SYNTHESIS, CHARACTERISATION AND ANTITUMOUR ACTIVITY OF 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*** *in Chemistry*

By

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DECLARATION

I hereby declare that the Thesis bound herewith is an authentic record of the research work carried out by me under the supervision of Dr. K. Krishnankutty, Professor, Department of Chemistry, University of Calicut, in the partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Chemistry of the University of Calicut and further that, no part thereof has been presented before for any other Degree.

John V.D.

CERTIFICATE

This is to certify that the Thesis bound herewith is an authentic record of the research work carried out by Mr. John V.D., under my supervision in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Chemistry of the University of Calicut, and further that no part thereof has been presented before for any other degree.

Dr. K. Krishnankutty

(Supervising Teacher)

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PREFACE

In recent years research in the field of coordination chemistry of biologically important materials has gained considerable momentum. This is because metal complexes play numerous vital roles in the chemistry of living matter. Similarly it has been revealed that the biological significance, especially medicinal importance, of many plant chemicals are associated with their ability to form complexes, with various inorganic species such as metal ions. However structural and biochemical aspects of these types of complexes has not received as much attention as they deserve. Thus chemical and biochemical examination of active plant products have considerable importance. The present investigation has been so designed as to provide some insight on various chemical and biochemical aspects of metal complexes of certain synthetic analogues of the active chemical components, present in the traditional Indian medicinal plant turmeric namely curcumionoids.

Structurally curcuminoids are 1,7-diarylhepta-1,6-diene–3,5-diones (1,7-diarylheptanoids) and are a group of naturally occurring 1,3-diketones in which the diketo function is directly linked to olefinic

groups. Present study is mainly on the synthesis and characterisation of a series of curcuminoid analogues (1,7-diarylheptanoids) and their typical metal complexes. The cytotoxic and antitumour activities of these compounds and their metal complexes were also studied.

The Thesis is dived into four parts.

Part I is a general introduction highlighting the importance of various aspects of coordination chemistry in biological processes particularly in medicinal inorganic chemistry and chemotherapeutic application of food phytochemicals. Since compounds considered in this study are typical 1,3-diketones some of the salient structural features of metal 1,3-diketonates are also included in this part. Importance of the present investigation has been interspersed at appropriate places.

Important chemical, biochemical and medicinal applications of curcuminoids and allied derivatives reported in the general literature have been briefly reviewed in **Part II**

Results of the present investigation are presented in **Part III.** For convenience this part of the **Thesis** is divided into four chapters based on the nature of the aryl groups of the 1,7-diarylheptanoids. Each chapter is divided into two **Sections**. Synthesis and characterization of the 1,7-diarylheptanoids and their metal complexes are presented in **Section 1**. In **Section 2** results of antitumour studies are discussed.

In **Chapter 1, Section 1** details on the synthesis and spectral characterisation of unsubstituted 1,7-diarylheptanoids are presented. Spectral data clearly indicate the existence of the compounds entirely in the intramolecularly hydrogen bonded *cis*-enol form. The behaviour of the compounds as monobasic bidentate ligands in complexes of VO^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, and Cu^{2+}, are clearly indicated. The use of uv, ir, nmr and mass spectral data in elucidating the structure and nature of bonding in these types of compounds are also illuminated in this section. The spectral data unequivocally showed that only the diketo function is involved in bonding with metal ion. In **Section 2** experimental details employed for the study of cytotoxic and other antitumour behaviour of the compounds are discussed. Results obtained on short term *in vitro* cytotoxicity studies and cytotoxicity of compounds towards L929 cultured cells are given. Determination of the effect of compounds in reducing ascites tumours and solid tumour volume in mices are also discussed. The observed activity of the compounds is correlated on the basis of their structural factors.

In **Chapter 2, Section 1** details on the synthesis and spectral characterisation of 1,7-diarylheptanoids having *ortho*-substituents on the aryl rings and their metal complexes are discussed. In **Section 2** results of

the antitumour activity of the compounds are given and the data are discussed on the basis of the *ortho*-aryl substituents.

Details on the synthesis and characterisation of 1,7-diarylheptanoids with different substitutents at the *para* position of the aryl rings and their typical metal complexes are included in **Chapter 3, Section 1**. In **Section 2** the results of *in vitro* and *in vivo* antitumour studies of the compounds are discussed.

Chapter 4, Section 1 is on synthesis and characterisation of certain curcuminoid analogues containing substituents at the *meta*- and *para*-positions of the aryl rings. Some of these compounds are the synthetic analogues of the compounds present in natural curcuminoids. Synthesis and structural characterisation of the compounds are given in **Section 1** and the results of their cytotoxic and other antitumour studies are presented in **Section 2**.

The structural aspects and antitumour activity of the fifteen-curcuminoid analogues considered in this investigation are summarised inorder to get an understanding on the structure activity relationships of the compounds. A comparative evaluation of the data shows that extended conjugation and presence of free –OH groups on the aryl rings increase the antitumour activities. The study also revealed that

complexation with Cu^{2+} ion significantly increases the antitumour activity of the curcuminoids.

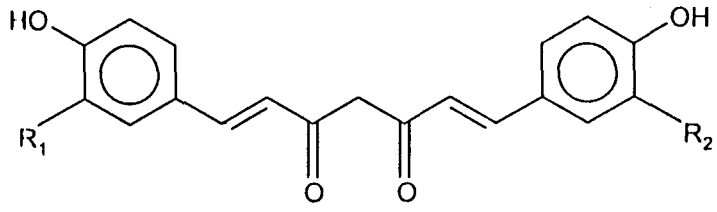
In **Part IV** references are given in serial order.

The work described in this **Thesis** has partially been published/accepted/communicated for publication as listed below.

1. 'Antitumour studies of metal chelates of synthetic curcuminoids'. *J. Exp. Clin. Cancer Res.*, 2002, **21** (2), 487.
2. 'Synthesis, Characterisation and Antitumour studies of Metal Chelates of some synthetic curcumionids'. *Synth. React. Inorg. Met-org.Chem.* (accepted).
3. 'Antitumour activity of four new synthetic curcuminoid analogues (1,7-Diaryl-1,6-heptadiene-3,5-diones) and their copper complexes'. *J. Clin. Biochem. Nutr.* (accepted).
4. 'Metal complexes of some 1,7-diheteroarylheptanoids'. (communicated).
5. 'Chemical and biochemical studies on Curcuminoids-A Review'. (communicated).
6. 'Metal chelates of some disubstituted 1,7-diarylheptanoids'. (communicated).

NOMENCLATURE AND ABBREVIATIONS

The yellow pigment, the most active chemical components of turmeric is named as 'curcuminoids'. Names indicating the source of the compounds is a usual practice of naming the active chemical compounds isolated from natural sources. The curcuminoids also conform to this practice indicating that its source is from the plant *curcumin longa* Linn (turmeric). Three major chemical components present in curcuminoid extract were identified as Curcumin I, Curcumin II and Curcumin III. Structurally they are 1,3-diketones of the type given below.



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

Systematically, these compounds are 1,7-diarylhepta-1,6-diene-3,5-diones and for brevity they are sometimes referred as 1,7-diarylheptanoids. In the present investigation both the names and the trivial name curcuminoid are used wherever necessary.

Important abbreviations used in the Thesis are:

ADI	average daily intake
Ar	aryl group
BM	Bohr Magneton
Dmf	dimethylformamide
DMBA	Dicmethyl benzanthracene
DLA Cells	Dalton's lymphoma ascites cells
EAC Cells	Ehrlich ascites carcinoma cells
FAB	Fast atom bombardment
h	hour
H_z	Hertz
ILS	Increase in Life Span
ip	intra peritonial
J	coupling constant
L	Deprotonated ligand
MEM	Minimum Essential Medium
M.P	Melting point

Ph	Phenyl group
ppm	parts per million
PBS	Phosphated buffer saline
ROS	Reactive Oxygen Species
SD	Standard deviation
tlc	thin layer chromatography
$\in$	molar extinction coefficient
μ_{eff}	effective magnetic moment in Bohr magnetons
μ_g	microgram (10^{-6}g)

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 isotpe of the concerned metal atoms.

CONTENTS

PART III

METAL CHELATES OF 1,7-DIARYLHEPTA-1,6-DIENE-3,5-DIONES AND THEIR ANTITUMOUR ACTIVITY

PART I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Importance of metal complexes in biological systems

The area of coordination chemistry is one of the most intellectually attractive and experimentally demanding frontiers in modern chemical science[1,2]. Its scope is well known in many fields including catalysis, biology and medicine where much progress has been made during the past few decades[2-4]. The importance of metal complexes, in the latter field is clearly indicated by the wide variety of metal complexes especially of organic ligands employed commercially in diverse types of medicinal formulations and various biochemical fields.

Many metals play a crucial role in living systems. This is mainly because of their ability to form positive ions which tend to be soluble in biological fluids[3,5,6]. Most biological molecules such as proteins, DNA, etc. possess electron rich sites. The positive metal ions can effectively bind to such biological molecules. The same principle applies to the affinity of metal ions for many small molecules and ions crucial to life such as O_2[7,8]. Considering the wide scope that exist for the interaction of metals in biological systems, it is not surprising that natural evolution has incorporated many metals into essential biological functions.

The active sites of a large number of proteins and enzymes contain one or more metal ions. Their structure and properties are often modulated by the coordination environment of the metal ions[9-14]. Examples like chlorophyll (a Mg complex) vitamin B_{12} (a Co complex), and haemoglobin and myoglobin (Fe complexes), etc emphasises this argument.

It has been variously estimated that approximately one-third of all proteins and enzymes require metal ions as cofactors for biological functions. Such protein bound metal sites can be classified into five basic types according to their functions[8].

1. *Structural*: Configuration (in part) of proteins, tertiary and/or quarternary structure;
2. *Storage*: Uptake, binding and release of metals in soluble form;
3. *Electron transfer*: Uptake, release and storage of electrons;
4. *Dioxygen binding*: Metal–O_2 coordination and de-coordination;
5. *Catalytic*: Substrate binding, activation and turnover. This is an extensive class subdivided by type of reactions catalysed such as dismutases, oxidases and oxygenases, nitrogenases, hydrogenases, oxotransferases, hydrolases, etc.

Metals which occur in the above five types of coordination sites includes Mg, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Mo, W, Cd and Hg. These "biological metals" are usually covalently bound to the polypeptide back-bone by endogenous ligands provided by amino acid side chains[15-16]. Protein structure and environment modulate properties such as electronic structure, redox potential, detailed stereochemistry, etc, and thus achieve its functions. In that sense metallobiomolecules can be considered as an elaborated inorganic complex equipped with the necessary protein structure for complimentarity and for all other functional aspects.

In general metal ions coordinate to biological ligands through nitrogen, oxygen and sulphur atoms. The general answer to the question, which, metal ion will tend preferentially to form a complex ion with which ligand was best summarized by the hard and soft acid–base concept developed by Pearson[17-18]. Biologically important groups containing nitrogen, oxygen and sulphur donor atoms are summarized in table 1, based on their hard-soft character. Hard metal ions are not readily polarized and are of small size and high charge density. While, soft metal ions are relatively large and easily polarisable ones.

In table 1, biologically significant metal ions are also classified as hard, soft and borderline.

Table 1

a) HSAB classification of biologically important cations

Hard	H^+, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cr^{3+}, Fe^{3+}, Co^{3+}, VO^{2+}, WO^{4+}, MoO^{3+}.
Soft	Cu^+, Ag^+, Au^+, TI^+, Pd^{2+}, Pt^{2+}, Cd^{2+}, Hg^{2+}.
Border line	Zn^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Fe^{2+}, Co^{2+}, Sn^{2+}.

b) HSAB Classification of biologically important ligands

Hard	H_2O, OH^-, ROH, RO^-, R_2O, NH_3, Cl^-, PO_4^{3-},SO_4^{2-}, F^-, NO^-_3, CO_3^{2-}, CH_3COO^-.
Soft	RSH, RS^-, R_2S, R_3P, R_3As, CO, $S_2O_3^{2-}$, H^-, I^-, CN^-.
Border line	Pyridine, R-NH_2, N_2, N_3^-, NO_2^-, Br^-.

It is logical to observe that evolution has selected elements for tasks that entirely consistent with chemical experience[19-24]. For example Fe and Cu with two stable oxidation states for electron transfer, binding and activation of O_2, oxidation–reduction of substrates; Mo with three stable oxidation states for oxygen atom transfer; Zn with its flexible

stereochemistry for non redox catalysis; Ni and Co for catalysis involving formation and rupture of metal – carbon bonds. Thus it can be stated that nature has made extensive use of metal ions in biological systems and most of their biological functions can be conveniently explained on the basis of various principles of coordination chemistry[25-29].

In this context it is logical to consider the extension of the use of coordination chemistry for medicinal purpose or the use of medicinal preparations incorporating metal ions. Investigations in this direction for the last four decades led to the development of a number of drugs containing metal ions and today medicinal inorganic chemistry has became a self-consistent branch of chemistry[30-32].

Metal ions in therapy

The ability of medicinal formulations containing metal ions and related materials to cure a number of diseases were well known for many ancient civilizations especially for Indian, Egyptian, Arabian and Chinese. The ability of Cu, Ag, Au, Hg, etc in combination with sulphur and similar non-metallic elements along with various plant extracts were extensively used in our traditional systems of medicine[33,34]. There are many other metallic elements that still

constitute a good number of drug formulations of the above traditional system of medicine. During the Renaissance era in Europe mercurous chloride was used as a diuretic. Nutritional essentiality of iron was also discovered[3,29]during this period. In the early twentieth century, $K[Au(CN)_2]$ was used against tuberculosis, various antimony compounds for leishmaniasis and gold compounds as antibacterial agents[35,36,37].

The biochemical literature of the last 40 years led to understand that many of the biological activity of proteins and enzymes can be ascribed to the metal centers and the role of metal ion is very crucial in effecting the requisite transformation. Ironically, the first structure–activity relationship developed by Paul Ehrlich in the first decade of the 20th century involved the organoarsenic compound, arsphenamine (salvarasan or Ehrlich 606), as a successful treatment for syphilis[35]. This Nobel laurate (1908) also established the preferential accumulations of lead in the central nervous system and above all the discoveries of chemotherauptic index and immunochemistry which are fundamental concepts in medical science.

Medicinal inorganic chemistry comprises the introduction of a metal ion into a biological system either by fortuity or by intention.

In the case of fortuitous introduction, the metal has to be removed from the biological system because excess metal causes poisoning. This is usually done by using suitable chelating agents like EDTA.

The intentional introduction of a metal ion into a biological system will be for either therapeutic or diagonestic purpose[31]. For instance γ-emitting radio pharmaceuticals based on Technetium-99m (^{99m}Tc) complex, the magnetic imaging control agents containing complexes of Gd and X-ray contrasting agents based on $BaSO_4$ are in routine clinical examination[37].

The use of chemotherapeutics involving metal complexes as anticancer agents, antibiotics, antibacterials, antivirals, antiparasitics, antiarthritics, etc. are well known and a huge growth of therapeutic applications of metal complexes will result in the near future. Thus medicinal inorganic chemistry is a vibrant and growing field of biotechnology. The essentiality of an element in biology is usually described in the form of a Bertrand diagram[38]. The Bertrand diagram (Fig. 1) indicate the relationship between benefit/detriment from an element and its concentration.

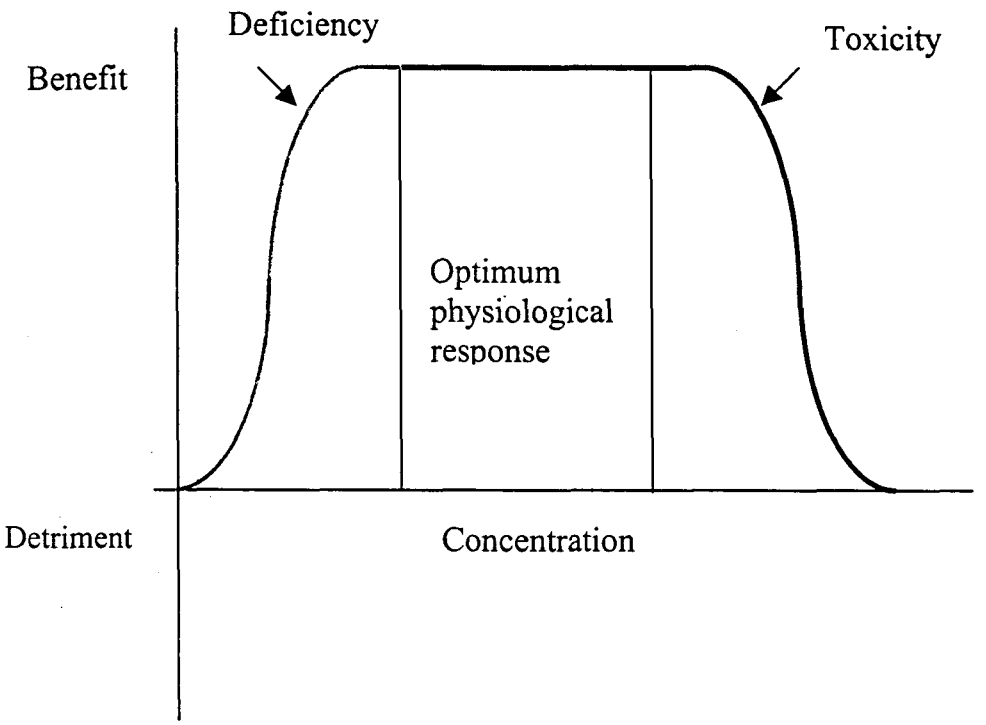


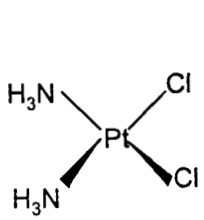
Fig 1. Bertand diagram indicating the relationship between benefit/detriment from an element and its concentration

The area of optimum physiological response will vary greatly according to the element, it's speciation, oxidation state and the biochemistry of the specific compound in which it is found. Thus by changing the variables optimum physiological response can be achieved and also to design suitable ligand system that can effectively regulate the functions of metal ions in biological systems.

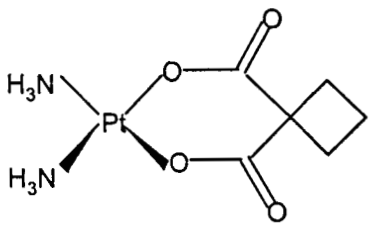
The need of redesigning ligand system, was established during early clinical trials of cisplatin (**1**) which is the most effective drug for the treatment of advanced cancer especially testicular and ovarian cancers[39-42]. The use of cisplatin as an antitumour agent caused drastic side effects like nausea and vomiting. This has been substantially controlled by design of a 'second generation' drug from cisplatin. Antitumour testing of a series of cisplatin analogues and kinetic studies on the loss of their leaving groups (chloride in case of cisplatin) showed that antitumour activity was retained across a range of reactivity, but that the nephrotoxicity (toxic side effects) were directly related to the rate of ligand loss. This discovery led to the preparation of a series of substituted malonate derivatives from which carboplatin was selected for clinical trials with minimum nephrotoxiciy[43].

Usually these drugs are given as intravenous hydration. A further objective in treatment of cancer would be development of a drug capable of oral administration as neither cisplatin nor carboplatin (**2**) were effective when given orally. A new class of Pt(IV) dicarboxylate complexes[44] has been developed (JM 216) (**3**), where the lipophilic nature of the compounds coupled with the inertness to substitution of

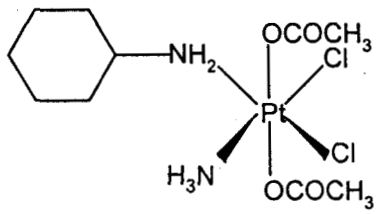
the Pt(IV) centre allows good absorption. After absorption, these compounds are reduced to Pt(II) species.



(1)
Cisplatin



(2)
Carboplatin



(3)
JM 216

Thus, the redesigning of ligand systems causes the refinement of drug properties especially in the case of platinum chemotherapeutics[45,46].

Despite the resounding success of cisplatin and closely related platinum antitumour agents, the development of other transition metal antitumour agents has been exceptionally slow mainly because of solubility problems. Actually non-platinum chemotherapeutic metallopharmaceuticals have at least four advantages than platinum compounds which may involve (i) additional coordination sites, (ii) changes in oxidation state (iii) alterations in ligand affinity and substitution kinetics and (iv) photodynamic approaches to therapy. Gallium, ruthenium, rhodium, titanium, vanadium, etc are considered as highly useful metals in this respect[46].

A part of the present investigation is on antitumour properties of metal complexes of a series 1,3-dicarbonyl compounds which are structurally related to certain important food phytochemicals. Therefore the importance of such studies on phytochemicals are briefly mentioned below.

Food phytochemicals in chemotherapy

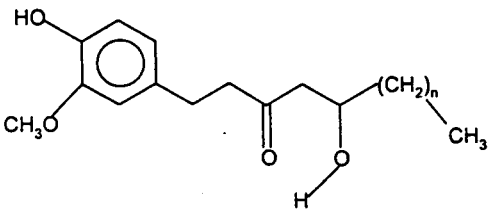
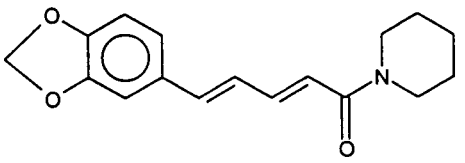
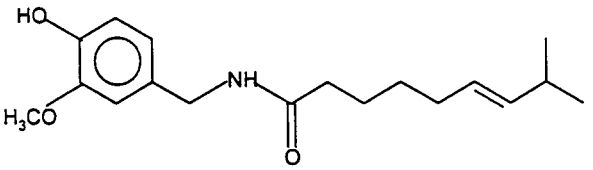
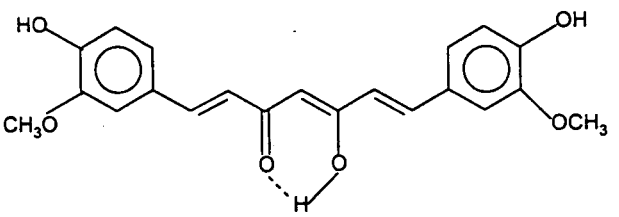
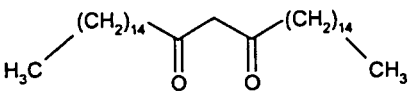
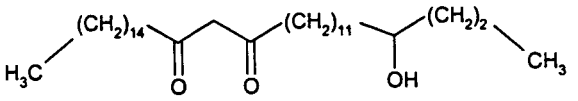
Synthetic organic compounds predominate modern drug formulations. However the human race since time immemorial has been using plants as a major source of medicine apart from their use as staple food. It can be seen that medicinal plants play a key role in the health care system of almost all countries[47]. This has been evident from the *materia medica* of the indigenous systems of medicine which has become extensive and heterogeneous during centuries[48,49]. 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. Many plant derived drugs used in modern medicine are developed by ethnomedical concepts and subsequent studies[50].

