexanoid is reproduced in **Fig. 5.6**. The thermogram exhibits a weight loss at temperature range 150-220°C. From the % wt. loss of 5.86, this can be attributed to the loss of two molecules of coordinated water, where the calculated value corresponds to 6.53%.

Another striking feature of the thermogram is that loss of ligands occurred in two steps. The first weight loss (39.6%) at temperature range 230-250°C, which is due to the loss of one ligand molecule (calculated weight loss 41.01%). The loss of second ligand took place at ~ 420°C and completed at ~ 450°C. The final product obtained was CuO and the % of residue obtained (found 13.55, calcd. 14.33) closely agree with the formulation of the complex $[CuL_2(H_2O)_2]$.

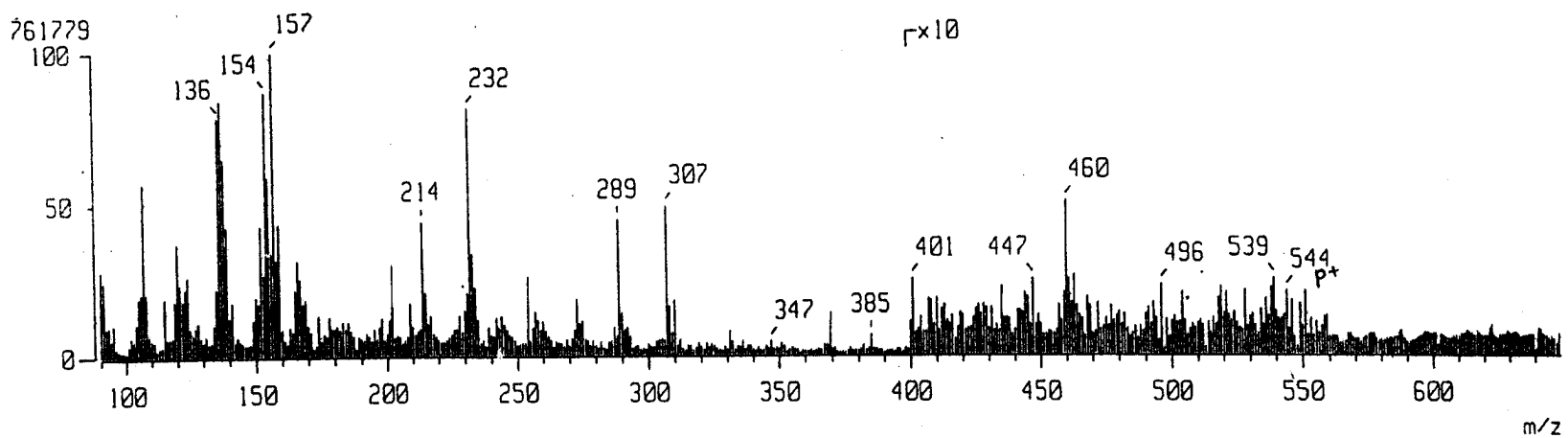


Fig. 5.5. Mass spectrum of Cu(II) complex of 6-indolylhexanoid

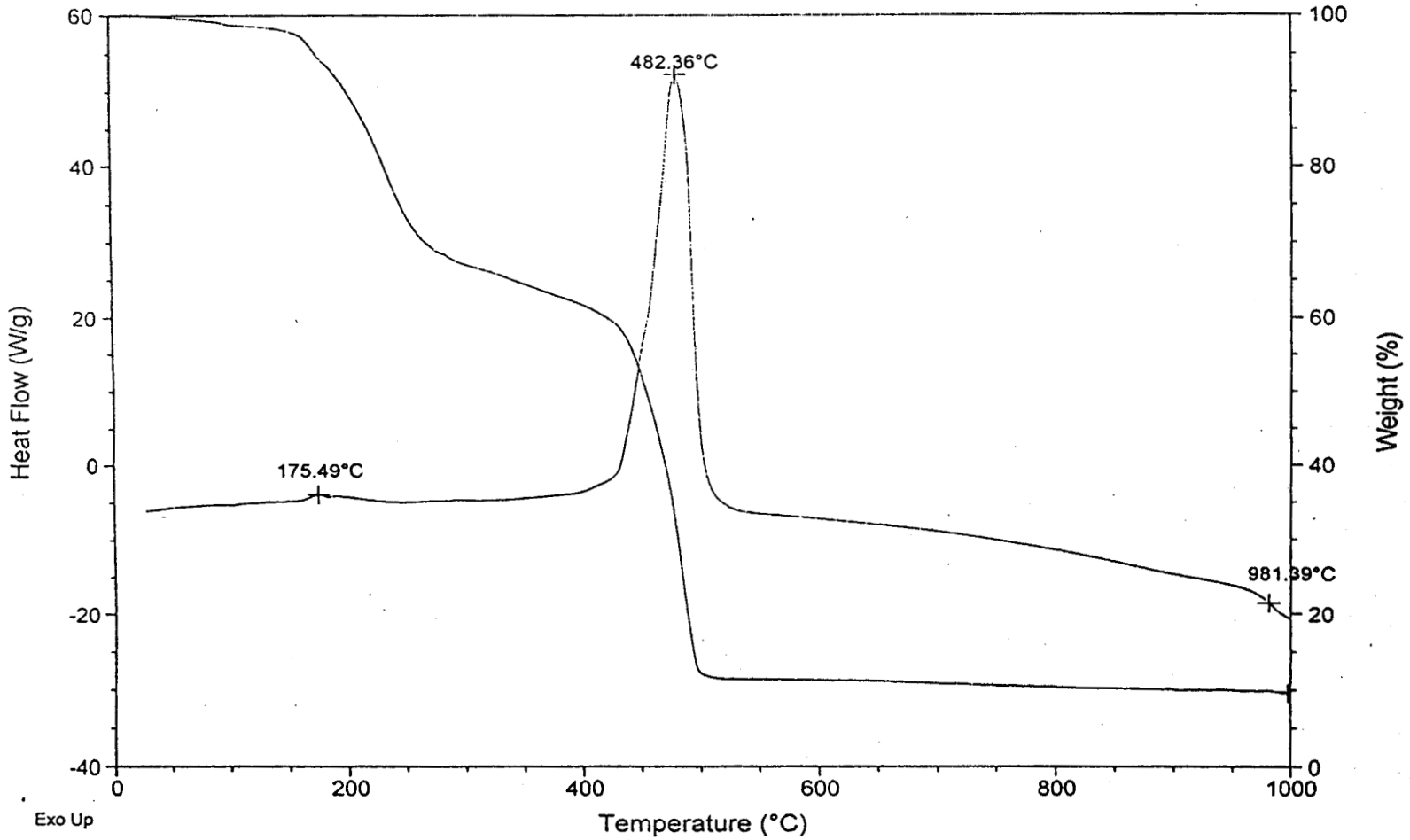


Fig. 5.6. Thermogram of Cu(II) complex of 6-indolylhexanoid

ESR spectral study of Cu(II) complex

The g and A values calculated from the esr spectrum (**Fig. 5.7**) of the Cu(II) complex of 6-indolylhexanoid have given below. The $g_{||}$ and $g_{\perp}$ values are 2.1468 and 2.0283 respectively, $A_{||}$ 80 x 10^4 cm^{-1} and $A_{\perp}$ 25 x 10^4 cm^{-1}. The A values indicate high degree of delocalisation in the chelate ring.

SUMMARY

6-Indolyl-4-pentene-2,4-dione was synthesised by the condensation of indole-3-carboxaldehyde and acetylacetone at 0°C. The metal chelates of Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Al(III) were also synthesised. The compound 6-indolylhexanoid and its metal complexes were characterised by various spectral and analytical techniques. These studies revealed that the ligand exists in an intramolecularly hydrogen bonded enol form. For the metal chelates a 1:3 stoichiometry was found in the case of Al(III) and Fe(III), while 1:2 stoichiometry in the case of other metal ions. These compounds did not have noticeable fluorescence property and biological activities.

