SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES ON METAL COMPLEXES OF 1,7-DIARYLHEPTA-1,6-DIENE-3,5-DIONES

THESIS

submitted to the Faculty of Science
University of Calicut in partial fulfilment of the requirements for the Degree of
DOCTOR OF PHILOSOPHY

By

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C E R T I F I C A T E

This is to certify that the thesis entitled **SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES ON METAL COMPLEXES OF 1,7-DIARYLHEPTA-1,6-DIENE-3,5-DIONES** is an authentic record of the research work carried out by **Sri. Babu Joseph**, under my supervision in partial fulfilment of the requirements for the award of the degree of the 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)
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Calicut University,
29.05.2007.

D E C L A R A T I O N

Certified that the thesis bound herewith is an authentic record of the research work on **SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES ON METAL COMPLEXES OF 1,7-DIARYLHEPTA-1,6-DIENE-3,5-DIONES**, 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,
29.05.2007. **Babu Joseph**

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C O N T E N T S

CHAPTER 3
BIOLOGICAL STUDIES

CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 2

METAL COMPLEXES OF 1,7-DIARYLHEPTA-1,6-DIENE-3,5-DIONES

CHAPTER 3

BIOLOGICAL STUDIES

P R E F A C E

Active chemical constituents of a number of medicinal plants contain functional groups such as –OH, C=O, OCH_3, etc. that can form stable bonds with various metal ions. Many of the biological significance of these plant chemicals are associated with their ability to form complexes with various biologically important metal ions and other inorganic species. Therefore, studies on the coordination behaviour of synthetic analogues of active chemical components of medicinal plants have considerable importance. The present investigation is mainly on these aspects of certain synthetic analogous of natural curcuminoids. Curcuminoids are the active chemical constituents present in the traditional Indian medicinal plant turmeric (*Curcuma longa*). Synthesis and characterisation of metal complexes of a series of curcuminoid analogues are considered in this study from a structural point of view. The antioxidant and antitumour activity of these compounds are also included in this work. The Thesis is divided into three chapters.

Structurally, curcuminoids are a group of naturally occurring β-diketones in which the diketo function is directly attached to olefinic groups. Therefore, some of the salient features β-diketones and metal β-diketonates are briefly discussed in **Chapter 1, General Introduction**. Importance of

coordinatin chemistry in biological processes has been highlighted. In this chapter, reported studies on synthetic and structural aspects of curcuminoids and their metal complexes are briefly mentioned. Therefore no separate review is included. Various biochemical and medicinal applications of turmeric and curcuminoids have been cited. Need for further studies and importance of the present investigation have been interspearsed at appropriate places.

In **chapter 2**, synthesis and characteristisation of curcuminoid analogues, (1,7-diarylheptanoids) and their metal complexes are included. The results are presented in three sections. Details on the synthesis and characterisation of Al(III), Cr(III) and Fe(III) complexes of 1,7-diarylheptanoids having no aryl substituents are presented in **section 1**. The spectral and analytical data clearly revealed the formation of $[ML_3]$ complexes in which the diketo oxygens are involved in bonding with the metal ion.

Synthesis and characterisation of a new curcuminoid analogue, 1,7-dianthryl-1,6-heptadiene-3,5-dione (**Hdah**) and its metal complexes are discussed in **section 2**. The existence of **Hdah** entirely in the intramolecularly H-bonded enol form has been established from the ir, nmr and mass spectral data. The monobasic bidentate coordination of the compounds in the $[ML_2]$ and $[ML_3]$ complexes also revealed from the spectral

and analytical data. In **section 3** complexes of some trivalent metals of 1,7-diarylheptanoids having various aryl substituents (OH, $N(CH_3)_2$, OCH_3, CH_3, etc.) are considered. Spectral and analytical data clearly indicated that the potential donor atoms of the aryl substituents are not involved in bonding with metal ion.

Results of studies on biological properties such as biodistribution assay and antitumour and antioxidant activities of the curcuminoids and their metal complexes are considered in **chapter 3**. Details of *in vitro* studies on inhibition of superoxide, hydroxyl and lipid peroxides are given in **section 1**. The result showed that metal complexation increases the radical scavenging activity of curcuminoids. In **section 2**, the cytotoxicity and solid tumour reduction studies are presented. Results indicated that both cytotoxicity and solid tumour reduction increased in complexes compared to free curcuminoids. Results of the biodistribution of the synthetic analogue of the natural curcuminoid (diferuloylmethane) in the form of ^{51}Cr labelled complex administered intraperitonially in mice are brought out in **section 3**.

References are given in the order cited.

The work described in this thesis and related studies have partially been published/accepted/communicated/to be communicated for publication as listed below:

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

2. Synthesis, characterisation and antitumour activity of Al(III) complexes of synthetic curcuminoid analogues. J. Maingroup Metal Chemistry (accepted).

3. Biodistribution study of curcuminoids in mice after radiolabelling with ^{51}Cr. Proceedings of the National Seminar on Frontiers in Chemistry at Cochin University of Science and Technology, 2006 March 24 & 25.

4. Antioxidant activity of synthetic analogues of curcuminoids and their metal complexes. Serbian J. Chem. (accepted).

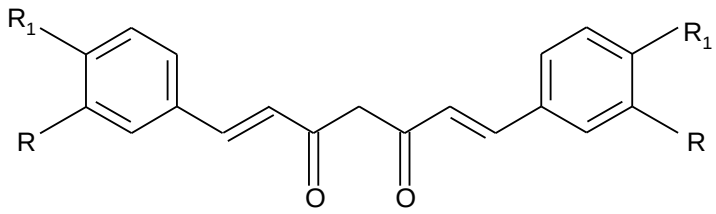
5. Synthesis and characterisation of Cr(III) and Fe(III) complexes of curcuminoids. J. Indian Chem. Soc. (accepted).

6. Cytotoxicity and antitumour activity of metal complexes of curcuminoids. Cancer Letts. (communicated).

7. Biodistribution assay of curcuminoids using ^{51}Cr labelled complexes. Applied Radiation and Isotopes (to be communicated).

NOMENCLATURE AND ABBREVIATIONS

Names indicating the source of the compounds is a usual practice of naming the active chemical constituents isolated from natural products. Thus the name curcuminoids was given to a group of structurally related compounds present in the yellow pigment of the medicinal plant *Curcuma longa* Linn. (turmeric). Structurally they are 1,3-diketones of the type given below.



Synthetic analogues of these "unsaturated" β-diketones are also known by the term curcuminoids. Systematically these compounds are 1,7-diarylhepta-1,6-diene-3,5-diones and for brevity they are sometimes referred as 1,7-diarylheptanoids. Both these and the trivial name, curcuminoid are freely used in the present investigation.

Important abbreviations used in the thesis are:

ADI	average daily intake
Ar	aryl group
BM	Bohr Magneton

DHP	disodium hydrogen phosphate
dmf	dimethylformamide
DLA	Dalton's lymphoma ascites
dmso	dimethylsulfoxide
FAB	Fast atom bombardment
Fig.	figure(s)
h	hour
Hdah	1,7-diantrhyl heptanoid
id	injected dose
ILC	Increase in Life Span
L	Deprotonated ligand
M	Central metal ion in a metal complex
M.P.	Melting point
NBT	Nitro blue tetrazolium
Ph	Phenyl group
PBS	Phosphated buffer saline
ppb	Parts per billion
ppm	Parts per million
TBA	Thiobarbituric acid
TBARS	TBA reacting substance
tlc	thin layer chromatography
μCi	micro Curie

μ_{eff}	Effective magnetic moment in Bohr magnetons
μL	microlitre (10^{-6} L)
μg	micro gram
5 : 2 : 1	H_2O : EtOH : NH_3

Uv-visible absorption maxima (λ_{max}) are given in nm/cm^{-1} as indicated. The infrared bands are given in cm^{-1}.

Chemical shifts in ^1H nmr spectra are expressed as δ values (ppm downfield from tetramethylsilane, TMS).

While reporting mass spectral data, P^+ represents the parent ion (molecular ion). In the case of metal complexes, the m/z of P^+ correspond to the most abundant isotope of the concerned metal atoms.

1,3-DIKETONES AND THEIR METAL COMPLEXES

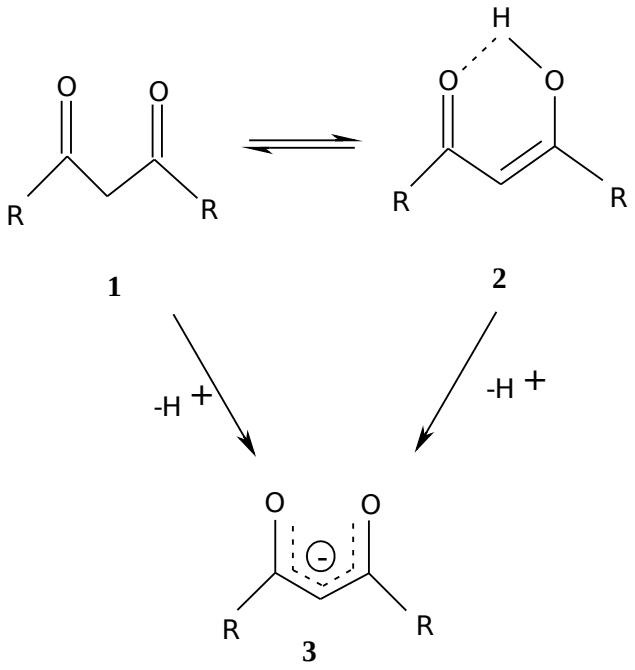
One of the fascinating features of modern coordination chemistry is the ever increasing academic, commercial and biological interest exhibited by metal complexes of organic molecules, both natural and synthetic.[1,2] This is mainly because of the ability of coordinated metal atom/ion to influence the structure, properties and applications of the organic compound. Thus numerous highly efficient catalytic systems based on metal complexes for the synthesis and manufacture of several industrial chemicals are available. Similarly, several synthetic metal complexes which mimic the behaviour of complex biomolecules are known and at present the study of such compounds are receiving much attention.[3,4] Although the results obtained so far do not always parallel those in nature, a knowledge of the chemistry is being built up and the biochemical role of metal ions in natural ligand systems is beginning to be better understood.[5]

The structure, properties and uses of metal complexes are dependent both on the nature of the metal ion and the type of the ligands coordinated. Thousands of new ligand systems have been developed in recent years because of the developments in modern synthetic organic chemistry. This trend is evident from the reports on numerous compounds based on 1,3-diketones and related compounds.

The 1,3-diketones have several interesting structural features. They serve as the best example of keto-enol tautomerism and intramolecular hydrogen bonding. For a coordination chemist, perhaps the most important aspect of β-diketones is their ability to form diverse types of complexes with various metal and metalloid elements. The 1,3-diketones still serves as the starting material for the design and synthesis of a large number of compounds having wide application in many fields. Therefore, investigation on metal complexes of different types of 1,3-diketones have considerable importance. The present investigation is mainly on certain structural and biological aspects of a series of 'unsaturated' 1,3-diketones and their metal complexes. Therefore, some of the salient features of 1,3-diketones and metal 1,3-diketonates which are quite pertinent to the present study are briefly discussed below.

Tautomerism of β-diketones

Since the preparation of acetylacetone and similar 1,3-dicarbonyl compounds in the later half of the 19th century, organic chemist has considerable interest in their properties, especially their ability to exhibit keto-enol tautomerism. The 1,3-diketones contain a methylene group or a substituted methylene group which is interposed between acyl or aroyl groups. Usually, 1,3-diketones exist as a mixture of keto **1** and enol **2** forms related by a 1,3-hydrogen shift.



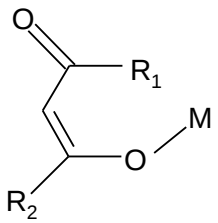
Usually, the enolic form is favoured in nonpolar solvents and simultaneous conjugation and chelation through hydrogen bonding is responsible for the stability of the enol tautomers The proportion of the enol tautomer is dependent on a number of factors like solvent, temperature and substituents.[6] In general, the amount of enol form decreases when a bulky alkyl substituent is present at the α-position. This can be attributed to the steric hindrance offered by the bulky group together with inductive effects of the alkyl groups.[6,7] Presence of electron withdrawing groups such as Cl^-, Br^-, CN^- & CH_3COO^- at the α-position increases the proportion of the enol tautomer. The enolization also increases[8,9] when the compounds are fluorinated or contain an aromatic ring.[10] The removal of the active hydrogen from the enol/keto forms generate the 1,3-diketonate anion **3**.

Metal complexes of β-diketones

The coordinating abilities of 1,3-diketones were recognized as early as in 1887 when, Combes reported the synthesis of beryllium acetylacetonates.[11] This was followed by the pioneering work of Werner[12], Morgan[13,14] and Sidgwick[15] and confirmed the bidentate chelating character of these ligands. The diketonate anion, being a powerful chelating agent, form complexes with virtually almost all the metal and metalloid ions in the periodic table. Literature on β-diketones and metal β-diketonates are so voluminous that even an attempt to summarise is purposefully avoided. However, since β-diketones can be bonded to metal ions in a variety of ways, the different coordination modes reported are briefly mentioned below.[16]

In general, metal β-diketonates can be divided into four categories. (1) Oxygen bonded (2) Carbon bonded (3) Both carbon bonded and oxygen bonded and (4) Olefin bonded. Typical examples are given in structures **4-14**.

1) Oxygen bonded β-diketonate complexes



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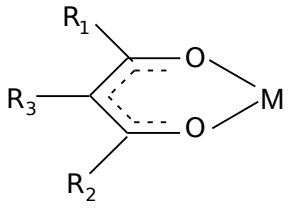

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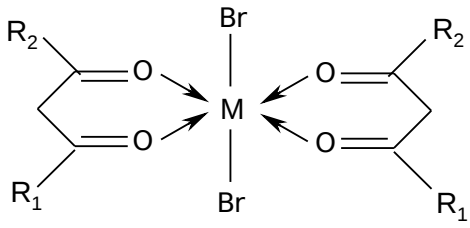

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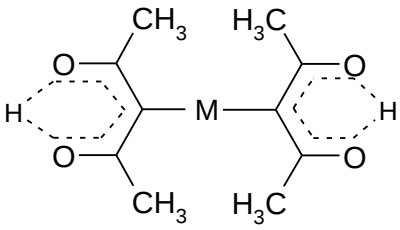
2) Carbon bonded complexes



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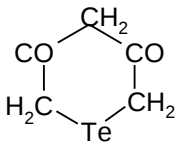
M = Sn(II), Se(II)



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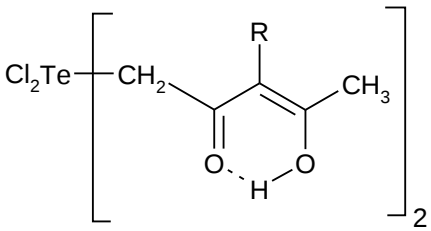

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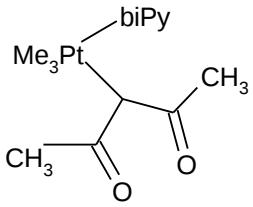

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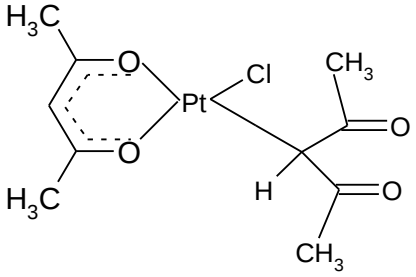
3) Both carbon and oxygen bonded complexes



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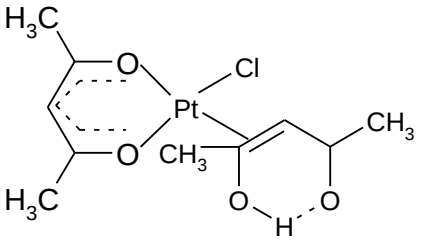
4) Olefin bonded complexes



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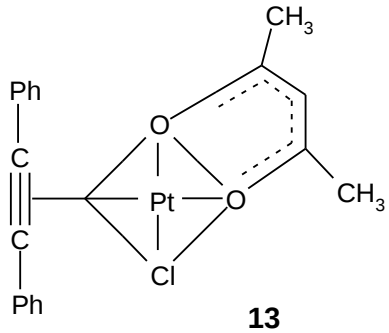

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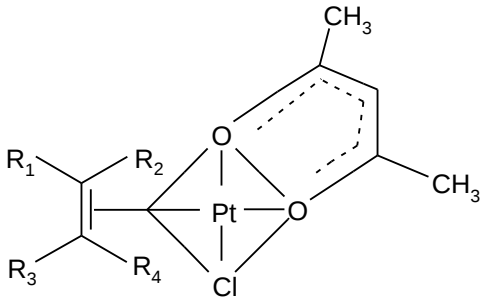


14

Structural characterisation of metal complexes of β-diketones

Almost all the available spectral techniques as well as diffraction data along with other physical and chemical methods have been extensively employed in studying the structure and nature of bonding in various metal 1,3-diketonates.[17] Thus, the uv-visible absorption spectral data together with magnetic moment values have been widely employed in establishing the structure and stereochemistry of the various metal diketonates. The importance of ir, nmr and esr spectral data in elucidating the structure and

nature of bonding in coordination compounds were infact started with the application of these techniques in metal β-diketonates.[17]

Electronic Spectra: Interpretation of electronic absorption spectral data in establishing the structure and nature of bonding of metal β-diketonates has been a major research activity in coordination chemistry for a long time. Thus, numerous reports exist on uv-visible spectral data of metal β-diketonates. Theoretical calculations based on SCF and LCAO-MO calculations of the various absorptions are also available, particularly in the case of metal acetylacetonates. Thus, for instance, the strong broad absorption bands appearing at ~ 34700 cm^{-1} and 49500 cm^{-1} of metal acetylacetonates have been assigned to various π-π* transitions. Similarly, almost all the observed electronic spectral bands have been justified on the basis of various MO calculations.[18-27]

IR spectra: Vibrational spectroscopy is one of the most important available technique for establishing the structure and nature of bonding of coordination compounds particularly metal complexes of organic ligands. This aspect of ir spectra of coordination compounds has been well illuminated from the reported ir studies on various metal β-diketonates.

Studies on the ir spectra of metal β-diketonates were initiated during the second half of the last century. Infrared spectra of metal β-diketonates

provide valuable information regarding the nature of bonding of the diketo group attached to the metal. In addition, conclusive evidence for the quasi-aromatic behaviour of the six membered C_3O_2M chelate ring and various other structural features of metal β-diketonate has also been deduced from ir data. Importance of ir spectra in establishing the keto-enol tautomers of β-diketones has been well established.[28,29] For example characteristic carbonyl band of the enol form of acetylacetone appeared at 1613 cm^{-1} and that of diketo form at 1725 cm^{-1}. Presence of a broad band at 2700-3000 cm^{-1} is an indication of the intramolecularly hydrogen-bonded enol form of β-diketones.[30,32] Upon complexation, the carbonyl stretching frequency of β-diketones shows a shift (10-50 cm^{-1}) to lower values and additional bands due to ν_{M-O} vibrations appear in the region 400-500 cm^{-1}.

NMR Spectra: NMR spectral studies of metal β-diketonates appear to have been initiated in 1958 by Holm and Cotton[33] who assigned the positions of methyl and methine (=CH-) protons in neutral metal acetylacetonates. They observed that the chemical shifts were close to those observed for olefinic protons and were nearly independent of the size, charge and π bonding ability of the metal ion. The position and nature of splitting of the signals depends on the mode of the coordination, nature of the substituents and the extent of delocalization in the chelate ring.[34-39] The *cis* enol proton chemical shift, δ(OHO)/ppm, of β-diketone and β-ketoaldehyde of general formulae

R'COCH (R'') COR[111] have been reported.[40] Nonhebel[41,42] showed that the bulky substituents on the α and β sites not only shifted δ(OHO) down field but produced a sharper line.

X-Ray Diffraction studies: The m-chloro and m-bromo derivatives of dibenzoyl methane were the first β-diketones to be investigated by X-ray methods.[28,29,43] The crystal and molecular structures of several metal β-diketonates have been determined by the 3-dimensional X-ray method.

Mass spectra: Mass spectroscopy is an efficient tool in the structure elucidation of coordination compounds.[44-46] Macdonald and Shammon[47] studied mass spectra of a series of metal acetylacetonates. The most intense peaks in the spectra are usually derived from the monomeric forms of the complexes, but rarely peaks due to dimer or even trimer have also been observed. These studies confirm the influence of the odd or even electron character of an ion on its dissociation reactions (McLafferty)[48] and provides an additional evidence[44-46] that odd electron ions can be changed to even electron ions and vice versa, by change of valency of the metal atom in the ions.

Thermogravimetric studies: Thermal anlysis is a well established method for the characterization of inorganic complexes. Sievers and co-workers[49,50] have made a detailed comparative study of the thermal stability of derivatives

of lanthanons with different β-diketones. These workers found that size and extent of fluorination of the ligand along with careful selection of substituents attached to the donors, enhances the volatility and stability of resulting complexes.[50]

Applications and use of metal complexes of β-diketones: The chemical reactivity coupled with volatility, thermal and solvolytic stability of metal β-diketonates have been exploited in solvent extraction studies of various ions and gas chromatographic separations of several metals. The application of certain coordinately unsaturated lanthanide chelates, called 'shift reagents' for nmr spectral elucidation has become an extremely useful analytical technique.[51-54] Addition of certain metal β-diketonates for measurements of carbon-13 nmr spectra is effective in reducing the normally long longitudinal relaxation times, thus minimizing saturation effects and allowing more rapid collection of data.[55-59]

The chemistry of lanthanide β-diketonates has assumed considerable importance because of their practical use as potential laser materials.[60-64] Since the development of gas chromatography as an efficient technique for separation and estimation of different species, volatile compounds of metals have assumed special significance.[65-69] Fluorinated β-diketones are highly useful in the solvent extraction of metals.[70-74] Metal complexes of β-diketones

are used as fuel additives,[75] as supercritical fluids for waste clean up[76] in superconducting thin film manufacturing[77] and in production of homogeneous and heterogeneous catalysts.[78,79] Iron(II) and iron(III) chelates of β-diketone are used as catalysts for the removal of hydrogen sulphide from natural gas.[80]

Time resolved fluorescence spectra of europium chelates of β-diketones is one of the most rapidly growing areas of application of fluorescence spectroscopy. Highy sensitive time-resolved fluorometric determination of estrogens by HPLC using europium β-diketonate are reported.[81] Microsecond time-resolved fluorimetry (TRF) of europium chelates was introduced in the area of nucleic acid hybridization assays and immunoassays of proteins.[82-89]

Naturally occurring β-diketones

Majority of the reported studies on metal β-diketonates are based on synthetic β-diketones in which, the diketo function is directly linked to alkyl/aryl groups. However active chemical components of several medicinal plants contain one or more carbonyl group as essential functional group. Many of the medicinal and other biological properties of these plants are due to the presence of these type of compounds.

Several plant species are known to exert wide range of beneficial physiological effects in addition to aroma and flavour. Even in the modern

world, nature is still the greatest source of drugs and pharmaceuticals. The Indian subcontinent is endowed with rich and diverse local health traditions which is matched with an equally rich and diverse plant genetic resources.[90] The classical systems of medicine are also based on herbal medicine.

Herbs and herbal constituents are found to be safe and function as natural remedies for many tragic illness. Powerful antioxidants originating from edible and medicinal plants have been extensively investigated as important inhibitory materials for the prevention of oxidative deterioration of lipids. Recently it has been shown that peroxidation in living organism is closely related to the initiation of some human diseases, such as cancer, coronary heart disease and Alzheimer's disease. Ingestion of antioxidants may possibly prevent these diseases.[91,92]

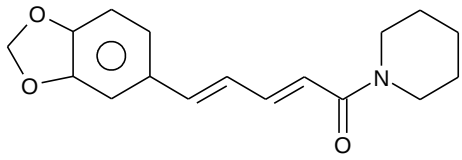
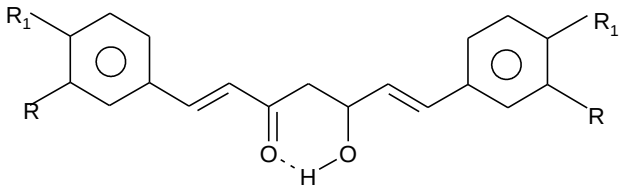
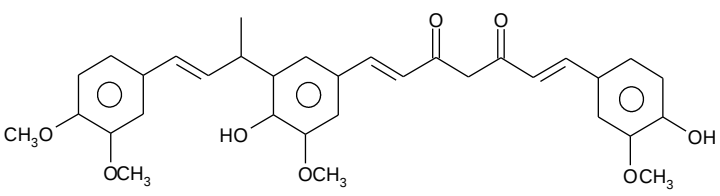
The state of health is a result of body's ability to recover from the continuous challenges posed by toxic substances entering through air, food and water. The most vivid example of this struggle, is our defence against chemicals that cause cancer. Most cancer causing compounds (carcinogens) undergo metabolic change in the body to 'activated' carcinogens. The 'activated' carcinogen binds to the cell DNA, and damages it forming the so called DNA adducts. Chemoprevention includes the use of pharmacologic or natural agents that inhibit the development of invasive cancer either by blocking the DNA damage that initiates carcinogenesis or by arresting or

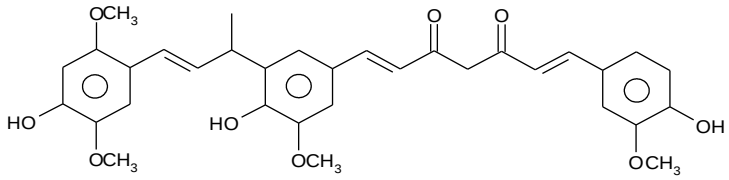
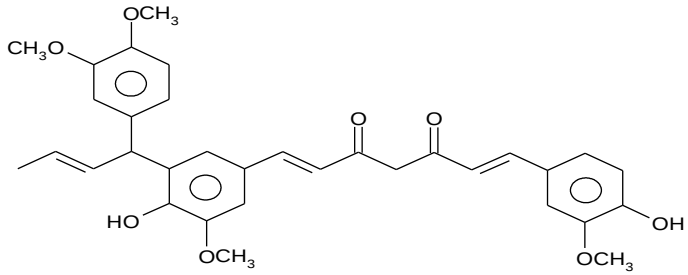
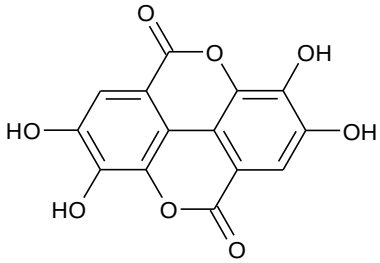
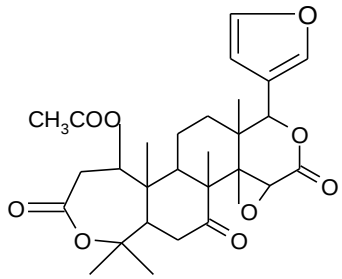
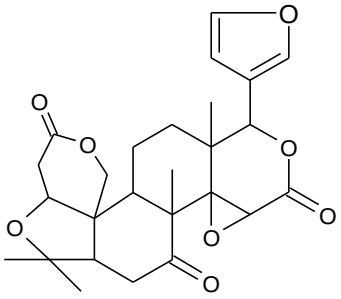
reversing the progression of premalignant cells in which such damage has already occurred.[93]

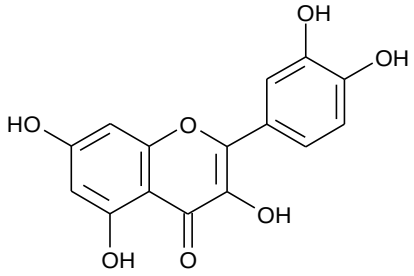
Foods of plant origin contain many bioactive compounds in addition to vitamins and minerals. These phytochemicals belongs to several classes of organic compounds such as sulphur containing compounds, terpenoids, flavanoids, polyphenols, carbonyl compounds, etc. Some of the naturally occurring carbonyl compounds and their main plant sources are given in **Table 1**. In addition to carbonyl group, several other functional groups are also present in these compounds.

Table 1

Active constituents of some common spices and medicinal plants

Spice (Plant species)/ Active Principle	Structure
Black pepper (*Piper nigrum*) piperine	
Turmeric (*Curcuma longa*) Curcuminoids	
Indonesian medicinal ginger (*Zingiber cassumunar*) Cassumunin A	

Spice (Plant species)/ Active Principle	Structure
Cassumunin B	
Cassumunin C	
Fruits and nuts (grapes, strawberries) Ellagic acid	
Terpenoids (Citrus fruits) Nomillin	
Limonin	

Spice (Plant species)/ Active Principle	Structure
Flavanoids and flavanones (Most vegetable fruits and cereal grains) Quercetin	

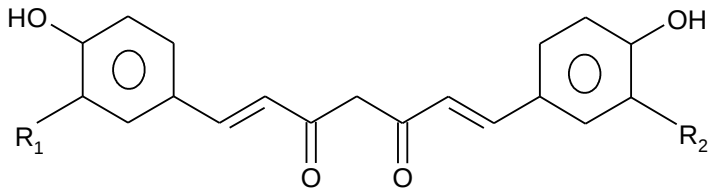
Among the various naturally occurring carbonyl compounds, curcuminoids possess several interesting structural features and numerous practical applications. Structurally they are typical 1,3-diketones in which diketo function directly attached to olefinic groups. Recently metal complexes of curcuminoids and several structurally related compounds have been synthesized and characterized.[94-98] Since the present investigation is mainly on metal complexes of synthetic analogues of natural curcuminoids, some of the chemical and biochemical aspects of curcuminoids are briefly mentioned below.

Chemical and biochemical aspects of curcuminoids

The main source of curcuminoids is the herbaceous Indian medicinal plant turmeric (*Curcuma longa* Linn.). Turmeric is used as a spice in Indian cooking and also as a household medicine. Its pharmacological properties are well documented in ancient Indian literature.[99,100] Turmeric is a common ingredient in many traditional Indian ceremonies and cosmetic preparations.

Also, turmeric occupies an important position in the life of Indian people as a common remedy for many diseases. A paste of turmeric and slaked lime is an household remedy for grains, muscular pain and inflamed joints. Turmeric powder is still used against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatitic disorders, rheumatism and sinusitis.[101-109]

Constitution of curcuminoids: Natural curcumin isolated from turmeric contains three well defined yellow compounds.[103] In 1953, Sreenivasan *et al* separated these three curcuminoids using column chromatography over silica gel.[104] Later these compounds were identified.[103,104] as curcumin I (diferuloyl methane) as the major component, curcumin II (feruloyl-p-hydroxy cinnamoyl methane) and curcumin III [bis-(*p*-hydroxy cinnamoyl methane] as in structure **15** given below.



15

	R_1	R_2
Curcumin I	OCH_3	OCH_3
II	OCH_3	H
III	H	H

The structure of the compounds were later confirmed by chemical degradation studies and by spectral techniques.[108]

Synthesis of 1,7-diarylheptanoids: The synthesis of curcumin was first reported in 1913 by Lampe and Milobedzka.[105] This method was further improved by Povoloni.[106] H.J.J. Pabon developed a general method of synthesis for curcuminoids[107] and related 1,7-diarylheptanoids in good yield. This method involve the condensation of an aromatic aldehyde and acetylacetone in presence of B_2O_3, tri(sec butyl) borate and n-butylamine. The reaction was carried out in dry ethyl acetate in the temperature range 85-110^{o}C. According to Pabon, an acetylacetone-boric oxide complex first formed prevent Knoevenagel type condensation and facilitate Claisen type reaction.