The Indian subcontinent is endowed with rich and diverse local health traditions which is matched with an equally rich and diverse plant genetic resources[51]. Medicinal uses of plants are well described in 'Rig Veda' and several other ancient literatures. The classical systems of medicine such as Ayurveda, Siddha, Unani, Amachi, etc. are largely based on herbal medicines which gained wide acceptance all over the world. This is clearly evident from the fact that the production and export of herbs and spices constitute a major source of income to our country.

Many plants and plant products are used as food and some as food additives from time immemorial. Spices are widely used as food additives because of their colour, flavour and preservative ability. Spices are mainly derived from the rhizomes, barks, seeds, fruits, leaves or flowers of plants and are pungent and aromatic. Spices in general increase salivary flow and secretion of neuramic acid and hexosamine which in turn helps digestion. Therapeutically, spices were used by ancient people for the treatment of several gastro-intestinal disorders[52-55]. Some of the major spices, medicinal plants and their active chemical constituents are given in table 2.

Table 2

Active constituents of some common spices and medicinal plants

Spice (Plant species)/ Active principle	Structure
Ginger (*Zingiber officinale*) Gingerols	 n = 2, 4, 6 and 8
Black pepper (*Piper nigrum*) Piperine	
Red pepper (*Capsicum annum*) Capsaicin	
Turmeric (*Curcuma longa*) Curcumin I	
Eucalyptus (*Eucalyptus globulus)* n-Tritri acontan-16,18-dione	
4-Hydroxy-tritri acontan-16,18-dione	

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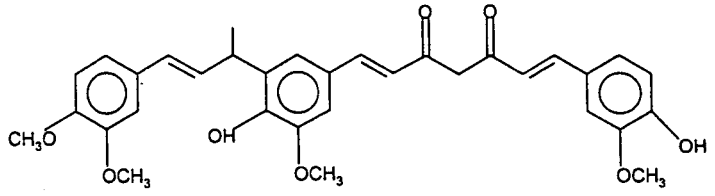
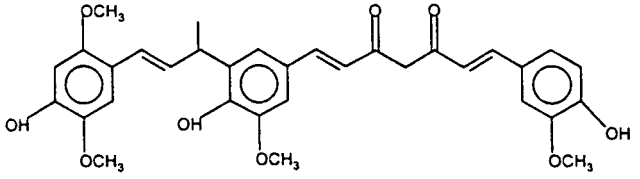
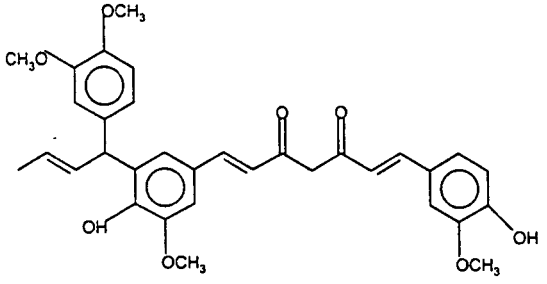
Spice (Plant species)/ Active principle	Structure
Indonesian medical ginger (*Zingiber cassumunar*)	
Cassumunin A	
Cassumunin B	
Cassumunin C	

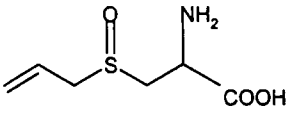
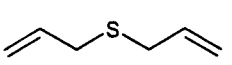
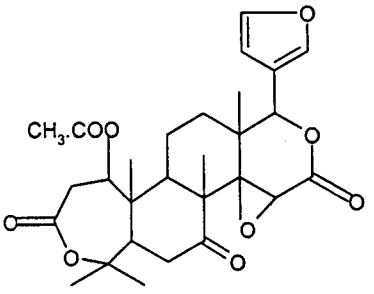
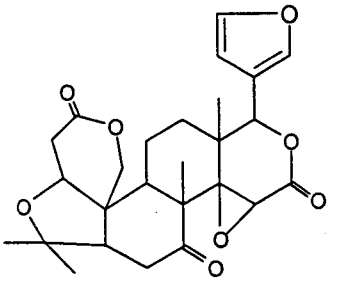
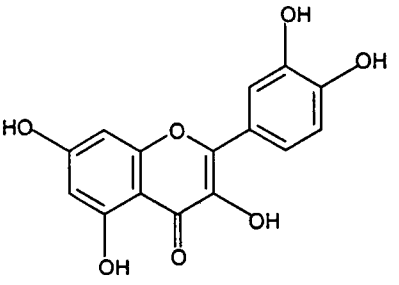
Research has identified herbs and herbal constituents that take us into a realm of safe and natural responses to many tragic illnesses. One of the fascinating aspects of herbal medicine is the finding of new ways that ancient remedies can help in modern medicine. It has been increasingly clear that plants and pharmaceutical drugs derived from them plays an important role in the treatment of diseases such as cancer, arthrites, alzhiemers and inflammation.

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[56]. Chemoprevention is an important defence strategy against human cancer since it is highly unlikely for one to avoid all carcinogenic insults. The objective of chemoprevention is to administer one or more chemical agents naturally occurring or synthetic, which may have multiple biological mechanisms to inhibit various stages of carcenogenesis.

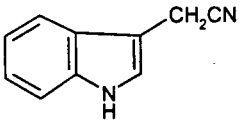
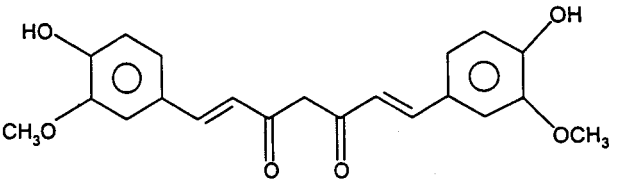
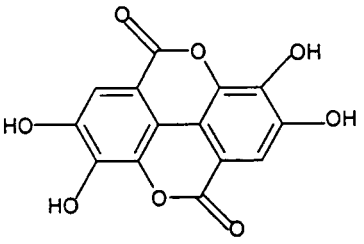
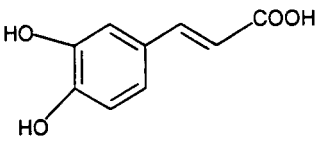
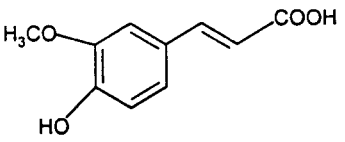
Foods of plant origin contain many bioactive compounds in addition to the vitamins and minerals. Some important phytochemicals that are able to inhibit carcinogenesis are given in table 3.

Table 3

Important Phytochemicals that are able to Inhibit Carcinogensis

Class of Compound/ Important plant source	Structure	Reference
Sulphur containing compound Allium sp. vegetables (garlic, onion and chives)	 allin	57-59
	 Diallyl sulfide	
Terpenoids Citrus fruits (lemon, lime, bitter orange)	 Nomilin	60
	 Limonin	
Flavanoids and flavanone Most vegetables fruits and cereal grains	 Quercetin	61,62

contd...

Class of Compound/ Important plant source	Structure	Reference
Indoles Cruciferous vegetables, (cabbage, cauliflower)	 3-Indole acetonitrile	63,64
Polyphenols Turmeric	 Curcumin	65-69
Fruits and nuts (grapes, strawberries)	 Ellagic acid	
Fruits, coffee bean and soybean	 Caffeic acid	
Fruits and soybean	 Ferulic acid	

The active chemical constituents of many medicinal plants possess one or more potential donor sites that can form stable metal complexes. Presumably their metabolic pathways may involve complexation with various metal ions in the biosphere. Although several studies exist on the wide range of physiological and various chemical aspects of the active chemical components of numerous medicinal plants, only very few reports are available on the synthesis, characterisation and biochemical aspects of their metal complexes.

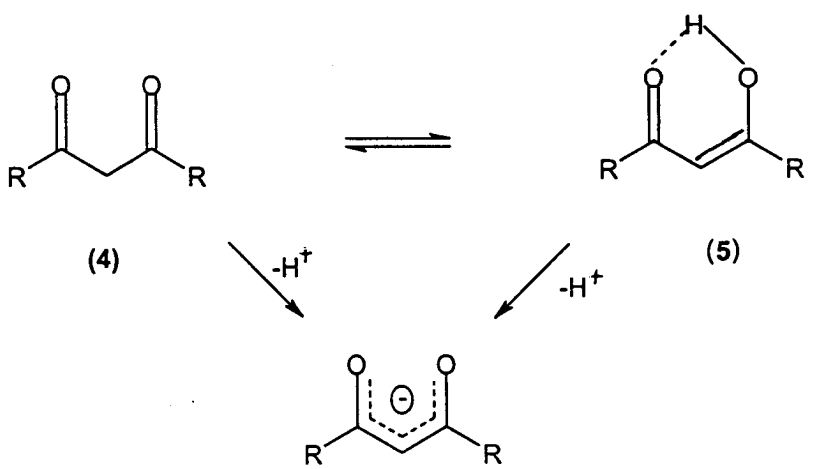
The present investigation is an attempt in this direction. Transition metal complexes of a series of synthetic analogues of curcuminoids were prepared and their structural characterisation were carried out. The *in vitro* and *in vivo* anticancer activity of typical metal complexes were also investigated. Curcuminoids, a group of structurally related compounds, are the most important active chemical components present in the herbaceous medicinal plant *curcuma longa* Linn (turmeric).

From a coordination chemist's point of view, perhaps, the most interesting feature of curcuminoids is that, structurally they are 1,3-diketones, in which the diketo function is directly attached to olefinic groups. This is because 1,3-diketones are known to form

complexes with almost all metal and metalloid ions in the periodic table[70-76]. Therefore it is quite appropriate to mention briefly some of the salient features of 1,3-diketones and their metal complexes which are quite pertinent to the present investigation.

Keto-enol tautomerism of 1,3-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. Compounds in which a methylene group is interposed between acyl or aroyl groups are designated as 1,3-diketones. Structurally, 1,3-diketones exist as a mixture of keto(**4**) and enol(**5**) forms related by a 1,3-hydrogen shift. The amount of enol form is



(6)

influenced by a variety of factors such as solvent, temperature, substituents at 1-, 2- and 3- positions and the presence of other species that are capable of hydrogen bonding. In general, bulky alkyl substituents on the 2-carbon, lead to decreased amount of the enol tautomer, while Cl^-, Br^-, CN^-, $^-COOCH_3$ and $^-SCH_3$ groups lead to almost 100% enol form[71,77-81]. The methine proton in the keto form and the hydroxyl proton in the enol form are acidic. The removal of these hydrogens generate 1,3-diketonate anion(**6**) which is the source of a broad class of coordination compounds referred to as metal 1,3-diketonates.

Metal complexes of 1,3- diketones

The coordinating ability of 1,3-diketones were recognised as early as in 1887 when, Combes reported the synthesis of beryllium acetylacetonates[82]. This was followed by the pioneering work of Werner[83], Morgan[84,85] and Sidgwick[86] who confirmed the *bifunctional* chelating character of these ligands. Being powerful chelating agents, the diketonate anion form complexes with virtually almost all the metal and metalloid elements. The literature on 1,3-diketones and metal 1,3-diketonates are so voluminous that even an attempt to summarise is

purposefully avoided. However, since 1,3-diketones exhibit a variety of coordination modes besides the usual behaviour as *monoanion* (**6**) these different types of coordination modes are briefly outlined below[87-89] with typical examples in order to exemplify the versatility of the diketo functions in their affinity towards metal ions.

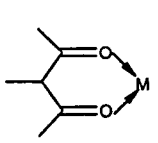
Different coordination modes of 1,3-diketones

The 1,3-diketones can coordinate to metal ions atleast in four different ways namely, (A) as neutral molecule (B) as monoanion

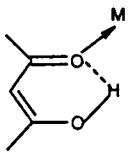
(C) as dianion and (D) as trianion. Typical examples of each category are given below.

A. Metal complexes of neutral 1,3-diketones: Three modes of coordination are proposed for neutral 1,3-diketone.

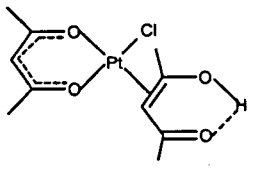
(a) O,O'-chelation of the keto tautomer, (**7**)
(b) O-unidentate coordination, (**8**)
(c) η^2 – (C,C^1) coordination of the enol form, (**9**)



(**7**)



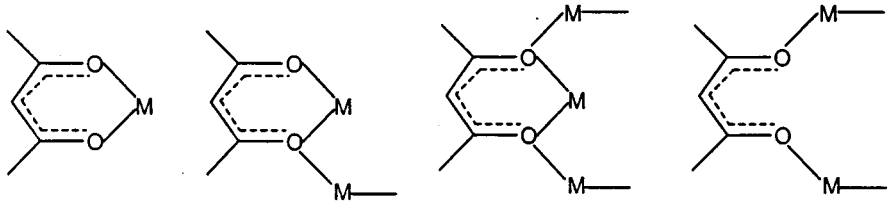
(**8**)



(**9**)

B. *Coordination modes of monoanion*

(a) O,O'– Chelation and bridging: This is the most popular mode of coordination of 1,3-diketones. Different structures possible are given below (**10-13**).

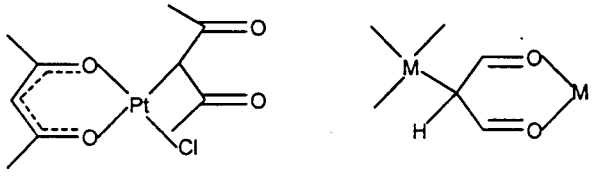


(10) **(11)** **(12)** **(13)**

(b) Central carbon bridging (**14**) and C,O,O' bridging, (**15**)

(c) O-unidentate linkage, (**16**)

(d) Terminal carbon bonding, (**17**)



(14) **(15)**

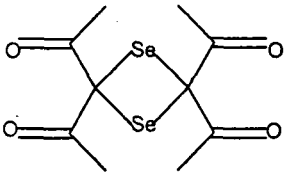
(16) **(17)**

C. *Coordination modes of dianions*

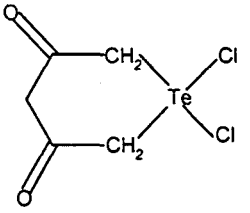
(a) Central carbon bonding, (**18**)

(b) Chelation through terminal carbons, (**19**)

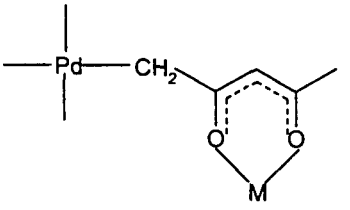
(c) C,O,O bridging, (**20**)



(18)



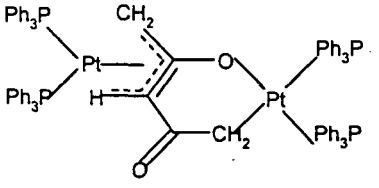
(19)



(20)

D. *Coordination modes of trianion*

An example of this rare coordination is the η^3, C, O complex (**21**)



(21)

Almost all spectral techniques as well as diffraction data along with other physical and chemical methods have been extensively employed in establishing the structure and nature of bonding in diverse types of metal 1,3-diketone complexes mentioned above.

Metal complexes of β-diketones have a number of practical applications. Lanthanide chelates of β-diketones are extremely useful as

nmr shift reagents[90-93]. Use of metal β-diketonates like $Cr(acac)_3$, in measurements of Carbon-13 nmr spectra of metal carbonyl compounds are well established[94-98]. Addition of metal β-diketonate reduces the normally long longitudinal relaxation times, thus minimizing saturation effects and allowing more rapid collection of data. Many of the rare earth metal β-diketonates find practical use as potential laser materials[99-103]. A number of metal β-diketone derivatives are suitable in vapour phase chromatographic separation of metals[104-108]. Fluorinated β-diketones are especially useful in the solvent extraction of metals[109-113].

Although literature is extensive on the chemistry and applications of 1,3-diketones and their metal complexes, it can be seen that majority of these studies are on 1,3 diketontes in which the diketo function is directly attached to alkyl/aryl groups. Only very few reports exist on metal complexes of 1,3-diketones in which the diketo function directly linked to olefinic groups. Such unsaturated 1,3-diketones and related compounds[114-118] constitute the active chemical principles of several medicinal plant, such as *curcuma longa*, *zingiber cassumunar*, leaf wax of *Eucalyptus globulus*[118](Table 2).

The present investigation, therefore, has been so designed as to provide ample opportunity for synthesis and structural investigation on metal complexes of a series of curcuminoid analogues.

Curcuminoids (1,7-diaryl-1,6-heptadiene-3,5-diones) have been reported to possess a number of medicinal uses particularly as a chemopreventive agent. Presence of phenolic group together with the conjugated β-diketone structure is suggested to be responsible for their high biological activity and this led to further studies using several structurally related compounds[119].

Metal complexation of these ∝,β-unsaturated 1,3-diketones may lead to dramatic changes in their biological activities including antitumour activity. Copper(II) is biologically active essential metal ion. Its chelating ability and positive redox potential allow participation in many biological reactions[20,120,121]. Copper(II) forms the active centres of more than a dozen metalloproteins. Literature suggests that copper(II) complexes possess a wide range of biological activity and are among the most potent antiviral, antitumour and antiinflammatory agents[122]. **The antitumour activity of a series of synthetic analogues of natural curcuminoids and their copper(II) complexes have also been investigated.**

PART II

CHEMICAL AND BIOCHEMICAL STUDIES ON CURCUMINOIDS AND ALLIED DERIVATIVES - A REVIEW

CHEMICAL AND BIOCHEMICAL STUDIES ON CURCUMINOIDS AND ALLIED DERIVATIVES - A REVIEW

Introduction

The use of medicinal plants and/or their active principles in the prevention and treatment of various human and animal ailments has a long history. Different ethnic societies have used plants and other natural resources in their own ways and developed different medicinal system mainly from their practical experience without any systematic scientific knowledge. However, during the last one hundred years, organic and plant chemists were seriously engaged in the isolation and structural characterisation, chemical synthesis and biochemical investigation of the active chemical constituents of various medicinal plants[47,123,124].

With the advent of modern chromatographic technique, it has become possible to isolate and characterise almost all chemical compounds present in medicinal plants. These studies have unmistakably shown that to a large extent the medicinal uses of a particular plant is due to the presence of certain specific chemical compound(s). The synthetic analogues of such chemical compound(s) also exhibit similar medicinal properties.

The Indian subcontinent is rich in diverse types of medicinal plants and a good number of our population still rely upon these herbs for their various diseases, despite the intentional publicity of the modern medicine, which is mainly based on synthetic organic chemicals[125]. In this context one of the best known example is turmeric. Turmeric occupies an unavoidable position in the life of Indian people not only as a spice but also as a common remedy for many diseases. It is employed also as a colouring agent[126-128] and as condiment entering into the composition of Indian pickles and curry powders. Indian women use turmeric powder to smear their hands and faces in ceremonial occasions. Such a use of turmeric is an inherent part of our society and no true references can be cited. However, some of the chemical and biochemical aspects of turmeric that are pertinent to the present investigation are briefly reviewed below.

Turmeric products

Turmeric, a native of South and South East Asia, probably originated in the sloppy hills of the tropical forests of the west coast of South India. Curing, drying and polishing are the three processes carried out in preparing the commercial grade turmeric. After harvesting, the

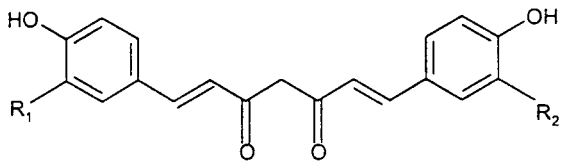
rhizomes of turmeric are boiled which allows the starch to gelatinise and helps in producing a product of fairly uniform colour due to the diffusion of the yellow pigments (curcuminoids) from individual cells into the surrounding tissues. The important commercial products derived from raw turmeric are turmeric powder, turmeric oleoresin, turmeric oil and curcuminoids[127,129]. Turmeric oleoresin is becoming increasingly important in the developed countries where the processed food industry has made phenomenal progress. Aroma of turmeric, caused by the steam volatile oil fraction ranges from 2.5-7.2% of the spice. Turmerone and ar-turmeron form more than 50% of the turmeric oil. The rest is made up largely of zingeberene, sesquiterpene alcohols, cineole, α-phellandrene, sabinene, and borneol[127,130-133].

The yellow colour of turmeric is due to the presence of curcuminoids. The dried rhizomes of *Curcuma longa* usually contain 1-5% of curcuminoids which gave the name 'yellow root' to this spice. Hot ethanol is a good extractant compared to other solvents like chloroform, acetone and benzene[134-137].

Constitution of curcuminoids

As early as in 1815, the crude pigment extracted from turmeric was found to contain at least three well defined yellow compounds[138].

Srinivasan *et al* in 1953 using column chromatography over silicagel separated these three curcuminoid compounds[134]. Later these compounds were identified[134,138] as curcumin I [diferuloylmethane] (**1**) or simply as curcumin, curcumin II [feruloyl-p-hydroxycinnamoylmethane] (**2**) and curcumin III [bis-(4-hydroxycinnamoylmethane] (**3**). Curcumin I which eluted first from the column is the major component (~ 40%) followed by curcumin II (~16%) and curcumin III (~10%).



	R_1	R_2
(1)	OCH_3	OCH_3
(2)	OCH_3	H
(3)	H	H

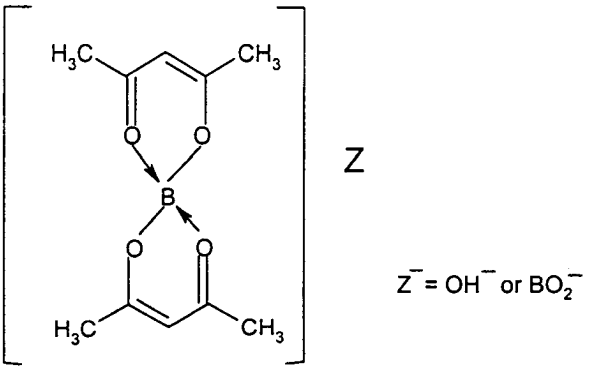
The structure of the compounds (**1,2,3**) were later confirmed by chemical degradation studies.