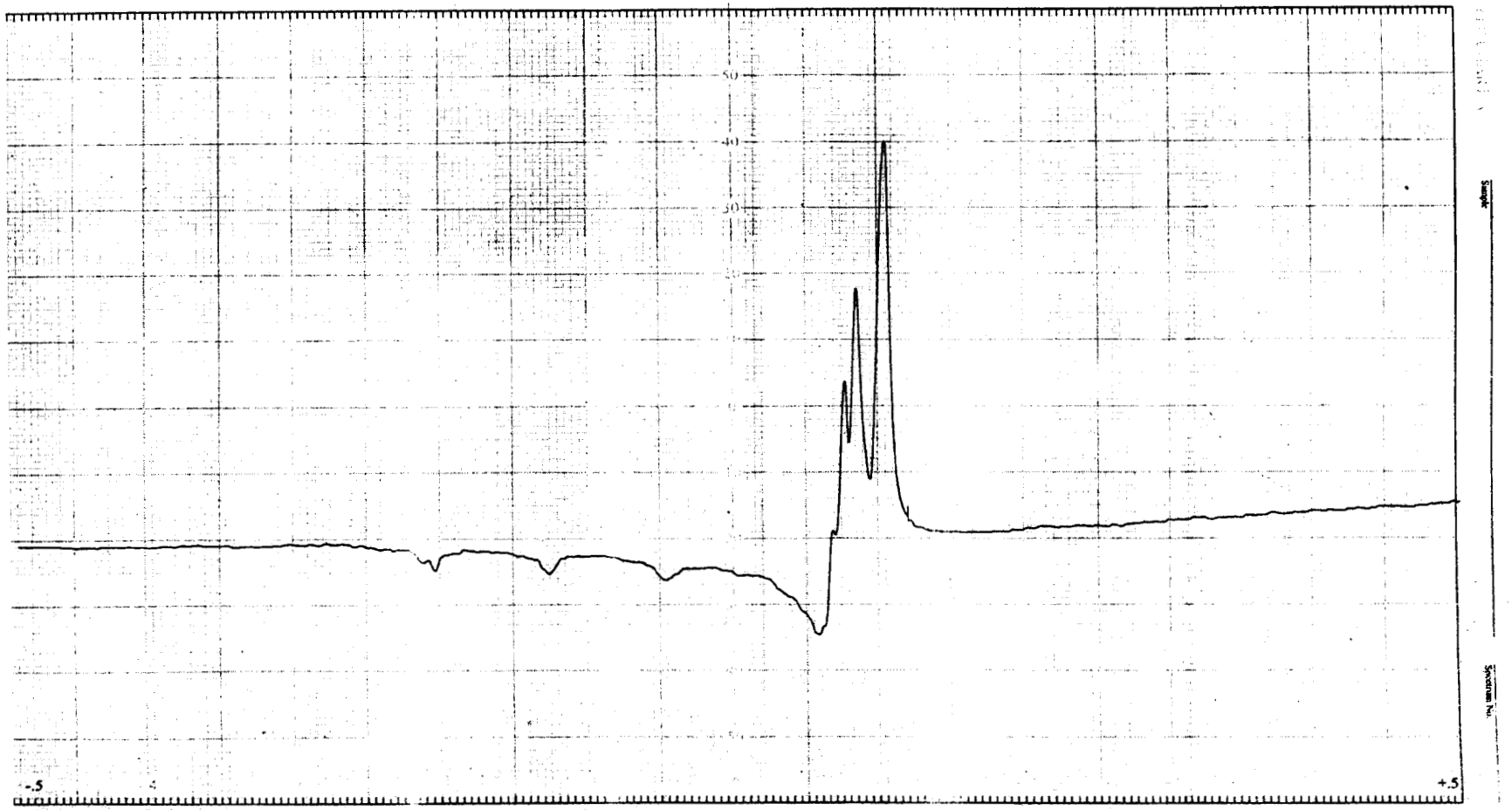


Fig. 5.7. ESR spectrum of Cu(II) complex of 6-indolylhexanoid

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