Structure of curcuminoids: Electronic, ir, nmr and mass spectral data of curcuminoids and a number of 1,7-diarylheptanoids have been reported.[99,108] Spectral analysis established that the curcuminoids exist predominantly in the intramolecularly hydrogen bonded enol form.[110] The crystal and molecular structure of curcumin **1** has been reported[111] by X-ray crystallographic methods. These data will be quoted at appropriate places while discussing the results of the present investigation.

Free radical scavenging and antioxidant efficiency of curcuminoids

Free radicals are produced in biological systems by the ionization of water by high energy radiations,[112] through metabolism, by triggered inflammatory phagocytes to reactive oxidants[113] and during oxidative

phosphorylation.[114] These free radicals are highly reactive species, which react with biological compounds causing tissue damage. Antioxidants can counteract against this free radicals.

Curcuminoids are natural phenolic compounds, with potent anti-oxidant properties.[115-117] Both turmeric and curcuminoids can inhibit generation of potent free radicals like superoxide and hydroxyl radicals.[118] The antioxidant properties of curcumin in prevention of lipid peroxidation, another process that generates free radicals is well recognized.[119-121] The primary role of curcumin as a lipid soluble antioxidant is to intercept peroxyl free radicals formed during lipid peroxidation. This prevents free radical chain reactions which deteriorate the lipid membrane.[122,123]

Medicinal uses of turmeric and curcuminoids

Turmeric is a traditional house hold Indian medicine.[124] Certain studies revealed that the topical applications of curcuminoids in patients improve wound healing significantly and protect tissues from oxidative damage.[125,126] It is used as an anthelmintic. Chemopreventive effect of turmeric against stomach and skin tumours have been studied.[127-130] Turmeric has anti-mutagenic,[131,132] property and prevents the DNA damage induced by smoke,[133,134] and lipid peroxidation and prevent BP-DNA adduct formation.[135] Studies have shown that many of the biological properties of turmeric are due to the presence of the curcuminoids.[111,136-147]

The pharmacological studies revealed that synthetic curcuminoids also have antimicrobial, antiinflammatory and anticarcinogenic activities.[137,138,148-163] Curcuminoids inhibits 4-nitroquinoline-1-oxide induced oral carcinogenesis,[164] azoxymethane induced small and large intestinal carcinogenesis[143] and azoxy methane induced colon carcinogenesis.[165] Antimutagenic and anticarcinogenic activity of natural and several synthetic curcuminoids have been studied.[166] Antitumour and free radical scavenging activity of some synthetic curcuminoids were analysed and reported.[96-98,167]

Turmeric and curcuminoids can exert protection either directly, by shielding the biomolecules, or indirectly by stimulating the natural detoxification and defence mechanisms of the body.[142,168,169] Curcuminoids also play a role in protecting some drugs from physico-chemical degradation.[115] Addition of curcumin to the cardiovascular drug nifedipine, prevented degradation of nifedipine due to uv light.[170]

One of the important protective mechanisms of turmeric extract and the curcuminoids is against side effects produced by drug therapy. A potential preventive role of curcumin on DNA adduct formation with the carcinogen has been studied in vitro.[171] Additionally, *in vivo* studies on rats were also performed. As compared to the control animals, rats fed with curcumin showed decrease in levels of DNA adduct in the liver cells.[172] This

decrease could be explained by the competitive binding of curcumin to the active site of benzopyrene, preventing cellular DNA adduct formation.

Because of the cytotoxic nature, anticancer drugs do not discriminate between cancer cells and normal cells, and could cause damage to non-cancerous tissue as well.[173] Curcumin administered to mice along with anti-cancer drug, cyclophosphamide, increased the life span of animals and reduced bone marrow and liver toxicity of cyclophosphamide.[174] Curcuminoids, by virtue of their antioxidant activity, scavenge free radicals as well as prevent their formation, thereby eliminating the toxic effects of the drug.

The effect of combining curcumin with cisplatin was evaluated.[175] Clinical use of cisplatin is limited because of its severe toxicity leading to kidney failure. Studies showed that curcumin administered to mice along with the drug decreased the side effects of the drug therapy. Additionally kidney lipid peroxidation was reduced. Curcumin inhibited the H_2O_2 and nitrite induced lipid peroxidation and haemolysis of erythrocytes *in vitro*.[176,177]

The hepatoprotective action of an alcoholic extract of curcumin against CCl_4-induced liver injury *in vitro*[178] and *in vivo*[179] was tested. Curcumin significantly reduced the urinary excretion of tobacco mutagens, and also enhanced the activity of enzymes to detoxify cigarette smoke mutagens and

carcinogens.[180,181] Curcumin also inhibits *in vitro* production of aflatoxins which causes injury to the liver.[182,183] Probably one of most discussed properties of curcuminoids is their anti-HIV effect demonstrated during *in vitro* and *in vivo* experiments, including a limited number of human studies.[184,185] Important beneficial physiological activities of turmeric and curcuminoids are listed in table 2.

TABLE 2

Important biological activities of Turmeric (Curcuminoids)

Sl. No.	Biological Activity	References
1	Antiinflammatory	102, 140, 136, 144, 148, 149, 150,
2	Antiarthritic	105, 151
3	Antispasmodic	152, 153, 154, 155, 163
4	Antihepatotoxic	154, 163
5	Antiulcerogenic	143
6	Anticoagulant	156, 157
7	Antiprotozoal	158
8	Antifertility	159
9	Antitumour	123, 129, 141, 145, 146, 147, 167
10	Antioxidant	119, 121, 160, 161
11	Antimutagenic	131, 132
12	Wound healing	162
13	Hypotensive	163

Metabolism of curcuminoids

The metabolic fate of curcuminoids has not been examined in detail though it is of relevance in view of their use as a food ingredient and as a medicine. Reports on the uptake, distribution and excretion of curcumin in rats have appeared only recently.[186-190] However, it is to be pointed out that the results obtained are contradictory. Wahlstrom and Blennow[186] observed that 65-85% of the oral administered dose of curcumin was excreted in the faeces, while negligible amounts were recovered in the urine.

Measurements of plasma levels and biliary excretion in anesthesised animals given curcumin revealed a very low absorption of curcumin into the blood.[189-191] The concentration of curcumin in the bile, liver, kidneys and body fat was negligible and the major part of the administered curcumin was found in the intestine. After intravenous injection, curcumin disappeared rapidly from the blood and excreted in the bile. Addition of curcumin to liver perfusion systems and isolated hepatocytes and liver microsomes showed that the sample was quickly metabolised and do not retain in the body over a prolonged period. This led to the conclusion that the liver was the major site of curcumin metabolism.[189,190]

Holder and co-workers[187] reported that following an oral dose, more than 90% of the dose was excreted in faeces as glucoronide conjugates of tetrahydrocurcumin (50%), hexahydrocurcumin (42%) and dihydroferulic

acid. The recovery in urine was only 6%. This again indicated that curcumin and its metabolites were undergoing biliary excretion.

Ravindranath and Chandrasekhara[188] studied the *in vitro* absorption of curcumin using everted intestinal sacs and found that 30 to 80% of the added sample disappeared from the mucosal side of the sacs whereas *in vivo* studies indicated that nearly 40% of the curcumin dose was excreted unchanged in the faeces and curcumin could not be detected in the urine, blood, liver or kidney.

Based on the reported works,[187-190] it is difficult to draw any conclusion about the fate of curcumin *in vivo*. After oral administration in rats, it seems likely that curcumin to a certain extent is metabolised in the liver and its metabolites are mainly excreted *via* the bile and the faeces. The amount of curcumin dose that is excreted unchanged and the exact metabolites of curcumin are not fully established.[99,100]

Toxicological studies on curcuminoids

Turmeric and curcuminoids are present in most habitual Indian diets as a part of the spices used in the traditional cooking and no ill effects have been observed. However, the FAO/WHO Expert Group did recommend that turmeric and curcuminoids should be properly evaluated when listed as a permitted food colourant. A temporary average daily intake (ADI) of 2.5 mg/kg body weight is set for turmeric.

Acute toxicity studies on turmeric in animals indicated no toxic effects of the drug even at high doses.[192-194] Further, short- and long-term studies in dogs, mice and rats did not reveal any adverse cytogenic and mutagenic effects compared with controls when curcumin was incorporated into the diets in amounts normally consumed by man.[195] Some attention has been given to mutagenicity studies recently and curcumin itself exhibited no mutagenic effects in the salmonella/mammalian microsome test.[190] The above studies thus indicate that both turmeric and curcuminoids are toxicologically safe even in doses far beyond the ADI given by FAO/WHO.[196]

Metal complexes of curcuminoids

In a typical Hindu religions ceremony, turmeric is mixed with $Ca(OH)_2$ and rice. In this process the natural yellow colour of turmeric turns to a deep red colour. The colour change may be due to the interaction of curcuminoids with calcium ions. The Ca^{2+} ions may replace either enolic/phenolic proton, and changes the chromophoric group. A gold(I) complex of curcumin 1 was reported to possess antiarthritic activity.[109] Recently synthesis and characterization of stable Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+} and Pd^{2+} complexes of some synthetic curcuminoids were appeared in the literature.[94,95] Antitumour studies of metal chelates of these synthetic curcuminoids were also reported.[197-199]

Importance of the present investigation

Coordination chemistry of biologically important plant products have gained considerable importance in recent years. This is evident from the numerous reports on medicinal and other aspects of curcuminoids and allied derivatives. However metal complexes of curcuminoids have not received as much attention as they deserve. **The present investigation is mainly on the synthesis and characterisation of metal complexes of a series of synthetic curcuminoid analogues. The antioxidant and cytotoxic activities of these curcuminoids and their metal complexes were also studied in detail.**

METAL COMPLEXES OF 1,7-DIARYLHEPTA-1,6-DIENE-3,5-DIONES

Synthesis, characterisation and cytotoxicity of a number of 1,7-diarylheptanoids and some of their divalent transition metal complexes were reported earlier. In this chapter the synthesis and characterisation of certain trivalent metal complexes of some of the 1,7-diarylheptanoids are presented. Many of the properties of these synthetic curcuminoids are associated with the nature of the aryl groups and also on the conjugated diketo function. In order to study the effect of conjugation on various properties, synthesis and characterisation of a new 1,7-diarylheptanoid having anthracene rings and their typical metal complexes are also considered in this chapter. For convenience this chapter is divided into three sections.

Section 1 Synthesis and characterisation of some trivalent metal complexes of 1,7-diarylhepta-1,6-diene-3,5-diones.

Section 2 Synthesis and characterisation of 1,7-dianthrylahepta-1,6-diene-3,5-dione and its metal complexes.

Section 3 Synthesis and characterisation of trivalent metal complexes of 1,7-bis (substituted aryl)hepta-1,6-diene-3,5-diones.

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[200] were employed for physical and physico-chemical measurements.

The metal salts employed for the synthesis of metal complexes are $CuSO_4.5H_2O$, $Al(NO_3)_3.9H_2O$, $CrCl_3.6H_2O$ and $FeCl_3.6H_2O$.

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

Instruments

Instruments used in this investigation are:

1. **UV-1-550** Jasco recording spectrophotometer.
2. Jasco 4100 FTIR spectrometer.
3. Varian 300 nmr spectrometer.
4. Jeol 400 nmr spectrometer

5. Jeol sx-102 (FAB) mass spectrometer

6. Heraeus CHN-O-rapid analyser

7. Perkin Elmer 2380-Atomic absorption spectrophotometer

8. 1001 spectronic, Bauch and Lomb spectrophotometer

9. Systronic pH meter

10. Toshniwal conductivity bridge

11. Sherwood magnetic susceptiblity balance

12. RIA well type γ-ray counter

Methods

Elemental analysis: Metal complexes were analysed by standard methods.[201] Metal percentages were recorded using atomic absorption spectrophotometer after decomposing them with concentrated sulphuric-nitric acid mixture. Carbon, hydrogen and nitrogen percentages reported are by microanalysis carried out at RSIC, CDRI, Lucknow.

Uv-visible spectra were recorded from solution (10^{-3}M) of compounds in ethanol unless otherwise mentioned.

Infrared spectra of compounds were recorded from discs with KBr. Bands were calibrated using the nearest polystyrene bands.

^{1}H nmr spectra were recorded using $CDCl_3$/dmso-d_6 as solvents and TMS as internal reference.

FAB mass spectra were recorded at room temperature using Argon (6KV, 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, 307. If metal ions such as Na^+ are present these peaks may be shifted accordingly.

EI mass spectra were recorded by imparting vapourised sample molecules with a beam of electrons at 70 eV.

Molar conductance[202] of the complexes were determined in dmf at 28 ± 1^oC using solution of about $1x10^{-3}$ M.

Magnetic susceptibility was determined at room temperature (28 ± 1^oC) using $Hg[Co(NCS)_4]$ as standard.[203]

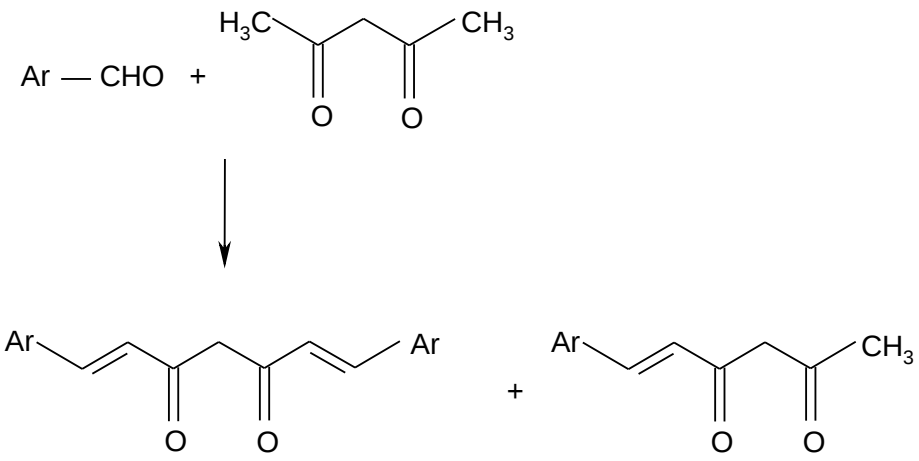
SECTION 1

Synthesis and characterisation of some trivalent metal complexes of 1,7-diarylhepta-1,6-diene-3,5-diones

Experimental

Synthesis of the 1,7-diarylheptanoids

The 1,7-diarylheptanoids were synthesised by the condensation of aromatic aldehydes (benzaldehyde, cinnamaldehyde, naphthaldehyde and furfural) with acetylacetone as reported for the synthesis of curcuminoids.[94] The reaction (scheme 2.1) leads to the formation of 1,7-diarylheptanoid as the major product (75%) along with small amount of 6-arylhexanoid (the monocondensation product). The two products were separated through column chromatography. A typical procedure for the synthesis and purification are given below.



Scheme 2.1

Acetylacetone (0.005 mol, 0.5 g) was stirred for ~ 1 h with boric oxide (0.0035 mol, 0.25 g) to obtain acetylacetone-boron complex. To this reaction mixture the aldehyde (0.01 mol) dissolved in dry ethylacetate (7.5 mL) containing tri(sec-butyl)borate (0.002 mol, 4.6 g) were added and the temperature was kept above 80°C. The reaction mixture was stirred and while stirring *n*-butylamine (0.1 mL dissolved in 1 mL dry ethylacetate) was added dropwise during 40 min. Stirring was continued for an additional period of ~ 4 h and the solution was set aside overnight. Hot (~ 60°C) hydrochloric acid (0.4 M, 7.5 mL) was added and the mixture again stirred for ~ 1 h. Two layers were separated and the organic layer was extracted (three times) with 5 mL of ethylacetate. The combined extracts were evaporated and the residual paste was stirred with HCl (50% 10 mL) for ~ 1 h. The solid product separated was collected, washed with water and dried in vacuum.

The product obtained was a mixture of 1,7-diarylheptanoid and 6-arylhexanoid and were quantitatively separated by column chromatography using silicagel (60-120 mesh) as detailed below.

The solid product obtained on acidification was dissolved in minimum quantity of ethylacetate and placed over the column (2 x 100 cm) densely packed with silicagel. The eluting solvent used was 1:5 (v/v) acetone : chloroform mixture. As the elution proceeds, two bands were developed in the column, a pale yellow lower band and an yellow to orange red upper band.

The eluate from the pale yellow lower region and the junction between the two bands were discarded.

The elution was then repeated by using a 1:2 (v/v) mixture of acetone and chloroform to recover the orange red band retained in the upper portion of the column. The eluates were collected in aliquots of 10 mL in separate tubes, checked by tlc and the combined extracts on removing the solvent in vacuum yielded 1,7-diarylheptanoids (50-70%). The isolated 1,7-diarylheptanoids were recrystallised twice from hot benzene to get chromatographcally (tlc) pure material.

Synthesis of metal chelates

The general procedure adopted for the preparation of aluminium(III), iron(III) and chromium(III) complexes is given below.

A methanolic solution (25 mL) of the metal salt (0.001 mol) was added slowly with stirring to a solution of the 1,7-diarylheptanoid (0.003 mol) in methanol (25 mL). The mixture was stirred well and refluxed gently for ~ 1 h and the volume was reduced to half. On cooling to room temperature the complex gets precipitated. The precipitated product was filtered, washed with 1:1 methanol-water mixture and dried. For purity, the compounds were recrystallised from hot methanol.

Results and Discussion

Characterisation of 1,7-diarylheptanoids

All the 1,7-diarylheptanoids synthesised are crystalline in nature, show sharp melting points and are freely soluble in common organic solvents. Synthetic details such as the aldehyde used, yield, systematic name, etc. are given in **table 2.1**. Synthesis and characterisation of some of these 1,7-diarylheptanoids have been reported. However in order to ease the characterisation of the metal complexes considered in this investigation, the electronic, ir, nmr and mass spectral data of the compounds were obtained and discussed below.

The UV spectra of the compounds in methanol shows two absorption maxima in the 350-400 nm and 250-290 nm assignable respectively to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions (**Table 2.2**). The λ_{max} values increases with increase in the degree of conjugation.

TABLE 2.1

Synthetic details of the 1,7-diarylheptanoids (1a-d)

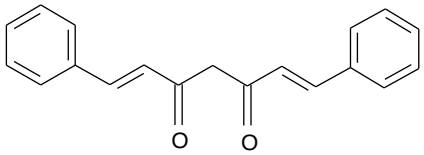
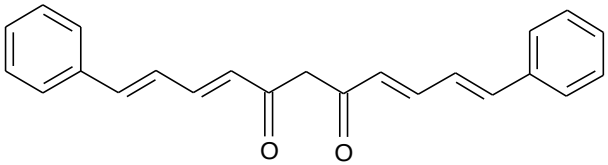
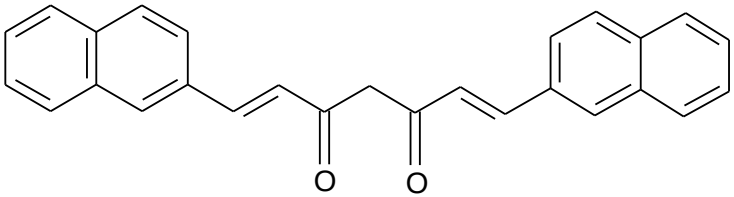
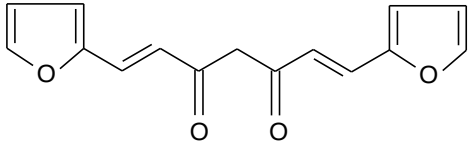
Compounds	Aldehyde used for synthesis	Structure	Systematic name	Yield
1a	Benzaldehyde		1,7-diphenyl-1,6-heptadiene-3,5-dione	62%
1b	Cinnamaldehyde		1,11-diphenyl-1,3,8,10-undecatetraene-5,7-dione	65%
1c	2-Naphthaldehyde		1,7-dinaphthyl-1,6-heptadiene-3,5-dione	68%
1d	Furfuraldehyde		1,7-difuryl-1,6-heptadiene-3,5-dione	60%

TABLE 2.2

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

Comp-ounds	MP (°C)	Elemental analysis (%)		Mol. weight	λ_{max} (nm)	log ∈
		C	H			
		Found / (Calcd)				
1a	160	82.41 (82.61)	5.76 (5.80)	275 (276)	258 358	4.05 4.39
1b	162	83.81 (84.14)	5.92 (6.08)	326 (328)	288 397	4.21 4.87
1c	165	85.70 (86.21)	4.98 (5.30)	373 (376)	260 386	4.09 4.72
1d	145	69.61 (70.28)	4.50 (4.69)	254 (256)	267 391	4.07 4.59

Infrared spectra

The problem of assigning correct tautomeric structure can be settled by means of ir spectra. In the compounds, 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 group is at ~ 1650 cm^{-1}. Hydrogen bonding decreases carbonyl frequency. A further shift of lower values can be observed in compound where C=O is in conjugation with C=C, etc. Thus in

the case of β-diketones and allied derivatives, the nature and position of carbonyl stretching bands can provide valuable information regarding their structure.[31,204]

The ir spectra of all the 1,7-diarylheptanoids are characterised by the presence of a strong band at ~ 1615 cm^{-1} (Table 2.3) and no other band is observed in the region 1600-1800 cm^{-1}. Since the compounds are highly conjugated, the carbonyl stretching frequency will shift to very low values. The possible enolisation of the carbonyl groups will also lower the ν(C=O) values. Thus considering the position and intensity, the band at ~ 1615 cm^{-1} can confidently be assigned to the enolised dicarbonyl function of the compounds.[205]

TABLE 2.3

Charactristic ir data (cm^{-1}) of 1,7-diarylheptanoids

Compounds				Probable assignments
1a	**1b**	**1c**	**1d**	
1612	1608	1620	1614	ν(C=O) chelated
1589	1574	1594	1578	ν(C=C) phenyl / ν(C=C) alkenyl
1559	1540	1581	1556	
1545	1528	1542	1544	
1520	1508	1506	1528	ν_{as} (C-C-C) chelate ring
1462	1448	1458	1444	ν_{s} (C-C-C) chelate ring
1109	1114	1134	1105	β (C-H) chelate ring
1076	1090	1080	1030	

976	995	969	972	ν(CH=CH) (*trans*)

Since there is no other band in the region assignable to free carbonyl group, it can be concluded that the compounds exist entirely in the enolic form. In the case of **1b**, where there is extension of conjugation compared to other compounds, the C=O stretching frequency further lowered to 1608 cm^{-1}. Several medium intensity bands observed in the region 1550-1600 cm^{-1} are due to various ν(C=C) vibrations.

Infrared spectra of the compounds are also charactrised by the *trans* –CH=CH– absorption which occurs in the region ~ 970 cm^{-1}. Band corresponding to free –OH of the enol group (~ 3600 cm^{-1}) is not observed in the spectra of the compounds. Instead a considerably broad band ranging from 2500-3600 cm^{-1} is found in the spectra of all the compounds. This suggests the presence of strong internal hydrogen bonding in all the compounds.

^{1}H nmr spectra

The ^{1}H nmr spectroscopy is perhaps the most efficient tool in studying the keto-enol tautomerism of 1,3-diketones. The resonance signal of a proton participating in strong intramolecular hydrogen bonding generally appears in the down field 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.[16]

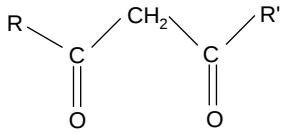
The chemical shift of methine and enolic proton is also indicative of the tautomeric nature of the compound under consideration. The chemical shift values of methylene proton and enolic proton of some substituted β-diketones (Table 2.4) show that these values are greatly influenced by the groups substituted.[41,42] 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 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. This shift is caused by strengthening of C=C bond attributable 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 group by resonance.

The ^{1}H nmr spectra of the 1,7-diarylheptanoids considered in this section displayed a one proton singlet at ~ 16 ppm and another singlet at ~ 5.9 ppm assignable respectively to the strong intramolecularly hydrogen bonded

enolic proton and to the methine proton. The observed downfield shift of the enolic protons of **1b** and **1c** may be due to the extended conjugation.

TABLE 2.4

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

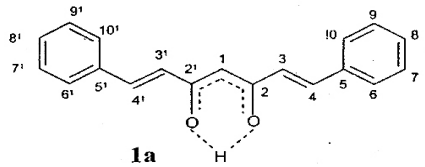
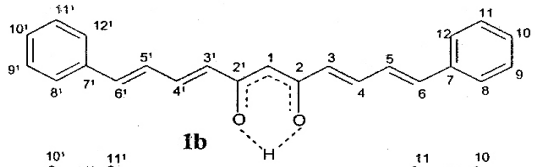
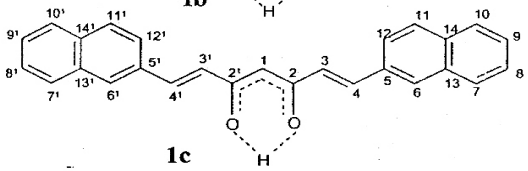
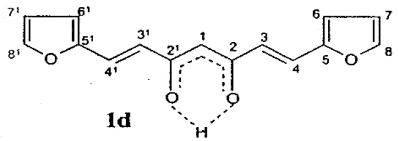


R	R'	Chemical shift (δ ppm)	
		Methylene proton	Enolic proton
CH_3	CH_3	5.44	15.40
CH_3	CF_3	5.90	14.24
CF_3	CF_3	6.43	13.00
C_6H_5	C_6H_5	6.80	17.13
CH_3	C_6H_5	6.08	16.24
CF_3	C_6H_5	6.56	15.23
2-C_4H_3S	CF_3	6.5	16.2

The *trans* orientation of the alkenyl protons are indicated from the observed J values (~ 16 Hz). The aromatic protons also showed characteristic chemical shifts. The characteristic ^{1}H nmr spectral data of the compounds are summarised in the table 2.5 and the spectrum of **1b** and **1c** are reproduced in **figures 2.1** and **2.2**.

Table 2.5

Characteristic ^{1}H NMR spectral data of 1,7- diarylheptanoids (1a-d)

Compound	enolic	1	3,3'	4,4'	5,5'	6,6'	7,7'	8,8'	9,9'	10,10'	11,11'	12,12'
 **1a**	16.86	6.8665	8.0063	7.9877	-	6.8638	-	-	6.8655	7.2598	-	-
 **1b**	16.043	5.7200	6.1760	6.9250	7.282	7.346	-	-	7.516	7.413	7.394	-
 **1c**	15.9892	5.9572	6.76	8.56	-	7.84	7.90	7.58	7.62	7.88	7.92	8.28
 **1d**	15.60	6.774	6.515	7.221	-	-	6.657	6.774	8.104	-	-	-

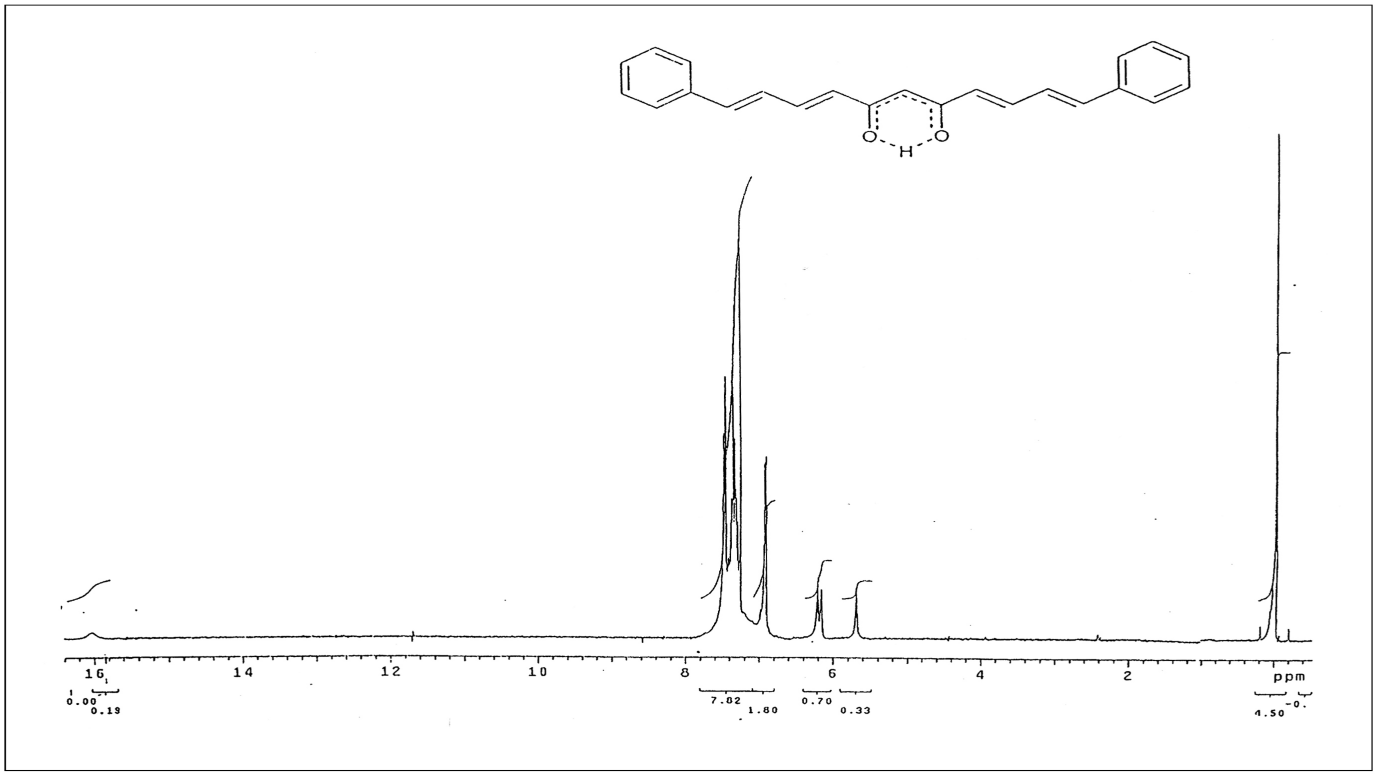


Fig.2.1 ^{1}H NMR Spectrum of 1b

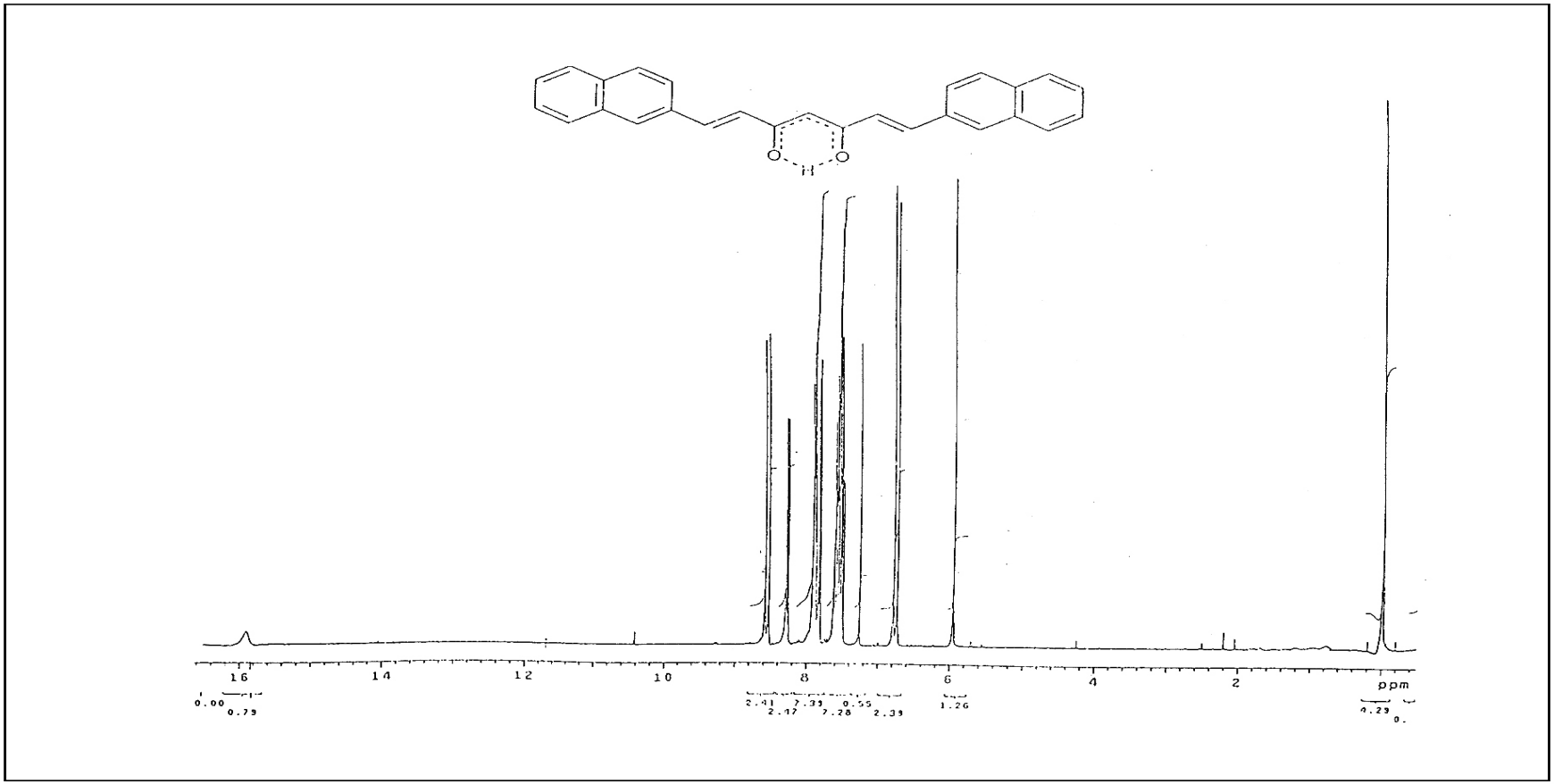


Fig.2.2 ^{1}H NMR Spectrum of 1c

Mass spectra

Several reports are available on mass spectral studies on diverse types of 1,3-dicarbonyl compounds. The fragmentation patterns depend mainly on the nature of groups attached to the diketo function. For example, elimination of CO, O, OH, CH_2=C=O (ketene), etc. are characteristic of acetylacetone and related 1,3-diketones. The mass spectral fragmentation pattern of 1,3-diketones are well established.[44-46]

Mass spectra of all the 1,7-diarylheptanoids show intense molecular ion peaks $P^+/(P+1)^+$. Elimination of O, OH, CO, CH_2O, $C_3HO_2^+$ from the molecular ion are clearly evident from the observed spectra. The spectra of **1b** and **1c** are brought out in **figures 2.3** and **2.4**. Important peaks appeared in the spectra of all the 1,7-diarylheptanoids can be conveniently accounted by the fragmentation patterns given in **Scheme 2.2**.