Synthesis of 1,7-diaryl heptanoids

In 1913 Lampe and Milobedzka first reported the synthesis of curcumin I. Their synthesis starting from vanillin builds up curcumin I in eight steps and had little practical value[139]. Later this method of synthesis

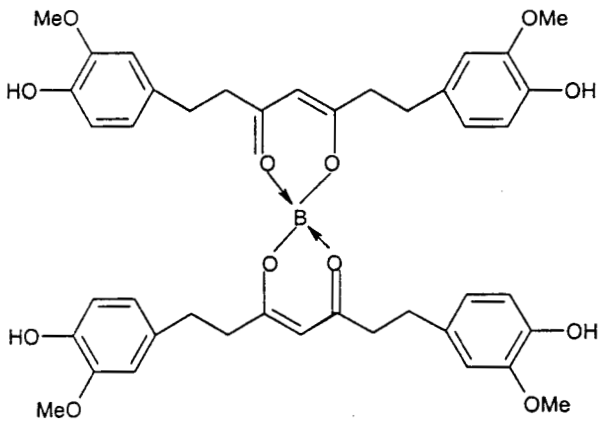
was improved by Pavalonie[140] who prepared curcumin I in a one step procedure by heating vanillin, acetylacetone and boric anhydride (2:1:2) over a free flame for 30 minutes and claimed a yield of 10%. H.J. J. Pabon in 1964 developed a general method for synthesising curcuminoids[141] and related 1,7-diarylheptanoids in ~80% yield from aromatic aldehyde, acetylacetone, B_2O_3 in presence of tri(sec-butyl) borate and *n*-butylamine. The reaction was carried out in dry ethylacetate at room temperature. It was found that the presence of tri(sec-butyl) borate is unavoidable for obtaining higher yield. The suitable temperature range for condensation was found to be 85-110^{0}C. At lower temperature mono condensation product will be formed.

As per the method developed by Pabon the acetylacetone-boric oxide complex (**4**) is first formed.



(4)

The complex formation with B_2O_3, provides an environment for coupling with free methyl group of acetylacetone-boron complex. This complex (**5**) has reported to be metallic green when formed, brick red in acid medium and deep purple in alkaline medium. The new complex is converted to free curcumin by acidification with con. HCl.

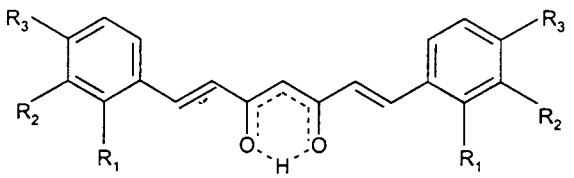


(5)

Using this method in recent years more than thirty curcuminoid analogues (1,7-diaryl-1,6-heptadiene-3,5-diones) were synthesised. These are given in table 1-3.

Table 1

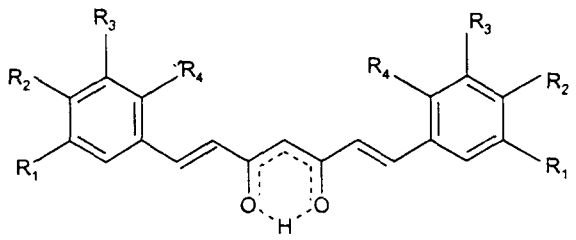
Synthetic curcuminoid analogues (mono and disubstituted)



No.	R_1	R_2	R_3	References
1	H	H	H	141,142,143
2	H	H	$N(CH_3)_2$	141,144
3	H	H	Cl	141,145
4	H	H	CH_3	145
5	H	H	OCH_3	141,142,143,144
6	H	H	OH	142,143,144,146,147
7	H	OCH_3	H	145
8	H	NO_2	H	141
9	OH	H	H	144
10	OCH_3	H	H	145
11	H	OH	OH	148
12	H	OCH_3	OCH_3	142,143,144,145,149,150
13	H	OCH_3	$OOCH_3$	143,149
14	H	$O–CH_2–O$		144
15	H	OC_2H_5	OH	151
16	H	OCH_3	OH	141,142,143,149,147
17	H	OCH_3	$OCH_2–C_6H_5$	149
18	H	OCH_3	$OOC–C_6H_5$	149

Table 2

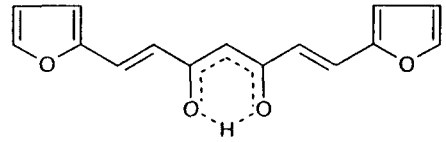
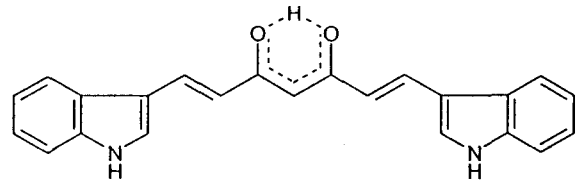
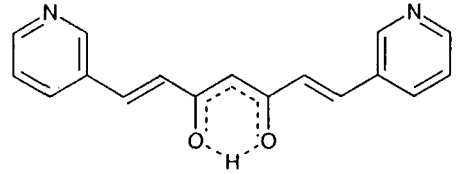
Synthetic curcuminoid analogues (trisubstituted)



No.	R_1	R_2	R_3	R_4	Reference
1	OCH_3	OH	H	$COCH_3$	152
2	OCH_3	OH	H	CH_3	152
3	OCH_3	OH	H	CH_3CH_2	152
4	OCH_3	OH	H	$CH_3CH_2CH_2$	152
5	OCH_3	OH	H	$(CH_3)_2CH$	152
6	OCH_3	OH	H	$CH_3CH_2CH_2CH_2$	152
7	OCH_3	OCH_3	OCH_3	H	153
8	CH_3	OH	CH_3	H	153
9	CH_3CH_2	OH	CH_3CH_2	H	153
10	$(CH_3)_2$ CH	OH	$(CH_3)_2CH$	H	153
11	$(CH_3)_3C$	OH	$(CH_3)_3C$	H	153
12	OCH_3	OH	OCH_3	H	153

Table 3

Synthetic curcuminoid analogues (Heteroaromatic)

Name of compound	Structure	Reference
1,7-bis(2-furyl)-1,6-heptadiene-3,5-dione		141, 144
1,7-bis(3-indolyl)-1,6-heptadiene-3,5-dione		141, 144
1,7-bis(3-pyridyl)-1,6-heptadiene-3,5-dione		144

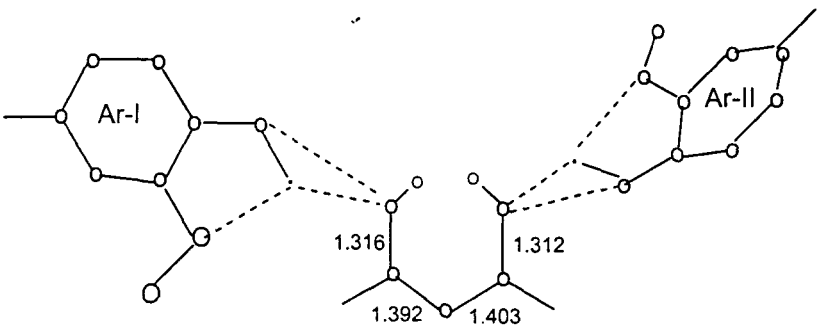
Structure of curcuminoids

Spectral characterisation: Electronic, ir, nmr and mass spectral data of curcuminoids and a number of 1,7-diarylheptanoids have been reported[127,154]. Spectral analysis established that the curcuminoids exist entirely in the intramolecularly hydrogen bonded enol form[155]. To avoid repetition, these spectral data will be quoted at appropriate sections while discussing the results of the present investigation.

Crystallographic data: The crystal and molecular structure of curcumin I (**6**) has been reported[156,157] by x-ray crystallographic methods. The crystals are monoclinic and space group P2/n with unit cell dimensions **a** = 20.028A^0, **b** = 7.073 A^0, **c** = 12.609 A^0, β = 94.94^0. The enolic hydrogen is found to be equally associated with two oxygens. There is no significant difference in the C-C or the C-O bonds in the enol ring giving a pseudo-aromatic character to the chelate ring system.

Conjugation between the aromatic ring I and the pseudo aromatic ring seems to be indicated by the distances between the atoms connecting the two ring systems which are also essentially coplanar, the angle between the two ring planes being only about 3^0. The interaction between

the π electron systems in pseudo aromatic and aromatic rings is probably some what less, as the angle between these two ring planes is about 45^0.



pseudo aromatic

(6)

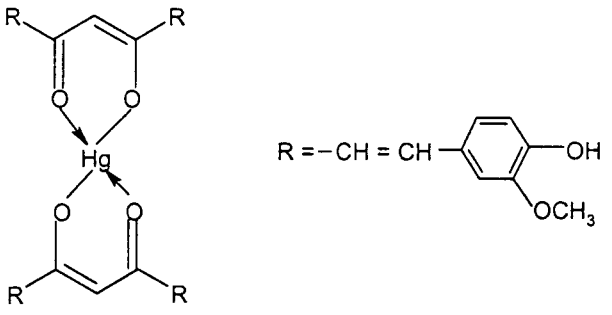
Metal complexes of curcuminoids

Considering the presence of the diketo moiety in curcuminoids, they are expected to form large number of coordination compounds. However only very few reports exist on the synthesis, characterisation and structural studies[158-160] of metal complexes of curcuminoids. Most of the reported reactions of curcuminoids[161,162] and metal ions were associated with various biological investigations.

The interaction of curcuminoids with slaked lime to from deep red product is utilised in many Hindu religious ceremonies. The colour change can be attributed to some kind of interaction between Ca^{2+} ions

and curcuminoids. The Ca^{2+} ions may replace either enolic/phenolic protons and changes the chromophoric group.

A gold(I) complex of curcumin I was reported to possess antiarthritic activity[163]. In this particular complex curcumin is acting as a neutral bidentate ligand. A Hg(II) complex(7) of curcumin I was characterised by Angulo (1986) in which curcumin I functions as a bidentate monobasic ligand[164].

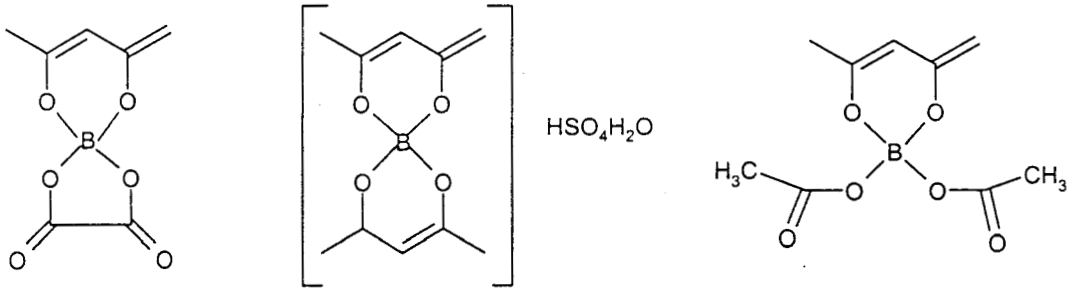


(7)

As per the method of Pabon for the synthesis of curcuminoids, an intermediate boron-curcuminoid complex was formed, which on acidification gave the free curcumin I. For the determination of small quantities of boric oxide by 'curcumin method', it was reported that two different species, one called rubrocurcumin and another called rosocyanin were formed[165-169]. Rubrocurcumin, a red complex was formed by the

reaction between boric acid, curcumin and oxalic acid in a 1:1:1 complex[165,166]. Roth and Miller[167] suggested structure (**8**) for the complex.

The formation of rosocyanin by the reaction between boric acid and curcumin in the presence of mineral acids (sulphuric acid) in combination with glacial acetic acid is the superior method in analytical procedures. Reported structure[167] of this complex is as in (**9**). Formation of a 1:1 boron-curcumin chelate of structure (**10**) has also been reported[169].



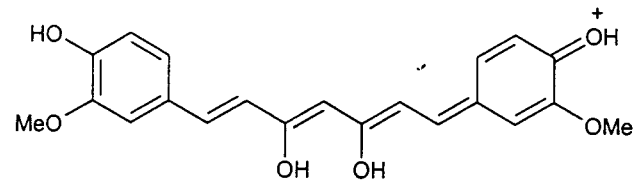
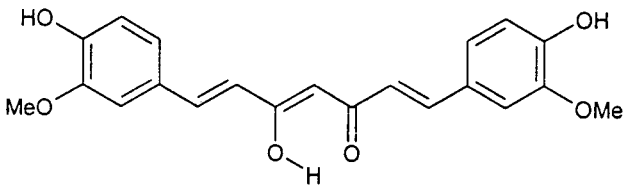
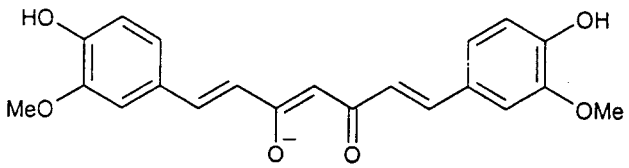
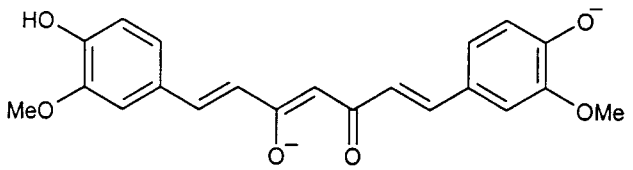
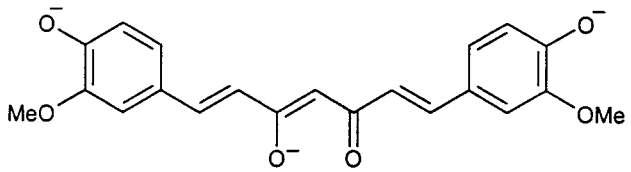
(8) **(9)** **(10)**

Recently stable Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+} and Pd^{2+} complexes of some synthetic curcuminoids were reported[142]. The structure and nature of bonding in these chelates were established on the basis of electronic, ir, nmr and mass spectral data. Potentiometric stability constants of these complexes were also reported[170,171].

Analytical applications of curcuminoid analogues

The colour of curcumin solution changes with pH[156,158,159] (**scheme 1**). The curcuminoids are insoluble in water, but freely soluble in alkali as well as in acid. Hence it is not easy to colour drug formulations and food items containing appreciable amount of water with this pigment. Hence a change of pH or use of a chemical emulsifier is necessary for making it soluble. In organic solvents and aqueous media its colour is not constant due to degradation.

At pH <1, the molecule exists in cationic form $(H_4A)^+$ with red colour. As pH increases (pH = 1-7) the molecule changes to a neutral species (H_3A) with yellow colour. At higher pH values (pH > 7) the molecule changes into the anionic forms (H_2A^-, HA^{2-}, and A^{3-}) and colour changes from yellow to brownish red and then to deep red[158]. The absorption maxima varies[160] in the pH and this has been attributed to various condensation products rapidly formed in alkaline medium from the feruloyl part of curcumin molecule.

	λ_{max} (nm)	form	pH
	555	H_4A^+	<1
	420	H_3A	1-5
	430	H_2A^-	5-8
	520	HA^{2-}	9-11
	480	A^{3-}	>11

Scheme 1

The change in colour of curcuminoids with pH change of the media has been utilised in designing indicators with this compound. 'Turmeric paper' available in market is a commercialised curcumin indicator system[172]. Curcúmin is also used as an effective spot reagent for identifying the presence of boron. This has been utilised in agricultural, waste water and metallurgical analysis[156,164,165].

For quantitative estimation of boron both rubrocurcumin and rosocyanin reactions were used[157,158]. This method is referred to as the 'curcumin method' in literature. For various reasons, rosocyanin method is considered as a superior method in analytical procedures.

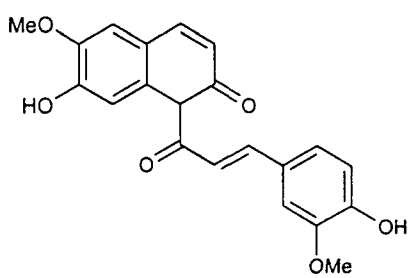
The *cis*-form of dicarboxylic acids can be separated from its *trans* form by the complex formation of curcumin and dicarboxylic acid with boron as in rubrocurcumin[127]. Complex formation similar to rubrocurcumin takes place with different α-hydroxy caboxylic acids and dicarboxylic acids instead of the oxalic acid part in rubrocurcumin[127].

Methods for the fluorometric estimations of B, Be and Si based on strong fluorescence of curcuminionds in organic solvents were also reported[156,159].

Photochemistry of curcuminoids

Curcuminoids exhibit a variety of photochemical activity including phototoxicity to bacteria[173,174]. Curcuminiods generally are less stable to uv radiation. So, yellow colour fades noticeably in turmeric products. The reaction mechanism and kinetics of the overall photochemical degradation of curcuminoids were investigated[159].

Curcumin I decomposes to form an yellow coloured cyclisation product (**11**) on exposure to visible radiation. Hence apparent yellow colour of curcuminoids remain when exposed to longer wavelength radiation. But when exposed to uv radiation rapid decomposition takes place.



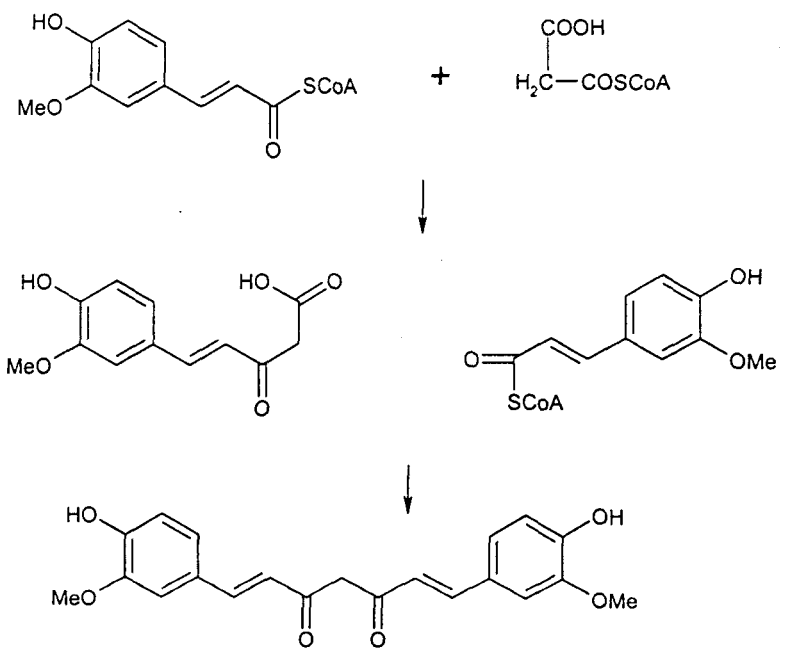
(11)

The photosensitizing mechanism of the compound suggests that it acts as a photosensitiser of singlet oxygen. Fading of colour also involves various other mechanisms independent of oxygen. Hence by excluding oxygen alone or by adding quenchers it is not possible to protect them from decomposition. Curcuminoids can be protected from light by use of brown glass if other singlet oxygen sources are excluded. The possibility that curcumin itself can act as a photosensitising agent is an intereseting

aspect in many drug formulations. Tonneson and co-workers have shown that the antibacterial activity of curcumin is greatly enhanced by visible radiation[175]. Photophysical properties of curcumin have been studied recently by Pill-Hoon Bong using time resolved fluorescence spectra[149].

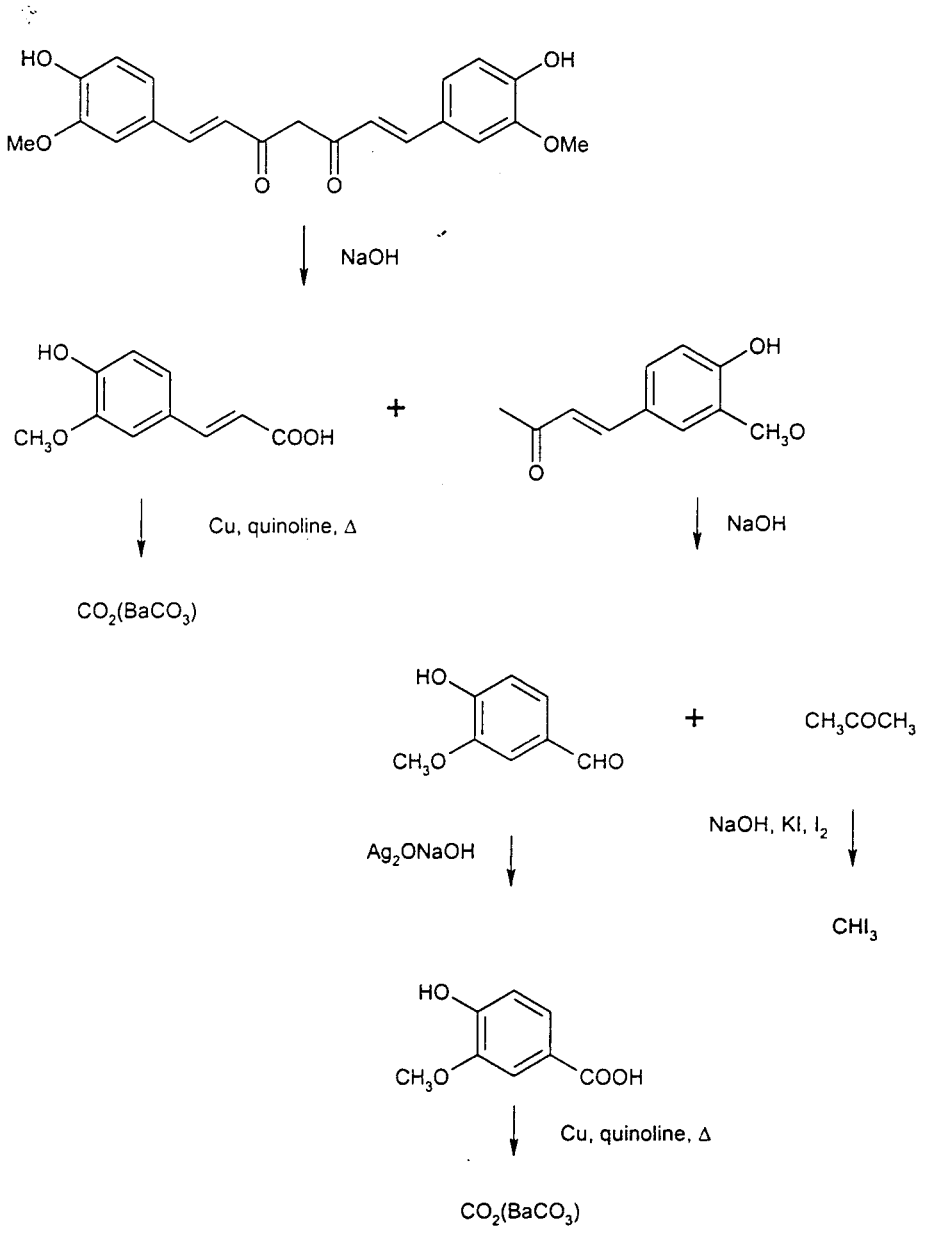
Biosynthesis of curcuminoids

Experimental observations predict that the biosynthetic path of curcuminoids involve two cinnamate units coupled to a central carbon atom provided by malonate group as shown in **scheme 2**.