Characterisation of metal chelates of the 1,7-diarylheptanoids

Elemental analysis (C, H and metal percentages) and physical data of the metal complexes are given in **tables 2.6-2.8**. The analytical data suggest 1:3 metal-ligand stoichiometry of the complexes. Conductometric studies show that all the complexes behave as non electrolytes in dmf (specific conductance < $10\Omega^{-1}cm^{-1}$ in 10^{-3}M solution) and do not contain the anion of the metal salt used for the preparation. The observed uv, ir, nmr and mass spectra are in agreement with the structure **2.1** of the complexes.

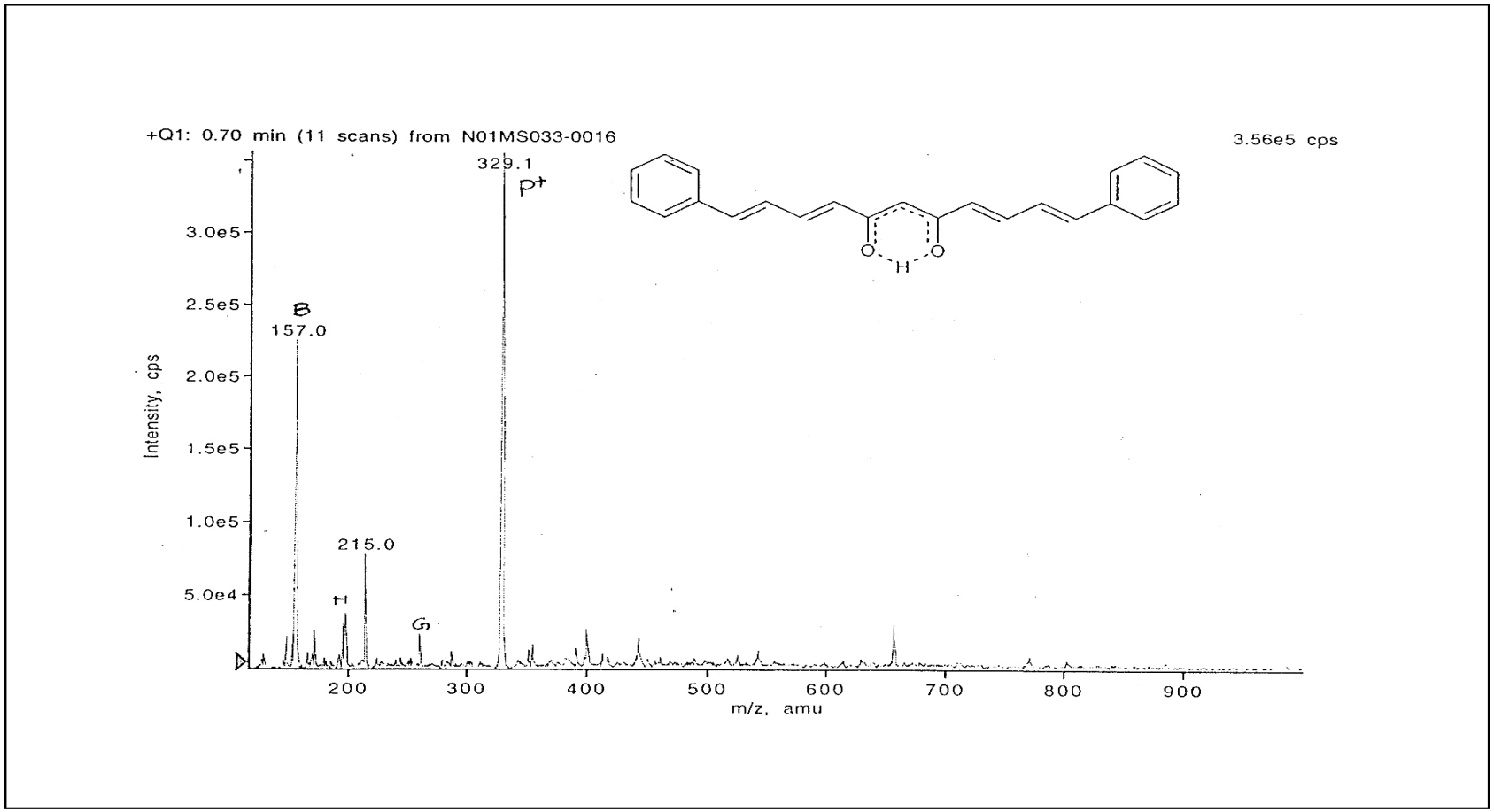


Fig.2.3 Mass Spectrum of 1b

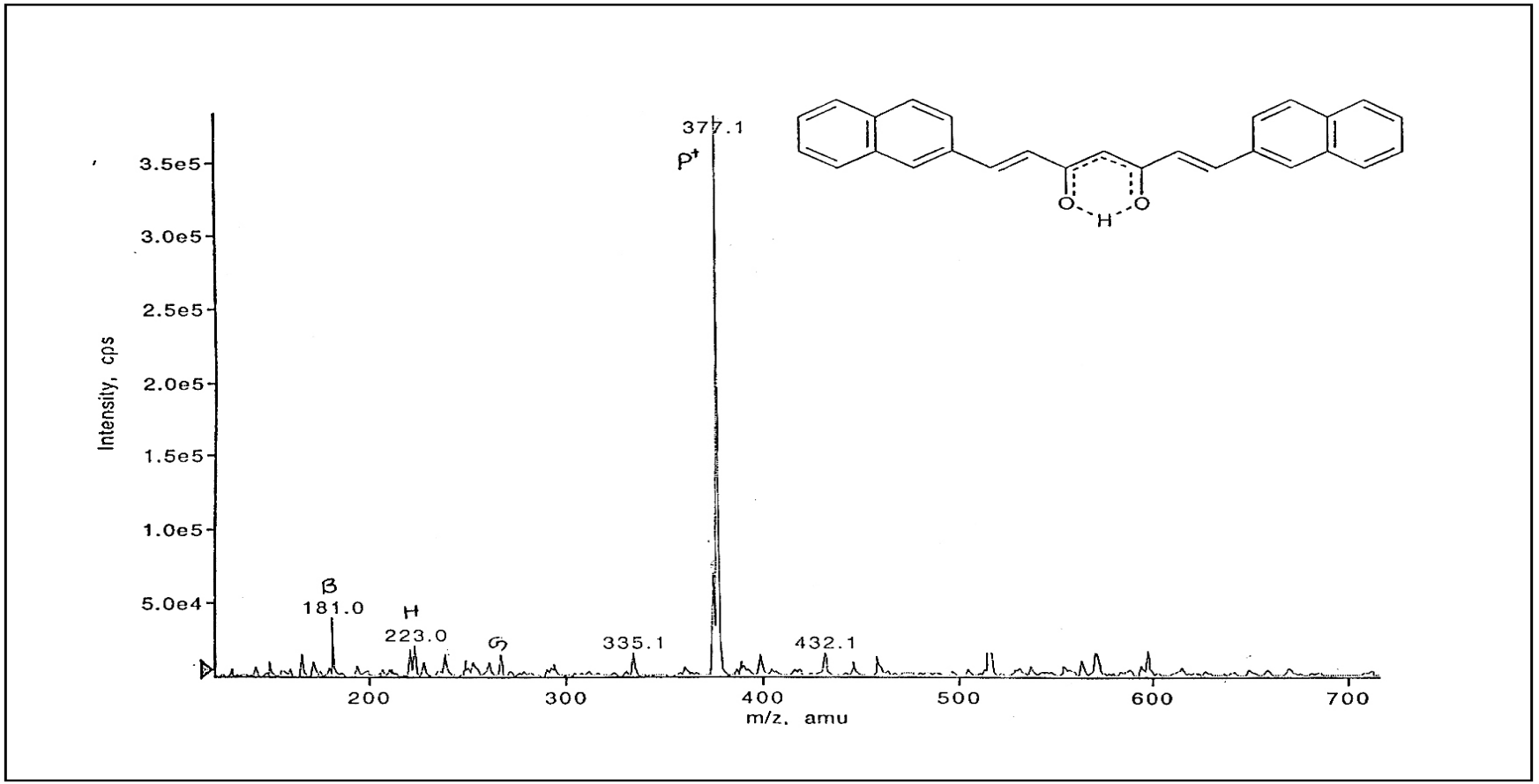
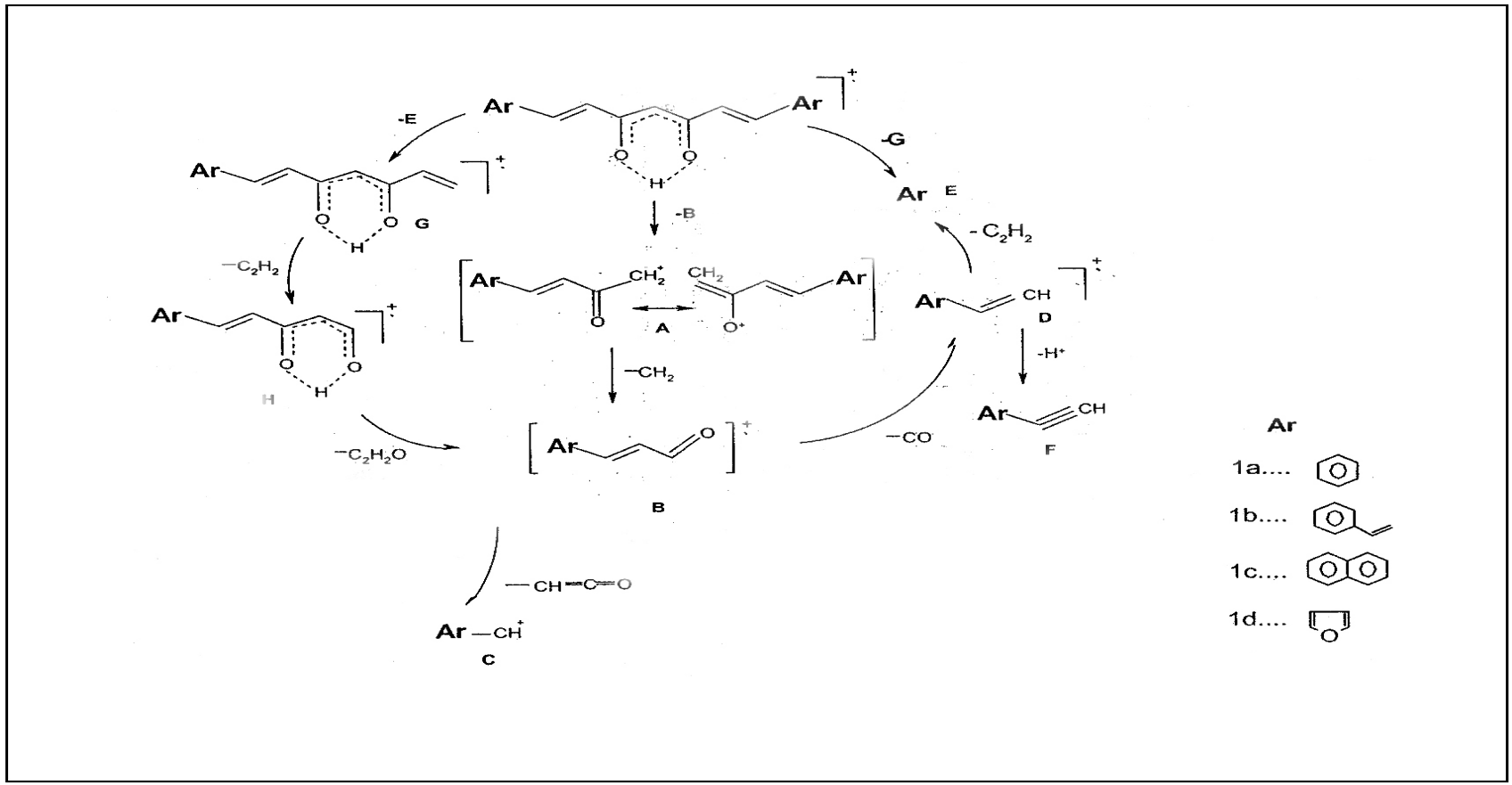


Fig.2.4 Mass Spectrum of 1c



Scheme 2.2 Fragmentation pattern of 1a-d

TABLE 2.6

Analytical and characteristic ir spectral data of Cr(III) chelates of 1,7-diaryl heptanoid (HL)

Chromium(III) chelates	M.P. (°C)	μ_{eff} B.M.	Elemental analysis (%) Found / (Calcd.)*			Characteristic ir stretching bands (cm^{-1})		
			C	H	Cr	ν(C=O)	(C=C)	(M-O)
1a	208	3.65	78.82 (77.99)	5.91 (5.13)	5.04 (5.93)	1595	1555, 1540, 1516	497 406
1b	217	3.71	80.98 (80.15)	5.03 (5.52)	5.64 (5.03)	1593	1562, 1545, 1514	440 492
1c	220	3.58	81.97 (82.58)	4.26 (4.84)	4.93 (4.18)	1578	1560, 1550, 1510	475 426
1d	197	3.70	66.62 (66.09)	4.28 (4.04)	6.96 (6.36)	1587	1565, 1548, 1513	475 416

*The calculated value corresponds to the $[CrL_3]$ composition where L stands for the deprotonated ligand.

TABLE 2.7

Analytical and characteristic ir spectral data of the iron(III) chelates

Iron(III) chelates of	M.P. (°C)	μ_{eff} B.M.	Elemental analysis (%) Found / (Calcd.)*			Characteristic ir stretching bands (cm^{-1})		
			C	H	Fe	ν(C=O)	(C=C)	(M-O)
1a	209	5.85	77.82 (76.29)	5.32 (5.11)	6.79 (6.34)	1594	1558, 1542, 1524	488 426
1b	219	5.95	80.14 (79.86)	5.68 (5.50)	5.58 (5.39)	1538	1552, 1550, 1518	469 424
1c	222	5.82	81.24 (82.31)	4.78 (4.83)	4.61 (4.73)	1576	1560, 1540, 1519	472 423
1d	186	5.78	66.84 (65.79)	4.12 (4.02)	6.76 (6.80)	1587	1568, 1555, 1516	482 428

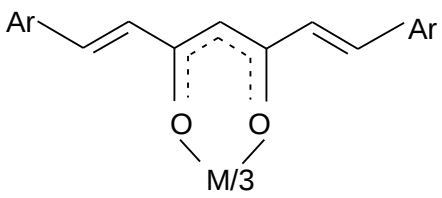
*The calculated value corresponds to the $[FeL_3]$ composition where L stands for the deprotonated ligand.

TABLE 2.8

Analytical and characteristic ir spectral data of the aluminium(III) chelates of 1,7-diaryl heptanoid

Aluminium(III) chelate of	M.P. (°C)	Elemental analysis (%) Found / (Calcd.)*			Characteristic ir stretching bands (cm^{-1})		
		C	H	Al	ν(C=O)	(C=C)	(M-O)
1a	194	80.10 (80.26)	5.01 (5.28)	3.20 (3.11)	1586	1565, 1550, 1516	486 416
1b	192	81.92 (82.24)	5.62 (5.65)	2.50 (2.67)	1584	1560, 1552, 1514	464 421
1c	197	83.51 (84.37)	4.56 (4.94)	2.20 (2.34)	1580	1568, 1555, 1513	478 416
1d	169	67.60 (68.18)	4.02 (4.16)	3.26 (3.41)	1592	1560, 1545, 1510	469 424

*The calculated value corresponds to the $[AlL_3]$ composition where L stands for the deprotonated ligand.



2.1

M = Fe(III), Cr(III) and Al(III)

Uv spectra

The uv spectra of the complexes clearly resembles to the respective ligands indicating that no structural alterations has taken place during complexation. The slight shift of absorption maxima to longer wavelength indicate the involvement of the carbonyl oxygens in metal complexation.

Infrared spectra

The use of vibrational spectra in establishing the structure and nature of bonding in numerous metal 1,3-diketonates has been well illuminated. 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 information can be derived. In general, upon complexation, the carbonyl stretching frequency of 1,3-diketones show a shift (10-50 cm^{-1}) to lower values and additional bands due to ν(M-O) vibrations appear in the region 400-500 cm^{-1}.

In the spectra of metal complexes of the 1,7-diarylheptanoids, the strong band due to hydrogen bonded carbonyl function at ~ 1615 cm^{-1} disappeared. Instead a prominent band appeared at ~ 1595 cm^{-1} which can be assigned as due to the metal bonded carbonyl group. Alkenyl and aromatic (C=C) stretching frequency appears between 1530-1585 cm^{-1} (Tables 2.6-2.8) The broad absorption of the free ligands in the region 2700-3500 cm^{-1} cleared up in the spectra of metal complexes, only weak band due to various ν(C-H) observed in the region. Metal-oxygen stretching frequencies ν(M-O) appeared at ~ 480 cm^{-1} and ~ 415 cm^{-1}. A band at ~980 cm^{-1} due to *trans*-CH=CH- remain unaffected in the metal complexes also.

^{1}H nmr spectra

In the ^{1}H nmr spectra of the diamagnetic aluminium(III) complexes of the 1,7-diarylheptanoids **1b** and **1c** (**figures 2.5 and 2.6**) the lowfield enolic proton singlet of the free ligand is absent. Methine proton singlet and aromatic proton signals shifted slightly to downfield. The doublets of *trans* alkenyl proton with higher J value also remain unaffected. The integrated intensities of all the protons agree with the formulation of the complexes.

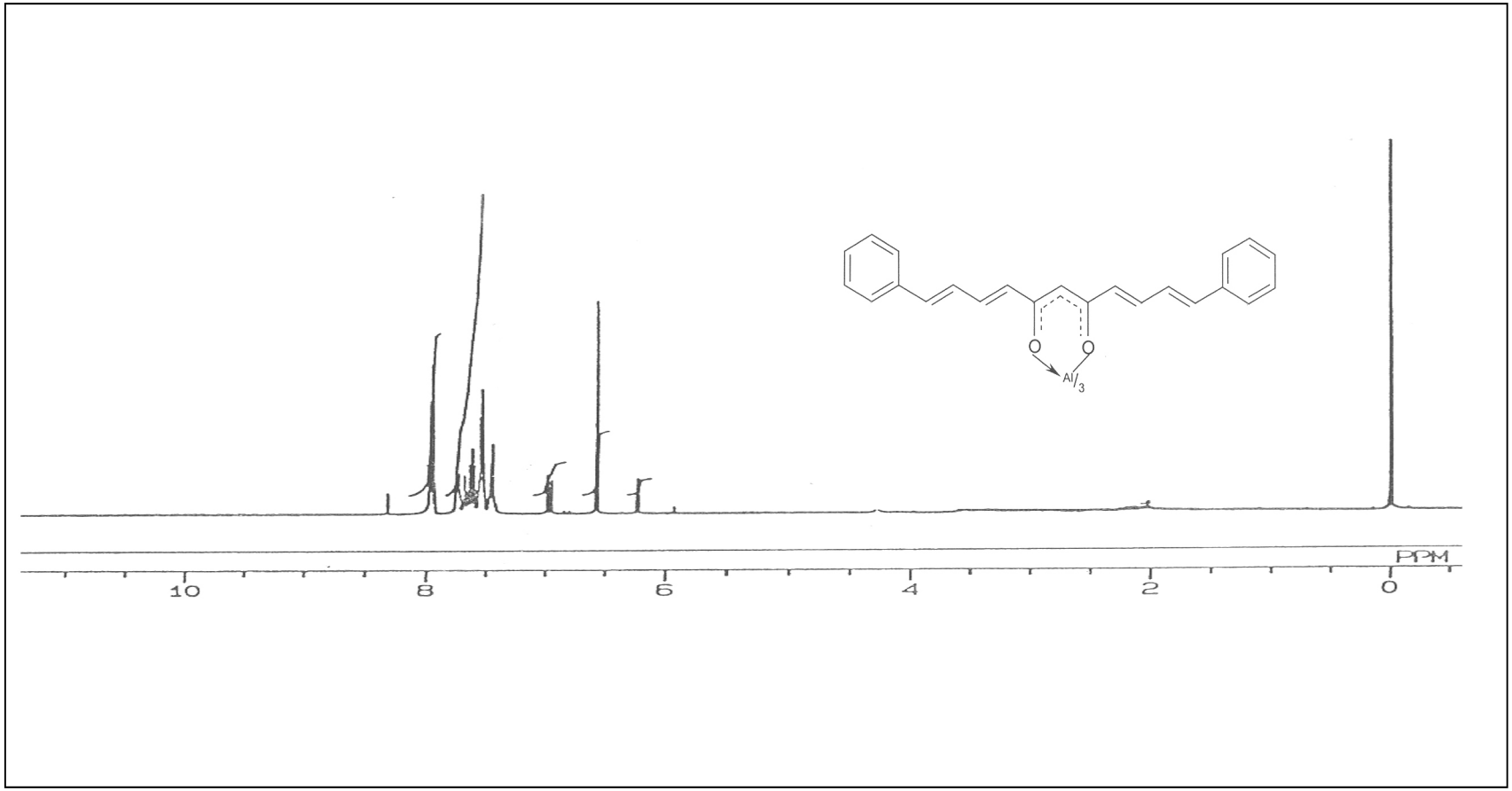


Fig.2.5 Mass ^{1}H NMR spectrum of Aluminium (III) complex of Ib

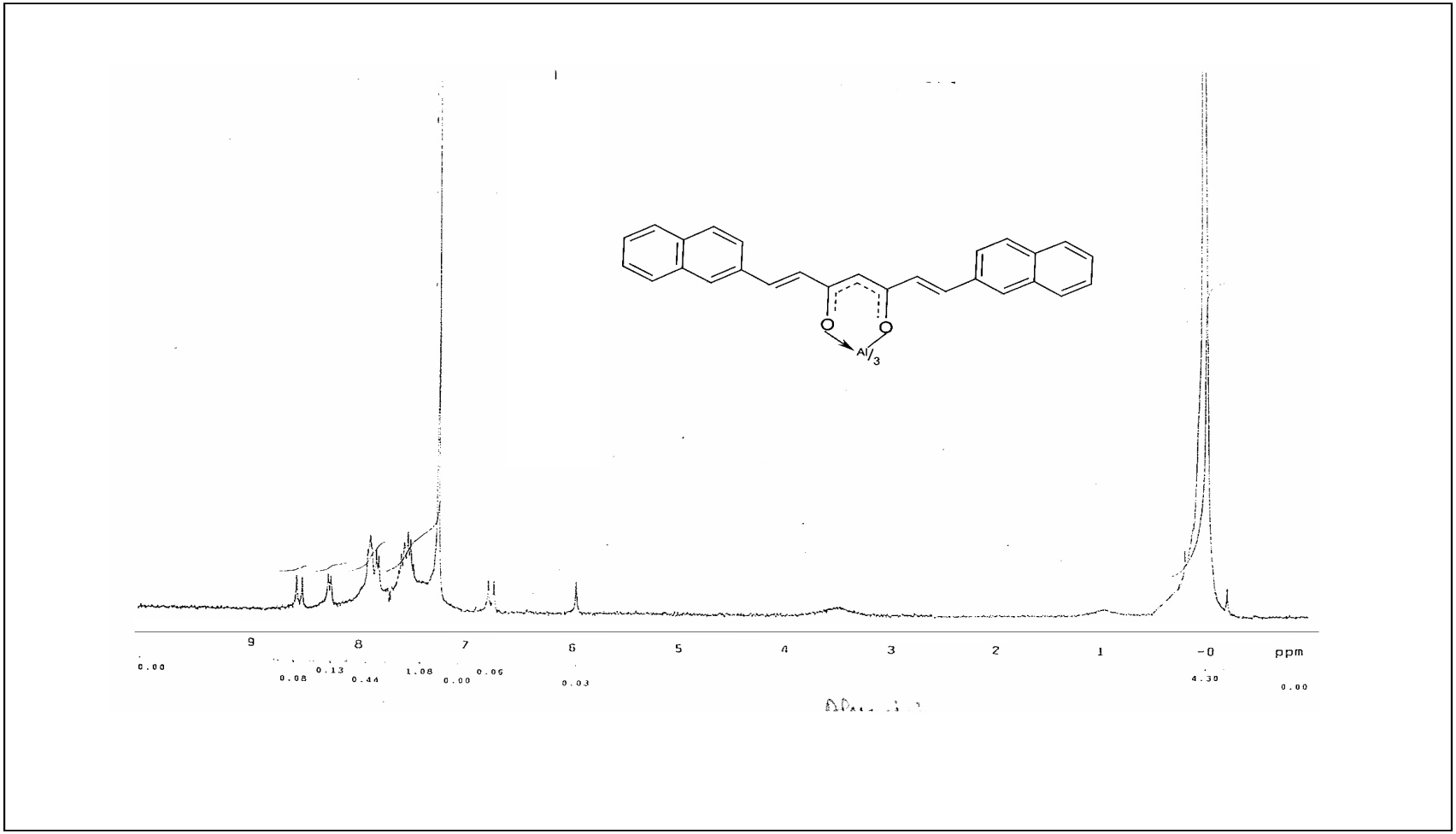


Fig.2.6 Mass ^{1}H NMR spectrum of Aluminium (III) complex of Ic

Mass spectra

The potential of mass spectrometry for the establishment of the stoichiometry and in structure elucidation of coordination compounds has been well demonstrated in the case of metal 1,3-diketonates and the mass spectral fragmentation patterns of metal 1,3-diketonates are well documented.[17] 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.[206] Electronic and steric effects of the group(s) attached to the dicarbonyl function also influence the stability of various fragments formed under mass spectral conditions. The mass spectrum of the iron(III) complex of **1a** given in **Fig. 2.7** clearly indicates $[FeL_3]$ stoichiometry of the compounds. Peaks due to $[ML_2]^{2+}$, $[ML]^{+}$, L^{+}, $[M_2L]^{+}$ and fragments of L^{+} are also detected in the spectra.

The spectrum of the Al^{3+} complexes of **1a** also support the formulation of the complexes (**figure 2.8**).

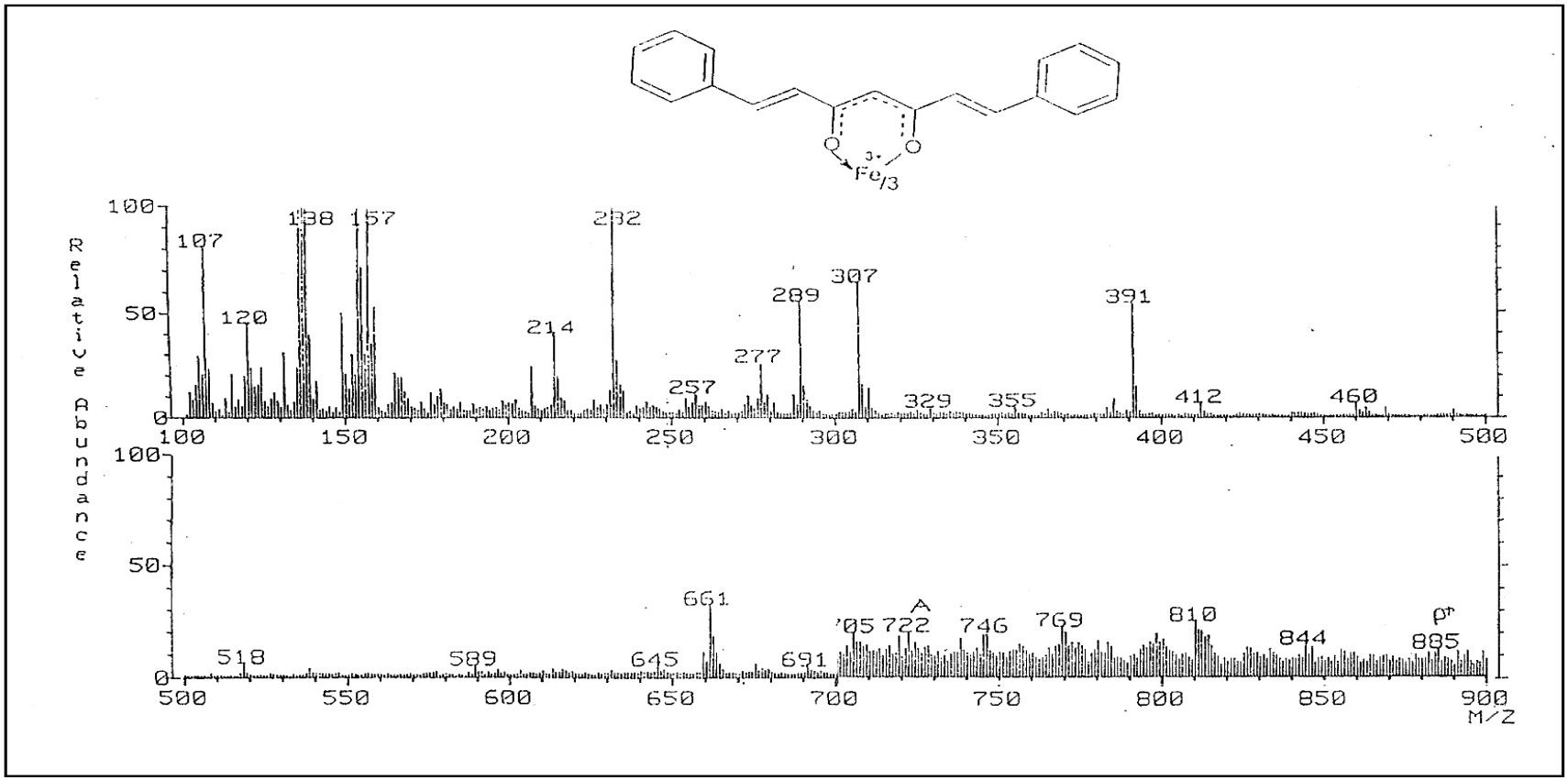


Fig.2.7 Mass spectrum of Iron (III) complex of 1a

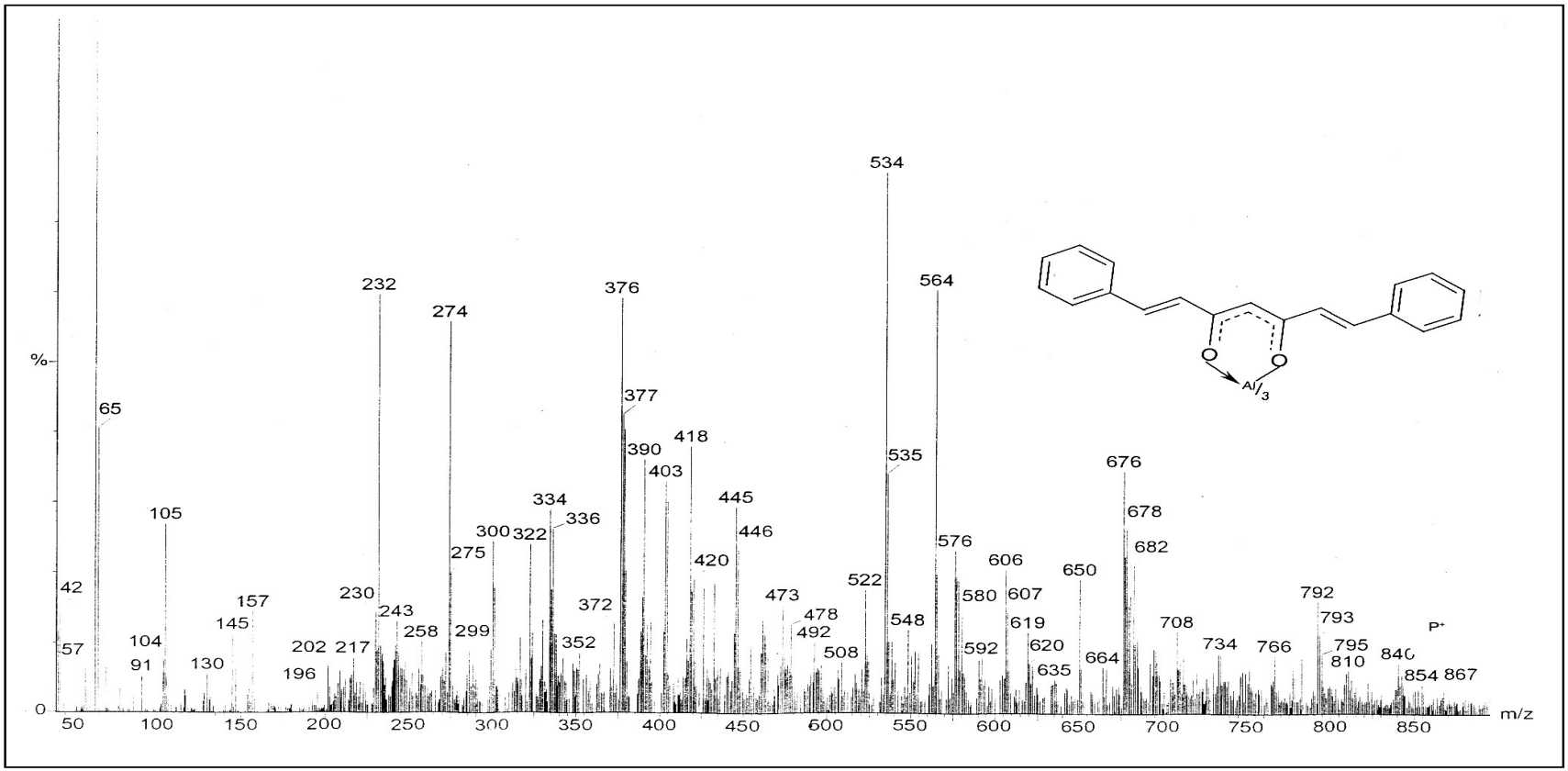


Fig.2.8 Mass spectrum of Aluminium (III) complex of 1a

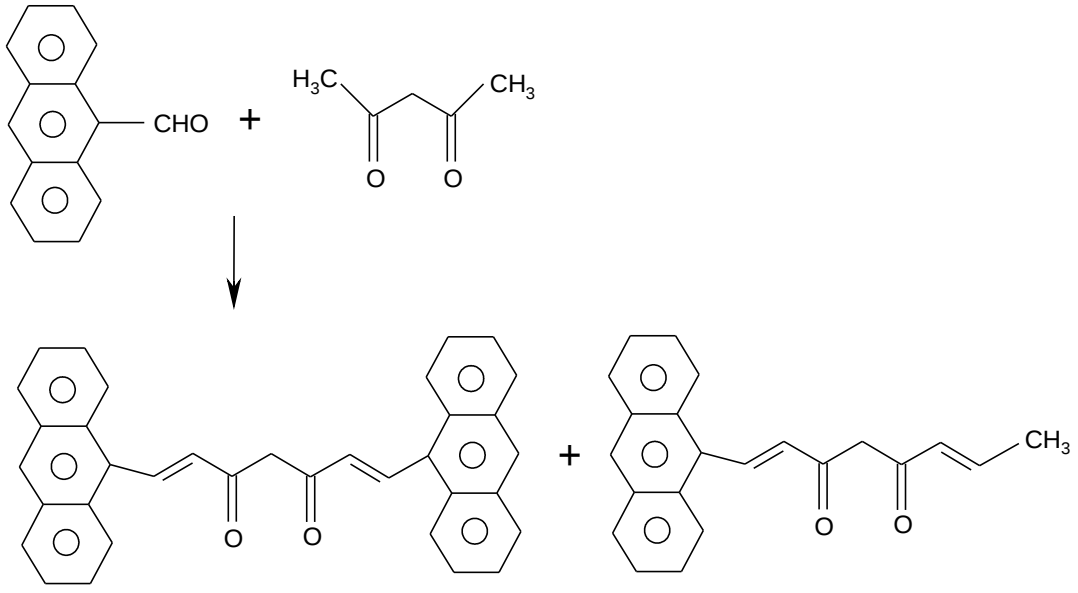
SECTION 2

Synthesis and characterisation of 1,7-dianthryl-1,6-heptadiene-3,5-dione and its metal complexes

Experimental

Synthesis of 1,7-dianthryl heptanoid, Hdah

The compound was synthesised by the condensation of anthracene-9-aldehyde with acetyl acetone in presence of boric oxide and tri(sec-butyl) borate using n-butyl amine as the condensing agent. The reaction leads to the formation of 1,7-dianthrylheptanoid **1** as the major product along with small amounts of 6-anthrylhexanoid **2**. Pure 1,7-dianthryl heptanoid was separated by column chromatography. The procedural details are outlined below:



1 **2**

Acetylacetone (0.5 ml, 0.005 mol) and boric oxide (0.25 g, 0.0035 mol) were stirred for ~ 1 h to yield acetylacetone-boron complex. To this, a solution of anthracene-9-aldehyde (0.01 mol) in dry ethyl acetate (7.5 ml) and tri(sec-butyl) borate (4.6 ml, 0.02 mol) were added and stirred while maintaining temperature at about 80^{o}C. n-Butyl amine (0.1 mL) was added dropwise with stirring and the stirring was continued for ~ 4 h and the solution was set aside overnight. The mixture was again stirred for ~ 1 h by adding hot (~ 60^{o}C) hydrochloric acid (0.4 M, 7.5 mL), and extracted with ethyl acetate. The combined extracts were evaporated and the residual paste was stirred with HCl (50%, 10 mL) for ~ 1 h. The solid product separated was collected, washed with water and dried in vacuum. The quantitative separation of the 1,7-dianthryl heptanoid from 6-anthrylhexanoid was done by column chromatography using silica gel (mesh 60-120) as detailed below.

The solid product obtained was dissolved in minimum quantity of ethyl acetate and placed over a column (2 x 100 cm) densely packed with silica gel. The elution was done by 1.5 v/v acetone-chloroform mixture. As the elution proceeds, two bands were developed in the column, a lower band of pale yellow and a upper band of yellow to orange red. The eluates from the lower band and the junction between the two bands were discarded.

The elution was then continued using a 2:1 v/v chloroform-aetone mixture to recover the upper orange-yellow band. The eluates were collected

at regular intervals, checked by tlc, and the combined extracts on removing the solvent in vacuum yielded 1,7-dianthrylheptanoid (**Hdah**). The compounds were further purified by recrystallisation from hot benzene.

Synthesis of metal complexes

The nickel(II), cobalt(II), copper(II), chromium(III), iron(III) and aluminium(III) complexes of the 1,7-dianthryl-1,6-heptadiene-3,5-dione (**Hdah**) were prepared as follows.

A methanolic solution (25 mL) of the metal salt (0.001 mol) was added slowly with stirring to a solution of the 1,7-dianthryl heptanoid (0.002 mol) in methanol (25 mL). The mixture was stirred well and refluxed gently for ~ 1 h and the volume was reduced to half. On cooling to room temperature, the complex gets precipitated. The precipitated product was filtered, washed with 1:1 methanol-water mixture and dried. For purity, the compound was recrystallised from hot methanol.

Characterisation of 1,7-dianthryl-1,6-heptadiene-3,5-dione and its metal complexes

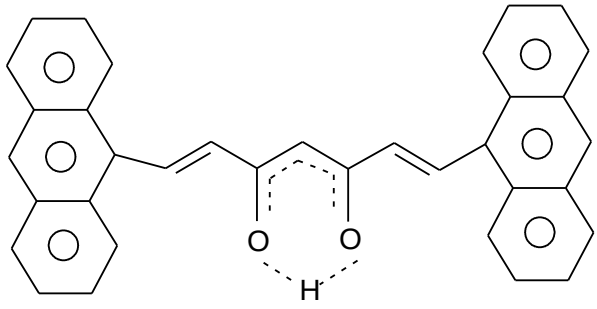
Physical and analytical data of 1,7-dianthryl-1,6-heptadiene-3,5-dione, **Hdah**, and its metal complexes are given in **table 2.9**.

TABLE 2.9

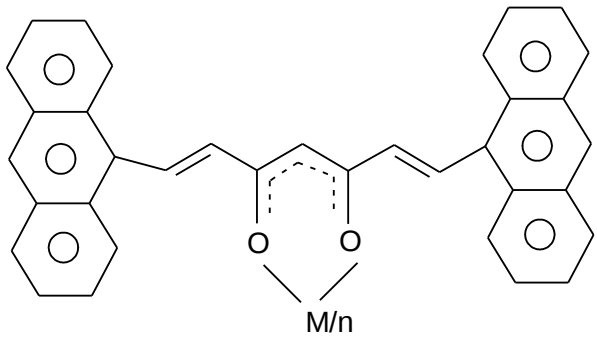
Physical and analytical data of Hdah and its metal complexes

Compound	M.P. °C	$\mu_{eff.}$ (B.M.)	Elemental Analysis (%) Found/(Calcd.)		Characteristic ir stretching bands (cm^{-1})		
			C	H	C=O	C=C–	M–O
Hdah	170		85.34 (86.81)	4.89 (5.41)	1610	1590	
$[Co(dah)_2(H_2O)_2]$	205	4.75	77.83 (78.64)	5.41 (5.35)	1595	1515	470 420
$[Ni(dah)_2]$	220	--	81.05 (81.72)	4.83 (4.68)	1585	1520	468 432
$[Cu(dah)_2]$	215	1.85	80.52 (81.13)	4.73 (4.55)	1578	1514	475 425
$[Fe(dah)_3]$	227	5.78	82.17 (83.12)	4.43 (4.52)	1590	1510	475 415
$[Cr(dah)_3]$	> 300	5.55	80.25 (81.55)	4.85 (4.77)	1580	1512	465 418
$[Al(dah)_3]$	> 300	--	75.45 (74.35)	4.75 (4.58)	1595	1520	470 420

All the complexes are non-conducting in DMF. The Cr(III), Fe(III), Co(II) and Cu(II) complexes are paramagnetic. These data conform to $[ML_2]$ stoichiometry of the Co(II), Ni(II) and Cu(II) complexes and $[ML_3]$ of Cr(III), Fe(III) and Al(III) complexes. The ir, nmr and mass spectral data of the complexes are in agreement with the structure **3** of the ligand and structure **4** of the $[ML_2]$ and $[ML_3]$ complexes. The spectral data are discussed below:



3



4

n = 2 for Co(II), Ni(II) and Cu(II)

n = 3 for Al(III), Cr(III) and Fe(III)

IR spectra

The ir spectrum of 1,7-dianthryl-1,6-heptadiene-3,5-dione is characterised by the presence of a strong band at 1610 cm^{-1} and no other bands appeared in the 1600-1800 cm^{-1} region. The band can confidently be assigned to the highly conjugated diketo function of structure **3**. The broad band in the 2800-3500 cm^{-1} region suggests strong intramolecular H-bonding of the enolic proton as in structure **3**. In the spectra of the metal complexes,

the broad free ligand band in the 2800-3500 cm^{-1} region cleared up and weak medium intensity bands appeared at ~ 3050 and ~ 2900 cm^{-1} due to various aliphatic and aromatic ν_{C-H} vibration. The band at 1610 cm^{-1} due to ν_{CO} of the ligand disappeared in the spectra of all the metal complexes and instead a very prominent band appeared at 1560 cm^{-1} assignable to metal bonded diketo function as in structure **4**. Further the presence of a medium intensity band at ~ 425 cm^{-1} due to ν_{M-O} vibrations also support structure **4** of the complex. Important ir bands and their probable assignments are brought out in table 2.1.

Nmr spectra

The ^{1}H nmr spectrum of the compound given in **figure 2.9** shows a one proton signal at 16.4 ppm assignable to the highly conjugated enolic proton of structure **3**. The olefinic, methylene and aromatic proton signals are shifted to low field compared to the corresponding signals of the 1,7-diarylheptanoids considered in section **1** as expected. In the spectra of the diamagnetic nickel(II) and aluminium(III) complexes the low field signal of the ligand disappeared indicating the replacement of the enolic proton by metal ion as in structure **4**. Integrated intensities and positions of all other protons agree well with the proposed structure of the complexes. The ^{13}C NMR spectrum of the compound (**figure 2.10**) show the number and position of signals as expected of structure **3**.

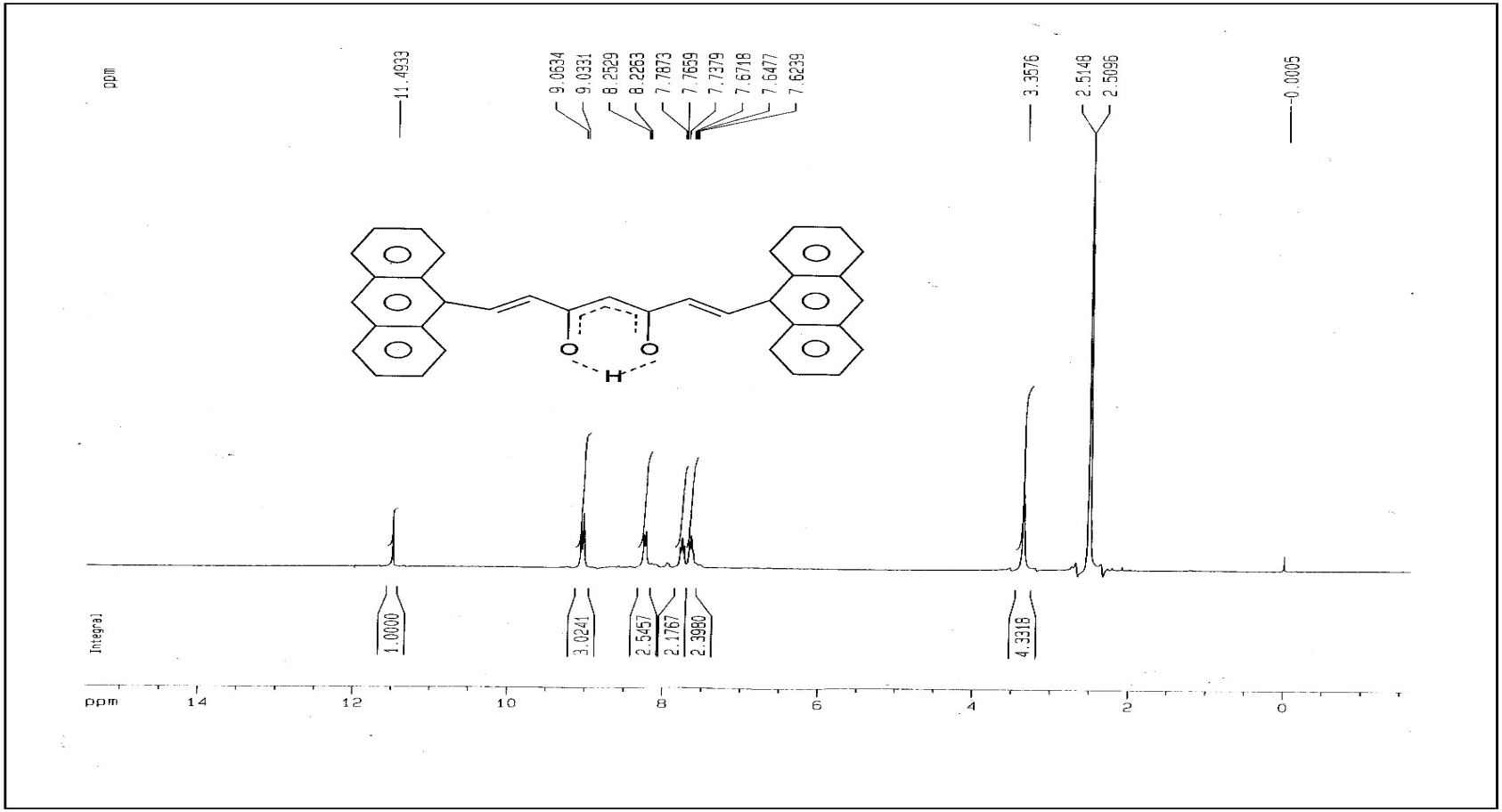


Fig.2.9 ^{1}H NMR spectrum of Hdah

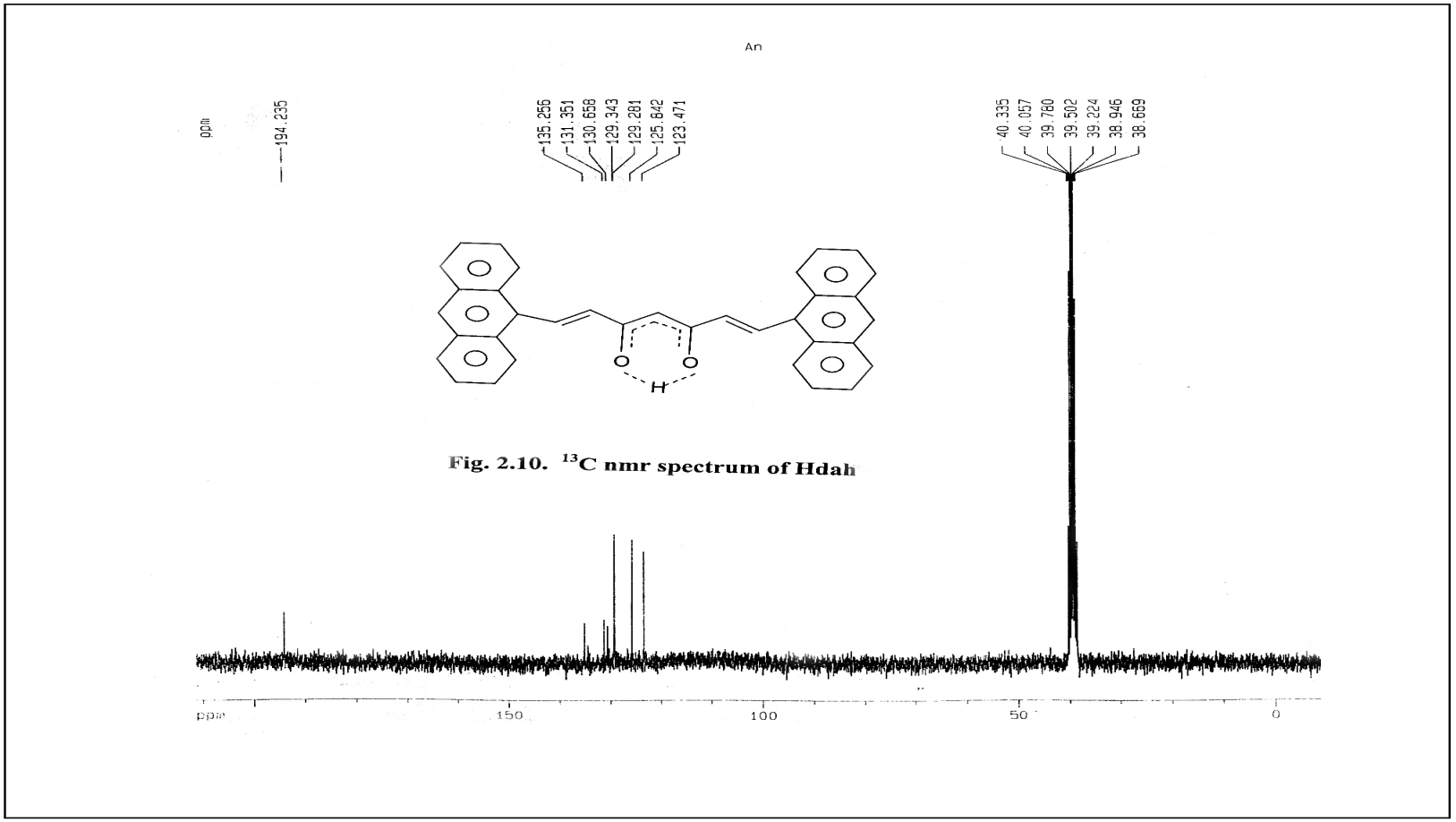


Fig. 2.10. ^{13}C nmr spectrum of Hdah

Fig.2.10 ^{13}C NMR spectrum of Hdah

Mass spectra

The FAB mass spectrum fully support the formulation and structure of the ligand. Thus peaks due to parent ion at m/z 477 and elimination of anthryl group, P-anthra $CH{=}CH^{+}$, etc. are all present in the spectrum (**Fig. 2.11**). Peaks due to CuL and fragments of L are all appeared in the spectra of complex. The spectrum of $Cu(dah)_2$ is given in **figure 2.12**.

Geometrical structure of the complexes

In the copper(II) complex, the presence of a broad visible band at ~ 15,000 cm^{-1} and the measured μ_{eff} values (1.75 B.M.) support the square-planar structure.[206] Square-planar copper(II) complexes undergo a change to octahedral symmetry in the presence of donor solvents. When the spectra of the chelate was measured in pyridine (10^{-3} M solution) a broad absorption band centred at ~ 11,200 cm^{-1} was observed which indicates the formation of pyridine adduct of the planar CuL_2 complex. The visible spectra of Co^{2+} complex is dominated by a broad band with maxima at ~ 19,200 cm^{-1}. In some cases a shoulder appeared on this band at ~ 20,800 cm^{-1}. These values together with the observed magnetic moment of 4.70 B.M. suggest the octahedral geometry of the complex.