Scheme 2

However, based on tracer studies, Roughley and Whiting[154,176] suggested a more satisfactory pathway based on the results of alkaline degradation as shown in **scheme 3**.



Scheme 3

Metabolism of Curcuminoids

Reports are available on the uptake, distribution and excretion of curcuminoids in rats[177-181]. It was found that 65-85% of the orally

administered dose of the compounds was excreted in the faeces, while negligible amounts were recovered in the urine[177].

The major part of the administered curcuminoids were found in the intestine and the concentrations in bile, liver, kidney and body fat were negligible. Low absorption of curcumin into the blood was revealed by the measurements of plasma levels and biliary excretion in anesthesised animals. Curcuminoids disappeared rapidly from the blood and excreted in the bile after intravenous injection. Addition of curcuminoids 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 is the major site of curcuminoid metabolism[180,181].

Curcuminoids and their metabolites were reported to undergo biliary excretion[178]. Following an oral dose, more than 90% of the dose was excreted in faeces. The recovery in urine was only 6%. The *in vitro* absorption of curcumin using everted intestinal sacs 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 dose was excreted unchanged in the faeces and could not be detected in the urine,

blood, liver or kidney. The available data suggest that to a certain extent the compounds are metabolised in the liver and the metabolites are mainly excreted through bile and faeces. Details regarding the quantity excreted unchanged and the exact metabolites are not still available.

Toxicological studies of curcuminoids

As a food ingredient the FAO/WHO expert group has fixed ADI (Average Daily Intake) of turmeric as 2.5 mg/kg body weight. However studies indicate that turmeric and curcuminoids are toxicologically safe even in doses far beyond the ADI given by FAO/WHO[182-184]. Toxicologically, the compound is inert; it does not appear to be toxic to animals or humans but is toxic to some bacteria at high concentrations[185]. Short and long term studies in dogs, mices and rats did not reveal any adverse cytogenic and mutagenic effects compared with controls when incorporated into the diets in amounts normally consumed by man. Curcuminoids exhibited no mutagenic effects in the salmonella/ mammalian microsome test[183].

Free radical scavenging and antioxidant efficiency of curcuminoids

Free radical intermediates are produced in living system from a number of sources such as the ionization of water by x-rays[186], through

metabolism, by triggered inflammatory phagocytes to reactive oxidants[187] and due to the partial reduction of oxygen during oxidative phosphorylation[188]. The resulting free radicals, such as superoxide anion and hydroxyl radicals, as well as the non radical hydrogen peroxide can damage macromolecules including DNA[189]. Likewise, other product of phagocyte oxygen metabolism such as hypochlorous acid, chloramine and oxidised lipids have all been related to DNA damage.

Antioxidants act as a major defence against these ROS (Reactive Oxygen Species) by protecting especially membrane and cytosolic components. Antioxidant family includes natural and synthetic antioxidant enzymes present in the biological system[188,190]. Catalases, glutathione peroxides and superoxide dismutase are the most important antioxidant enzymes within the body.

Powerful antioxidant originating from the edible and medicinal plants have been extensively investigated as important inhibitory materials for the prevention of oxidative deterioration. Recently, it has been shown that peroxidation in living organisms 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. In edible and medicinal plants, various types of antioxidants exist, and their chemical investigation is still in progress. Curcuminoids have been recognised for its useful biological activities that are related to its antioxidant property.

Medicinal uses of turmeric and curcuminoids

Turmeric: Turmeric has been employed in the indigenous systems of medicine of the orient since time immemorial[50]. Juice of the fresh rhizome is applied to recent wounds, bruises and leech bites. Internally it is used as an anthelmintic. According to Materia Medica[48,50] in doses of 15 to 20 grains (1 mg = 0.01643 grains) twice a day is given for flatulence, dyspepsia and weak state of the stomach. Turmeric is used both externally and internally in skin diseases due to impurity of the blood. Mixed with gingelly oil, it is applied on the body to prevent skin eruptions. Turmeric paste mixed with a little lime and salt petre when applied hot is a popular application to sprains, bruises, wounds and inflammatory troubles to the joints. In small-pox and chicken-pox patients a coating of thin paste of turmeric powder facilitate the process

of scabbing. Turmeric powder sprinkled on ulcers stimulate them to healthy action. Ghee mixed with powdered turmeric relieve cough. A paste of turmeric and the pulp of neem leaves is used for the treatment of eczema, obstinate itching and other parasitic skin diseases like ring worm infection. Smoke produced by sprinkling powdered turmeric over burnt charcoal relieve scorpion sting when the part affected is exposed to the smoke for a few minutes. Turmeric and alum powder in the proportion of 1:20 blown into the ear as a remedy for chronic otorrhoe. Milk boiled with turmeric rhizome and then sweetened with sugar is a popular remedy for cold.

Turmeric also enjoys the reputation as an antitumour agent. Dietary administration of turmeric protects tumour induction by chemical carcinogens of diverse structures and inhibits spontaneous mammary tumours in virgin C3H(Jax) CRI mice[191]. Turmeric has been found to be cytotoxic to tumour cells[192] and useful in cancer therapy[193]. Chemopreventive effect of turmeric against stomach and skin tumours have been studied[194-196] and found to reverse the aflatoxin induced liver damage and carcinogenesis induced by DMBA and croton oil. Turmeric

is non-mutagenic and by itself can antagonize the mutagenecity of other substances[197]. It is a potent antimutagen *in vivo* against carcinogens such as benzo(α)pyrene and methylcholanthrene[198]. Aqueous turmeric extract has been shown to protect against mutagenecity of direct acting carcinogens as well as benzo(α)pyrene induced genotoxicity and carcinogenicity[199]. Studies on human smokers have also revealed the strong antimutagenic potential of dietary turmeric[200]. Turmeric also protects against the DNA damage induced by smoke[201] and lipid peroxidation and prevent BP-DNA adduct formation[202].

Curcuminoids: It has been well established that many of the biological activities of turmeric are due to the presence of the curcuminoids. All the three curcuminoids are pharmacologically important. As a result, numerous studies exist on various biological activities of these curcuminoids, both natural[148,152,153,203-208] and synthetic[150,157,209-211]. Similarly, in recent years, a number of compounds structurally related to curcuminoids have been synthesised[142-147] and the important among them are brought out in tables 1-3. These compounds exhibit a broad spectrum

of medicinal activities. Most of such studies are on antimicrobial, antiinflammatory antioxidant and anticarcinogenic activities[212-232].

Earlier studies were mainly among the curcuminoids extracted from turmeric. Ramaprasad *et al* in 1956 reported[204] the antimicrobial activity and Srimal *et al* the antiinflammatory activity of the natural curcuminoids[153,206]. Curcuminoids extracted from the rhizomes of turmeric has been found to be cytotoxic and tumour reducing[203]. Chemopreventive efficiency of natural curcuminoids have been studied in several model systems and found to be very effective[207]. Antioxidant ability of natural curcuminoid have been well established[148]. It's fungicidal[152] and anti-ulcerogenic activity[208] were also been identified. It has been reported that synthetic curcuminoids when tested for various ailments were more potent than their natural counterparts[151,154,209].

The pharmacological studies revealed that synthetic curcuminoids are inhibitors of cancer. Curcumin I has been shown to inhibit tumour initiation produced by benzopyrene and DMBA[210]. Synthetic curcuminoids have been reported to be anticarcinogenic[211] and as antimutagenic[197]. It is being tried as a chemopreventive drug by

National Cancer Institute, USA[142]. It inhibits 4-nitroqunoline-1-oxide induced oral carcinogenesis[207], azoxymethane induced small and large intestinal carcinogenesis[208] and azoxymethane induced colon carcinogenesis[233]. Ruby John Anto *et al* studied[209] antimutagenic and anticarcinogenic activity of three natural curcuminoids and five synthetic curcuminoids. Anti-tumour and free radical scavenging activity of eight synthetic curcuminoids were also analysed and reported[234]. The compounds with free –OH group on the phenyl ring were found to be more active.

Thus phenolic group together with the β-diketone moiety present in curcuminoids are suggested to be responsible for their high biological activity[235,236] and this led to further studies using compounds having structural similarity to curcuminoids. For brevity and to enable quick survey important medicinal applications reported for curcuminoids and allied derivatives are given in table 4.

Table 4

Important medicinal properties of Turmeric/ Curcuminoids

	Medicinal properties	References
1	Antiallergic	212
2	Antiarthritic	139,213
3	Antibacterial	204,214,215
4	Anticarcinogenic	193-196, 207-209
5	Anticoagulant	216,217
6	Antifertility	218
7	Antifungal	152,219
8	Antihepatotoxic	220,221
9	Antiinflammatory	205,152,150,145,222,223
10	Antimutagenic	197-200
11	Antineoplastic	224
12	Antioxidant	148,225,227
13	Antiprotozoal	228
14	Antirheumatic	229
15	Antispasmodic	214,215,220,221,230
16	Antithrombatic	229
17	Antitumour	192,203,206,209,210,211
18	Antiulcerogenic	208
19	Nematocidal	231

PART III

METAL CHELATES OF 1,7-DIARYLHEPTA-1,6-DIENE- 3,5-DIONES AND THEIR ANTITUMOUR ACTIVITY

1,7-Diaryl-1,6-heptadiene-3,5-diones are typical 1,3-diketones in which the diketo function is directly attached to olefinic groups. The chemical and biochemical (particulary in the field of medicinal inorganic chemistry) importance of these types of 1,3-diketones are discussed in Part I and Part II of this Thesis.

The main objectives of the present investigation are

1. Synthesis and characterisation of a series of aryl substituted 1,7-diaryl-1,6-heptadiene-3,5-diones and their metal complexes.
2. Antitumour activity of these diketones and their metal complexes.

The results of these studies are presented in this Part. For convenience, this part is divided into four chapters based on the position of the aryl substituents of the diketones. The synthesis and characterisation of the compounds are presented in **Section 1** of each chapter and details of antitumour studies in **Section 2**. Towards the end a comparative evaluation of the antitumour activity of the compounds are given.

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

The following metal salts were used for the synthesis of metal complexes. Vanadyl(IV) sulphate, iron(III) chloride hexahydrate, cobalt(II) acetate tetrahydrate, nickel(II) acetate tetrahydrate and copper(II) acetate monohydrate.

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-silicagel).

Materials, animals, chemicals etc. employed for antitumour studies are given separately in section 2 of chapter 1.

Instruments

Instruments used in this investigation are

1. UV-1601 Schimadzu recording spectrophotometer.
2. 8101 Schimadzu-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. Varian E 112 ESR spectrometer.
9. 1001 spectronic, Bauch and Lamp spectrophotometer.
10. Systronic pH meter.
11. Toshniwal conductivity bridge.
12. Gouy type magnetic balance.
13. Wilovert inverted microscope.
14. Trazon's 96 well flat bottom titre plate.
15. Deep freezer, Quene system.

Methods

Elemental analysis: Metal complexes were analysed by standard methods[238]. Metal percentages were recorded using atomic absorption

spectrophotometer after decomposing them with concentrated sulfuric-nitric acid mixture. Carbon, hydrogen and nitrogen percentages reported are by microanalysis carried out at RSIC, CDRI, Lucknow.

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

Infrared spectra of compound 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 reocrded by imparting vapourised sample molecules with a beam of electrons at 70 eV.

ESR spectra (*x- band*) of copper complexes were recorded at 77 K in glassy state between 8.5-9.5 GHz and calibrated with diphenylpicryl hydrazil (DPPH) free radical for which g= 2.0036.

Molar conductance[239] of the complexes were determined in dmf at 28 ± 1^0C using solution of about 10^{-3} M.

Magnetic susceptibility was determined at room temperature ($28+1^0$C) using $Hg[Co(NCS)_4]$ as standard[240].

Molecular weights of compounds reported were determined by Rast's method[241] using naphthalene/ camphor as medium.

CHAPTER I

SYNTHESIS, CHARACTERISATION AND ANTITUMOUR ACTIVITY OF 1,7-DIARYLHEPTA-1,6-DIENE-3,5-DIONES AND THEIR METAL CHELATES

SECTION 1

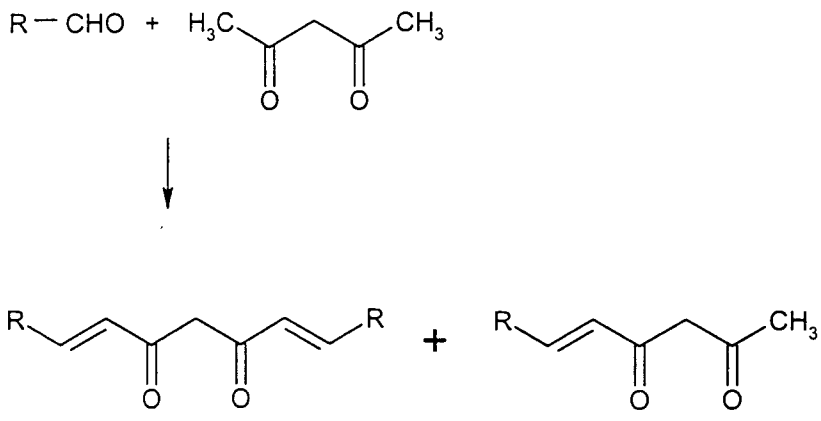
SYNTHESIS AND CHARACTERISATION OF 1,7- DIARYLHEPTANOIDS AND THEIR METAL COMPLEXES

Synthesis and characterisation of 1,7-diphenyl-1,6-heptadiene-3,5-diones (1,7-diphenylheptanoids) and some of its complexes were reported earlier[142]. Introduction of styryl and napthyl ring systems instead of the phenyl rings in the unsaturated diketone moiety may modify the chemical and biochemical properties of the compounds. Similarly heteroaryl ring system such as furyl, pyridyl, etc may also yield compounds having interesting properties. With this intention, synthesis and characterisation of three new highly conjugated 1,7-diarylheptanoids and a heteroarylheptanoid, and their typical metal complexes are presented in this section. In **Section 2** the *in vitro* and *in vivo* antitumour activity of the compounds are given.

Synthesis of the 1,7-diarylheptanoids

The 1,7-diarylheptanoids were synthesised by the condensation of aromatic aldehydes (benzaldehyde, cinnamaldehyde, naphthaldehyde and furfuraldehyde) with acetylacetone-boron complex (which was formed by stirring acetylacetone and B_2O_3 for ~ 1 h) in the presence of tri(sec-butyl)borate and *n*-butylamine as the condensing agent as in the

reaction **scheme 1** below. The reaction leads to the formation of 1,7-diarylheptanoid as the major product (75%) along with small amounts of 6-arylhexanoid (monocondensation product). Pure 1,7- diarylheptanoids were obtained through column chromatographic separation of the products formed. A typical procedure for the synthesis and purification of the 1,7-diarylheptanoids are given below.



Scheme 1.1

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 80^0C. 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 (~ 60^0C) 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 soild product obtained on acidification was dissolved in minimum quantity of ethylacetate and placed over the column (2x100 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 chromatographically (tlc) pure material.

Synthesis of metal chelates

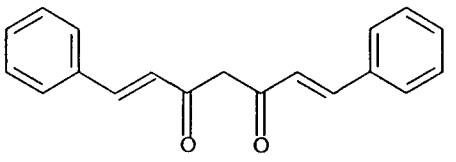
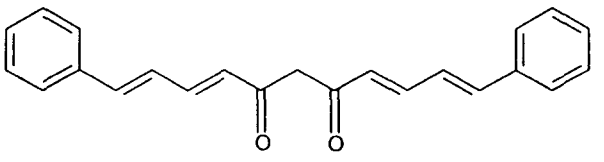
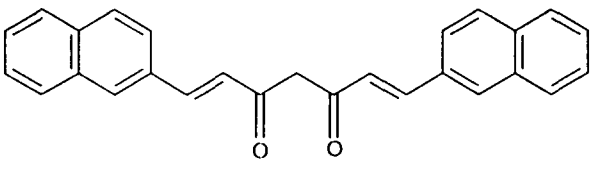
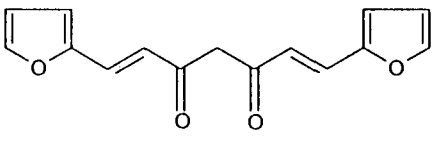
The general procedure adopted for the preparation of oxovanadium(IV), iron(III), cobalt(II), nickel(II) and copper(II) complexes is given below.

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 (0.002 mol) in methanol (25mL). The mixture was stirred well and refluxed gently for ~l 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.

Results and Discussion

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 1.1.

Table 1.1
*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%

The observed C,H percentages and molecular weight determination (Table 1.2) together with mass spectral data of the compounds clearly suggest the formation of the bis-condensation product in which two equivalents of aldehyde has condensed with one equivalent of acetylacetone as in reaction **Scheme** 1.1.

Characterisation of the 1,7-diarylheptanoids

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

Uv spectra

The uv spectra of the compounds in methanol show two absorption maxima (Table 1.2). Based on earlier reports[71,142,242-244] the band at 380-460nm corresponds to the n→π* transition and the one at 250-270nm is due to π→π* transition. The n→π* absorption values of simple diketones are much lower than that of the 1,7-diarylheptanoids. The increase in the values for 1,7-diarylheptanoids may be due to the ∝,β-unsaturation of the keto group.

Table 1.2

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

Compounds	MP (^{0}C)	Elemental analysis (%) Found/ (calcd) C	H	Mol. weight	λ_{max} (nm)	log ∈
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 carbonyl 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[127].

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 1,3-diketones and allied derivatives, the nature and position of carbonyl stretching bands can provide valuable information regarding their structure[71,166,245-246].

The ir spectra of all the 1,7-diarylheptanoids are characterised by the presence of a strong band at ~1615 cm^{-1} 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.

Table 1.3

Characteristic 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
1559	1540	1581	1556	
1545	1528	1542	1544	ν (C=C) alkenyl
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 characterised by the *trans* -CH=CH- absorption which occurs in the region ~ 970 cm^{-1}.

In compound **1b,** this region is shifted to ~995 cm^{-1} due to extension of conjugation.

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[127,154].

^1H nmr spectra

The ^1H nmr spectroscopy is perhaps the most efficient tool in studying the keto-enol tautomerism of 1,3-diketones.[71,247-249]. 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.