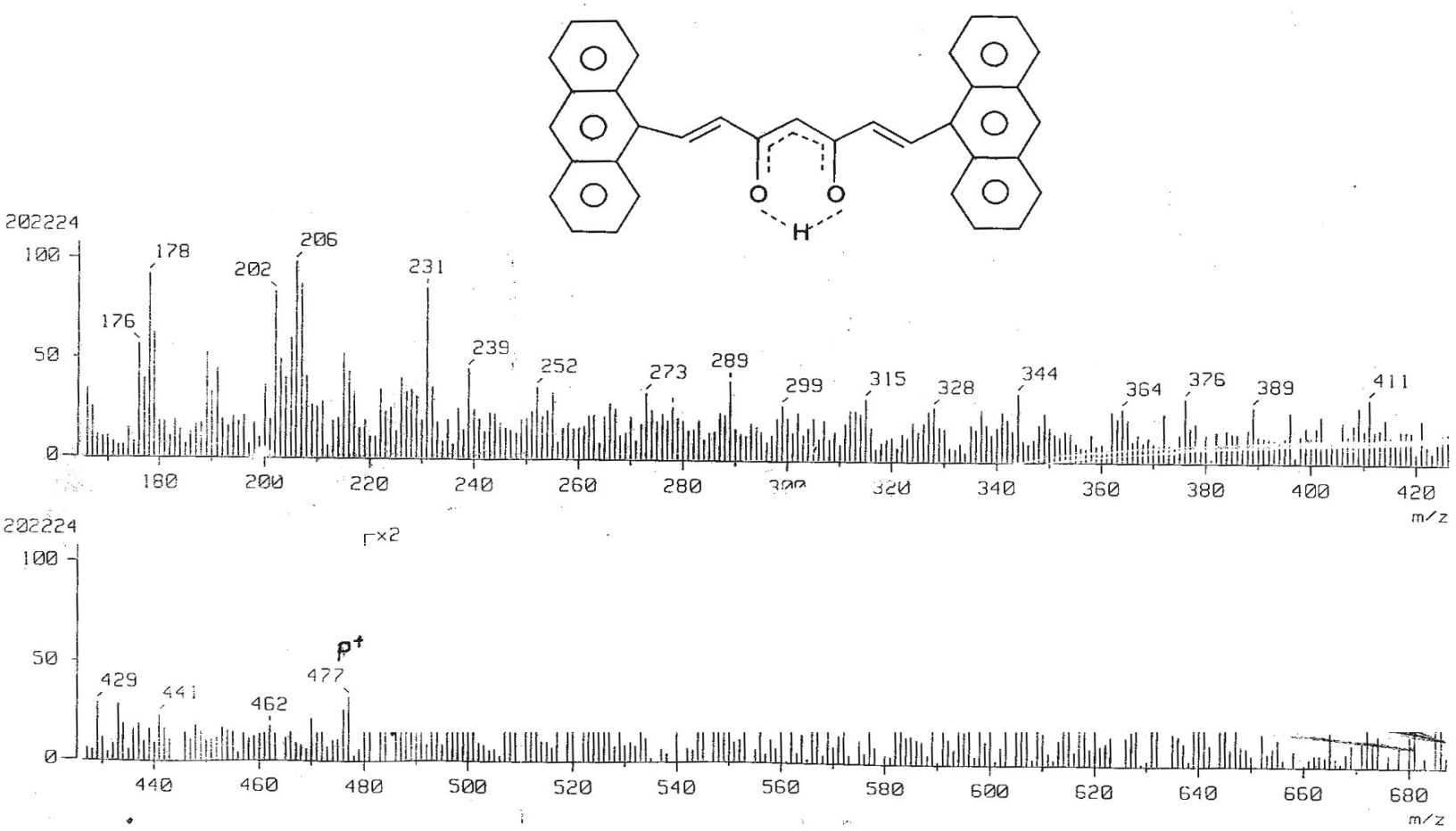


Fig.2.11 Mass spectrum of Hdah

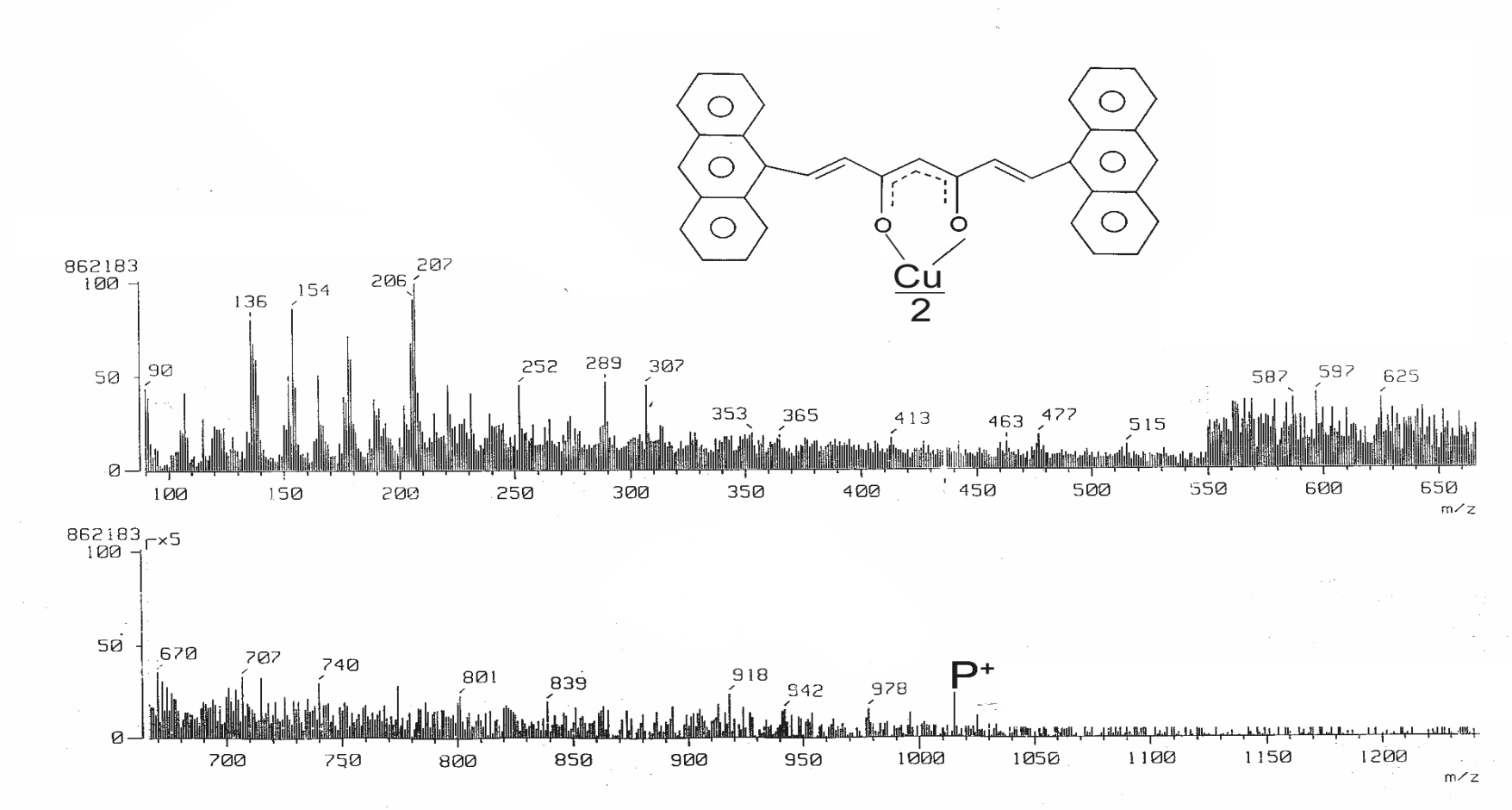


Fig.2.12 Mass spectrum of Copper(II) complex of Hdah

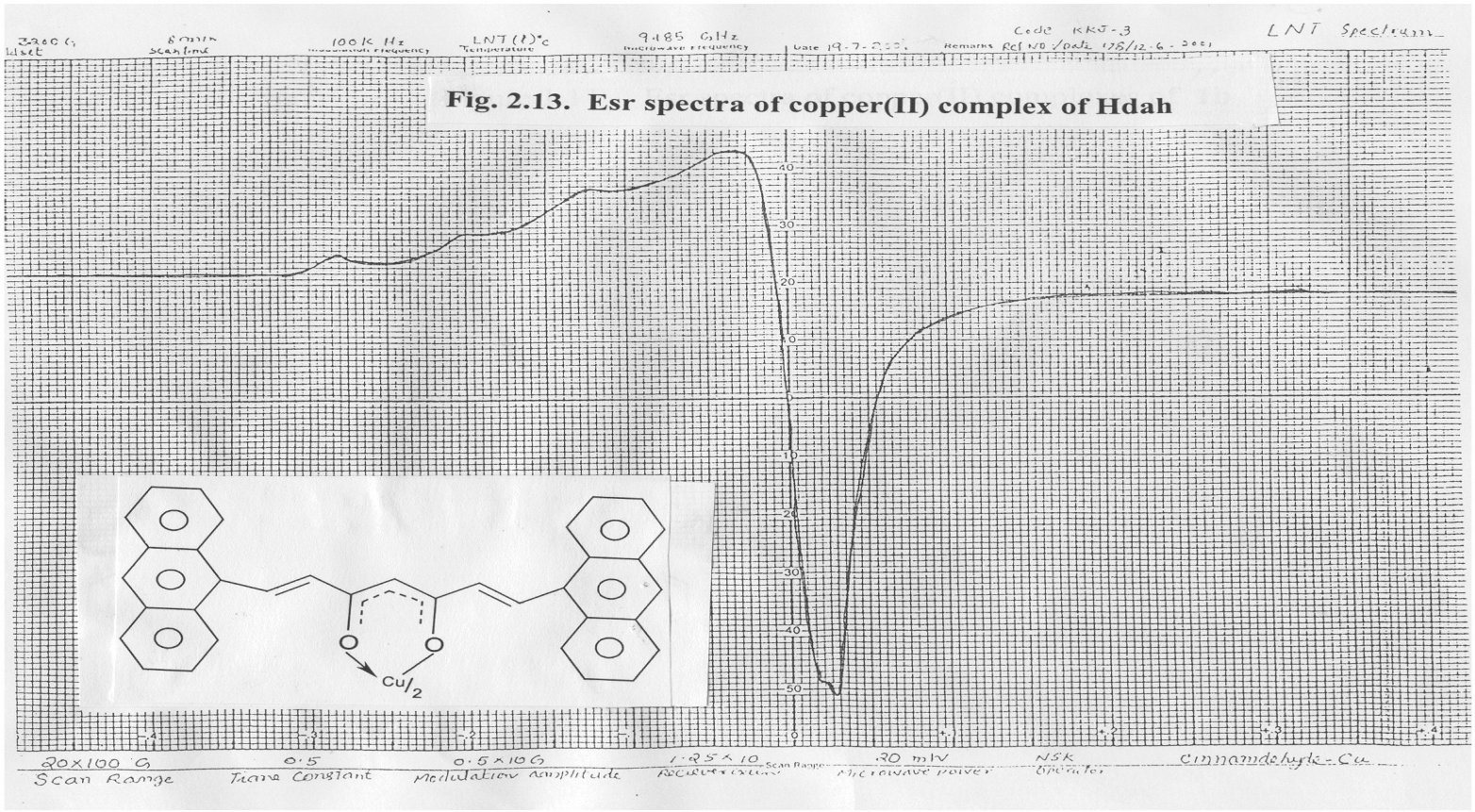
The observed diamagnetism and broad medium-intensity band at ~ 17,800 cm^{-1} in the visible spectra of the nickel(II) chelate undoubtedly suggest their square-planar geometry. In conformity with this observation the visible spectra of the chelate in pyridine solution (10^{-3} M) showed three bands corresponding to configurational change from square-planar to octahedral due to the association of pyridine. The three well-separated absorption bands at λ_{max} ~ 8,024, ~ 13,560 and ~ 24,356 cm^{-1} correspond to the transitions $^3A_{2g} \rightarrow {}^3T_{2g}$; $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g} \rightarrow {}^3T_{1g}(P)$, respectively.

Esr spectral studies of copper(II) complex

Electron spin resonance studies of paramagnetic transition metal complexes yield valuable 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.[207] 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 the copper(II) complex in DMF solution at 77K is given in **figure 2.13**. In general complexed Cu^{2+} ion in solution exhibits four hyperfine lines in its esr spectrum with varying line width. Spectra in the glassy state are 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.


3200 G
Offset
8 min
Scan time
100 K Hz
LNT (R) °C
Temperature
9.185 GHz
Microwave frequency
Date 19-7-2001
Code KKJ-3
Remarks Ref NO / Date 178/12-6-2001
LNT Spectrum
Fig. 2.13. Esr spectra of copper(II) complex of Hdah
40
30
20
10
0
10
20
30
40
50
-4
-3
-2
-1
+1
+2
+3
+4
O
O
Cu/2
20×100 G
Scan Range
0·5
Time Constant
0·5×10 G
Modulation Amplitude
1·25×10
Receiver Gain
Scan Range
30 mW
Microwave Power
NSK
Operator
Cinnamaldehyde-Cu

The spectrum of the copper complex of **Hdah** shows anisotropic pattern. The g and A values evaluated are given in **table 2.10**. For comparison the values reported for copper acetylacetonates[208] and $CuCl_2$ are also included in **table 2.10**.

TABLE 2.10

Esr spectral data of copper (II) complex of Hdah

Compound	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}$	$A_{\perp}$	Solvent
$[Cu(dah)_2]$	2.2734	2.06180	--	--	DMF
$[Cu(acac)_2]$	2.264	2.036	14.55	29	60% Toluene 40% $CHCl_3$
$CuCl_2$	2.340	2.05	1.12	--	60% Toluene 40% $CHCl_2$

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

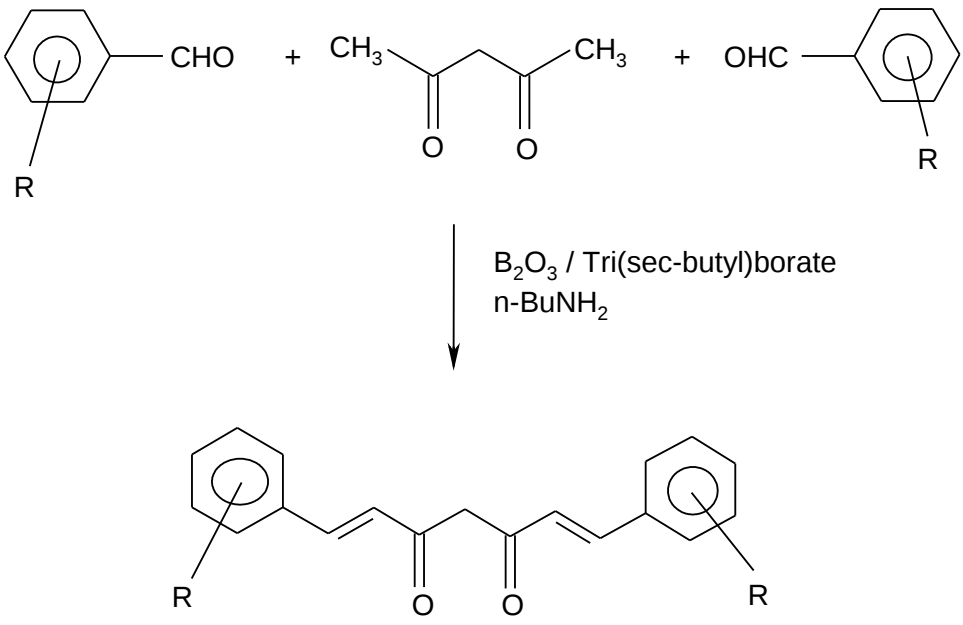
SECTION 3

Synthesis and characterisation of 1,7-bis(substituted aryl) hepta-1,6-diene-3,5-diones

Experimental

Synthesis of substituted 1,7-diarylheptanoids

The substituted diarylheptanoids were prepared by the condensation of substituted aromatic aldehydes with acetylacetone in presence of boric oxide, tri(sec-butyl) borate and *n*-butylamine (scheme 2.2). Experimental details and purification procedures are similar to that of the compounds considered in Chapter 2, Section 1.



Scheme 2.2

Synthesis of metal chelates

Aluminium(III), iron(III) and chromium(III) complexes were prepared by the following general method.

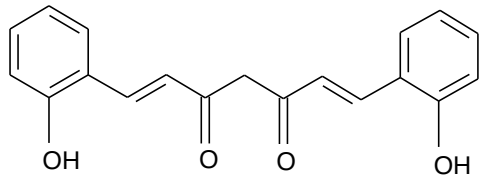
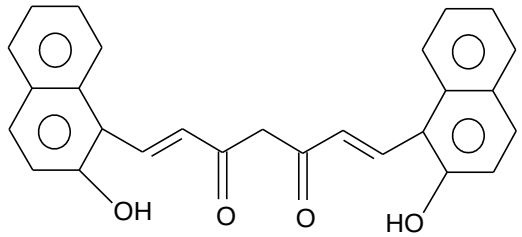
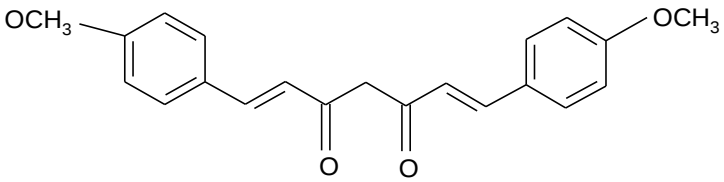
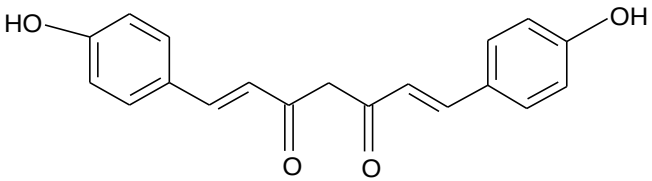
The metal salt solution was added with stirring to a methanolic solution (25mL) of the diketone and refluxed for ~1h and the volume was reduced to half. On cooling to room temperature the complex gets precipitated, which was filtered, washed with cold ethanol and recrystallised from hot methanol.

Results and Discussion

The aldehydes used for synthesis, expected structure of the products, their systematic name, colour and yield are given in table 2.11. The elemental analysis results of the compounds are given in table 2.12. The data suggest that two equivalents of aldehydes have condensed with one equivalent of acetylacetone to form the 1,7-diarylheptanoids.

TABLE 2.11

Synthetic details of the substituted 1,7-diarylheptanoids (2a-g)

Compounds	Aldehydes used for synthesis	Structure	Synthetic name	Colour	Yield
2a	Salicylaldehyde		1,7-bis(2-hydroxyphenyl)-1,6-heptadiene-3,5-dione	brown	51
2b	2-Hydroxynaphth-aldehyde		1,7-bis(2-hydroxynaphtyl)-1,6-heptadiene-3,5-dione	dark green	72
2c	4-methoxy benzaldehyde		1,7-bis(4-methoxy-phenyl)-1,6-heptadiene-3,5-dione	orange	68
2d	4-hydroxy benzaldehyde		1,7-bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione	Red	70

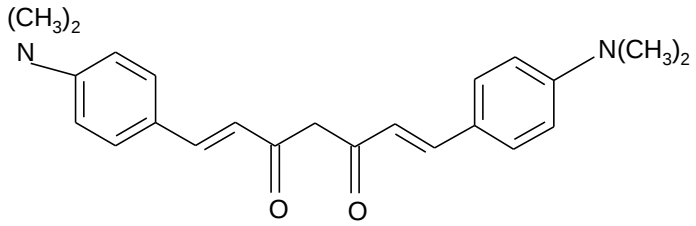
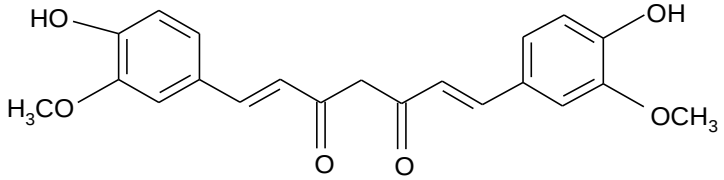
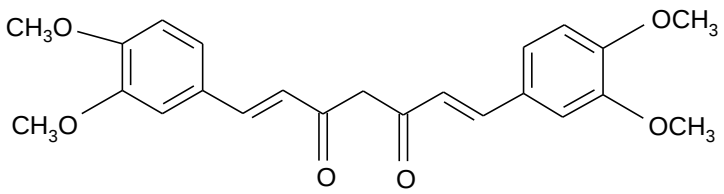
Compounds	Aldehydes used for synthesis	Structure	Synthetic name	Colour	Yield
2e	4-dimethylamino benzaldehyde		1,7-bis(4-N,N-dimethylaminophenyl)-1,6-heptadiene-3,5-dione	Red	75
2f	4-hydroxy-3-methoxybenz-aldehyde (vanillin)		1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	Orange red	76
2g	3,4-dimethoxy benzaldehyde (veretraldehyde)		1,7-bis(3,4-dimethoxy phenyl)-1,6-heptadiene-3,5-dione	Brownish red	72

TABLE 2.12

Physical, analytical and uv spectral data of substituted 1,7-diarytheptanoids (2a-g)

Compound	MP °C	Elemental analysis (%) Found/(Calcd)		Mol. Weight Found/(Calcd)	λ_{max} (nm)	log ∈
		C	H			
2a	120	72.89 (74.01)	5.35 (5.19)	306 (308)	266 390	4.08 4.67
2b	101	80.01 (79.41)	4.32 (4.91)	410 (408)	260 385	4.05 4.57
2c	108	74.98 (75.10)	5.83 (5.95)	330 (336)	261 398	4.09 4.59
2d	132	73.86 (74.03)	5.10 (5.19)	305 (308)	263 408	4.08 4.67
2e	142	78.10 (76.24)	7.91 (7.18)	364 (362)	256 420	4.12 4.71
2f	183	69.10 (68.48)	5.85 (5.43)	372 (368)	265 431	4.09 4.74
2g	218	70.11 (69.69)	5.97 (6.06)	399 (396)	268 445	4.12 4.79

Characterisation of the substituted 1,7-diarylheptanoids

The substituted 1,7-diarylheptanoids were characterised on the basis of their uv, ir, ^{1}H nmr and mass spectral data.

Uv spectra of the compounds **(2a-g)** are characterised by the presence of two absorption maxima (Table 2.12); the low energy band corresponds to n→π* transition (380-460nm) and the high energy band (360-280nm) due to the π→π* transitions. The n→π* absorption values of *o*-substituted 1,7-

diarylheptanoids are of lower energy compared to the corresponding unsubstituted compounds, discussed in section 1.

The ir spectra of the 1,7-diarylheptanoids are characterised by the presence of a strong band in the range of 1605-1640cm^{-1} due to the enolised conjugated 1,3-diketo group. No other band is observed in the region 1600-1800 cm^{-1} assignable to free or bound carbonyl group indicating that the compounds exist entirely in the intramolecularly hydrogen bonded enolic form. The spectra of the compounds are also characterised by the *trans*-CH=CH-absorption which occurs at ~ 970cm^{-1}. The intramolecular hydrogen bonded enolic group gives a broad band in the region 2600-3800 cm^{-1}. Characteristic ir data and their probable assignments are given in **table 2.13**.

TABLE 2.13

Characteristic ir data (cm^{-1}) of substituted 1,7-diarylheptanoids

Compounds							Probable assignments
2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	
1607	1637	1618	1616	1631	1619	1617	ν(C=O) chelated
1596 1585 1535	1602 1593 1541	1587 1561 1548	1576 1563 1541	1599 1560 1545	1583 1566 1540	1568 1568 1542	ν(C=C) phenyl / alkenyl ν(C–C)
1510	1514	1522	1528	1529	1526	1529	νas (C–C–C) chelate ring
1454	1464	1458	1450	1458	1448	1453	νs (C–C–C) chelate ring
1109 1037	1167 1078	1096 1063	1101 1068	1101 1065	1093 1065	1096 1062	p(C–H) chelate ring
978	985	966	981	980	988	979	ν(CH=CH) (trans)

^{1}H nmr spectra

Further evidence for the tautomeric nature of the compounds is provided by the ^{1}H nmr spectra. The ^{1}H nmr spectra of all the compounds displayed a single (1H) downfield at $\delta \sim 16$ ppm and another singlet at $\delta \sim 6.0$ ppm due to the enol and methine protons respectively. The position of methine proton signal is varied because it is also influenced by the electronic effects of the groups attached to the carbonyl function. *Trans* orientation of the alkenyl groups of the compounds are evident from their observed J values (16 Hz). The spectra of **2a, 2b** and **2e** are brought out in **figures 2.14-2.16**. The phenolic protons on the aryl rings of the compounds **2a, 2b** and methyl group on nitrogen of the compound **2e** showed signals as expected. The assignments of various proton signals observed in the ^{1}H nmr spectra of the compounds are assembled in **table 2.14**. The assignments are in agreement with the earlier data on related compounds discussed in **section 1**.

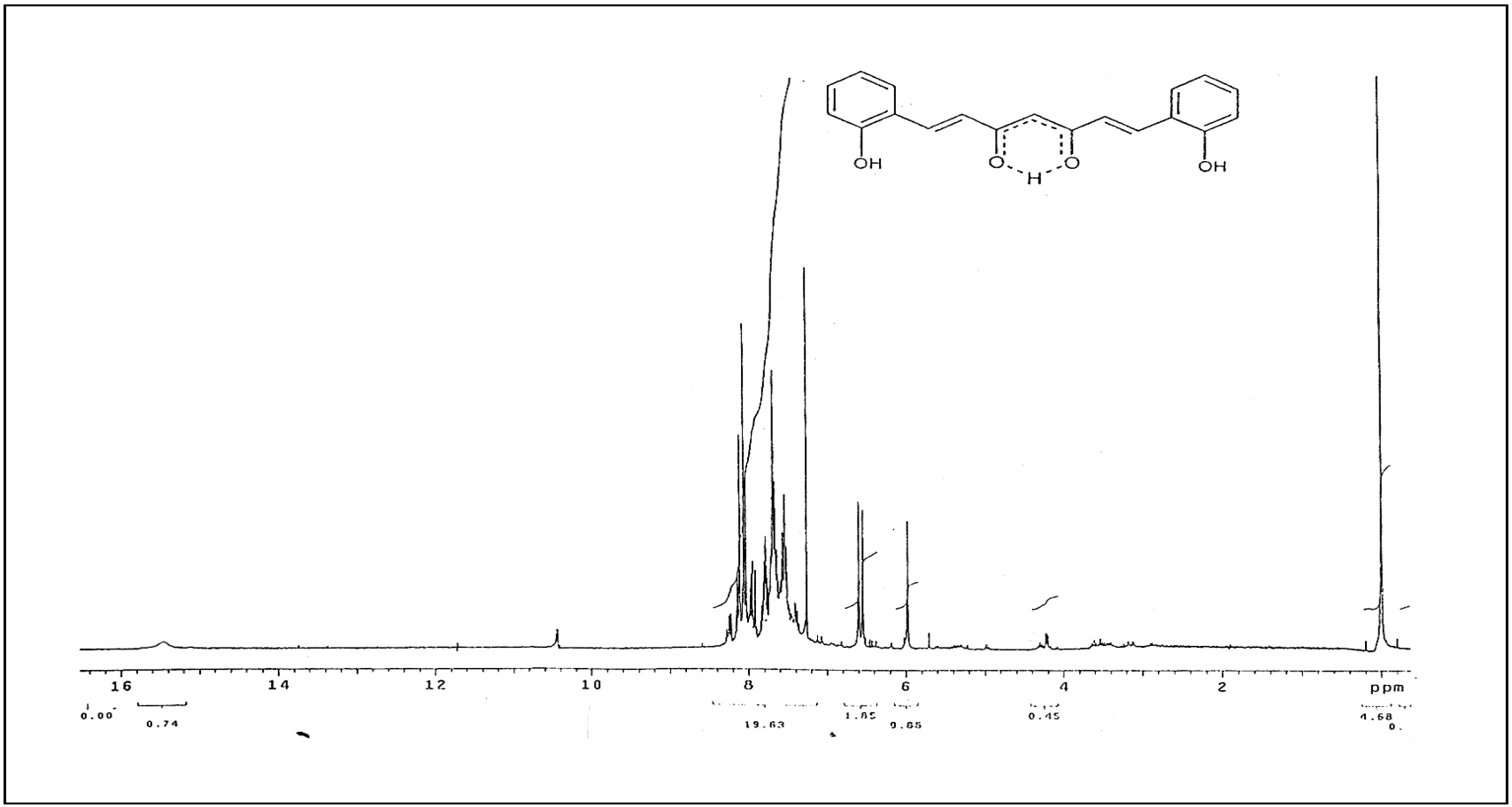


Fig.2.14 ^{1}H NMR spectrum of 2a

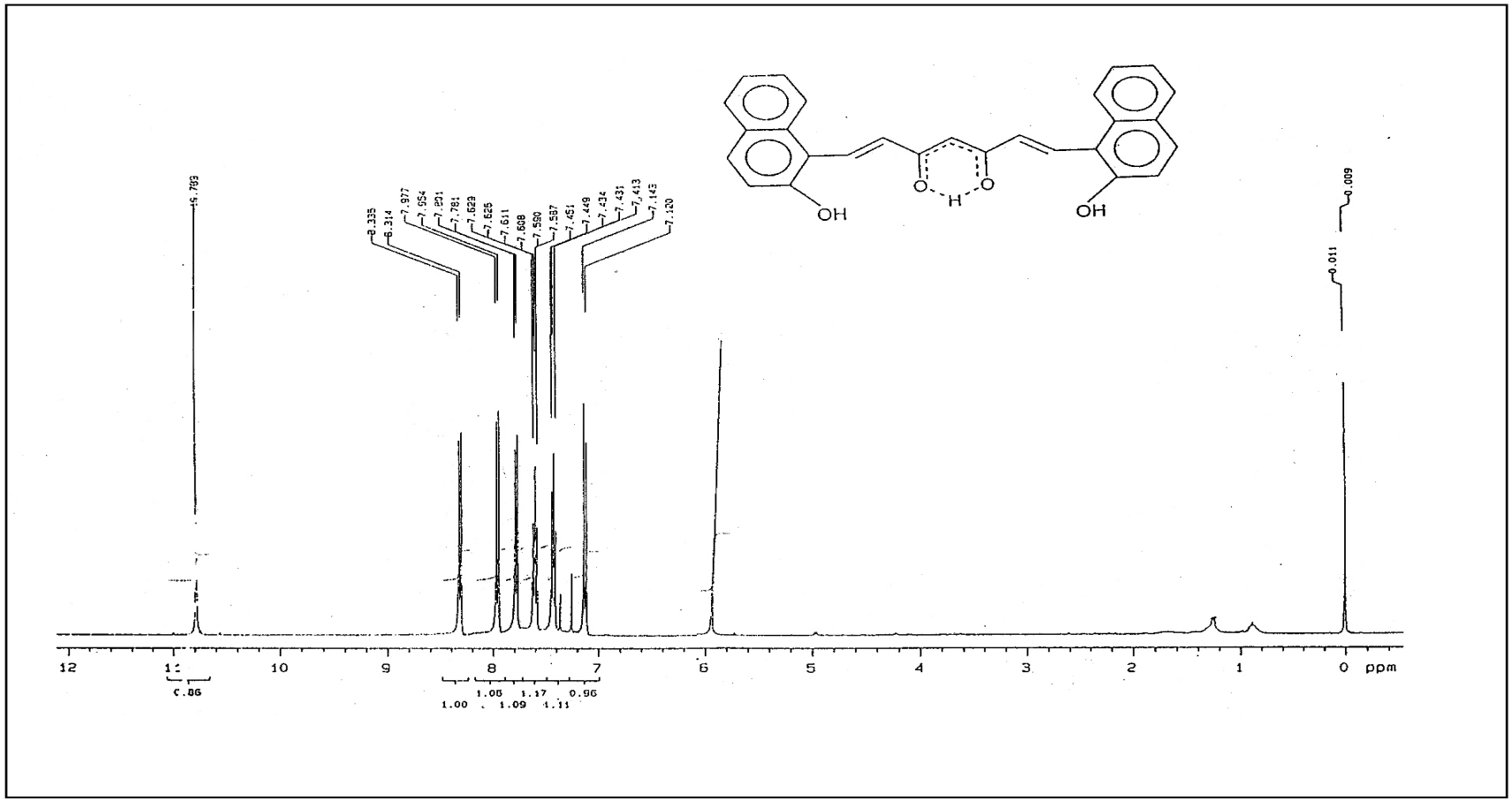


Fig.2.15 ^{1}H NMR spectrum of 2b

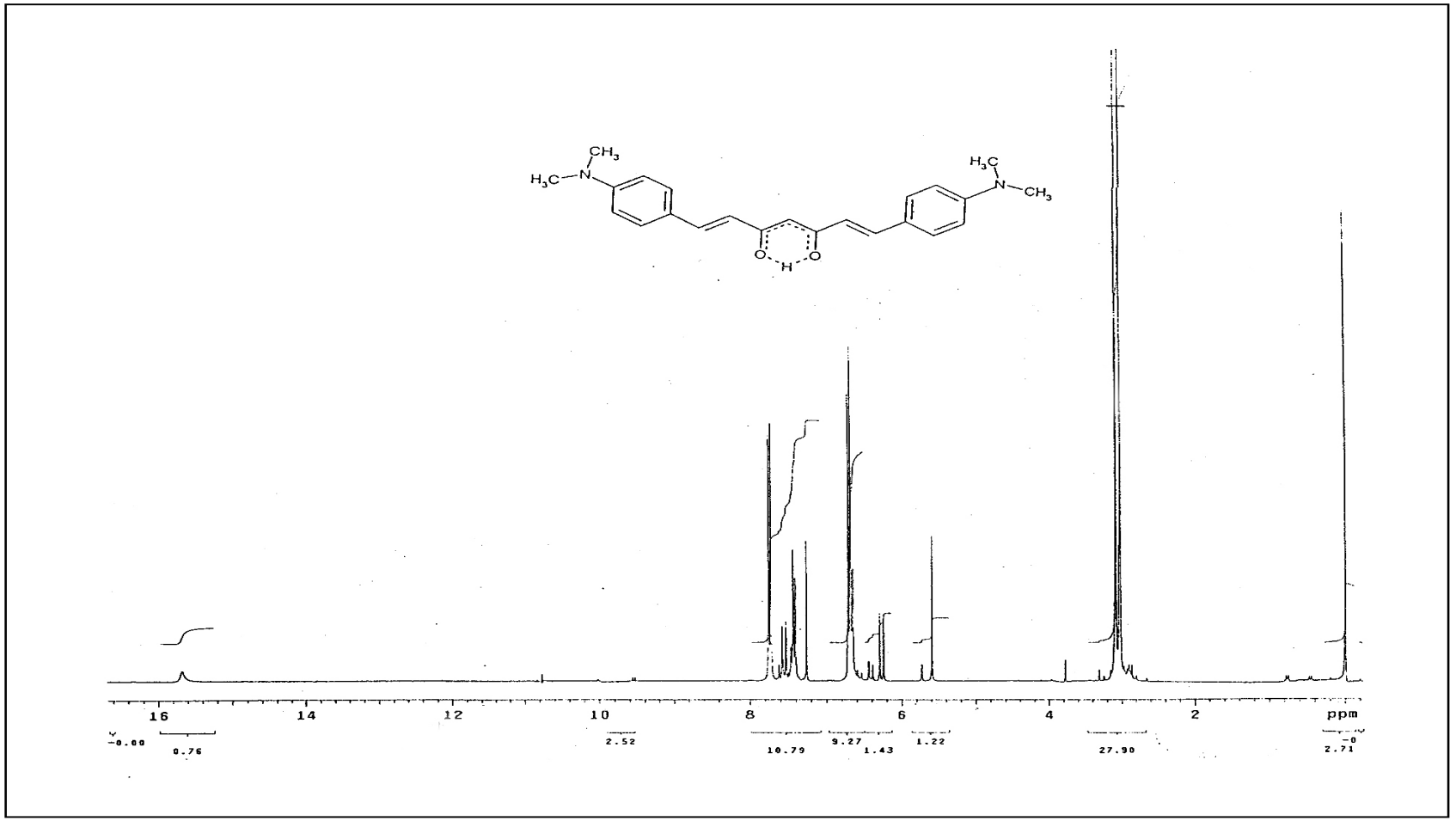


Fig.2.16 ^{1}H NMR spectrum of 2e

TABLE 2.14

Characteristic ^{1}H nmr spectral data of disubstituted 1,7-diarylheptanoids

Compounds	Chemical shifts (δ ppm)				
	Enolic	Methine	Alkenyl	Aryl	Substituents
2a	15.559	6.083	6.552 – 7.580	7.400 – 8.220	10.439 (Hydroxy)
2b	16.041	5.909	7.1420 – 7.977	7.413 – 8.335	10.789 (Hydroxy)
2c	16.43	6.83	7.78 – 7.90	6.833 – 7.09	3.822 (Methoxy)
2d	17.21	6.84	8.18 – 8.20	6.82 – 7.36	10.043 (Hydroxy)
2e	15.68	5.59	6.27 – 7.55	6.65 – 7.75	3.089 [$N(CH_3)_2$]
2f	17.08	6.84	8.16 (2H) 8.12 (2H)	6.84 – 7.36 (6H)	3.92 (Methoxy) 10.04 (Phenolic)
2g	16.35	6.77	7.96 (2H) 8.05 (2H)	6.90 – 7.05 (6H)	3.86 (methoxy) 3.83 (methoxy)

Mass spectra

Mass spectra of all the substituted 1,7-diarylheptanoids show molecular ion peaks $P^+/(P+1)^+$. Peaks corresponding to m/z $(P-O)^+$, $(P-OH)^+$, $(P-HR)^+$, etc. are characteristic of all the compounds. Peaks corresponding to m/z $(P\text{-}Ar.CH.\ CH.CO)^+$ and $(P\text{-}Ar.CH.CH.CO\ CH_2)^+$ are also prominent in the spectra of these compounds brought out in **figures 2.17-2.19**.