The percentage of enol tautomer present in a β-diketone, which can exist in the keto-enol tautomeric forms can be roughly calculated

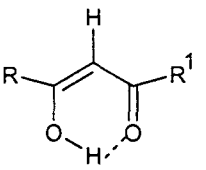
from the integrated intensity of the downfield enolic proton signal. The chemical shift of methine and enolic proton is also indicative of the tautomeric nature of the compound under consideration. The chemical shift values of methylene proton and enolic proton of some substituted β-diketones (Table 1.4) show that these values are greatly influenced by the groups substituted[248]. 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[248]. 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 1.4

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

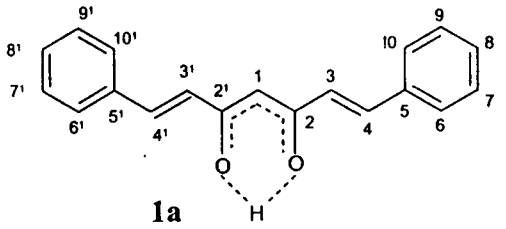
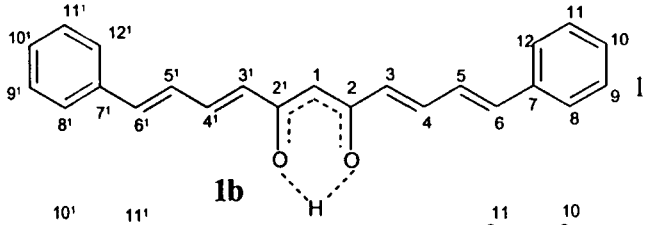
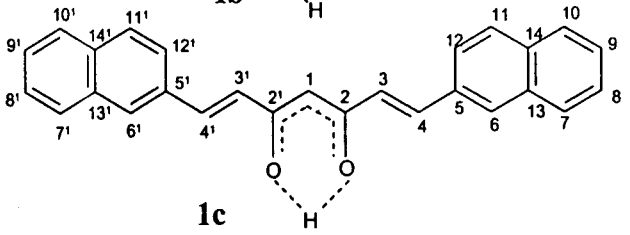
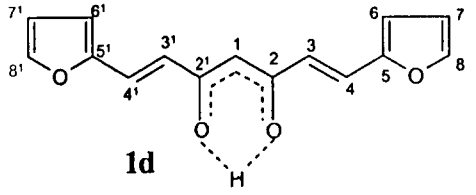


R	R^1	Chemical Shift (δ ppm)	
		Methylene proton	Enolic proton
CH_3	CH_3	5.44	15.40
CH_3	CF_3	5.90	14.24
CF_3	CF_3	6.43	13.00
C_6H_5	C_6H_5	6.80	17.13
CH_3	C_6H_5	6.08	16.24
CF_3	C_6H_5	6.56	15.23
2-C_4H_3S	CF_3	6.5	16.2

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 ^1H nmr spectral data of the compounds are summarised in the table 1.5 and the spectrum of **1b** and **1c** are reproduced in **figures 1.1** and **1.2**

Table 1.5

*Characteristic ^1H nmr spectral data of 1,7-diarylheptanoids (**1a-1d**)*

Compound	enolic	1	3,3^1	4,4^1	5,5^1	6,6^1	7,7^1	8,8^1	9,9^1	10,10^1	11,11^1	12,12^1
 **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	-	-	-

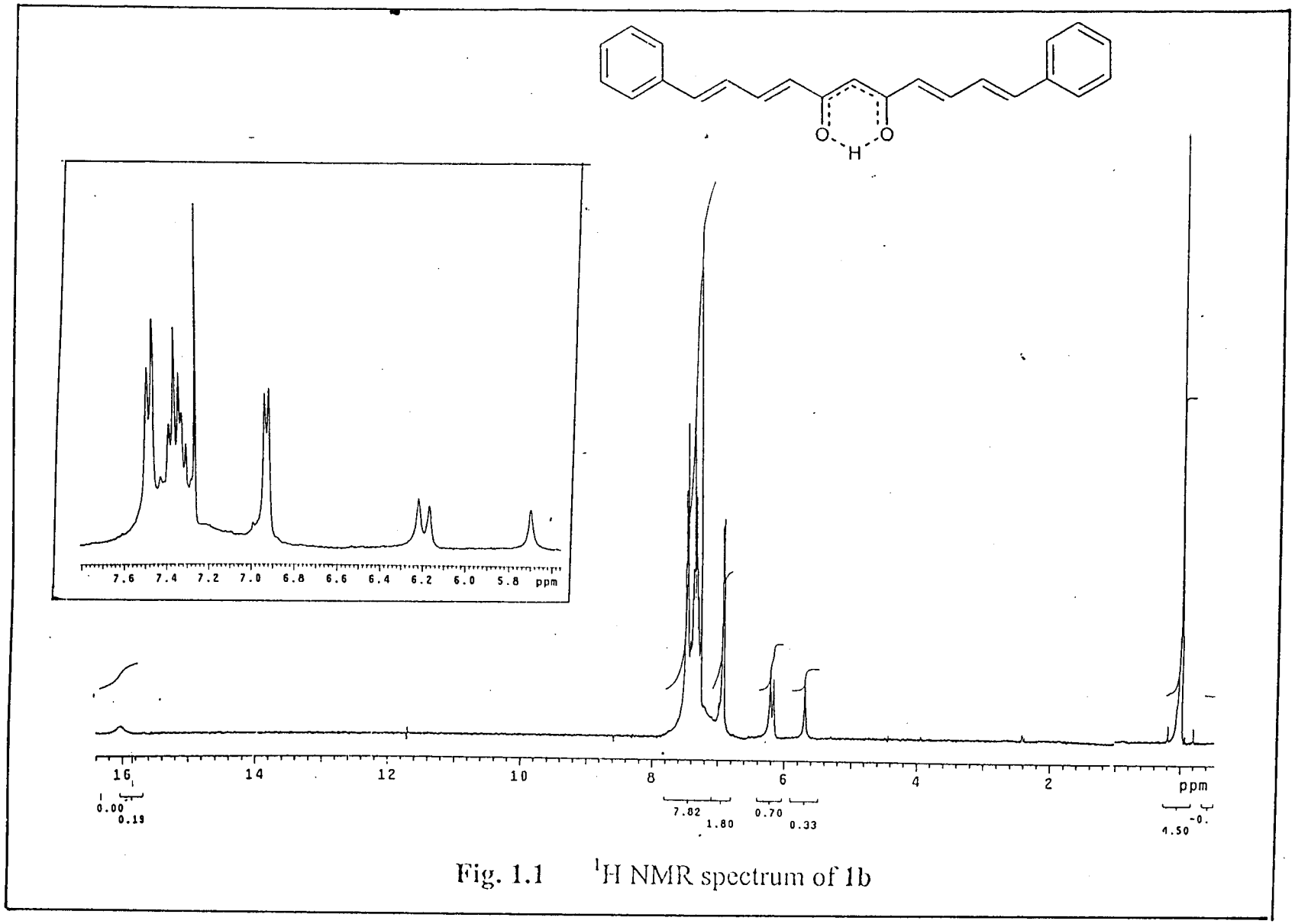


Fig. 1.1 ^{1}H NMR spectrum of 1b

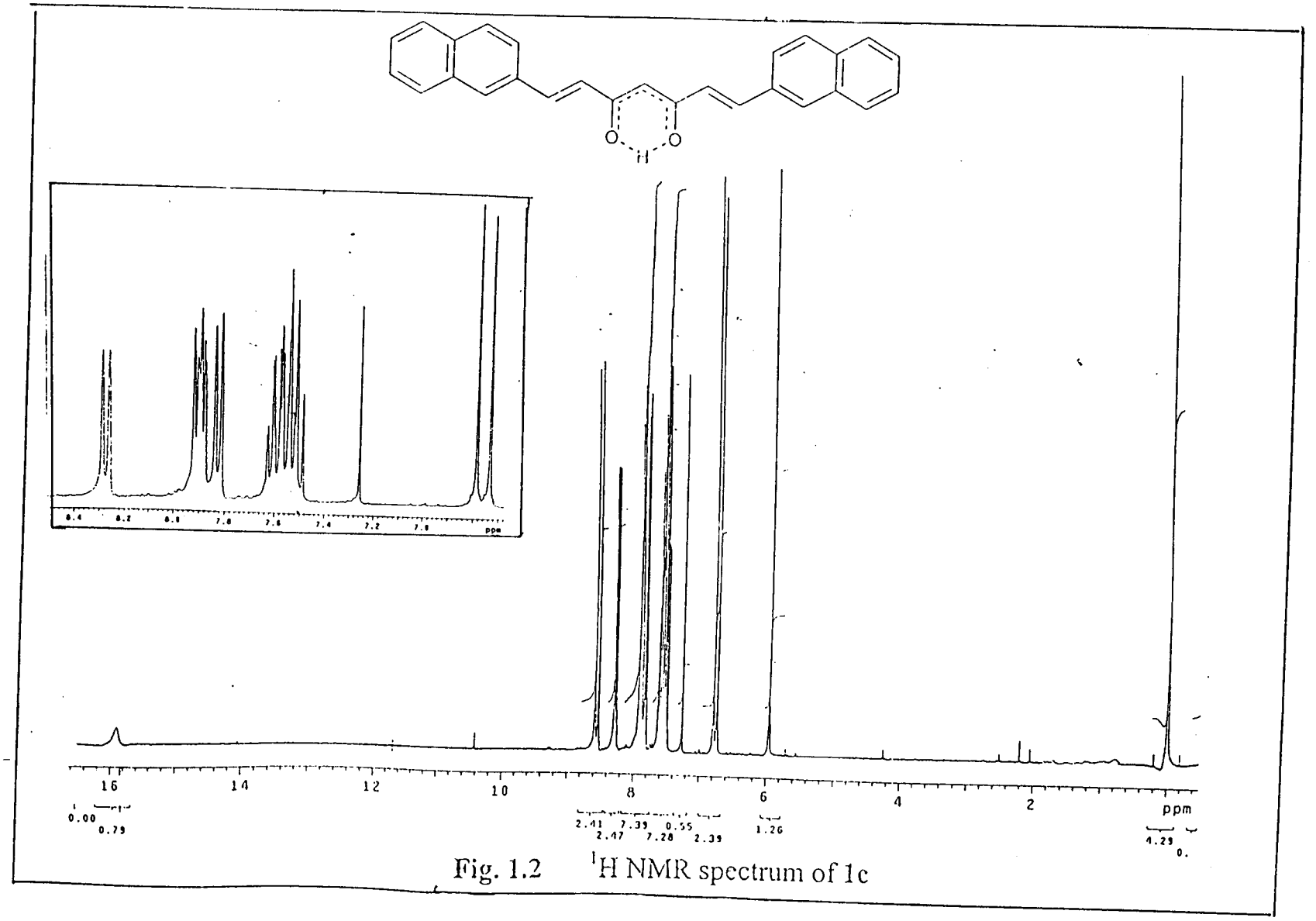


Fig. 1.2 ^{1}H NMR spectrum of 1c

Mass spectra

Several reports are available on mass spectral studies on diverse types of 1,3-dicarbonyl compounds[250]. The fragmentation patterns depend mainly on the nature of groups attached to the diketo function.[251] 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.

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**, **1c** and **1d** are brought out in **figures 1.3-1.5**. Important peaks appeared in the spectra of all the 1,7-diarylheptanoids can be conveniently accounted by the fragmentation patterns given in **Scheme 1.2**.

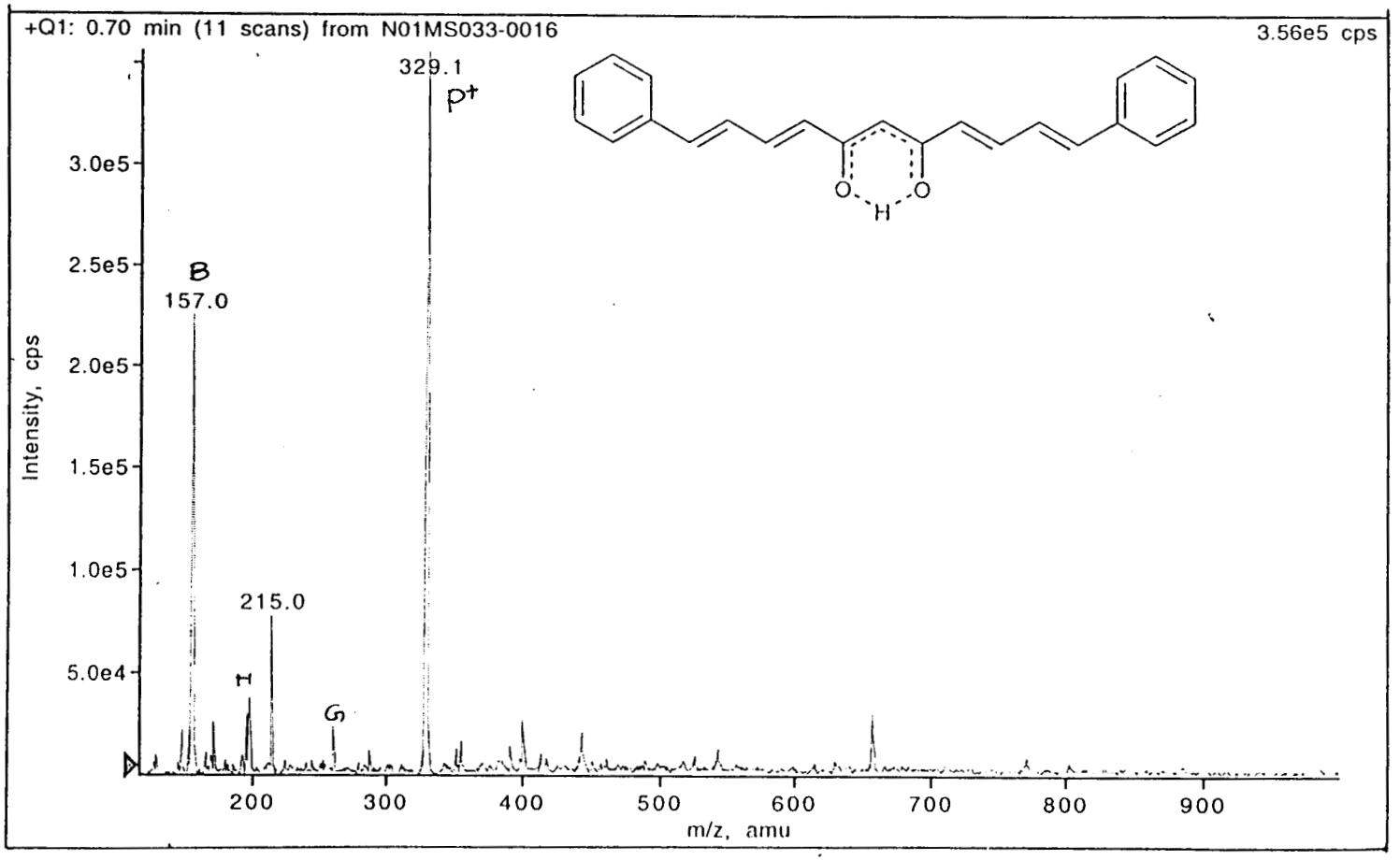


Fig. 1.3 Mass spectrum of 1b

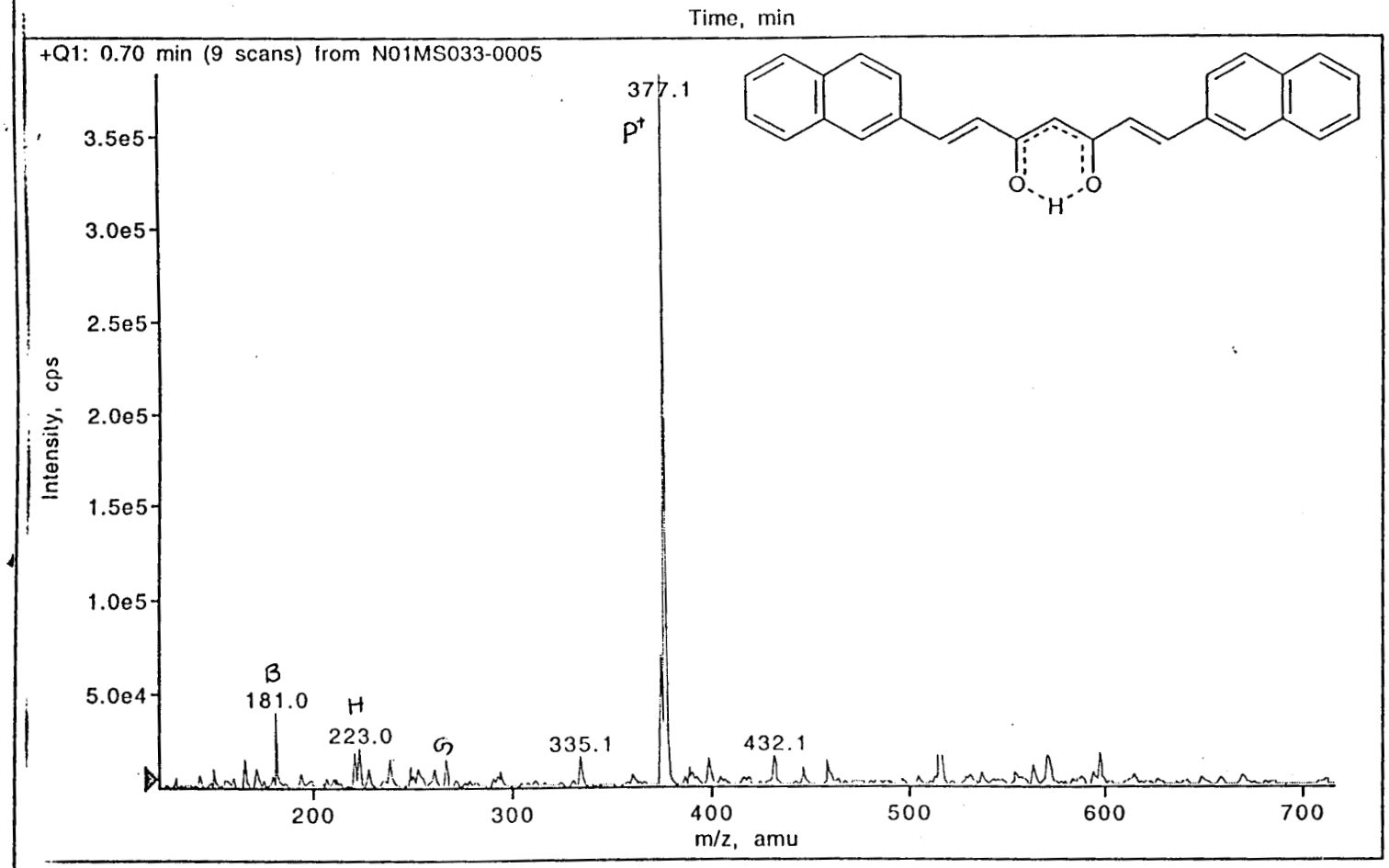


Fig. 1.4 Mass spectrum of 1c

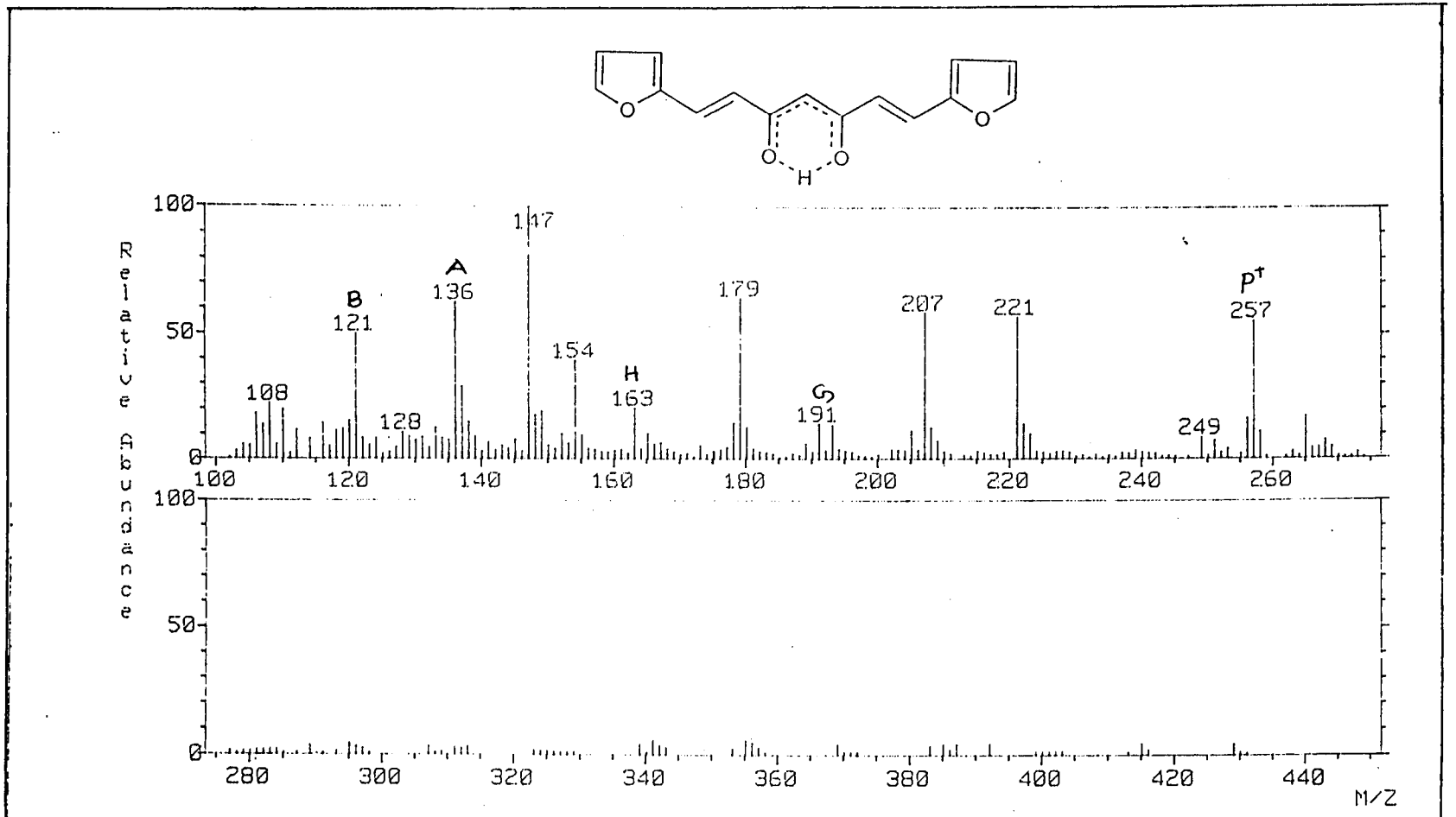
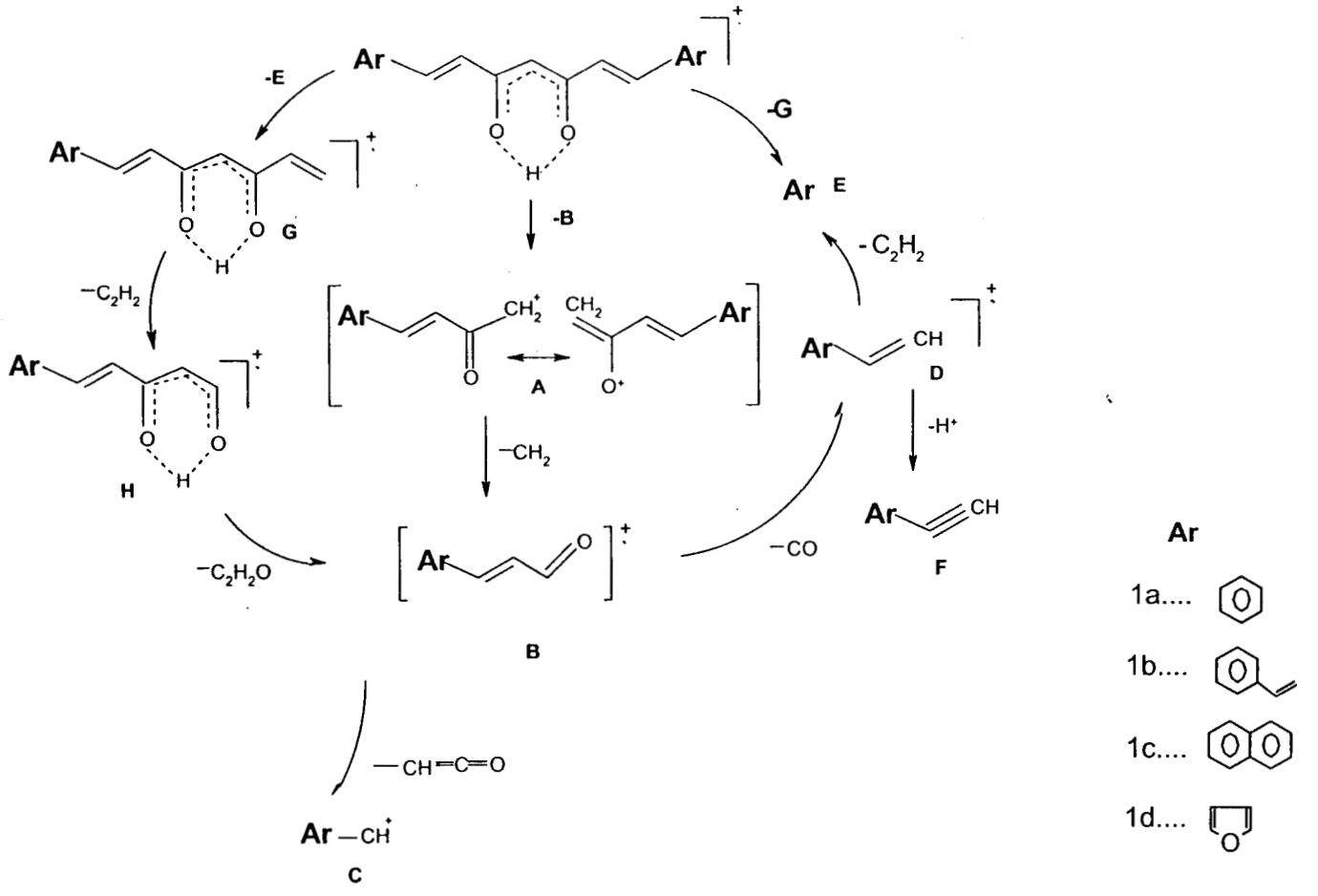


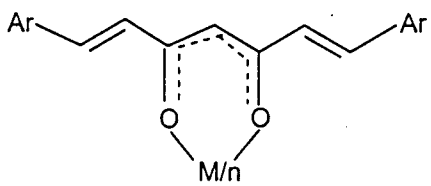
Fig. 1.5 Mass spectrum of 1d



Scheme 1.2 Fragmentation pattern of **1a-d**

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 1.6-1.10. The analytical data suggest 1:2 metal-ligand stoichiometry for VO(IV), Co(II), Ni(II) and Cu(II) complexes and 1:3 for Fe(III) 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. Magnetic moment measurements show that nickel complexes are diamagnetic and all other complexes are paramagnetic. The observed uv, ir, nmr, mass and esr spectra are in agreement with the structure **1.1** of the complexes.



1.1

n=2 for VO(IV), Co(II), Ni(II) & Cu(II)
n=3 for Fe(III)

Uv Spectra

The uv spectra of the complexes clearly resembles to the respective ligands indicating that no structural alteration has taken place

during complexation. The slight shift of absorption maxima to longer wavelength indicate the involvement of the carbonyl oxygens in metal complexation[70,252,253].

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.[70,73,74,76,87]. This is because in metal 1,3-diketonates, the most important functions available for structural studies are the carbonyl groups, O-H, C=C and M-O bonds and all these groups show characteristic ir absorption and from their position and nature, various structural informations can be derived. 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.

Table 1.6

Analytical and characteristic ir spectral data of the oxovanadium (IV) chelates of 1,7-diarylheptanoids (HL)

Oxovanadium (IV) chelates of	MP	μ_{eff}	Elemental analysis (%) Found/(calcd)*			Characteristic ir stretching bands (cm^{-1})		
	(^{0}C)	BM	C	H	V	(C=O)	(C-C-C)	(V-O)
1a	201	1.73	73.81 (72.61)	4.90 (4.86)	8.52 (8.27)	1596	1517	491.8 407
1b	215	1.78	78.10 (76.56)	6.12 (5.27)	7.01 (7.07)	1590	1515	418
1c	230	1.76	78.35 (79.31)	5.02 (4.65)	6.01 (6.24)	1561	1493	479 422
1d	169	1.74	63.10 (62.39)	4.02 (3.81)	8.12 (8.84)	1586	1514	470 419

** The calculated value corresponds to the [VOL_2] composition where L stands for the deprotonated ligand.*

Table 1.7

Analytical and characteristic ir spectral data of the iron(III) chelates of 1,7-diarylheptanoids (HL)

Iron (III) chelates of	MP	μ_{eff}	Elemental analysis (%) Found/(calcd)*			Characteristic ir stretching bands (cm^{-1})		
	(^{0}C)	BM	C	H	Fe	(C=O)	(C-C-C)	(Fe-O)
1a	210	5.85	77.81 (76.29)	5.25 (5.11)	6.85 (6.34)	1597	1516	482 419
1b	218	5.93	80.52 (79.86)	5.60 (5.50)	5.75 (5.39)	1545	1512	471 419
1c	220	5.86	81.11 (82.31)	4.25 (4.83)	4.95 (4.73)	1585	1512	479 419
1d	188	5.76	66.65 (65.79)	4.00 (4.02)	6.95 (6.80)	1580	1514	480 428

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

Table 1.8

Analytical and characteristic ir spectral data of the cobalt(II) chelates of 1,7-diarylheptanoids (HL)

Cobalt(II) chelates of	MP	μ_{eff}	Elemental analysis (%) Found/(calcd)*			Characteristic ir stretching bands (cm^{-1})		
	(^{0}C)	BM	C	H	Co	(C=O)	(C-C-C)	(Co-O)
1a	197	4.76	71.21 (70.69)	4.99 (5.58)	9.23 (9.15)	1590	1514	489 418
1b	189	4.85	74.11 (73.69)	5.85 (5.87)	7.93 (7.88)	1582	1512	466 419
1c	201	4.70	76.37 (77.23)	5.38 (5.24)	7.15 (7.03)	1600	1511	472 419
1d	164	4.83	60.88 (59.31)	4.77 (4.61)	10.01 (9.72)	1598	1472	465 420

** The calculated value corresponds to the $[CoL_2(H_20)_2]$ composition where L stands for the deprotonated ligand.*

Table 1.9

Analytical and characteristic IR spectral data of the nickel (II) chelates of 1,7-diarylheptanoid (HL)

Nickel (II) chelates of	MP	Elemental analysis (%) Found/(calcd)*			Characteristic ir stretching bands (cm^{-1})		
	(^{0}C)	C	H	Ni	(C=O)	(C-C-C)	(Ni-O)
1a	209	74.56 (74.91)	4.80 (4.93)	9.55 (9.64)	1582	1512	455, 423
1b	219	78.67 (77.45)	5.88 (5.33)	9.15 (8.24)	1572	1510	453, 421
1c	212	80.47 (80.13)	4.93 (4.69)	8.13 (7.26)	1598	1520	474, 437
1d	170	64.30 (63.31)	3.85 (3.87)	11.02 (10.32)	1596	1516	484, 430

**The calculated value corresponds to the $[NiL_2]$ composition where L stands for the deprotonated ligand.*

Table 1.10

Analytical and characteristic ir spectral data of the copper(II) chelates of 1,7-diarylheptanoids (HL)

Copper (II) chelates of	MP	μ_{eff}	Elemental analysis (%) Found/(calcd)*			Characteristic ir stretching bands (cm^{-1})		
	(^{0}C)	BM	C	H	Cu	(C=O)	(C-C-C)	(Cu-O)
1a	183	1.76	74.11 (74.33)	4.18 (4.89)	10.18 (10.35)	1580	1518	461, 425
1b	204	1.77	77.56 (76.93)	5.42 (5.29)	9.10 (8.85)	1585	1512	440, 419
1c	209	1.75	78.10 (79.66)	4.85 (4.67)	7.70 (7.81)	1581	1518	484, 428
1d	162	1.80	60.95 (62.77)	3.50 (3.84)	10.90 (11.07)	1598	1486	480, 422

** The calculated value corresponds to the [CuL_2] composition where L stands for the deprotonated ligand.*

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}. 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 ~ 480cm^{-1} and ~ 415cm^{-1}. A band at ~980cm^{-1} due to *trans*-CH=CH- remain unaffected in the metal complexes also. Characteristic ir bands of the complexes are given in tables 1.6 - 1.10. Spectra of all the VO^{2+} complexes showed a medium intensity band at ~960 cm^{-1} presumably due to the stretching of the V=O group. Presence of weak bands in the spectra of the Co^{2+} complexes in the region 3200-3400 cm^{-1} suggest that water molecules are also coordinated to the metal ion.

^{1}H nmr spectra

In the ^{1}H nmr spectra of the diamagnetic nickel(II) complexes of the 1,7-diarylheptanoids **1b** and **1c** (**figures 1.6 and 1.7**) the lowfield enolic proton singlet of the free ligand is absent. Methine proton singlet and aromatic proton signals shifted slightly to downfield[142]. 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.

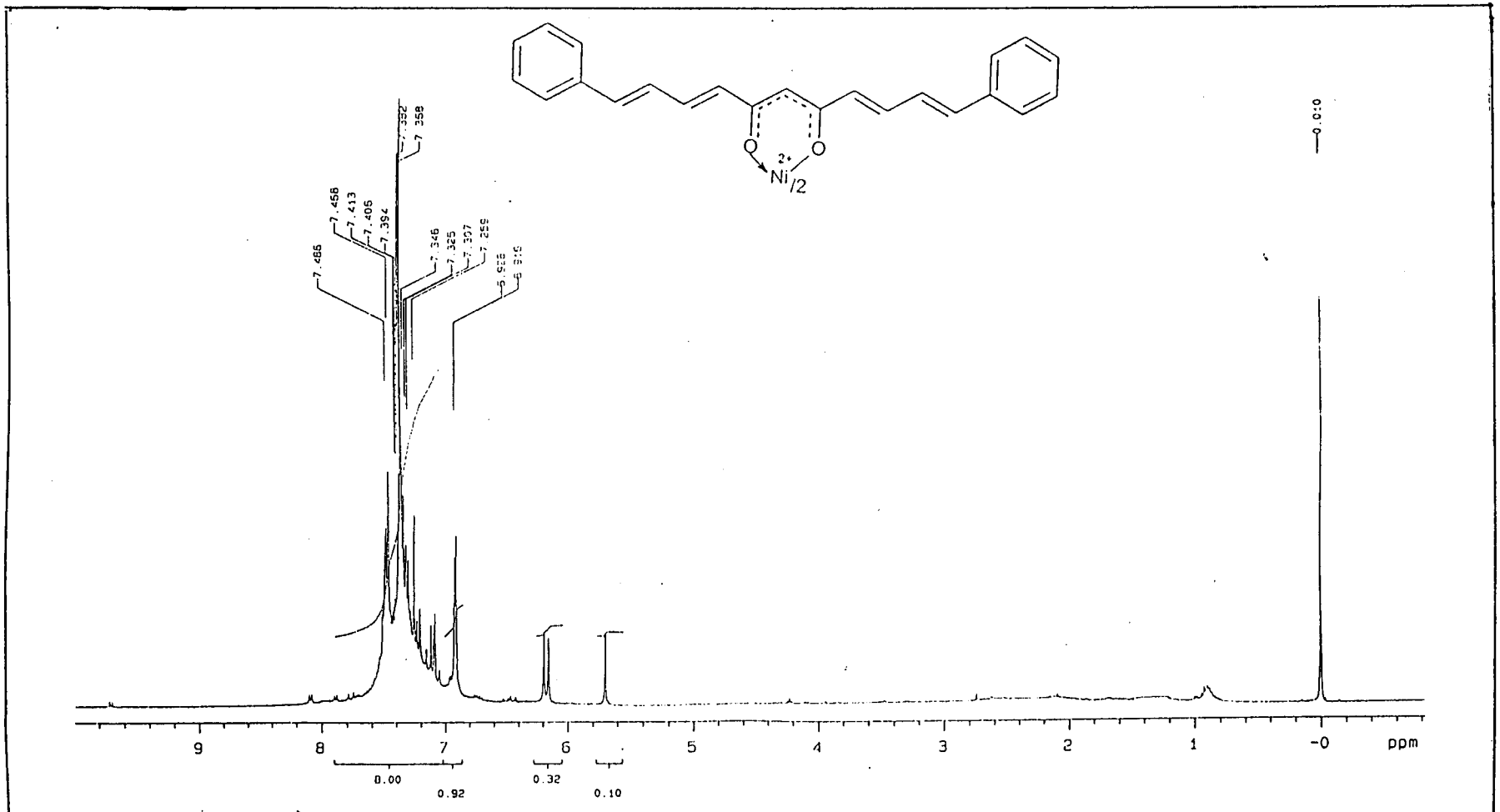


Fig. 1.6 ^{1}H NMR spectrum of nickel(II) complex of **1b**

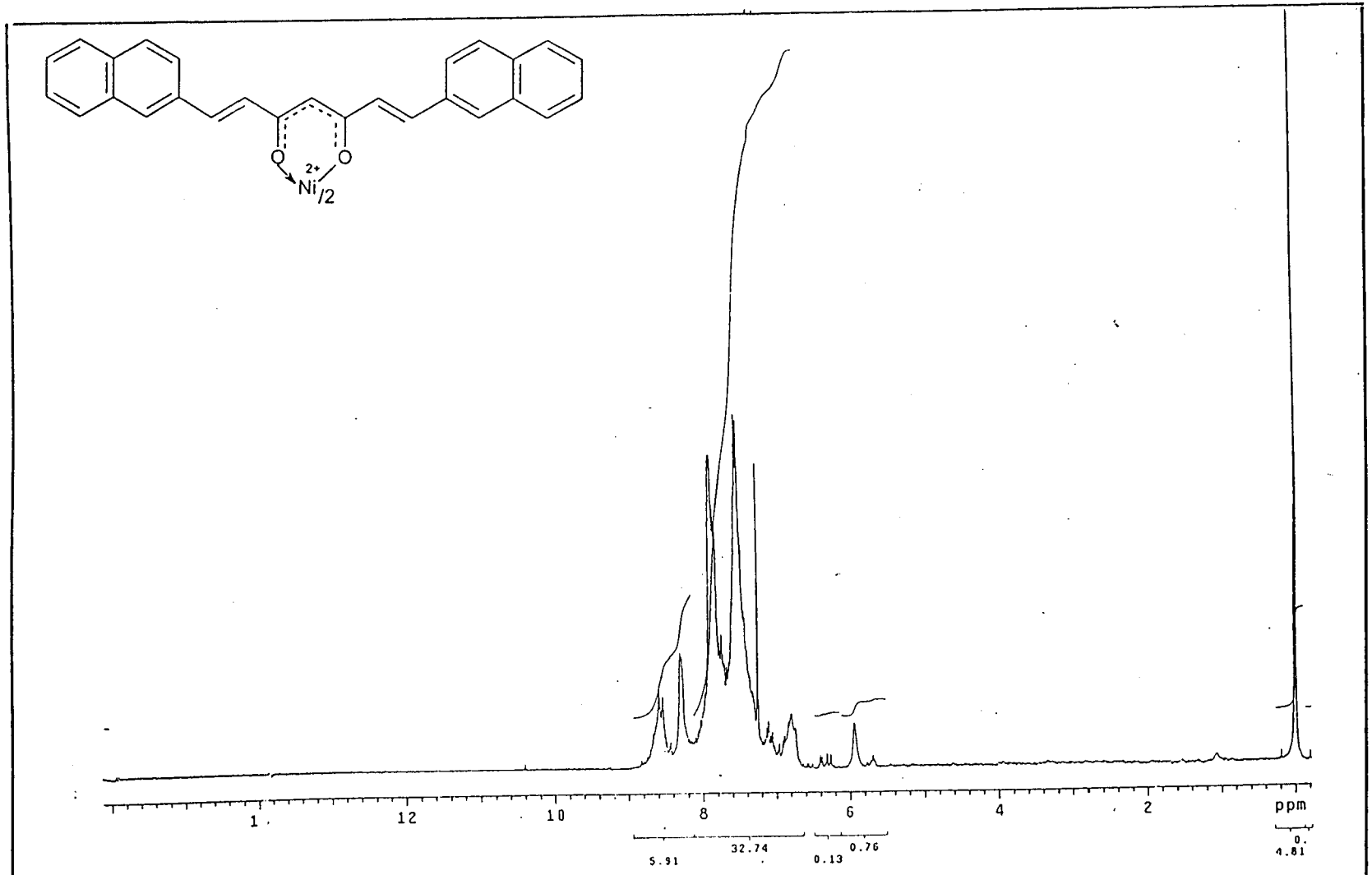


Fig. 1.7 ^{1}H NMR spectrum of nickel(II) complex of **1c**

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[70,254-256] and the mass spectral fragmentation patterns of metal 1,3-diketonates are well documented. 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[254]. 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 FAB mass spectra of copper chelates of the 1,7-diarylheptanoids **1b** and **1c** (figure 1.8-1.10) show the stepwise removal of aryl groups. All the chelates show relatively intense $(P+1)^+$ peaks corresponding to the $[CuL_2]$ stoichiometry. Peaks due to $[ML]^+$, L^+, $[M_2\ L]^+$ and fragments of L^+ are also detected in the spectra. The mass spectrum of the iron (III) complex of **1a** given in Fig. 1.10 clearly indicates $[FeL_3]$ stoichiometry of the compounds.

It was found that some fragments rearrange to form stable cyclic species. The mass spectral fragmentation of **(1a-1c)** are similar and these fragments rearrange to stable cyclic species as illustrated in **scheme 1.3**.