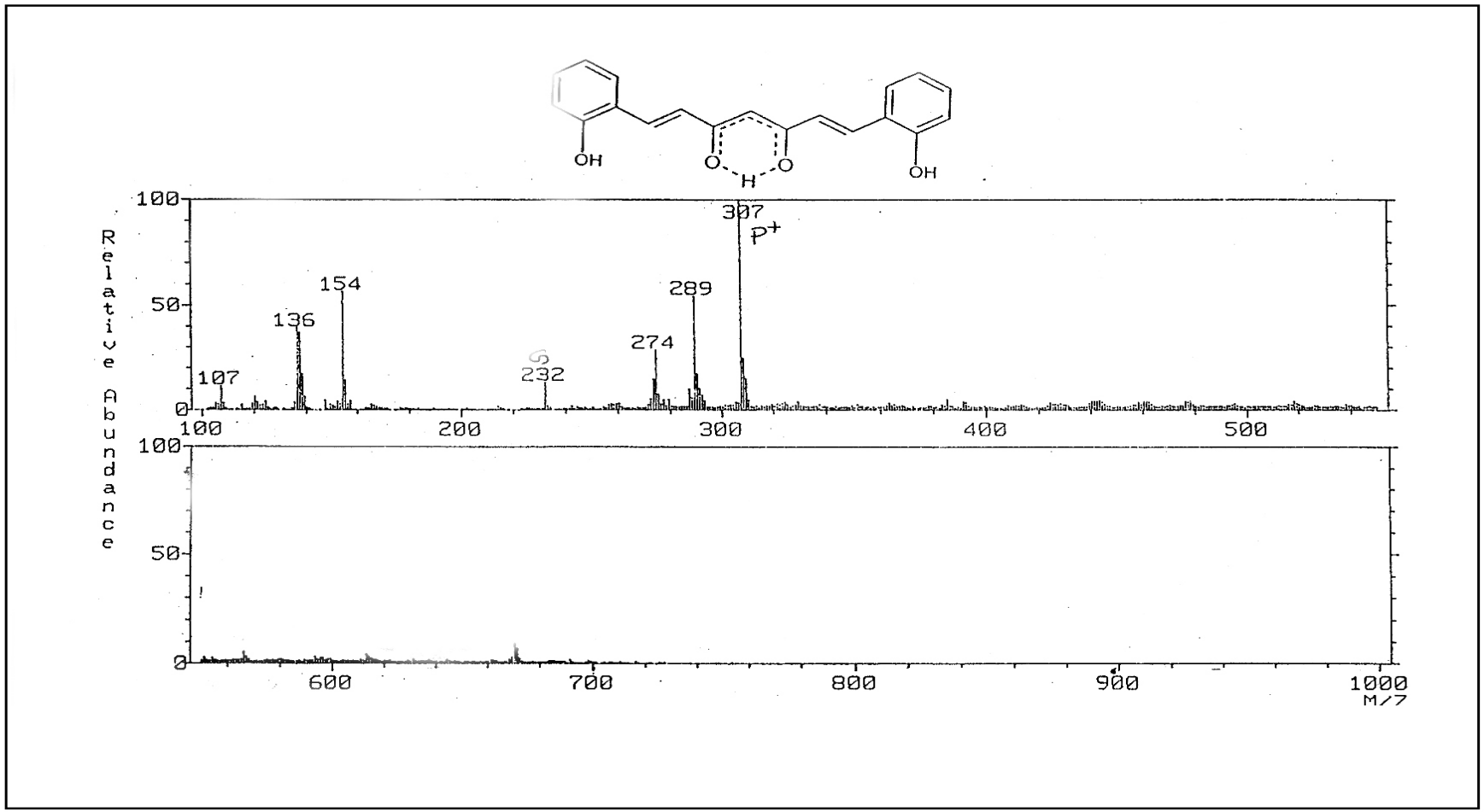


Fig.2.17 Mass spectrum of 2a

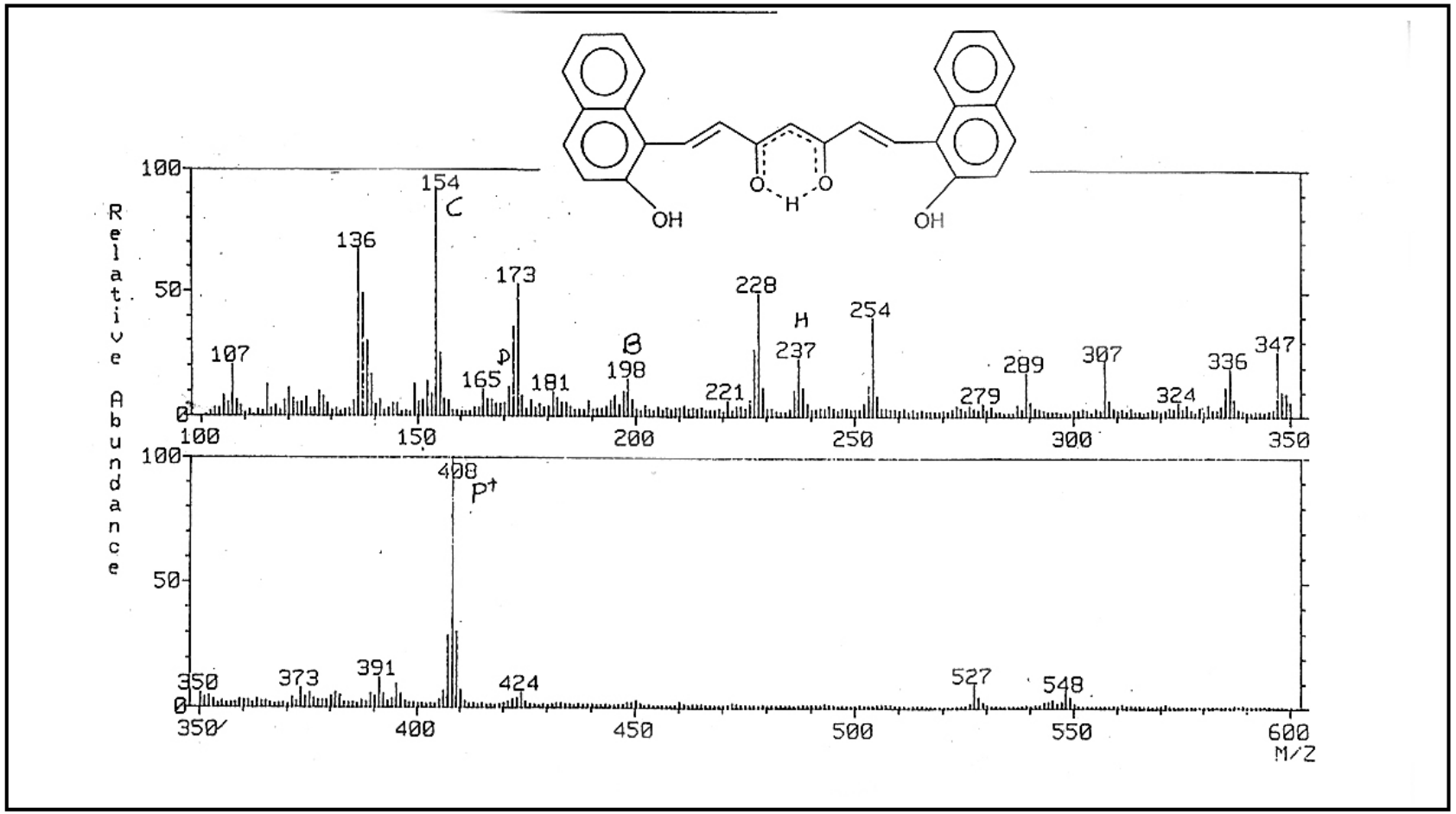


Fig.2.18 Mass spectrum of 2b

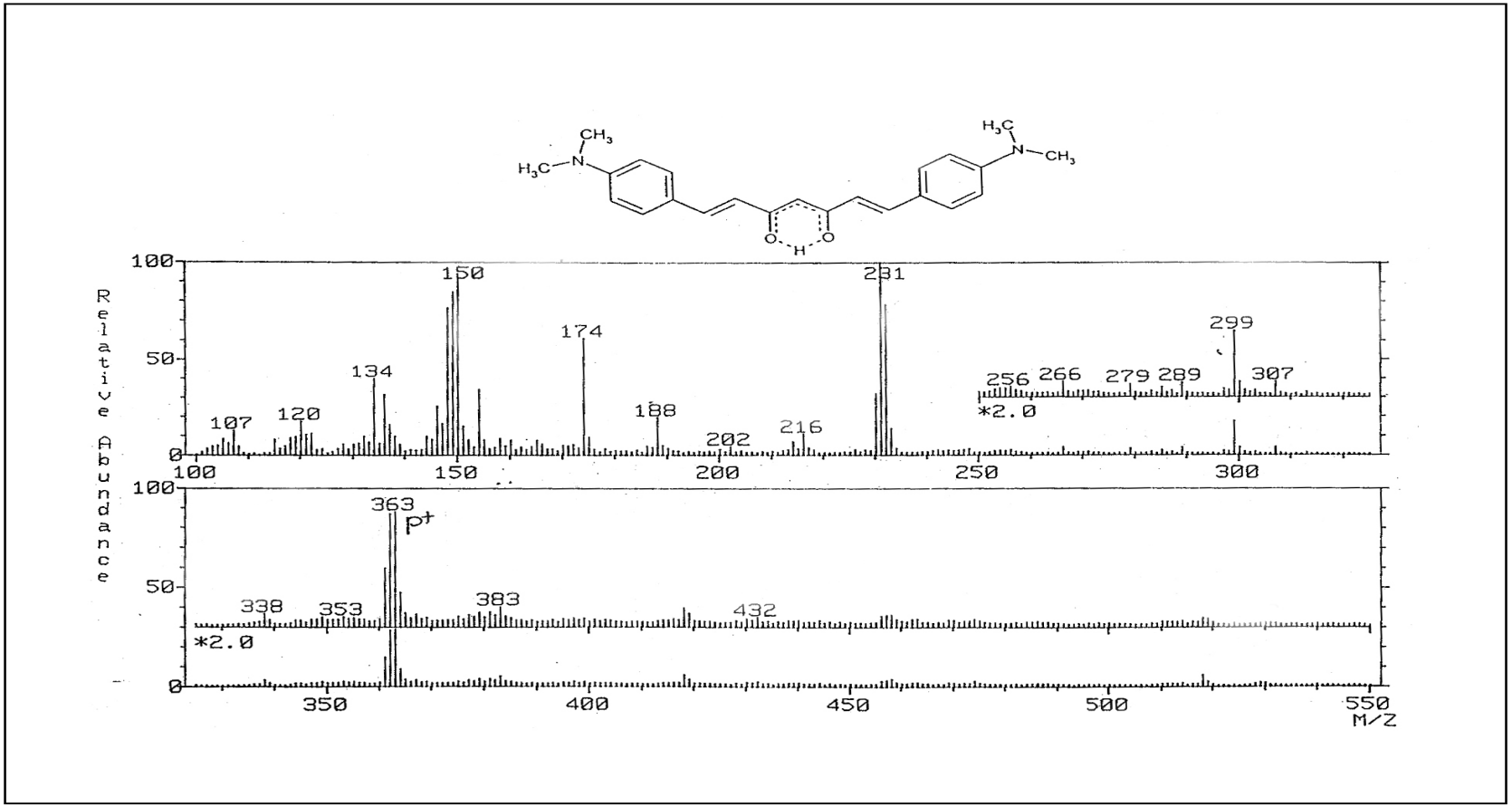
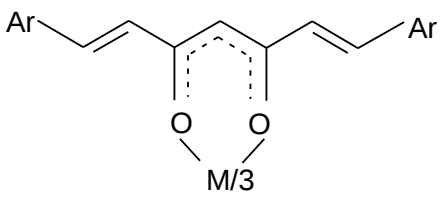


Fig.2.19 Mass spectrum of 2e

Characterisation of metal chelates

Elemental analytical data of the complexes suggests their [ML_3] stoichiometry for the Fe(III), Cr(III) and Al(III) complexes. Analytical and physical data of the metal complexes are given in tables 2.15 – 2.17.

Conductometric studies show that all the complexes behave as non electrolytes in dmf (specific conductance < 10 Ω^1 cm^{-1} in 10^{-3} M solution) and do not contain the anion of the metal salt used for the preparation. Cr^{3+} and Fe^{3+} chelates show normal paramagnetic moment. The uv, ir, nmr and mass spectral data suggest structure **5** of the complexes. The data are discussed below.



5

M = Fe(III), Cr(III), Al(III)

Uv spectra

The characteristic uv absorption maxima of the diketones due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions show only slight bathochromic shifts in the spectra of the metal complexes. It is, therefore, evident that no structural alterations has occurred to the ligand during complexation.

Infrared spectra

In the spectra of the metal complexes, the band due to hydrogen bonded dicarbonyl function of the free ligands disappeared but instead, a strong band assignable to the stretching of the metal coordinated dicarbonyl group of the β-diketone moiety appeared at ~ 1595 cm^{-1}. Similarly the broad band due to the X-H stretching of the free ligands in the region 3400-3800 cm^{-1} also cleared up in the spectra of all the complexes. However spectra of complexes of **2b** and **2d** show prominent band at ~ 3400 cm^{-1} and at ~ 3600 cm^{-1} attributable to the stretching of the phenolic OH groups. This indicate that the phenolic groups are not involved in complex formation. Bands due to the stretching of various C-H groups are present in the region 2500-3200 cm^{-1}. In agreement with this structure spectra of all complexes show additional bands at ~ 465 cm^{-1} and at ~ 420 cm^{-1} assignable to ν(M-O) vibrations. Important ir bands of the complexes are included in **tables (2.15-2.17)**.

^{1}H nmr spectra

In the ^{1}H nmr spectra of the diamagnetic Al(III) complexes, the enolic proton singlet (δ ~ 16 ppm) of the free ligands disappeared. However the phenolic –OH signals of **2a** and **2d** remain as such indicating that the chelate formation has occurred only through the 1,3-diketo moiety of the ligands.

TABLE 2.15

Analytical and ir spectral data of Fe(III) chelates of substituted 1,7-diaryl heptanoides (HL)

Fe(III) chelates of	M.P. (°C)	μ_{eff} B.M.	Elemental analysis (%) Found / (Calc.)*			Characteristic ir stretching bands cm^{-1}	
			C	H	Fe	C=O	Fe–O
2a	160	5.89	70.55 (70.01)	5.01 (4.60)	5.95 (5.73)	1595	459 417
2b	167	5.87	75.67 (76.11)	4.83 (4.46)	3.93 (4.39)	1582	454 422
2c	180	5.78	69.87 (71.25)	5.90 (5.65)	5.01 (5.27)	1588	462 417
2d	179	5.86	71.87 (70.01)	5.02 (4.91)	5.33 (5.73)	1575	470 417
2e	201	5.85	73.01 (72.69)	7.08 (6.85)	5.11 (4.92)	1580	488 425
2f	216	5.89	66.23 (65.34)	4.21 (4.94)	4.82 (4.84)	1600	485 430
2g	239	5.9	61.27 (60.92)	4.93 (4.59)	5.00 (4.51)	1609	480 419

*The calculated value corresponds to the $[FeL_3]$ composition where L stands for the deprotonated ligand.

TABLE 2.16

Analytical and ir spectral data of Cr(III) chelates of substituted 1,7-diaryl heptanoides (HL)

Cr(III) chelates of	M.P. (°C)	μ_{eff} B.M.	Elemental analysis (%) Found / (Calc.)*			Characteristic ir stretching bands cm^{-1}	
			C	H	Cr	C=O	Cr–O
2a	182	3.82	77.81 (78.67)	6.32 (5.93)	5.07 (5.41)	1584	454 423
2b	196	3.61	70.64 (70.29)	4.98 (4.62)	5.02 (5.34)	15.86	461 421
2c	201	3.78	62.37 (63.09)	4.02 (3.59)	4.56 (4.79)	1596	48 420
2d	178	3.89	77.12 (76.35)	4.08 4.47	3.75 (4.08)	1592	459 418
2e	185	3.70	74.45 (75.15)	4.55 (4.32)	4.85 (4.62)	1580	470 425
2f	190	3.58	78.34 (77.05)	4.23 (4.01)	4.25 (4.02)	1575	455 430
2g	192	3.68	72.34 (70.51)	4.65 (4.35)	4.98 (4.34)	1588	462 420

*The calculated value corresponds to the $[CrL_3]$ composition where L stands for the deprotonated ligand.

TABLE 2.17

Analytical and ir spectral data of Al(III) chelates of substituted 1,7-diaryl heptanoides (HL)

Al(III) chelates of	M.P. (°C)	Elemental analysis (%) Found / (Calc.)*			Characteristic ir stretching bands cm^{-1}	
		C	H	Cr	C–O	Al–O
2a	> 320	72.47 (72.15)	4.93 (4.75)	2.98 (2.85)	1575	465 418
2b	> 340	78.01 (77.88)	4.83 (4.56)	2.43 (2.16)	1580	458 418
2c	260	70.45 (68.89)	4.77 (4.48)	2.57 (2.37)	1563	470 415
2d	210	75.46 (73.88)	4.57 (4.32)	2.85 (2.67)	1585	488 430
2e	235	69.87 (67.54)	4.97 (4.67)	2.77 (2.57)	1592	480 425
2f	> 300	67.52 (67.02)	5.28 (5.05)	2.63 (2.39)	1577	490 417
2g	160	74.54 (74.19)	6.43 (6.18)	2.72 (2.42)	1583	477 419

*The calculated value corresponds to the $[AlL_3]$ composition where L stands for the deprotonated ligand.

The spectrum of the complex of **2e** is given in **figure 2.20.** Integrated intensities of the various proton signals agree well with the $[AlL_3]$ stoichiometry of the complexes.

Mass spectra

The mass spectra of the aluminium(III) complex of **2b** showed intense P^+ ion peak corresponding to [AlL_3] stoichiometry (**Fig. 2.21**) prominent peaks due to AlL_2, AlL, etc. are also present in the spectra.

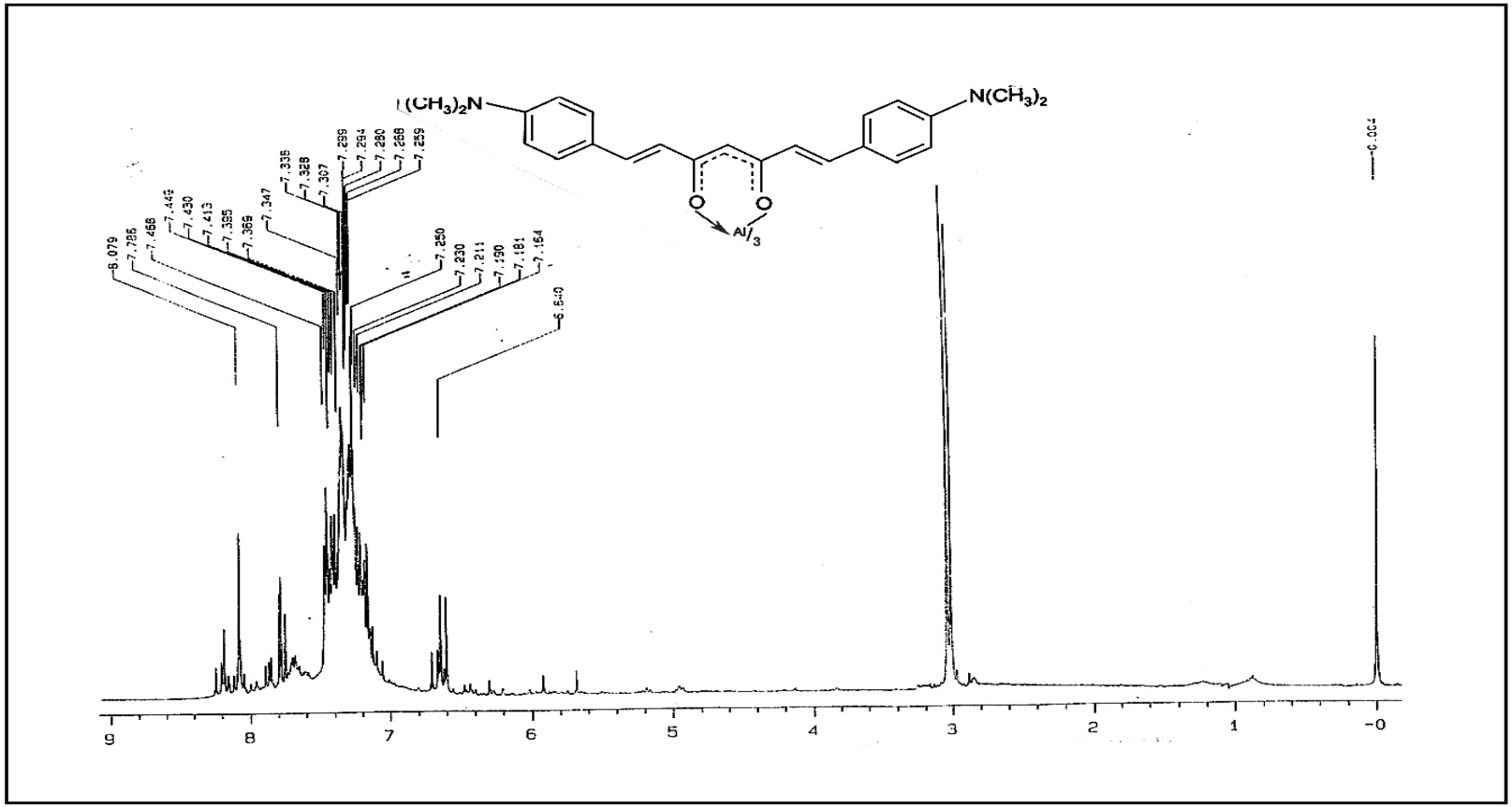


Fig.2.20 ^{1}H NMR spectrum of Aluminium (III) complex of 2e

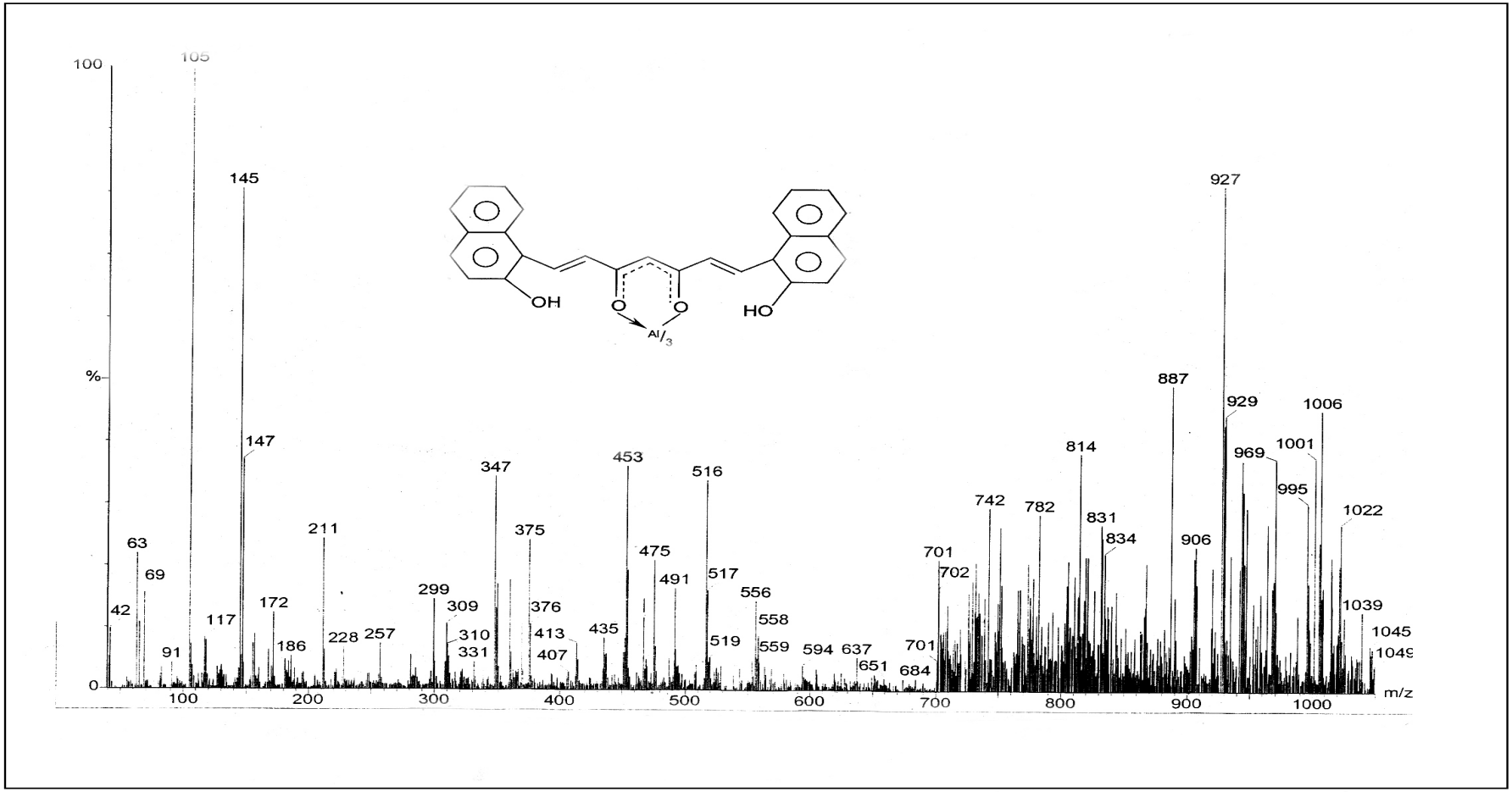


Fig.2.21 Mass spectrum of Aluminium (III) complex of 2b

BIOLOGICAL STUDIES

Some of the synthetic curcuminoids discussed in chapter 2 were investigated for their antitumour and antioxidant activity. Typical metal complexes of the compound were also examined for their antioxidant and antitumour activities. Biodistribution assay of one of the curcuminoids was carried on mice using ^{51}Cr radioisotope labelling of the chromium complex. The details on these studies are presented in three sections.

Section 1. Studies on cytotoxicity and antitumour activity.

Section 2. Studies on antioxidant activity.

Section 3. Biodistribution assay of curcuminoids.

SECTION 1

Studies on Cytotoxicity and Antitumour Activity

Recently several reports appeared on cytotoxicity and anticancer properties of both natural and synthetic analogues of curcuminoids. Different investigators have attributed these properties to the diketo function, olefinic linkages, aryl substituents such as phenolic group, etc. of the compounds. It has also been reported that certain metal complexes enhance the activity of curcuminoids. In the present study, the cytotoxic and antitumour activities of dianthrylheptanoid, **Hdah** (**chapter 2, section 2**) and its typical metal complexes were investigated. The results are discussed below.

Experimental

Materials

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

Animals: Swiss albino mice (female, 6-8 week old weighing 25 $\pm$ 3 g) were obtained from Veterinary College, Thrissur, Kerala. They were kept in polypropylene cages under controlled temperature (25-27^{o}C) and humidity (60%) and fed with pellet diet (Lipton, India Ltd.) and water *ad libitum*.

Chemicals: Ascorbic acid, deoxy ribose, nitroblue tetrazolium (NBT) and riboflavin were purchased from Sisco Research Laboratories Pvt. Ltd. Bombay. Thiobarbituric acid (TBA) from Himedia Laboratories Pvt. Ltd., Bombay. All other chemicals and reagents used were of analytical grade. Tissue homogenizer from Yorco Delhi.

Preparation of Reagents

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

Phosphated 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 (0.2 g) $Na_2HPO.2H_2O$ (1.15 g) and KH_2PO_4 (0.2g) in one litre distilled water.

Trypan blue: Cell viability was determined using this dye which penetrates in to 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 1.00 g of trypan blue in 100mL of distilled water.

Maintaining Cell Lines: DLA cell lines were maintained as ascites tumours 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 cell were suspended in saline or PBS so as to get a cell suspension of one million cells/mL. One mL of the cell suspension was injected into the peritonial cavity of Swiss albino mice. 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 grow with this tumour and 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.

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

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

NaCN (0.0015%) in 0.1M.EDTA was prepared by dissolving 1.5 mg NaCN in 100mL of 0.1 M EDTA (3.7224 g EDTA in 100 mL water).

Phosphate buffer: pH 7.8 (0.06M) was prepared by mixing 3.5 mL 0.06 M $NaH_2 PO_4.2H_2O$ (0.936g/100 mL) and 91.5 mL 0.06M Na_2HPO_4 (1.068/100 mL) solutions in water.

Deoxyribose (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.

Tris buffer (0.2mM) was prepared by dissolving this buffer 2.42g in 100 ml water and adjust the pH with HCl.

Tissue Homogenate was prepared by the following method. Liver tissue from healthy mice was cut in to small pieces and 25% homogenate was prepaed 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.

Determination of *in vitro* cytotoxicity of compounds

The short term *in vitro* cytotoxic activity of the compounds were analysed by using DLA cells. The synthetic curcuminoid 1,7-dianthrylhepta-1,6-diene-3,5-dione and its Fe(III), Cr(III) and Cu(II) complexes (Chapter 2, Section 2) were dissolved in minimum quantity of DMSO. Different concentrations (1-100 μg/mL) of the 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 the different concentrations of the compounds and volume was finally made up to 1 mL using PBS and mixture was incubated for ~ 3h at 37^{o}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.

Control		Test sample
Cell line	- 100 μL	100 μL
PBS	- 8000 μL	800 μL
Dye	- 100 μL	100 μL
Drug	- Nil	Various concentrations of test compounds

Determination of the effect of compounds on solid tumour development

Solid tumours were induced in groups of Swiss albino mice (6 nos/group) by subcutaneous infection 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 μ mol/kg body weight) and continued for 10 days. Tumour diameter was measured every fourth day for one month and tumour volume was calculated using the formula $V = \frac{4\pi r_1^2 r_2}{3}$ where r_1 and r_2 are the major and minor radii respectively.[209-210]

Determination of the effect of compounds in reducing ascites tumour development

Five groups of Swiss albino mice (6 nos/group) were injected ip with DLA cells (1x10^6 cells/animal). One group was kept as control and the other groups, were simultaneously injected ip with the test compounds (160 mg/kg body weight) dissolved in DMSO and injection of the compounds 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:

Percentage $\text{ILS} = \frac{(T - C) \text{ x } 100}{C}$, where T and C are the mean number of days survived by the treated and control animals respectively.[211]

Results and Discussion

Short term *in vitro* cytotoxicity studies

Short term *in vitro* cytotoxicity of the compound **Hdah** and its Cu(II), Cr(III), Fe(III) complexes are shown in the **table (3.1)**. These preliminary experiments were carried out with five different concentrations of the compounds. The compound, **Hdah** produced 50% cell death at a concentration 26 μg/mL, while the Cu(II) and Cr(III) complexes produced the same effect at concentrations 13 μg/mL, 18 μg/mL and Fe(III) complex has 50% cell death greater than 20 μg/mL. Among the various compounds Cu(II) complex was found to be most cytotoxic. The increased activity of the Cu(II) and Cr(III) complexes than the ligand may be due to the increased reaction site over the ligand.