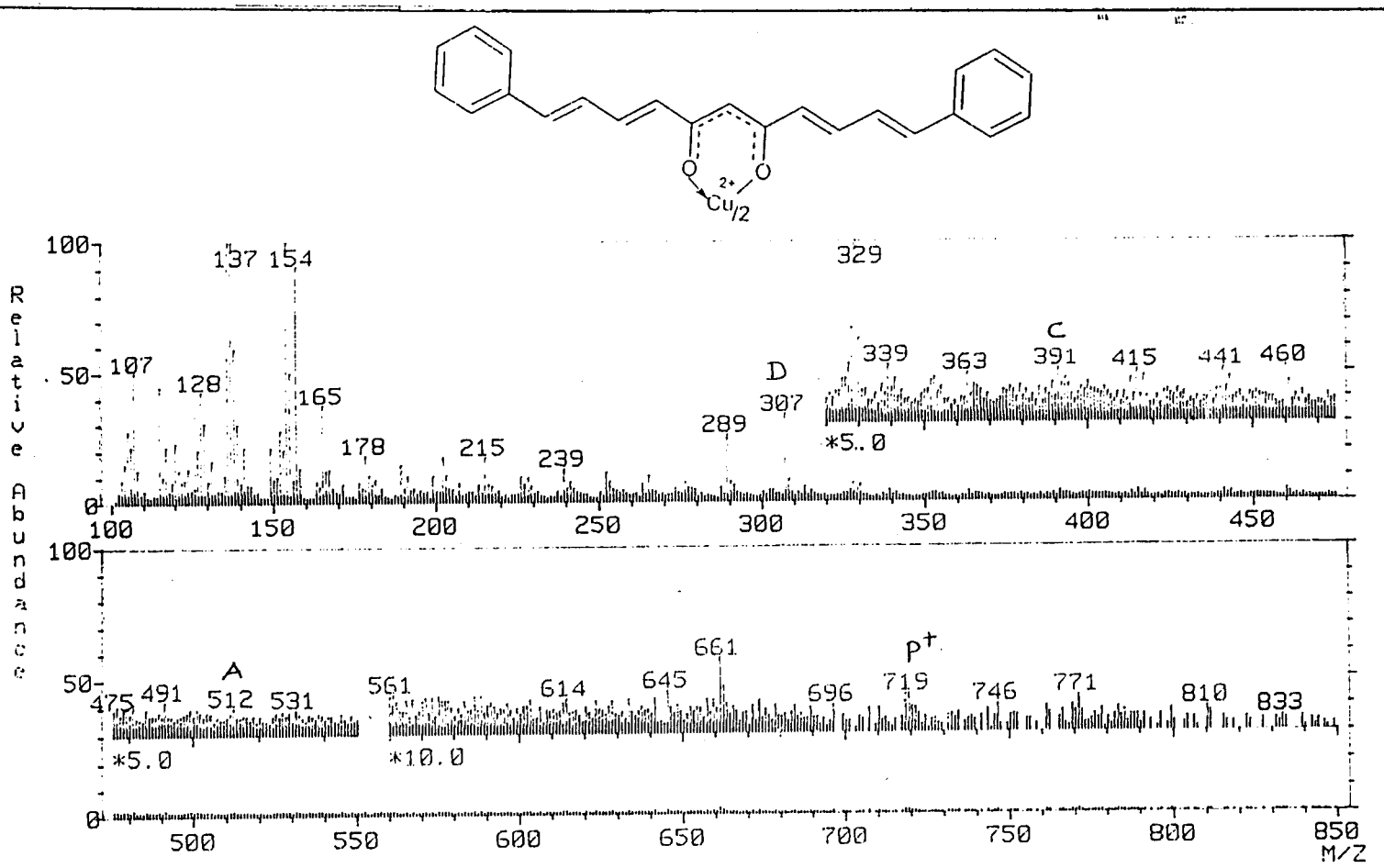


Fig. 1.8 Mass spectrum of copper(II) complex of **1b**

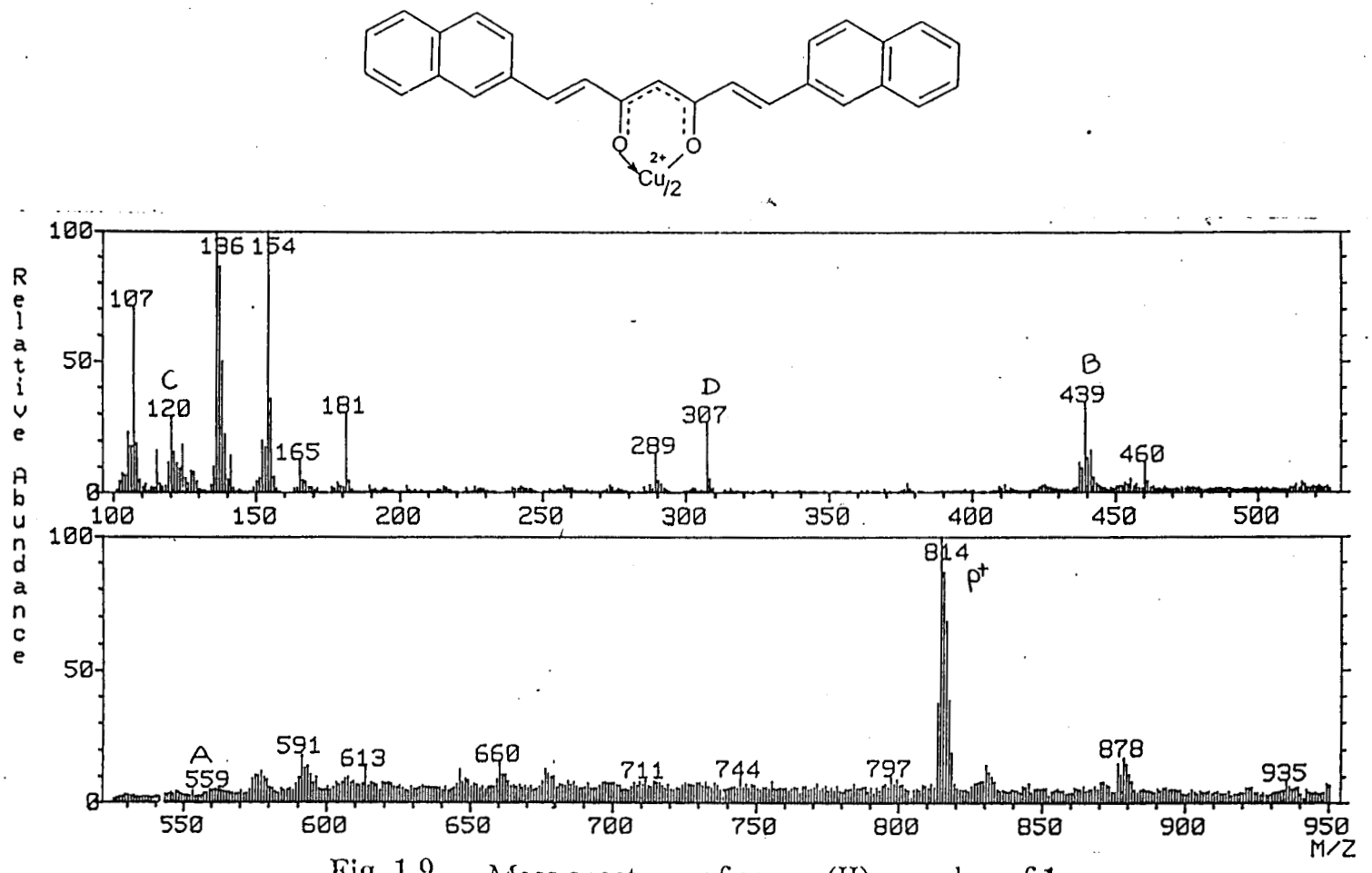


Fig. 1.9 Mass spectrum of copper(II) complex of **1c**

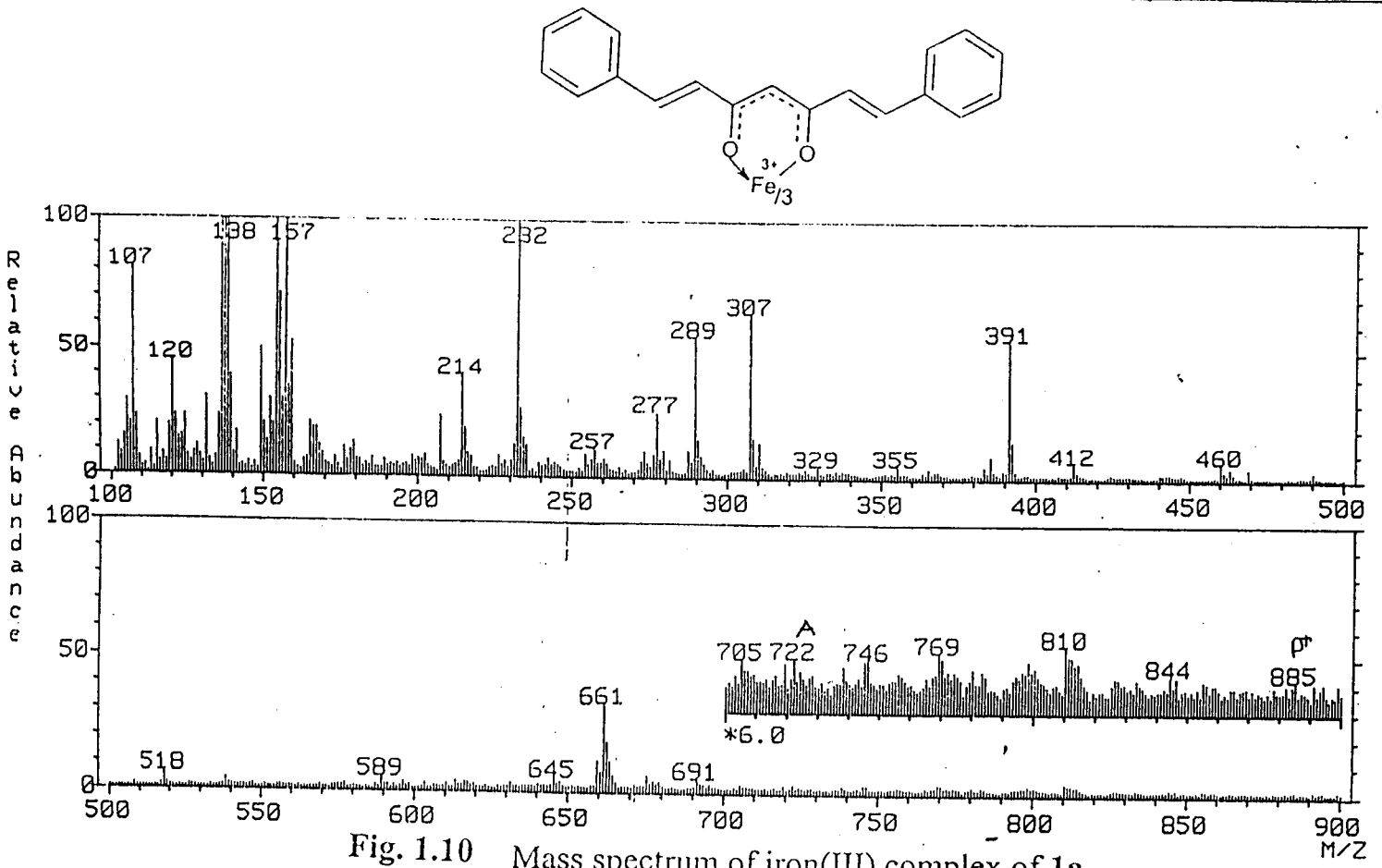
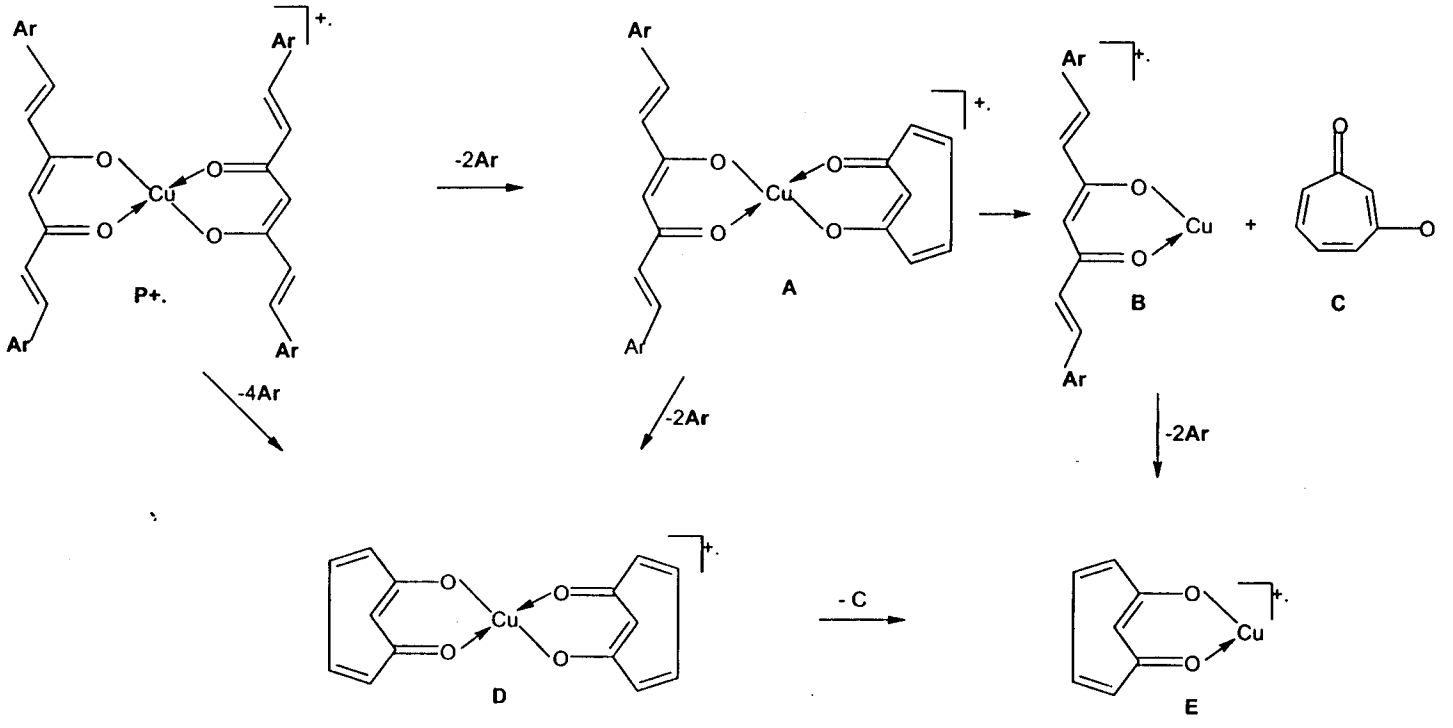


Fig. 1.10 Mass spectrum of iron(III) complex of **1a**



Scheme 1.3

Geometrical structure of the complexes

In the copper(II) complexes the presence of a broad visible band at ~15,000 cm^{-1} and the measured μ_{eff} values (1.75-1.81B.M.) support the square-palanar structure[73]. Square-planar copper(II) complexes undergo a change to octahedral symmetry in the presence of donor solvents. When the spectra of the chelates were 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 adducts of the planar CuL_2 complexes. The observed μ_{eff} values of the vanadyl complexes are in the range 1.68-1.72 B.M. Their visible spectra appears to be complex probably due to the presence of strong V=O π bonding. The visible spectra of Co^{2+} complexes are 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 in the range 4.70 – 5.00 B.M. suggest the octahedral geometry of the complexes.

The observed diamagnetism and broad medium-intensity band at ~17,800 cm^{-1} in the visible spectra of the nickel(II) chelates undoubletedly suggest their square-planar geometry. In conformity with this observation the visible spectra of the chelates 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-seperated 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) complexes

Electron spin resonance studies of paramagnetic transition metal complexes yield valuable information regarding the magnetic properties of the unpaired electrons and there by some understanding about the nature of the bonding between the metal ion and the ligands[257]. 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 of **1b** in DMF solution at 77K is given in **figure 1.11**. 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 lignad interactions. It has been reported that the g values are very sensitive to the covalent nature of the metal-ligand bond[257-259].

The spectrum of the copper complex of **1b** shows anisotropic pattern. The g and A values evaluated are given in table 1.11. For comparison the values reported for copper acetylacetonates[260] and $CuCl_2$ are also included in the table 1.11

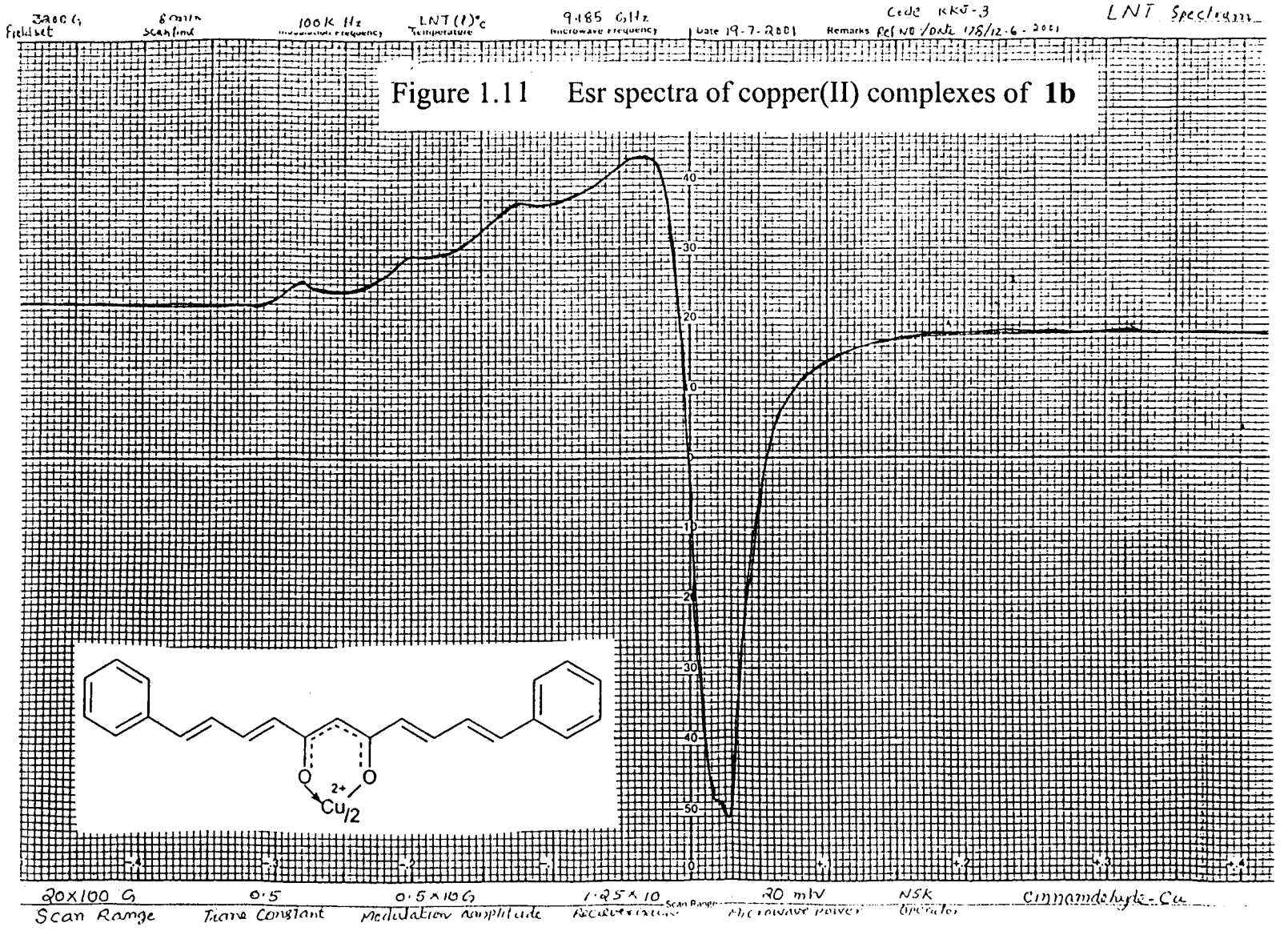


Figure 1.11 Esr spectra of copper(II) complexes of **1b**

Table 1.11

Esr spectral data of copper(II) complex of ***1b*** *(HL)*

Compound	$g_{\|}$	$g_{\perp}$	$A_{\|}$	$A_{\perp}$	Solvent
$[Cu(L)_2]$	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	112	--	60% Toluene 40% $CHCl_3$

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 g values suggest the presence of appreciable delocalisation of π electron cloud of the chelate ring C_3O_2Cu and the metal-ligand bond has considerable covalent character[260].

SECTION 2

ANTITUMOUR ACTIVITY OF 1,7-DIARYLHEPTANOIDS AND THEIR COPPER(II) COMPLEXES

The four synthetic curcuminoids (1,7-diarylheptanoids) were investigated for their *in vivo* and *in vitro* antitumour activities. All the metal complexes discussed in **Section 1** were also examined for their cytotoxic and antitumour activities. Since copper(II) complexes were found to be most active, only results of copper(II) complexes are discussed.

Materials

Reagents

Eagle's minimum essential medium (MEM) and trypsin were obtained from Himedia Laboratories. All other reagents and chemicals are of AnalaR grade.

Cell lines

Ehrlich ascites carcinoma (EAC) cells were obtained from the Cancer Research Institute, Mumbai, India. Dalton's Lymphoma ascites

(DLA) cells, from the Cancer Institute, Adayar, India, and L929 cells from National facility for animal cell and tissue culture, Pune, India.

Animals

Inbred strains of swiss albino mice (7-8 weeks old weighing 20-27 g obtained from Veterinary College, Thrissur, Kerala, India) were used for the animal experiments. The animals were fed with normal mouse chow (Lipton India) and water *ad libitum* and were housed in ventilated cages in air controlled room.

Methods

Preparation of Reagents

Normal saline: Normal saline was prepared by dissolving A.R. NaCl (0.85 g) in 100 mL of distilled water. Normal saline is essential for preparing cell suspension, as the osmotic pressure due to it 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 rapture during experiments. It is prepared by dissolving NaCl (8 g), KCl (0.2 g), $Na_2HPO_42H_2O$ (1.44 g), and KH_2PO_4 (0.2 g) in one litre distilled water.

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

PBS – EDTA: This solution is essential for washing the tissue culture bottles after removing the medium from them. It is prepared by dissolving 0.02 g EDTA in 100 mL PBS.

Trypsin: Prepared by dissolving 0.02 g EDTA, 0.02 g glucose and 0.20 g trypsin in 100 mL distilled water. The solution was then filtered and transferred into bottles under sterile conditions. In tissue culture experiments it is employed for extracting the cells that adhere inside the culture bottles.

Medium: Minimum essential medium is used for maintainance and growth of tumour cell lines which is prepared by the following method.

To 445 mL of double distilled water, 50 mL goat serum and 5.37 g MEM were added. To prevent bacterial infection 0.5 mL each of pencillin and streptomycin were also added. Stirred magnetically and pH was adjusted to 7.2 by adding the $NaHCO_3$ and sterilised by bacterial filtration.

Maintaining cell lines

EAC and 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 cells were suspended in saline or PBS so as to get a cell suspension of 1 million cells/mL. One mL of the cell suspension was injected into the peritonial cavity of fresh swiss albino mice. The test animals were given normal diet and within 10-14 days ascites fluid that contains cancer cells were accumulated in the abdomen. The animals grow with this tumour and die within 18-25 days. These cells are propogated regularly by transferring it, as mentioned above, to other normal mice and thus the cell lines were maintained.

L929 cells were maintained in culture using MEM and subcultured according to the procedure given below. Medium from a confluent bottle of cells was removed and the cells were rinsed 2-3 times with PBS-EDTA solutions. The cells were then trypsinized by adding 0.1mL of 0.2% trypsin and incubated for 10 minutes at 37^0C. The cells were detached by tapping the bottle and to this fresh medium containing 10% goat serum and antibiotics were added. A single cell suspension was made and 5,000 cells were added to bottles containing fresh medium and incubated at

37^0C. Subculturing was repeated every third day in order to get healthy fresh cells[192].

Determination of *in vitro* cytotoxicity of compounds

The short term *in vitro* cytotoxic activity of the compounds were analysed using Ehrlich ascites cells[192, 261]. The synthetic curcuminoids and their copper complexes were dissolved in minimum quantity of DMSO. Different concentrations (1-50 μg/mL) of the compounds were prepared by diluting this solution with PBS. 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 upto 1 mL using PBS, and the mixture was incubated for ~3 h at 37^0C. 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.

Determination of the cytotoxic activity of the compounds towards tissue cultured L929 cells

In the case of compounds that are incapable of acting directly as antitumour agents, long term incubation is required to evaluate their cytotoxic action against the cell lines. In culture experiments the

compounds were subjected to long term incubation and they may be metabolised into derivatives that are powerful carcinostatic agents.

cytotoxic activity of compounds in culture were determined using L929 cells[192,261]. The cells ($5x10^3$cells/cell) were plated in 96 well flat bottom titre plates and incubated for 24h at 37^0C in 5% CO_2 atmosphere. After incubation, the cells were stained with crystal violet and the cytotoxicity was calculated by measuring the optical density at 540 nm after eluting the dye from the cells.

Determination of the effect of compounds in reducing ascites tumour development

Nine groups of swiss albino mice (6nos/group) were injected intraperitonially with Ehrlich ascites cells ($1x10^6$ cells/animal). One group was kept as control and the other groups of mice, were simultaneously injected (ip) with the test compounds (200 μmole/Kg body weight) suspended in gum accasia 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 %ILS=[100(T-C)]/C where T and C are the mean number of days survived by the treated and control animals respectively[192, 261, 262].

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Determination of the effect of compounds on solid tumour development

Solid tumours were induced in groups of swiss albino mice (6nos/group) by subcutaneous injection of DLA cells ($1x10^6$cells/animal) on the right hind limbs. One group was kept as control and other groups were simultaneously injected (ip) with the test compounds (200μmol/Kg body weight) and continued for 10 days. Tumour diameter was measured every third day for one month and the tumour volume was calculated using the formula, $v = {}^4/_3\pi r_1^2.r_2^2$; where r_1 and r_2 are the minor and major radii respectively[192, 261-263].

Results and Discussion

Short term incubation studies

Results of the short term *in vitro* cytotoxicity of the curcuminoids (**1a-d**) and their copper complexes are shown in table 1.12. These preliminary experiments were carried out mainly with six different concentration of the compounds. Copper complexes were found to be more cytotoxic than corresponding curucminoids. **1b** produced 50% cell death at a concentration of 12.5 μg/mL, **1a, 1c** and **1d** produced the same effect at 25 μg/mL, 16.0 μg/mL and 20.5 μg/mL respectively. However the copper complexes of **1a, 1b, 1c** and **1d** showed the same effect at

lower concentrations 10 μg/mL, 4.8 μg/mL, 7.5 μg/mL, and 12.5 μg/mL respectively. Copper complex of **1b** was found to be the most cytotoxic one among this group of compounds.

Table 1.12

Short term in vitro cytotoxicity of compounds towards EAC cells

Compounds	Percentage cell-death at different concentrations				
	1μg/mL	5μg/mL	10μg/mL	25μg/mL	50μg/mL
1a (HL^1)	14	29	35	50	66
1b (HL^2)	20	36	48	62	84
1c (HL^3)	19	31	41	57	74
1d (HL^4)	17	28	39	55	71
$[(Cu(L^1)_2]$	17	37	49	59	78
$[(Cu(L^2)_2]$	22	52	71	100	100
$[(Cu(L^3)_2]$	25	43	57	78	100
$[(Cu(L^4)_2]$	23	40	47	63	94

Ehrlich ascites carcinoma cells (1 million cells/mL) in presence of various concentrations of compounds (1-50 μg/mL) were incubated for 3h at 37^0C. The short-term cytotoxicity was determined by trypan blue dye exclusion method.