TABLE 3.1

Short term *in vitro* cytotoxicity of Hdah and its metal complexes

Compounds	Percentage cell-death at different concentration				
	5 μg/mL	10 μg/mL	20/μg mL	50 μg/mL	100 μg/mL
Hdah	11	20	54	72	92
$[Cu(dah)_2]$	21	35	47	84	100
$[Cr(dah)_3]$	28	48	59	87	100
$[Fe(dah)_3]$	12	23	32	45	69

L = Deprotonated Hdah

TABLE 3.2

Effect of Hdah and its complexes on solid tumor reduction

	7 days	12 days	16 days	20 days	24 days	28 days	32 days	36 days
Control	0.1302	0.3083	0.5374	0.8405	1.1936	1.5027	2.0581	2.4062
Hdah	0.0955	0.1089	0.1974	0.3175	0.4587	0.6394	0.8765	1.0435
$[Cr(dah)_3]$	0.0741	0.0985	0.1374	0.2051	0.3446	0.5398	0.7793	0.9082
$[Fe(dah)_3]$	0.1215	0.1803	0.2762	0.4063	0.5764	0.7912	1.0735	1.2342
$[Cu(dah)_2]$	0.0925	0.0975	0.1081	0.1538	0.2576	0.4374	0.6791	0.8684

L = Deprotonated Hdah

Effect of the compounds on solid tumour development

The curcuminoid, **Hdah** and its chromium(III), iron(III) and copper(II) complexes were studied for their antitumour activity. All compounds showed a significant reduction of solid tumour volume, in mice when injected intraperitoneally. The results are given in **figure 3.1**. The tumour volume on

35th day was found to be 2.42 cm^3 in the case of control and 1.32, 1.24, 0.98 and 0.88 cm^3 respectively, for Hdah, $Fe(dah)_3$, $Cr(dah)_3$ and $Cu(dah)_2$. However the administration of the compound did not prevent solid tumour death in these animals.

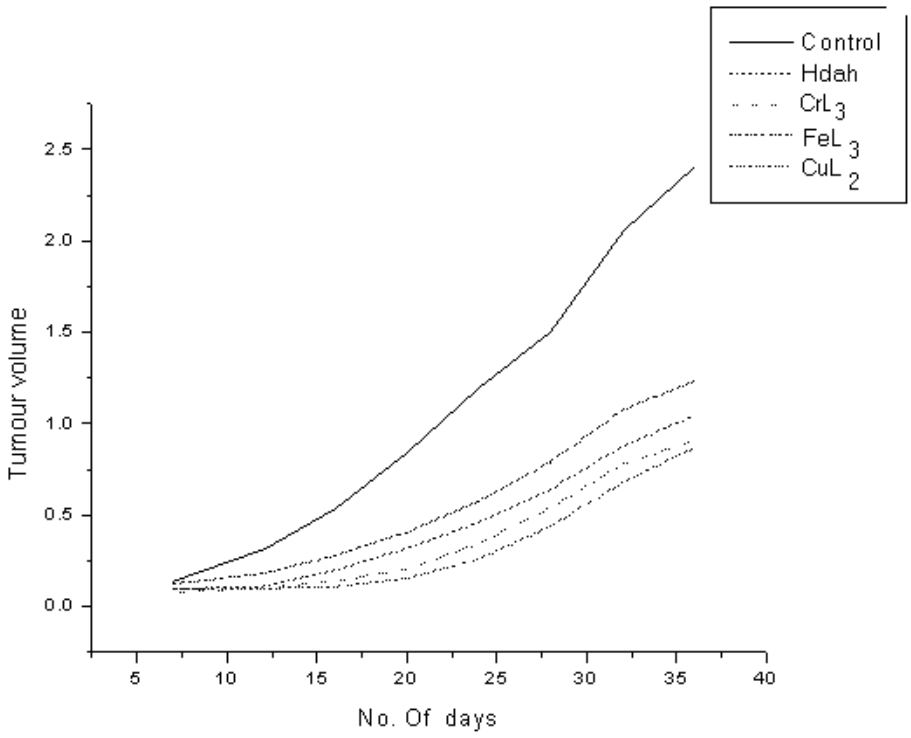


Fig. 3.1. Effect of compounds, Hdah, $Cr(Hdah)_3$, $Fe(Hdah)_3$ and $Cu(Hdah)_2$ on solid tumour volume

Effect of compounds on ascites tumour development

All the compounds when administered intraperitonially could produce increase in the life span of tumour bearing mice (**Table 3.3**). It was found that the Cu, Fe and Cr complexes showed slight increase in the activity than

the ligand towards the tumour cell. However the effect on the ascites tumour reduction was not so marked as in the case of solid tumour.

Development of tumour is mainly due to the action of various free radicals generated in the living system, on the DNA strand. Antitumour activity of the curcuminoid analogues correlates with their ability to protect biomolecules against singlet oxygen and other prooxidants. The ability in turn depends on the nature of the curcuminoid analogues, especially the presence of double bond in conjugation with aryl rings. Compounds which possess substituted aryl rings and extended conjugation show moderately high antitumour activity, than unsubstituted diaryl heptanoids.[212] The study also revealed that complexation with metals, significantly increases the antitumour activities, compared to free curcuminoid analogues. In certain cases, an anomaly was observed between the *in vitro* and *in vivo* studies. Certain compounds exhibit high activity in *in vitro* studies, but they are less effective *in vivo*. This may be due to the transformation of the compound into different metabolites in the living cell, which can either enhances or retard the activity depending on the nature of the metabolic products.

TABLE 3.3

Effect of compounds on ascites tumour reduction

Compound	No. of animals with tumour	No. of days survived	Increase in Life Span (%)
Control	6/6	17.3 ± 1	
Hdah	6/6	21.2 ± 1.2	22.54*
$[Cr(dah)_3]$	6/6	24.4 ± 1.7	41.04*
$[Fe(dah)_3]$	6/6	23.7 ± 1.6	36.99*
$[Cu(dah)_2]$	6/6	26.9 ± 1.7	55.49*

*P < 0.001; L = Deprotonated.

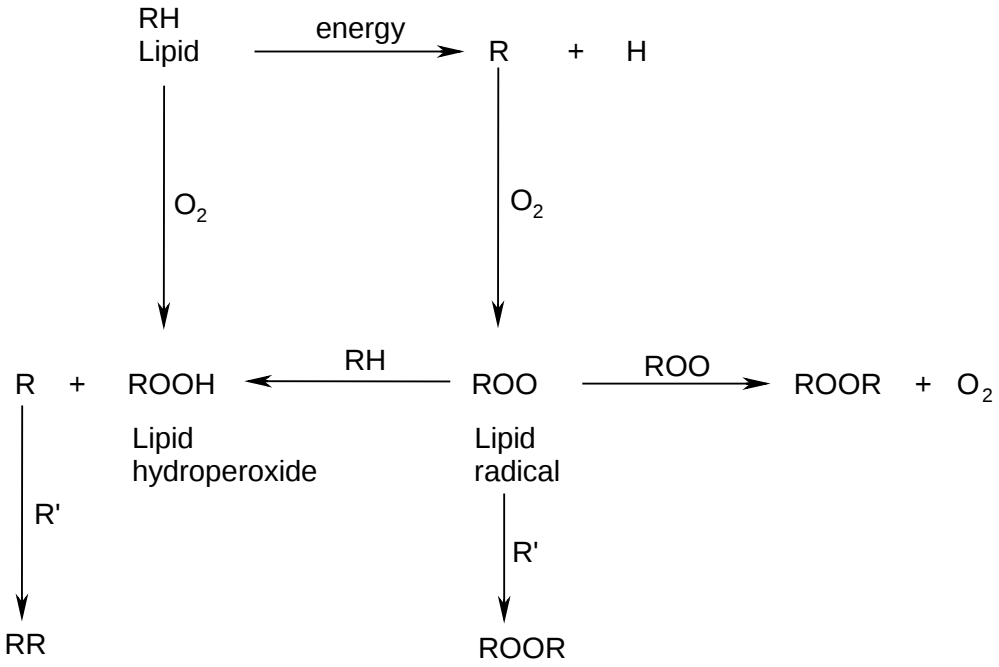
Values are means of ± SD of six determinations.

SECTION 2

Studies on Antioxidant Activity

Introduction

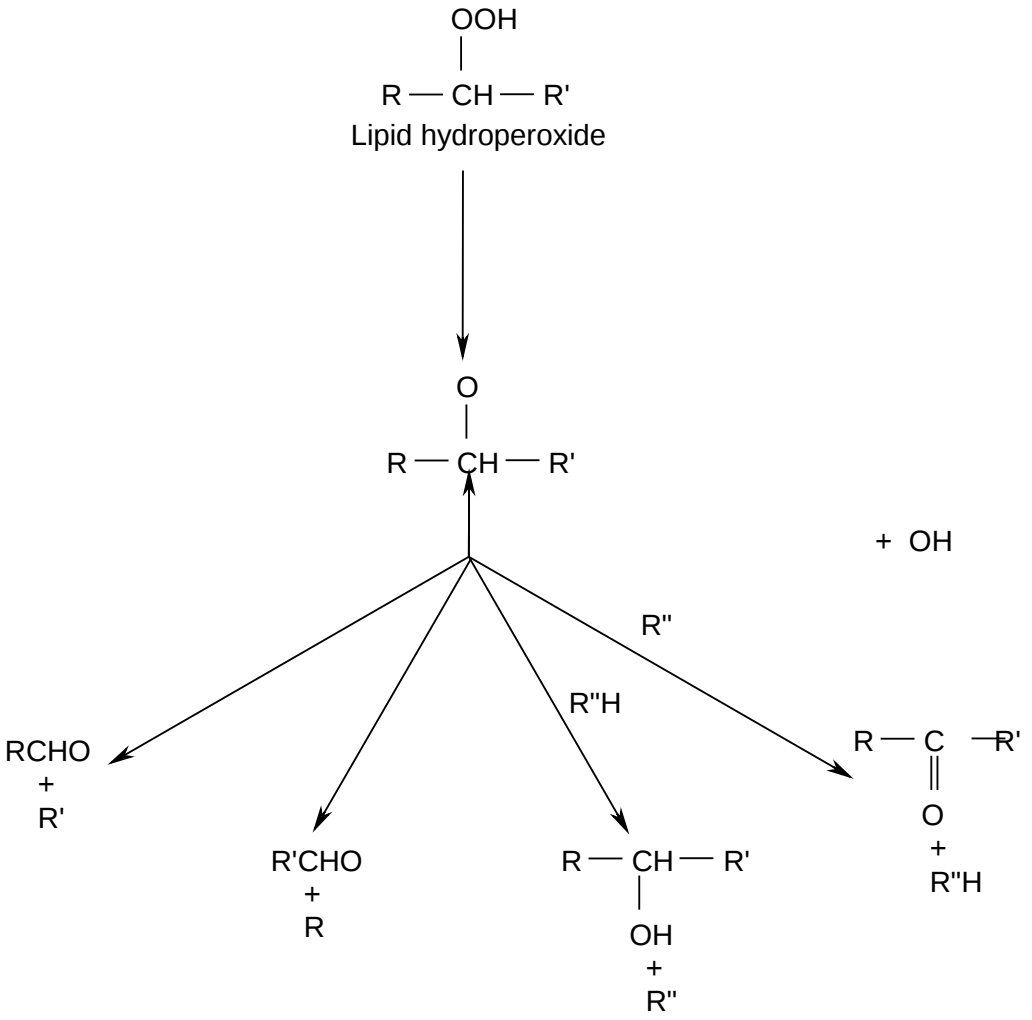
Oxygen molecule, a diradical in its ground state, can enter into energetically favourable chain reactions with many organic free radicals. A direct consequence of this is found in the rancidifcation of certain lipids. This autoxidation of unsaturated fatty acid components of lipids is known[213] to proceed by a free radical mechanism as shown in **scheme 3.1**.



Scheme 3.1

The hydroperoxides formed as the primary products are quite unstable and can produce more free radicals by degenerate branching and numerous

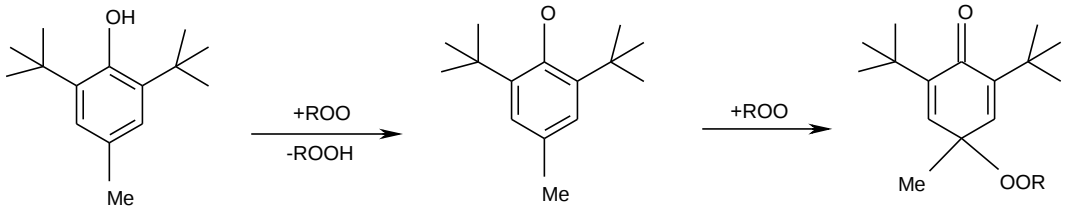
unwanted secondary degradation products (**Scheme 3.2**) of which the volatile aldehydes play significant role in imparting off-flavours to foodstuffs even at ppb levels.[214]



Scheme 3.2

Synthetic organic chemists have developed many effective antioxidants for rubber, hydrocarbon fuels, plastics and foodstuffs which rapidly react with the free radical intermediates of an autoxidation chain and prevents it from further progressing. An excellent example is the synthetic

hindered phenol, 2,6-di tert-butyl-4-methylphenol, often called BHT (a most common ingredient of several baked foods) which can effectively scavenge peroxy radicals. This reaction is depicted in **scheme 3.3**.



Scheme 3.3

Hydroxyl radical: Hydroxyl radical can be generated in biologically relevant systems by multiple reactions. One is iron catalysed Fenton reaction.

$$H_2O_2 \xrightarrow[\text{Catalyst}]{Fe^{2+}\ \text{salt}} Fe^{3+} + OH + OH$$

or

$$H_2O_2 \xrightarrow[\text{Catalyst}]{Fe^{2+}} \tfrac{1}{2}O_2 + H_2O$$

Uv induced homolytic fission of the O–O bond in H_2O_2 makes OH.

$$H - O - OH \xrightarrow{uv} 2OH$$

This could conceivably happen to H_2O_2 generated in sunlight exposed skin. OH has been suggested to arise during ethanol metabolism and during peroxynitrous acid decomposition.

Another source of OH free radical is the ionising radiation. Since the major constituent of living cell is water, exposure to high-energy radiation such as γ-rays will result in OH production. Hydroxyl radicals are responsible for a large part of the damage done to cellular DNA, proteins, and lipids by ionizing radiation. DNA damage, especially, as double-strand breaks cannot easily be repaired by the cell. Oxygen, normally present in most biological systems, aggravates the damage done by ionizing radiation. Since living organisms are exposed to background levels by radiation from cosmic rays, natural radioactivity in rocks, release of radioactive radon gas and pollution of the environment with man-made radioactive isotopes, it is likely that some OH is always formed *in vivo*.

Superoxide radical: By comparison with OH, superoxide is far less reactive with non radical species in aqueous solution. Enzymes and photochemical reactions often generate O_2^-. Mixtures of Xanthine or hypoxanthine with xanthine can generate O_2^- in biological system. Iron(II) ions can take part in electron-transfer reactions with oxygen.

$$Fe^{2+} + O_2 \rightleftharpoons Fe^{3+} + O_2^-$$

This superoxide can dismutate to form H_2O_2, giving all the essential ingredients for formation of OH radicals.

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe(III) + OH^- + OH$$

Thus the addition of Fe^{2+} salt (or a ferric salt plus a reducing agent, such as ascorbate) to a peroxide free unsaturated lipid in the presence of O_2 should initiate lipid peroxidation (H abstraction by OH). The resulting peroxidation should be inhibitable by H_2O_2 removing enzymes (eg. catalase), scavengers of OH and chelating agent that bind iron and prevent its participation in free radical reactions.

It has been recognised that several plant products have significant antioxidant activity and public awareness on the harmful side effects of synthetic food additives increased their demand in food processing. Although reports are available on the antioxidant activity of ethanolic/acetone turmeric extracts,[213-219] no systematic investigation exist on individual curcuminoids and related 1,7-diarylheptanoids.

In this investigation, hydroxyl scavenging activity, inhibition of lipid peroxidation and inhibition of superoxide radical generation, activity of two typical 1,7-diarylheptanoid considered in chapter 2, section 3 namely, 1,7-bis(hydroxynaphthyl) hepta-1,6-diene-3,5-dione, **HL**, **2b** and dianthryl heptanedione, **Hdah**, chapter 2, section 2 and their metal complexes were studied. The experimental details and results obtained are discussed below.

Experimental

Assay for antioxidant activity

Inhibition of superoxide, scavenging of hydroxyl radical and inhibition of lipid peroxidation by the curcuminoids, Hdah and their metal complexes were examined. The ligand and its complexes of Cr(III), Cu(II) and Fe(III) were dissolved in dimethyl sulphoxide (DMSO) and various test concentrations were prepared by diluting this solution to study their antioxidant activity.

Inhibition of superoxide radicals by riboflavin photoreduction method: Superoxide scavenging was determined by the NBT reduction method of McCord and Fridovich.[220] The reaction mixture contained EDTA (0.1M) containing 0.0015% NaCN, riboflavin (0.12 mM) NBT (1.5 mM) and various concentrations of test sample and total volume was adjusted by adding phosphate buffer (67 mM, pH 7.8) to 3mL in a test tube. The tubes were uniformly 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 absorbance of the control and experimenal tubes. The percentage inhibition was calculated by using the formula $\frac{C-T}{C} x100$ where C and T are the optical density of the control and test sample.

Assay Protocol

Control			**Test Sample**
NBT	-	100 µL	100 µL
NaCN/EDTA	-	200 µL	200 µL
Drug	-	0 µL	Variable concentration
Riboflavin	-	50 µL	50 µL
Buffer	-	2650 µL	Volume adjusted to 3000 µL
		---	---
Total	-	3000 µL	3000 µL

The effect on the hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the test sample for hydroxyl radical generated from Fe^{3+} ascorbate - EDTA - H_2O_2 system. The hydroxyl radicals attack deoxy ribose that eventually result in thiobarbituric acid reacting substances formation (TBARS). The reaction mixture containing deoxyribose (2.8 mM) $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1.0mM), ascorbate (0.1 mM), KH_2PO_4 - KOH buffer (20 mM, pH7.4) and various concentrations (range 2.5, 50µg/mL of the test sample in a final volume of 1 mL was incubated and deoxyribose degradation was measured on TBARS as illiustrated below. 0.4mL of this reaction mixture was mixed with 200 µL sodium dodecyl sulphate (SDS), 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 hr. cooled and then 1 mL of water was added. Then 5mL of 15:1 mixture of butanol : pyridine mixture was added, mixed 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 $\frac{C-T}{C} x100$ where C and T are the optical density of the control and test samples respectively.

Assay Protocol

	Control	**Test sample**
Deoxy ribose	- 100 µL	- 100µL
$FeCl_3$	- 100 µL	- 100 µL
Ascorbic acid	- 100 µL	- 100 µL
EDTA	- 100µL	- 100 µL
Test sample	- 0 µL	- Variable value
H_2O_2	- 100 µL	- 100 µL
Buffer	- 500 µL	- Volume adjusted to 1 mL
Total	- 1000 µL	- 1000 µL

The effect on the inhibition of lipid peroxide formation: The lipid peroxide formation was measured by the method of Ohkawa *et al.*[221] 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 (40mM, 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 volume of .5mL was incubated for 1 hour at 37°C. From this reaction mixture 0.4mL was mixed with SDS (0.2mL, 8.1%), TBA (1.5ml, 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 1 hour. After cooling 1mL distilled water, 5 mL of a mixture of n-butanol and pyridine 15:1 v/v) were added and shaken vigorously. 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 $\frac{C-T}{C} x100$.

Assay Protocol

	Control	**Test sample**
Tissue Homogenate	- 100 μL	- 100μL
Tris buffer	- 100 μL	- Vary accordingly
Ascorbic acid	- 100 μL	- 100 μL
Ferrous Amm. sulphate	- 100μL	- 100 μL
KCl	- 100 μL	- 100 μL
Test sample	- 0 μL	- Variable concentration
Total	500 μL	500 μL

Results and Discussion

The results of antioxidant activity observed for the two curcuminoids **2b** and **Hdah** and their metal complexes are discussed below separately.

Antioxidant activity of hydroxynaphthyl curcuminoid, 2b and its metal complexes

Inhibition of superoxide radical generation

The results obtained on the inhibition of superoxides produced by the photosensitization of riboflavin by the curcuminoid, **2b** and its Cu(II), Cr(III) and Fe(III) complexes are given in **table 3.4**. All the complexes produced enhanced superoxide scavenging activity compared to free ligand. Cr(III), Cu(II) complexes produced 50% inhibition at 17 μg/mL and 28μg/mL respectively. All other compound required higher than 30 μg/mL to produce 50% inhibition. The results showed that the metal complexes are more active than the free ligand (**Fig. 3.2**).

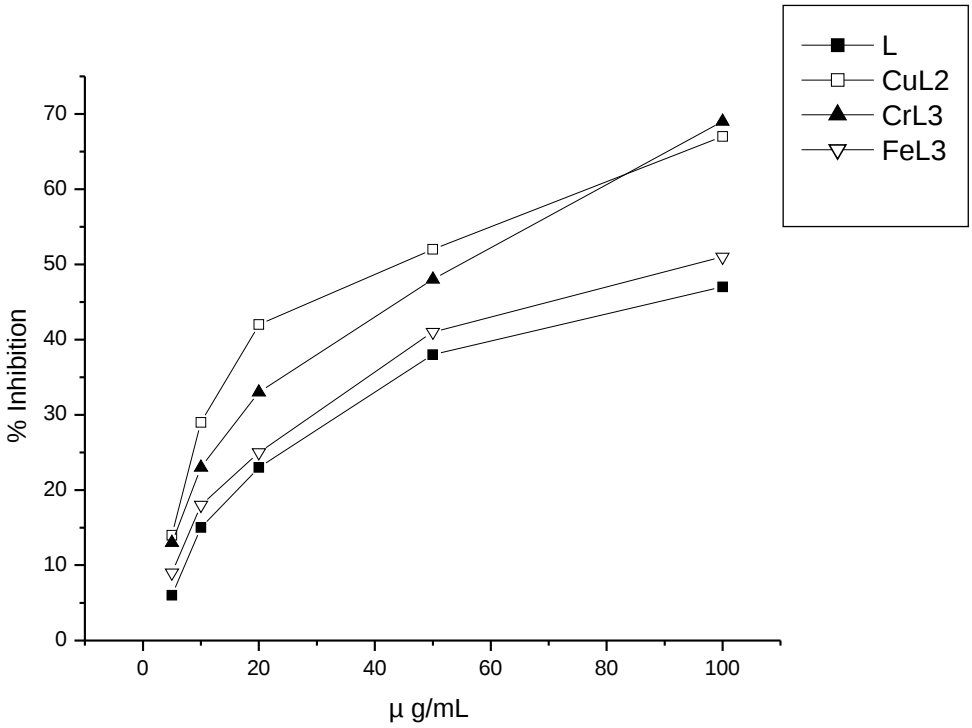


Fig. 3.2. Superoxide inhibition of 2b, HL and its metal complexes

Hydroxyl radical scavenging activity

The effect of hydroxynaphthyl curcumin (**2b**) and its Cu(II), Cr(III) and Fe(III) complexes are shown in the **Fig. 3.3**. The data indicated that all the compounds are good hydroxyl radical scavengers. The metal complexes are more active than the free ligand. At lower concentration Fe(III) possess maximum activity, but at higher concentration region it showed lesser activity. Fe(III), Cr(III) and Cu(II) complexes showed 50% inhibition at concentrations 1.5 µg/mL 4.8µg/mL and 5µg/mL respectively. The free ligand produced 50% inhibition at concentration 18 µg/mL (**Table 3.5**).

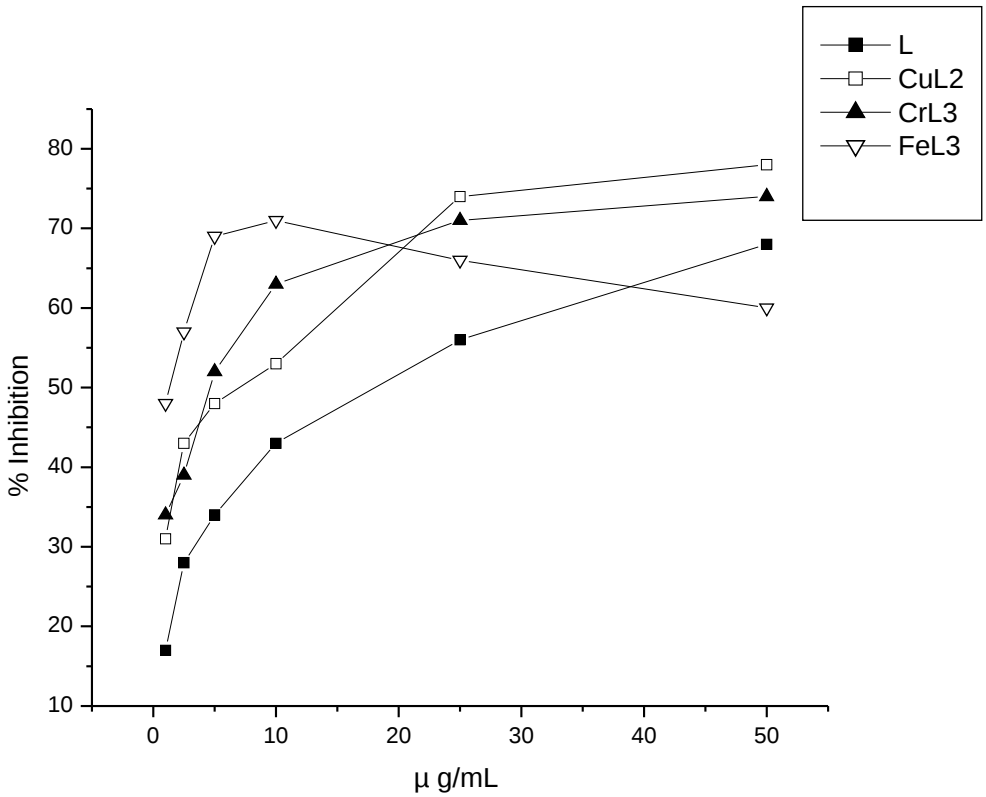


Fig. 3.3. Hydroxyl inhibition of 2b, HL and its metal complexes

Inhibition of lipid peroxidation

All the four compounds were tested for their ability to inhibit the ferrous ion induced lipid peroxidation or rat liver homogenate. Cu(II) and Cr(III) complexes produced 50% lipid peroxidation at concentration of 8.5 and 12.5 μg/mL respectively. The concentration required for 50% inhibition by other compounds was around 20 μg/mL (**Fig. 3.4**).

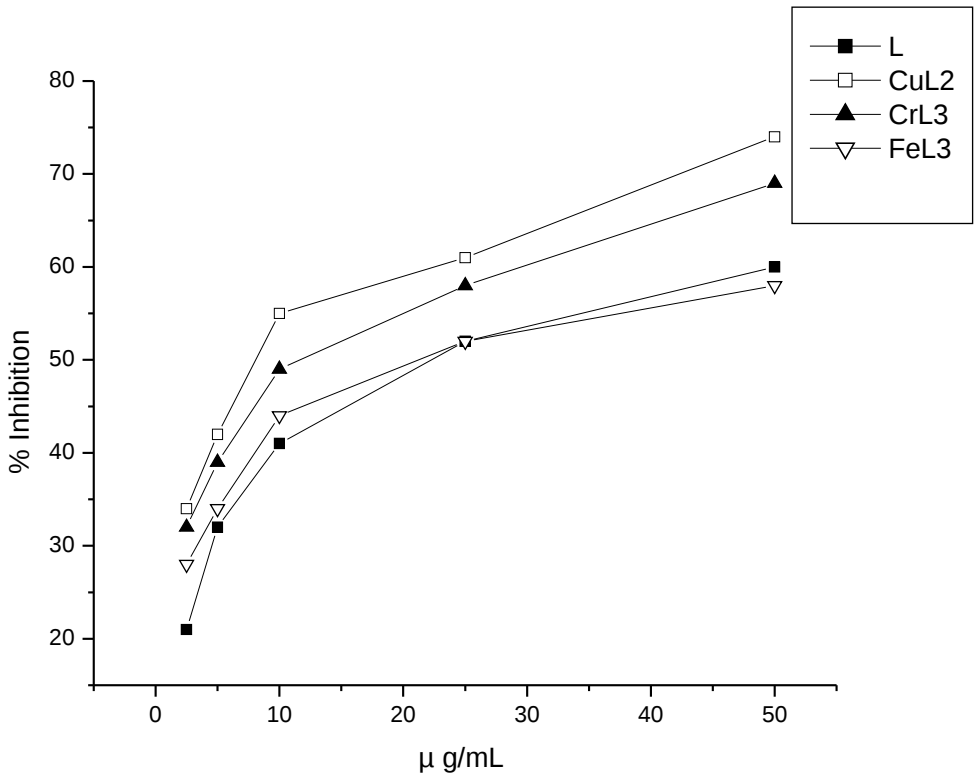


Fig. 3.4. Lipid peroxide inhibition of 2b, HL and its metal complexes

The ligand hydroxynaphthyl curcumin and its metal chelates possess considerable antioxidant property. Generally the metal complexes are more effecient antioxidant than the free ligand. The high activity of this compound may be due to the presence of phenolic –OH, which can scavenge the free radicals by a hydrogen atom abstraction mechanism. The increased activity of the metal complexes may be attributed to the presence of the metal ion which provide extra site for the attack of the free radicals or it may facilitate the hydrogen atom abstraction from the OH group (**Table 3.6**).

TABLE 3.4

Superoxide scavenging effect of 2b, HL and its metal complexes

Compound	Percentage inhibition				
	5 µg/mL	10 µg/mL	20/µg mL	50 µg/mL	100 µg/mL
2b (HL)	8	17	27	45	54
$[CuL_2]$	13	24	42	73	81
$[CrL_3]$	24	43	53	69	76
$[FeL_3]$	11	28	33	56	64

TABLE 3.5

Hydroxyl radical scavenging activation of 2b, HL and its metal complexes

Compound	Percentage inhibition					
	1 µg/mL	2.5 µg/mL	5 µg/ mL	10 µg/mL	25 µg/mL	50 µg/mL
2b (HL)	17	28	34	43	56	78
$[CuL_2]$	31	43	48	53	74	78
$[CrL_3]$	34	39	52	63	71	74
$[FeL_3]$	48	57	69	56	54	49

TABLE 3.6

Lipid peroxide scavenging of 2b, HL and its metal complexes

Compounds	Percentage inhibition				
	2.5 µg/mL	5 µg/mL	10/µg mL	25 µg/mL	50 µg/mL
2b (HL)	21	32	41	52	60
$[CuL_2]$	34	42	55	61	74
$[CrL_3]$	32	39	49	58	69
$[FeL_3]$	28	34	44	52	58

Antioxidant activity of anthryl curcumin, Hdah and its metal complexes

Inhibition of Superoxide radical generation

Data obtained on the inhibition of superoxide generation by **Hdah** and its metal complexes are given in the **Fig. 3.5**. All the four compounds produced considerable superoxide radical inhibition. Cr(III) and Cu(II) complexes produced 50% inhibition at 44 μg/mL and 48 μg/mL respectively. The ligand Hdah and the Fe^{3+} complex produced the same effect at higher concentrations.

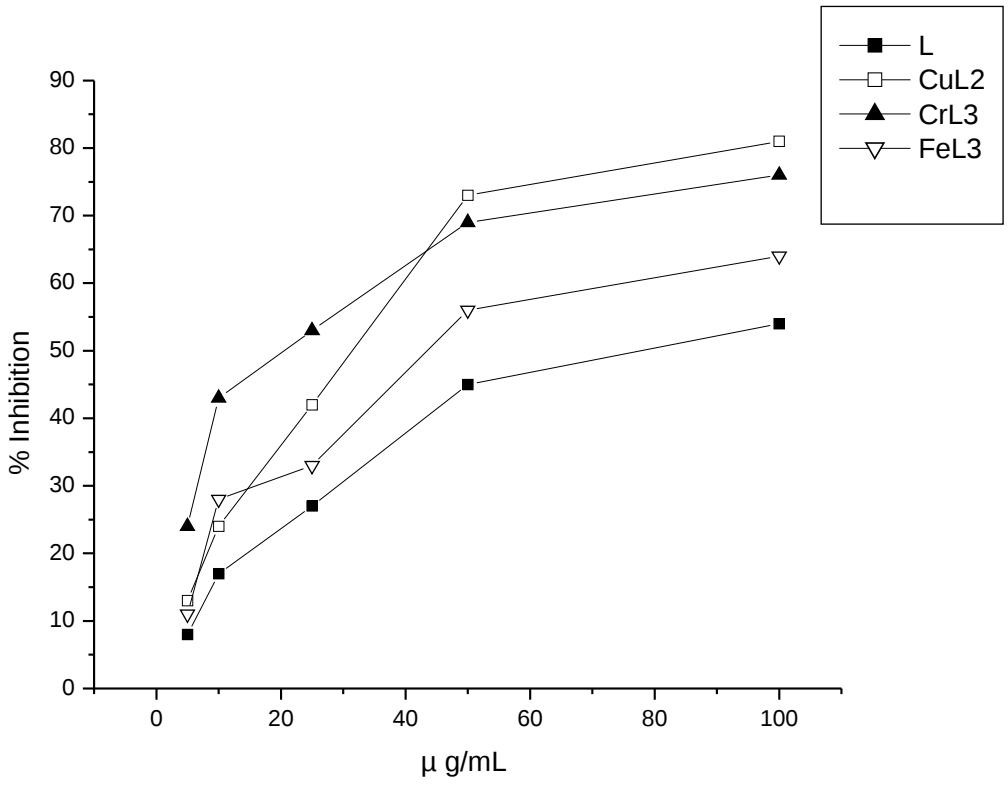


Fig. 3.5. Superoxide inhibition of Hdah and its metal complexes

The results showed that at moderate concentration Cr(III) complex is most effective towards superoxide scavenging and all the three metal complexes showed activity higher than the free ligand. But at higher concentration, Cu(II) has higher activity than the Cr(III) complex (**Table 3.7**).

Hydroxyl radical scavenging activity

The effect of the ligand and the metal complexes on scavenging hydroxyl radicals is given in **Fig. 3.6**. All the metal complexes show activity higher than the ligand. At lower concentration Fe(III) possesses maximum activity, but at higher concentrations Cu(II) and Cr(III) showed more activity.

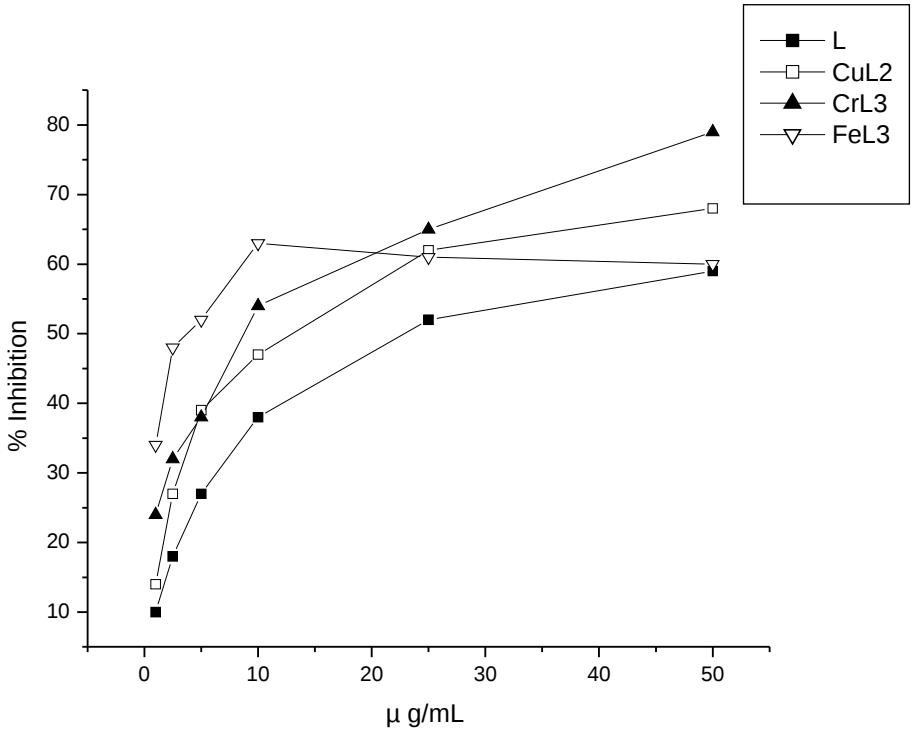


Fig. 3.6. Hydroxyl inhibition of Hdah and its metal complexes

The Fe(III), Cr(III) and Cu(II) produced 50% inhibition at concentrations 3.5, 7.5 and 12.5 μg/mL respectively. Compared to the metal complexes the ligand is less active towards scavenging hydroxyl radicals. The low activity of Fe(III) at higher concentration may be due to some catalytic effect of the complex itself or some Fe^{3+} ion that may be formed due to the decomposition of the complex which can assist the production of hydroxyl radicals by Fenton reaction (**Table 3.8**).

Inhibition of lipid peroxidation

All the four compounds were investigated for their ability to inhibit the ferrous ion induced peroxidation of rat liver homogenates. Cu(II), and Cr(III) complexes produced 50% lipid peroxidation at concentrations of 8 and 21 μg/mL respectively. The concentration required by all other compounds was around 30 μg/mL (**Fig. 3.7**).

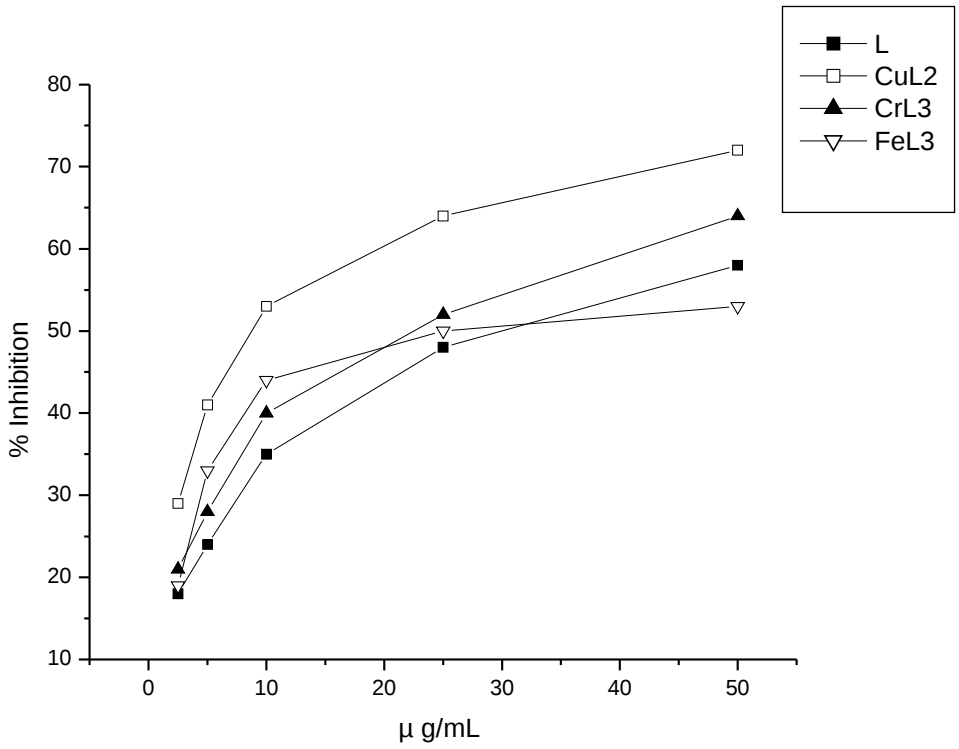


Fig. 3.7. Lipid peroxide inhibition of Hdah and its metal complexes

The two curcuminoids and their metal complexes, produced superoxide and hydroxyl radicals scavenging activity. Among the ligands **2b** is more active than **Hdah**. Generally the metal complexes showed more activity than corresponding free ligands. Among the various metal complexes Cu complexes showed more activity.

In the inhibition of ferrous ion induced peroxidation of rat liver homogenates, the ligand hydroxynaphthyl curcumin, **2b** was found to be more active than anthrylcurcumin, **Hdah**. As expected the metal chelates showed more activity than the free ligands towards inhibition of lipid peroxidation.

The *in vitro* studies of these metal chelates further revealed that Cu chelates are most effective in scavenging various free radicals (**Table 3.9**).

TABLE 3.7

Superoxide inhibition by Hdah and its metal complexes

Compound	Percentage inhibition				
	5 μg/mL	10 μg/mL	20 μg/mL	50 μg/mL	100 μg/mL
Hdah	6	15	23	38	47
$[Cu(dah)_2]$	14	29	42	52	62
$[Cu(dah)_3]$	13	23	33	48	69
$[Fe(dah)_3]$	9	18	25	41	51

TABLE 3.8

Hydroxyl scavenging activity of Hdah and its metal complexes

Compound	Percentage inhibition					
	1 μg/mL	2.5 μg/mL	5 μg/mL	10 μg/mL	25 μg/mL	50 μg/mL
Hdah	10	18	27	38	52	59
$[Cu(dah)_2]$	14	27	39	47	62	68
$[Cr(dah)_3]$	24	32	38	54	65	79
$[Fe(dah)_3]$	34	48	52	63	61	60

TABLE 3.9

Lipid peroxide scavenging of Hdah and its metal complexes

Percentage inhibition					
Compounds	2.5 µg/mL	5 µg/mL	10 µg/mL	25 µg/mL	50 µg/mL
Hdah	18	24	35	48	58
$[Cu(dah)_2]$	29	41	53	64	72
$[Cr(dah)_3]$	21	28	40	52	64
$[Fe(dah)_3]$	19	33	44	50	53

SECTION 3

Biodistribution assay of curcuminoids

Introduction

Numerous reports on the various aspects of the pharmacology of curcuminoids particularly their activity as chemopreventive agents are available.[222] However, the absorption pharmokinetics and metabolism in human and experimental animals have not been fully studied. The metabolism of these compounds has mostly been studied in mice *in vivo* and *in vitro*. Recently information on the metabolism of curcuminoids in human has been obtained from *in vivo* studies with hepatic and intestinal cells and subcellular fraction as well as from clinical studies with cancer patients.[223] These studies showed that the metabolism of curcuminoids involve the successive reduction of the olefinic bonds. The enzymes responsible for the reduction have been found to reside in the cytosol of liver and intestine and include alcohol dehydrogenase. However, no systematic data are available on the biodistribution of curcuminoids in various body organs. In the present study, a preliminary attempt has been made in this direction.

Several classical and instrumental techniques are available for the biodistribution and metabolism studies of both natural and synthetic pharmaceuticals. Among these, the most reliable information are often

obtained from radioisotope labelling studies (radiopharmaceuticals). This is because of the precision, accuracy and reliability which are inherent of the technique.

Since curcuminoids from stable metal complexes with various transition and non-transition elements, the possibility of using metal complexes of curcuminoids for the study of their biodistribution was attempted. The results obtained are promising and are discussed in this section.

Radiopharmaceuticals: Radiopharmaceuticals are the results of worldwide efforts to bring nuclear energy in a tangible form to the health care needs of people. The most popular example is cobalt-60 and cesium-137 used for the treatment of cancer and the well known technique of radioimmunoassay. In general, radiopharmaceuticals are formulations that incorporate radionucleide for the diagnosis, therapy, imaging or biodistribution studies. For this, short lived radionucleids having γ-ray emission to 100-200 KeV are preferred as they give low radiation dose and facilitate efficient external detection by suitable detectors. Apart from nuclear property, the chemical and biological properties of the radionucleide are important in developing such radiopharmaceuticals.

Of all the radionucleids used in radiopharmaceuticals, ^{99m}Tc is the one employed in more than 80% of the studies.[234] This is mainly because of the

excellent nuclear and chemical characteristics such as: (a) Its short half life ($t_{1/2}$ ~ 6 h), (b) Its decay by isomeric transition and involves very little particulate radiation, (c) It emits monoenergetic γ-rays of 140 KeV suitable for the detection by γ-camera imaging systems, (d) Its availability through the $^{99}Mo/^{99m}Tc$ generator and (e) Its versatile chemistry – being a transition metal which can exist in variable oxidation state, can form complexes with different ligand systems, many of which are found to concentrate on specific organs.

The biodistribution of radiopharmaceuticals are dependent on many factors. The overall size, charge and lyophilicity/hydrophilicity determine their biological fate. For example, the pertechnetate ion mimics iodide and is taken up by thyroid.

In 1959, Brookhaven National Laboratory developed the $^{99}Mo/^{99m}Tc$ generator and in 1964 the first ^{99m}Tc radiotracers were developed at the University of Chicago by Harper et al.[225] The generator yielded Technetium as pertechnetate ion, TcO_4^-. After injection into mice, the activity was found to localize in the thyroid, salivary glands, stomach and urinary bladder, similar to iodide ion. The vast range of technetium compounds grouped, mostly organwise such as imaging agents of liver, hepatobiliary systems, born, lungs, renal, cardiac, brain, etc.[226]

Radioisotope of iodine ^{123}I, ^{125}I and ^{131}I are widely used in nuclear medicine. ^{131}I ($t_{1/2}$ = 8 days) for chemical diagnosis; ^{125}I, to evaluate superficial organs such as the thyroid gland. Its lack of beta radiation, however, makes it a good choice for labelling organic compounds to avoid the problem of radiolytic decomposition encountred with ^{131}I. Its 60 day half-life is desirable for long term studies of the metabolism of its compounds and also provides a long shelf life. ^{123}I is an ideal isotope for chemical studies because of the 159 KeV gamma radiation, 13.3 h half life and lack of beta radiation.[226]

A widely used random label for erythrocytes is ^{51}Cr as sodium chromate. When Cr(VI) is incubated with anticoagulated whole blood at room temperature, it promptly crosses the red cell membrane and enters the cells. Within the red cell, chromate is promptly reduced to the chromium(III) ion, the majority of which binds to the beta chain of hemoglobin. Labelling efficiency with ^{51}Cr from chromate is approximately 90% and the label resists cell washing and dialysis. Whole blood is used routinely for ^{51}Cr labelling, but if the white blood cell count exceeds 25,000/mm^3 or if the platelet count exceeds 500,000/mm^3, a significant fraction of ^{51}Cr attaches to these cells and is unavailable to the red cells.

In the present study ^{51}Cr was successfully employed for the biodistribution assay of curcuminoids. Technetium (^{99m}Tc) labelling was also attempted however the percentage of labelling was below the required level.

So biodistribution assay could not be carried out using ^{99m}Tc, the ideal radioisotope for these type of studies. Experimental details and results obtained are discussed below.

Experimental

Labelling of the curcumin with ^{99m}Tc

The study was carried out at BRIT (Board of Radiation and Isotope Technology), BARC, Trombay.

The isotope ^{99m}Tc was made available from a ^{99}Mo generator. Technetium exists in oxidation state from +1 to +7. Tc obtained from the generator was in the form of sodium pertechnetate [Tc(VII)], the most stable oxidation state. The sodium pertechnetate from the generator obtained as a column chromatographic elute, was calibrated for radioactivity with dose calibration.[228]

Inorder to complex Tc with ligands, it has to be reduced to a more reactive lower oxidation state, for example +5 state. The reduction is carried out using reducing agents such as stannous chloride, ferric chloride/ascorbic acid, ferrous iron, sodium borohydride, etc. Stannous chloride is the most commonly used reducing agent, because it can efficiently reduce the pertechnetate. It can be relatively easily handled and has low toxicity.

The curcuminoid, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-hepta-diene-3,5-dione (**2f** in chapter 2, section 3) was used for labelling. The compound (1 mg) in NaOH (1 M) was mixed with 0.5 μCi ^{99m}Tc (in the form of pertechretate from a column chromatographic generator). To this different amount (100 μg, 200 μg, 300 μg and 500 μg) of $SnCl_2$ was added to reduce pertechnetate to lower oxidation state and to facilitate complex formation with the curcumin and ^{99m}Tc. The solution was then spotted after 10 minutes on a 6 x 0.5 cm paper chromatographic strip and chromatograms prepared in methanol (85%), 0.9% saline and acetone. The counts of the chromatograms at different height intervals were measured. From that the percentage labelling was determined. The result revealed that maximum labelling (60%) occurred when 300 μg of $SnCl_2$ used as reductant. Since a minimum of 80% labelling is essential for assay studies, biodistribution experiments was not carried out using the ^{99m}Tc labelled curcumin.

Labelling of curcumin with ^{51}Cr

The method employed for the labelling of the curcuminoid with ^{51}Cr involve first the preparation of a Cr(III) complex as follows.

Chromium(III) chloride hexahydrate (5 mg) and the curcumin (15 mg) were dissolved in 10 ml ethanol and heated to 60^{o}C for 3 h in nitrogen atmosphere. The precipitated complex was filtered and purified by column

chromatography using solvent system of petroleum ether : ethyl acetate in the ratio 8:2.

The pure chromium complex in 0.9% saline (500 μL) was mixed with $Na^{51}CrO_4$ (ImCi) and heated at ~ 60°C for 2 h. The reaction mixture was spotted on 15 cm long Whatman No.1 chromatographic paper after 1 h and 2 h. The chromatograms were developed in the solvent systems, H_2O : EtOH : NH_3 in the ratio 5:2:1 and also in 0.05 M disodium hydrogen phosphate. Electrophoresis studies of the developed chromatograms for both solvent systems gave 80% labelling after 1 h and 88% labelling after 2 h. The chromium complex with 88% labelling with ^{51}Cr was employed for biodistribution studies.

Biodistribution studies of the ^{51}Cr labelled curcumin in mice

This study was carried out at animal house of BARC, Trombay. Nine Swiss Albino mice were selected for the biodistribution studies. They were given 0.1 ml of 0.9% saline form of the radiolabelled complex intraperitonially and sacrificed after 1 h, 3 h and 24 h in a group of 3.

The initial weight of each mice was taken before giving the injection. After sacrificing the animals, different organs were separated and weighed accurately. Gama counts were measured in a well type counter. Background count was also noted.

The different organs separated for measuring the counts were liver, kidneys, stomach, intestine (small and large), brain, spleen, lungs and heart. Other parts measured for counts were femur, blood, tail and muscle.

The standard count was measured by placing about 0.1 mL saline solution of radiolabelled sample of complex and subtracting the background count which was measured without the sample. The measurement was in number of gamma ray counts per 10 sec. The injected dose (id) was calculated by measuring the tail count by the following equation

$$X = \frac{\text{Corrected standard count x Wt. of injected dose}}{\text{Wt. of standard}}$$

Injected dose count (id) = X – corrected tail count

By comparing the organ count with injected dose count, the percentage of injected dose (% id) on each organ was calculated.

In the case of muscle, the whole muscle count was calculated by assuming that only 0.4 part by weight of the animal contributed to the weight of muscle. Thus the percentage of injected dose at the muscle

$$= \frac{\frac{\text{Counts by muscle}}{\text{Wt. of muscle}} \text{ x } 0.4 \text{ x } 100 \text{ x Wt. of animal}}{\text{Standard injected dose}}$$

In the case of blood, the whole blood count was calculated by assuming that only 0.07 parts by weight of blood contributed to the total weight of the animal. that is, the percentage of injected dose in the blood

$$= \frac{\frac{\text{Counts by blood}}{\text{Wt. of blood}} \text{ x } 0.07 \text{ x } 100 \text{ x Wt. of animal}}{\text{Standard injected dose}}$$

Results and discussion

The calculated percentage injected dose for different organs after 1 h, 3 h and 24 h, tail and standard count are given in the **tables 3.10-3.16**. From the weight of organs, the percentage of injected dose per gram of organs were calculated and are also given in **tables 3.10-3.16**. The data show that most of the activity was in liver, about 60% which indicate the accumulation of the chromium complex in liver. Lungs and spleen are the important other organs where activity was shown prominently.

Measurement of activity at different time intervals showed that the activity of liver was almost constant after 1 h, 3 h and 24 h. The activity of the lungs was slightly decreases with time. But in the case of spleen, there is marked decrease in the activity with time which shows a better clearance of the complex from spleen.

TABLE 3.10

Variation of activity with time for organs, liver, lungs and spleen

Organ	Activity		
	After 1 h	After 3 h	After 24 h
Liver	61.51	59.83	54.82
Lung	13.66	10.40	8.97
Spleen	14.71	8.90	5.74

TABLE 3.11

Percentage id for different organs after 1 h giving the radiolabelled complex

Organs	Average counts – Background			Percentage injected dose			Mean	Standard deviation
	1	2	3	1	2	3		
Liver	17174	17459	17992	60.436	63.43	60.26	61.51	2.02
Kidneys	865	911	1094	3.575	3.331	3.66	3.45	0.17
Stomach	47.5	102.5	81.6	0.196	0.375	0.27	0.28	0.09
Intestine – small	204	261.5	134.5	0.843	0.956	0.45	0.75	0.27
Intestine – large	185	250	361	0.765	0.914	1.21	0.96	0.23
Brain	18	18	50	0.074	0.066	0.17	0.10	0.06
Spleen	2710	4398.5	5030.5	11.200	16.084	16.85	14.71	3.07
Lungs	2710	4398.5	4001.5	11.200	16.084	13.40	13.66	2.45
Heart	93.5	113	122.5	0.386	0.413	0.41	0.46	0.01
Corrected Standard	28416	29620	31634					
Corrected tail	24196	27347	29854					

id wt.	0.146	0.151	0.161					
Std. wt.	0.144							

TABLE 3.12

Percentage id for different organs after 3 h giving the radiolabelled complex

Organs	Average counts – Background			Percentage counts per injected dose			Mean	Standard deviation
	1	2	3	1	2	3		
Liver	13870	20200	17039	43.759	70.456	65.27	59.83	14.16
Kidneys	551	653.5	540	1.898	2.279	2.07	2.09	0.19
Stomach	53.5	33.5	50	0.184	0.117	0.19	0.16	0.04
Intestine – small	265	266.25	190	0.913	0.929	0.73	0.86	0.11
Intestine – large	149	289	93	0.513	1.008	0.36	0.63	0.34
Brain	15	30.5	33	0.052	0.106	0.13	0.09	0.04
Spleen	2688.5	2618	2619.5	9.263	9.132	8.31	8.90	0.52
Lungs	2688.5	2618	3345	9.263	9.132	12.81	10.40	2.09
Heart	83.5	101.5	63	0.288	0.354	0.24	0.29	0.06
Corrected Standard	31697	29905	29376					
Corrected tail	29025	28670	26107					
id wt.	0.157	0.149	0.149					
Std. wt.	0.144							

TABLE 3.13

Percentage id for different organs after 24 h giving the radiolabelled complex

Organs	Average counts – Background			Percentage counts per injected dose			Mean	Standard deviation
	1	2	3	1	2	3		
Liver	16979.5	13591	16761	51.839	50.561	62.07	54.82	0.2
Kidneys	682.5	427.5	272	2.266	1.590	1.01	1.93	0.61
Stomach	561.5	55	58	1.864	0.205	0.21	0.76	0.52
Intestine – small	99.5	125.5	82.5	0.330	0.467	0.31	0.37	0.07
Intestine – large	442.5	120.5	153	1.469	0.448	0.57	0.83	0.51
Brain	8	17.5	13.5	0.027	0.065	0.05	0.05	0.08
Spleen	1701	2150.5	960.5	5.648	8.000	3.56	5.74	2.21
Lungs	1701	2150.5	3583	5.648	8.000	13.27	8.97	3.91
Heart	65.5	53	65.5	0.217	0.197	0.24	0.22	0.02
Corrected Standard	32754	29143	29595					
Corrected tail	30116	26880	27004					
id wt.	0.155	0.14	0.142					
Std. wt.	0.144							

TABLE 3.14

Percentage id for different organ wt. of mice after 1 h giving the radiolabelled complex

Organs	Weight			Percentage per gram			Mean	Standard deviation
	1	2	3	1	2	3		
Liver	1.499	1.430	1.752	40.32	44.65	35.11	40.02	4.77
Kidneys	0.378	0.348	0.438	9.46	9.57	8.37	9.13	0.67
Stomach	0.497	0.465	0.964	0.39	0.81	0.28	0.49	0.28
Intestine – small	1.402	1.517	1.142	0.60	0.96	0.39	0.65	0.28
Intestine – large	1.958	1.993	2.715	0.39	0.60	0.45	0.48	0.11
Brain	0.31	0.435	0.366	0.24	0.15	0.46	0.28	0.16
Spleen	0.102	0.079	0.09	109.8	203.6	187.22	166.87	50.10
Lungs	0.187	0.141	0.178	59.89	114.07	75.30	83.09	27.92
Heart	0.145	0.147	0.154	2.66	2.81	2.66	2.71	0.08

TABLE 3.15

Percentage id for different organ wt. of mice after 3 h giving the radiolabelled complex

Organs	Weight			Percentage per gram			Mean	Standard deviation
	1	2	3	1	2	3		
Liver	1.635	1.575	1.302	26.76	44.73	45.95	39.15	10.74
Kidneys	0.356	0.411	0.376	5.33	5.55	5.50	5.46	0.11
Stomach	0.415	0.335	0.526	0.44	0.35	0.36	0.39	0.05
Intestine – small	1.923	1.402	2.07	0.47	0.93	0.35	0.59	0.30
Intestine – large	1.506	2.403	1.162	0.34	0.72	0.31	0.46	0.23
Brain	0.392	0.393	0.269	0.13	0.27	0.47	0.29	0.17
Spleen	0.082	0.117	0.079	112.96	78.05	105.19	98.73	18.33
Lungs	0.201	0.184	0.19	46.08	49.63	67.44	54.38	11.44
Heart	0.142	0.129	0.101	2.03	2.74	2.39	2.39	0.36

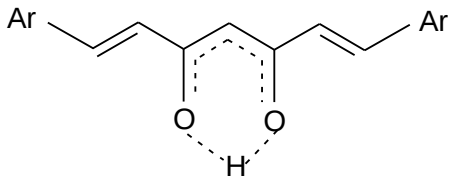
TABLE 3.16

Percentage id for different organ wt. of mice after 24 h giving the radiolabelled complex

Organs	Weight			Percentage per gram			Mean	Standard deviation
	1	2	3	1	2	3		
Liver	1.659	1.410	1.562	31.25	35.86	35.10	34.07	2.47
Kidneys	0.493	0.327	0.342	4.60	4.86	2.95	4.14	1.04
Stomach	2.681	1.241	1.382	0.70	0.16	0.16	0.34	0.31
Intestine – small	1.756	1.711	1.642	0.19	0.47	0.19	0.28	0.16
Intestine – large	2.711	1.352	2.184	0.54	0.26	0.26	0.35	0.16
Brain	0.435	0.408	0.451	0.06	0.16	0.11	0.11	0.05
Spleen	0.082	0.106	0.099	68.88	75.47	35.93	60.09	21.19
Lungs	0.2	0.106	0.181	28.24	75.47	73.30	59.01	26.67
Heart	0.204	0.138	0.203	1.07	1.43	1.19	1.23	0.18

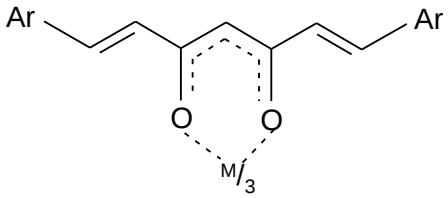
S U M M A R Y

The present investigation is mainly on the synthesis, characterisation and biological studies on metal complexes of some synthetic analogues of natural curcuminoids, the active chemical constituents of the Indian medicinal plant turmeric. Curcuminoids (structurally, 1,7-diaryl-1,6-heptadiene-3,5-diones) considered in the study are given below.



	Ar
1a	phenyl
1b	cinnamoyl
1c	naphthyl
1d	fuoryl
Hdah	anthryl
2a	2-hydroxy-1-phenyl
2b	2-hydroxy-1-naphthyl
2c	4-methoxyphenyl
2d	4-hydroxyphenyl
2e	4-dimethyl amino phenyl
2f	4-hydroxy-3-methody phenyl
2g	3,4-dimethoxy phenyl

These "unsaturated" 1,3-diketones formed well defined complexes of $[ML_3]$ stoichometry with Cr(III), Fe(III), Al(III) and $[ML_2]$ stoichiometry with Cu(II). Analytical and spectral data are consistent with the monobasic bidentate coordination of the ligands with the metal ions in which the diketo oxygens are involved in the formation of C_3O_2M ring system as in structure.



1,7-Dianthrylheptanoid and its Cu(II), Cr(III) and Fe(III) complexes were examined for their cytotoxic and antitumour activities. All the compounds showed cytotoxicity and possesses antitumour activity. The metal chelates showed more activity than the ligand.

In order to test the efficiency of the compounds as a successful drug against cancer, the percentage increase in life span of tumour bearing mice, administered with the curcuminoid analogue and its metal complex were studied. The results obtained clearly suggest that the compound **Hdah** and its metal complexes when administered intraperitonially could produce increase in the life span of tumour bearing mice. It was found that the metal complexes showed slight increase in the activity than the ligand **Hdah** towards tumour cell. However the effect on the ascites tumour reduction was not so marked as in the case of solid tumour.

The curcuminoids **2b** and **Hdah** and their Cu(II), Cr(III) and Fe(III) complexes were subjected to *in vitro* antioxidant studies. These studies help us to assess their ability to scavenge various free radicals like superoxide, hydroxyl and to inhibit lipid peroxidation. The results obtained showed that all the ligands and their metal complexes produced superoxide and hydroxyl radicals scavenging ability. Among the ligands, **2b** is more active than **Hdah**. Generally, metal complexes showed more activity than corresponding free ligands. Among the various metal complexes, copper complexes showed more activity.

In the inhibition of ferrous ion induced peroxidation of rat liver homogenates, the ligand **2b** was found to be more active than **Hdah**. As expected, the metal chelates showed more activity than the free ligands towards inhibition of lipid peroxidation. The *in vitro* studies of these metal chelates further revealed that copper chelates are most efficient in scavenging various free radicals.

The biodistribution studies of the synthetic curcuminoid (diferuloyl methane) in Swiss albino mice were carried out using radioisotope labelling technique. The chromium complex of the compound labelled with ^{51}Cr was used for the study. The measurement of activity at different time intervals showed that the activity of liver was almost constant, while that of the lungs decreases slightly with time. But there was a marked decrease in activity with time in spleen, which shows a better clearance of the complex from spleen.

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