Cytotoxicity of compounds in tissue culture

Cytotoxic activity of curcuminoids (**1a-d**) and their copper complexes in culture towards L929 cells are shown in figure 1.12 and 1.13.

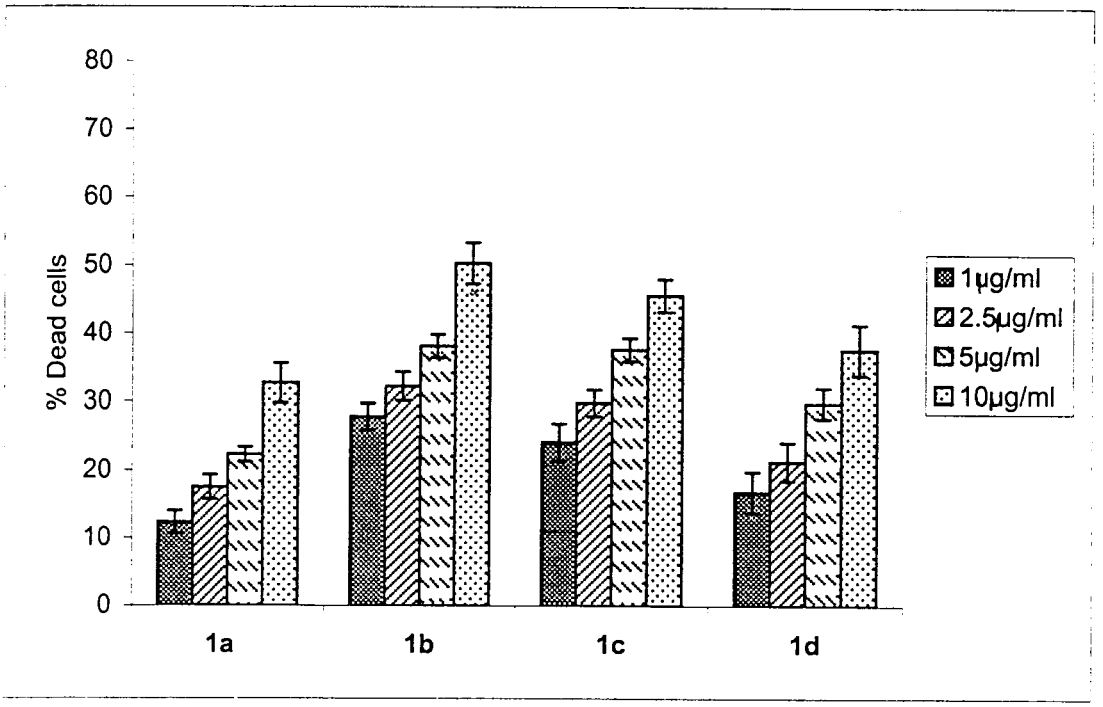


Fig. 1.12 Cytotoxicity (1a-1d) in culture towards L929 cells

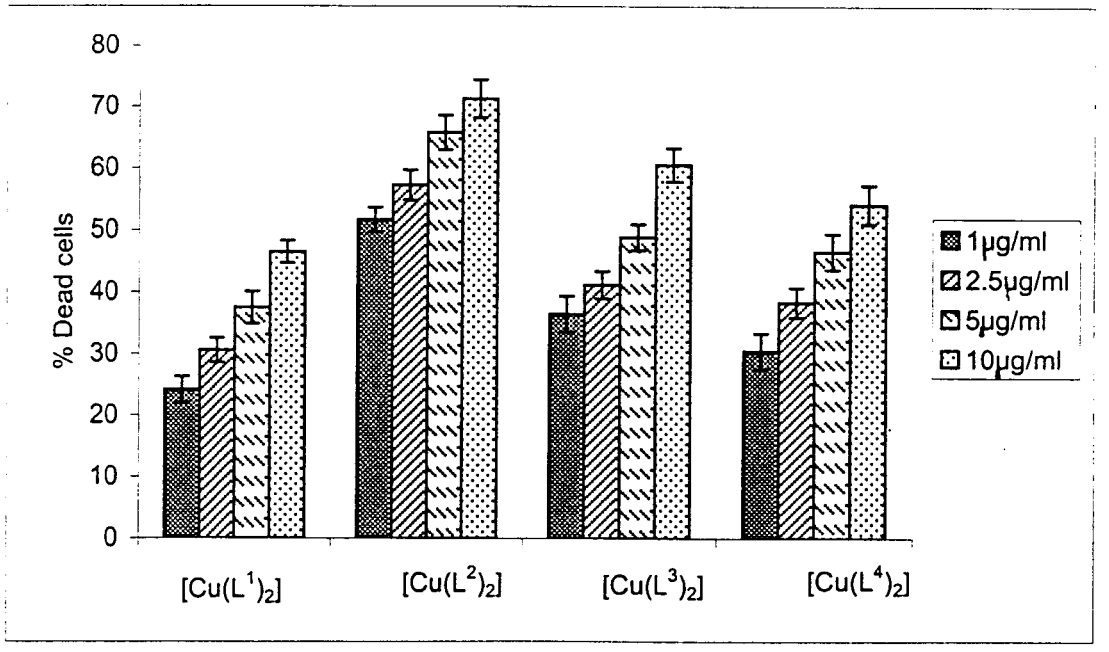


Fig. 1.13 Cytotoxicity of Copper complexes (1a-1d) in culture towards L929 cells

In culture studies copper complexes are proved to be more cytotoxic than the corresponding curcuminoids. Compound **1a** (12.3 ± 1.7% cell death at 1 μg/mL concentration) is the least active compound and copper complex of **1b** (51.7 ± 2.0% cell death at 1 μg/mL concentration) is the most active compound.

Effect of compounds on ascites tumour reduction

All the compounds when administered intraperitonially could produce significant increase ($P < 0.001$ from normal) in the life span of tumour bearing mices (Table 1.13). The percentage increase in life span of tumour bearing mice were 29.47, 60.71, 45.73 and 39.31 by the administration of **1a, 1b, 1c** and **1d** respectively, where as their respective copper chelates produced 43.93, 78.62, 66.47 and 54.34 percentage increase in life span.

Table 1.13

Effect of compounds on ascites tumor reduction

Compound	No. of animals with tumour	No. of days survived	Increase in Life Span (%)
Control	6/6	17.3±1.1	
1a (HL^1)	6/6	22.4±1.9	29.47*
1b (HL^2)	6/6	27.8±3.1	60.71*
1c (HL^3)	6/6	25.2±2.1	45.73*
1d (HL^4)	6/6	24.1±2.6	39.31*
$[Cu(L^1)_2]$	6/6	24.9±2.1	43.93*
$[Cu(L^2)_2]$	6/6	30.9±2.2	78.62*
$[Cu(L^3)_2]$	6/6	28.8±2.7	66.47*
$[Cu(L^4)_2]$	6/6	26.7±2.4	54.34*

*$P < 0.001$

Values are means of ±SD of six determinations. Animals were injected (ip) with Ehrlich ascites tumour cells ($1x10^6$ cells/animal), compounds (200μmol/Kg body wt) were injected (ip) for 10 days.

Effect of compounds on solid tumour development

Reduction of solid tumour volume in mice by the intraperitional administration of compounds are given in figure 1.14 and 1.15.

Fig. 1.14 Effect of compounds (1a-1d) on solid tumour volume

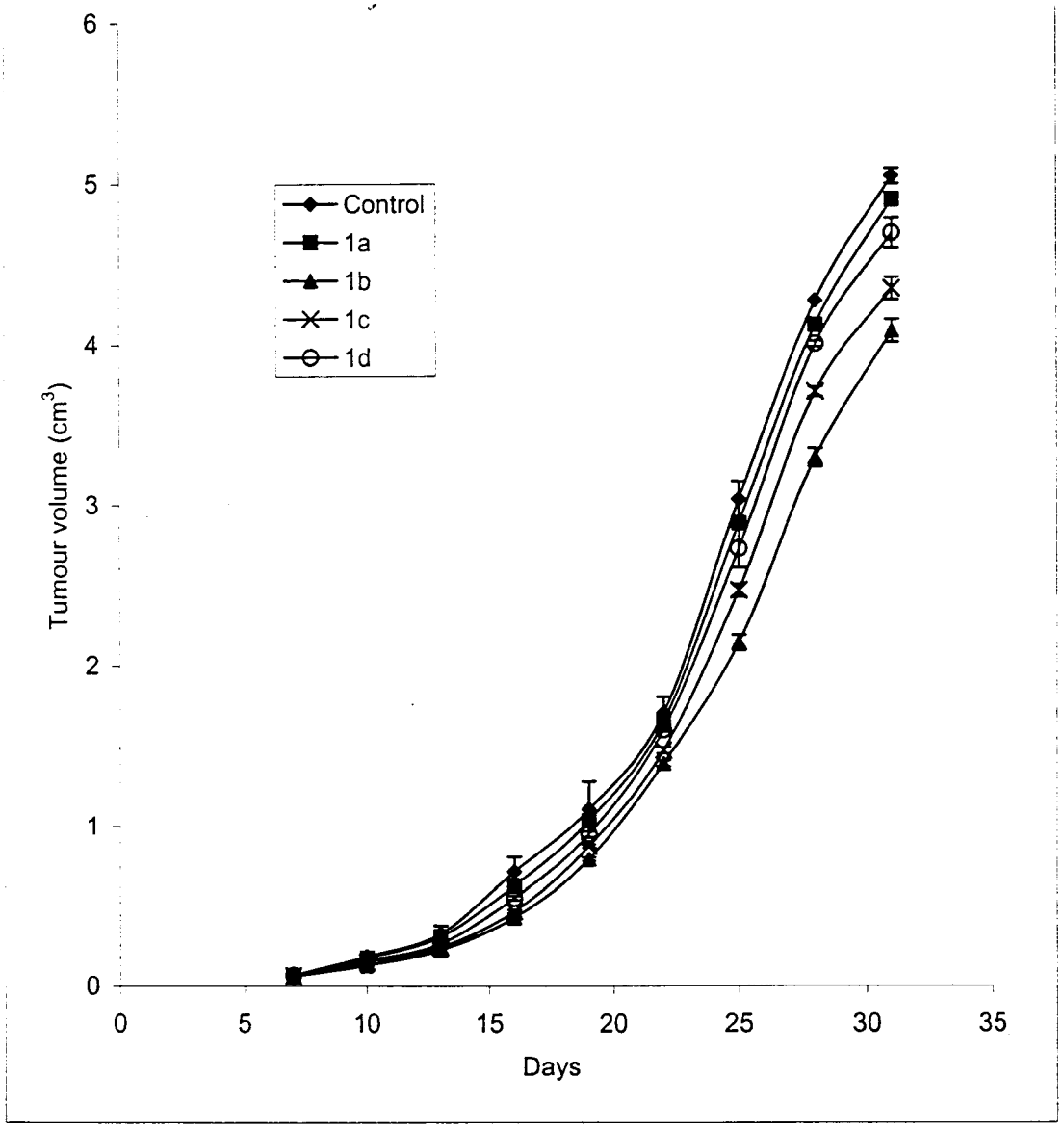
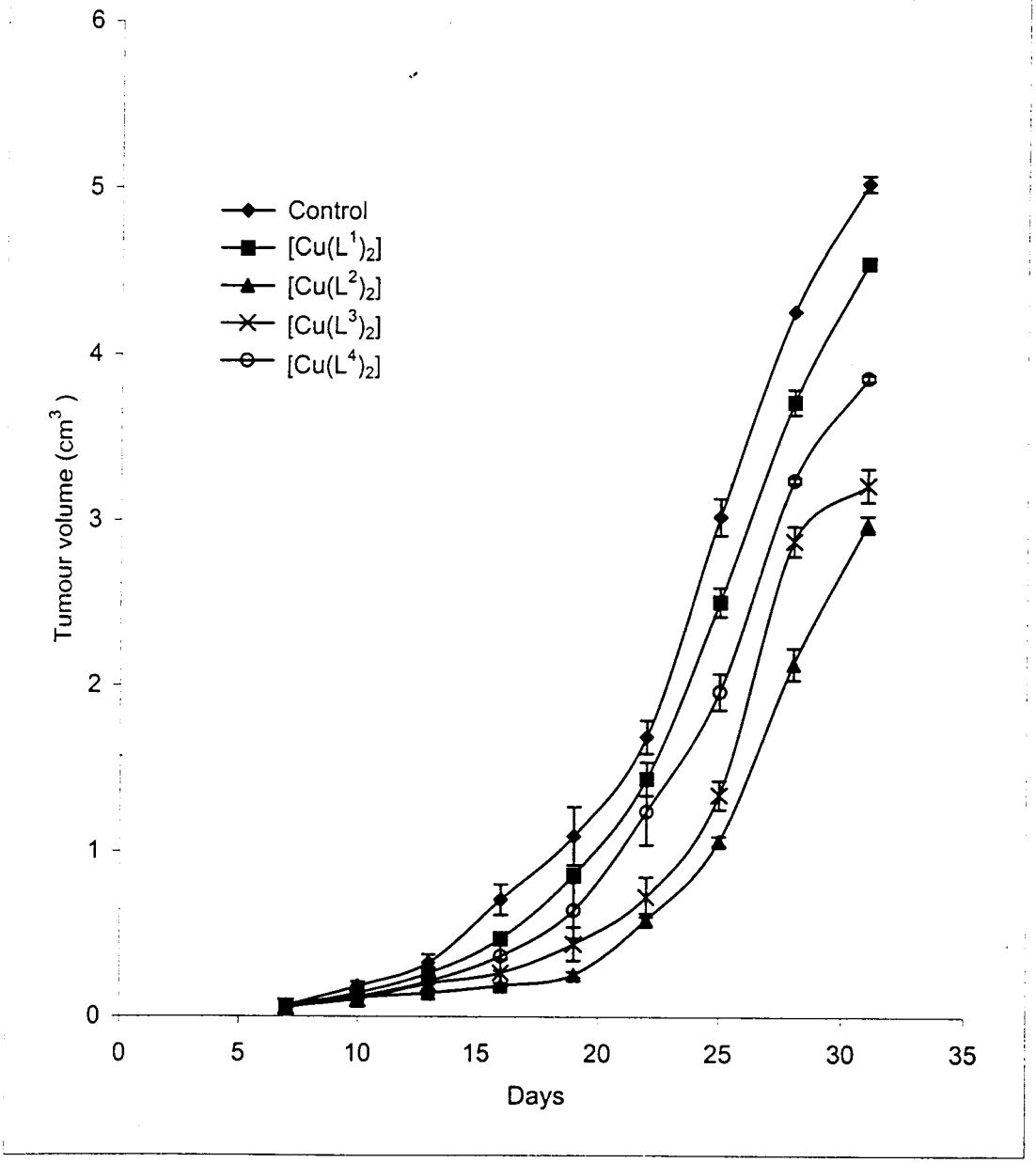


Fig. 1.15 Effect of copper complexes of (1a-1d) on solid tumour volume



All the compounds produced a significant reduction of solid tumour volume in mice ($P < 0.001$ from normal) on day-31. Compared to curcuminoid analogues, their respective copper chelates are remarkably active in reducing tumour volume. Tumour volumes were 5.042 cm^3, 4.90 cm^3, 4.08 cm^3, 4.34 cm^3 and 4.68 cm^3 on day-31 for control, **1a, 1b, 1c** and **1d** respectively. The tumour volumes on day 31 for copper complexes of **1a, 1b, 1c** and **1d** were 4.56 cm^3, 2.98 cm^3, 3.22 cm^3 and 3.87 cm^3 respectively. However the administration of curcuminoids did not prevent solid tumour induced death in these animals.

Antitumour activity of the curcuminoid analogues correlates with their ability to protect biomolecules against singlet oxygen and other pro-oxidants[151]. This ability in turn depends on the nature of curcuminoid analogues, especially the presence of a double bond in conjugation with each of the phenyl rings. Cinnamoyl methanes and chalcones with single conjugated diene structure had much lower antitumour activity[264]. Compound **1b** and its copper complex which possess an unsubstituted phenyl ring and extended conjugation shows moderately high antitumour activity than other unsubstitued diarylheptanoids.

CHAPTER II

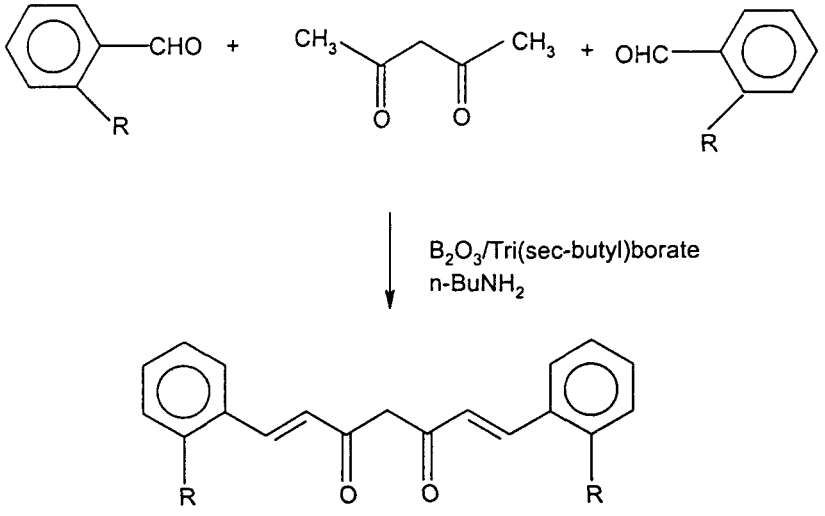
SYNTHESIS, CHARACTERISATION AND ANTITUMOUR ACTIVITY OF 1,7-BIS (*ORTHO*- SUBSTITUTED ARYL) HEPTA-1,6-DIENE-3,5-DIONES AND THEIR METAL CHELATES

SECTION 1

SYNTHESIS AND CHARACTERISATION OF *ORTHO*-SUBSTITUTED –1,7–DIARYLHEPTANOIDS AND THEIR METAL COMPLEXES

Synthesis of *ortho*-substituted 1,7-diarylheptanoids

The *ortho*-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 2.1**). Experimental details and purification procedures are similar to that of the compounds considered in **Chapter 1, Section1**



Scheme 2.1

Synthesis of metal chelates

Oxovanadium(IV), iron(III), cobalt(II), nickel(II) and copper(II) complexes were prepared by the following general method.

The metal salt solution was added with stirring to a methanolic solution (25 mL) of the diketone and refluxed for ~ 1 h 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 2.1. The yield was maximum for **2d** which is beautifully green coloured, crystalline and freely soluble in common organic solvents.

The elemental analysis results and molecular weight determination of the compounds are given in table 2.2. The data suggest that two equivalents of aldehydes have condensed with one equivalent of acetylacetone to form the 1,7-diarylheptanoids.

Table 2.1

*Synthetic details of the ortho-substituted 1,7-diarylheptanoids (**2a-d**)*

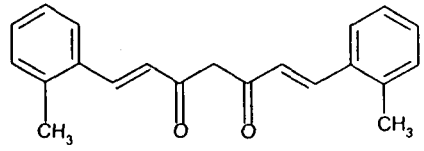
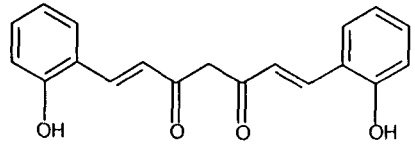
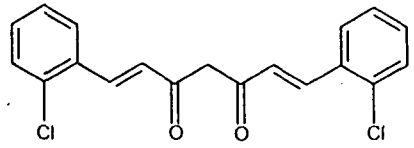
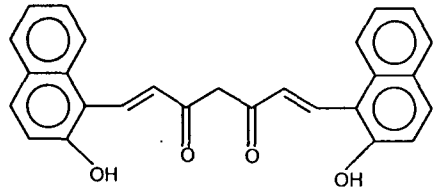
compounds	Aldehydes used for synthesis	Structure	Systematic name	Colour	Yield (%)
2a	2-Methyl benzaldehyde		1,7-Bis (2-methyl phenyl) -1,6-heptadiene-3,5-dione	Yellow	70
2b	Salicylaldehyde		1,7-Bis(2-hydroxy phenyl) -1,6-heptadiene-3,5-dione	brown	51
2c	2-Chlorobenzaldehyde		1,7-Bis(2-chloro phenyl) -1,6-heptadiene-3,5-dione	dark red	59
2d	2-Hydroxynaphthaldehyde		1,7-Bis(2-hydroxy naphthyl) -1,6-heptadiene-3,5-dione	dark green	72

Table 2.2

*Physical, analytical and uv spectral data of ortho substituted 1,7-diarylheptanoids (**2a-d**)*

Compound	MP	Elemental analysis (%) Found/(Calcd)		Mol. Weight Found/(Calcd)	λ_{max} (nm)	log ∈
	^{0}C	C	H			
2a	121	82.18 (82.90)	6.12 (6.58)	302 (304)	278 (397)	4.11 (4.70)
2b	120	72.89 (74.01)	5.35 (5.19)	306 (308)	266 (390)	4.08 (4.67)
2c	90	67.10	4.12 (4.07)	345 (344)	269 (376)	4.03 (4.39)
2d	101	80.01 (79.41)	4.32 (4.91)	410 (408)	260 (385)	4.05 (4.57)

Characterisation of the *ortho*-substituted 1,7-diaryl heptanoids

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

Uv spectra

Uv spectra of the compounds (**2a-d**) are characterised by the presence of two absorption maxima (Table 2.2); the low energy band corresponds to n→π* transition (380 – 460 nm) and the high energy band (260 – 280 nm) due to the π→π* transitions. The n→π* absorption values of *ortho*-substituted 1,7-diarylheptanoids are of lower energy compared to the corresponding unsubstituted compounds, discussed in Chapter I.

Infrared spectra

The ir spectra of *ortho*-substituted 1,7-diarylheptanoids are characterised by the presence of a strong band in the range of 1605 – 1640 cm^{-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 ~ 970 cm^{-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 2.3.

Table 2.3

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

Compounds				Probable assignments
2a	**2b**	**2c**	**2d**	
1625	1607	1620	1637	ν (C=O) chelated
1598	1596	1595	1602	ν (C=C) phenyl
1570	1585	1580	1593	
1545	1535	1540	1541	ν (C-C) alkenyl
1516	1510	1506	1514	ν_{as} (C-C-C) chelate ring
1452	1454	1465	1464	ν_{s} (C-C-C) chelate ring
1134	1109	1128	1167	β (C-H) chelate ring
1041	1037	1043	1078	
970	978	974	985	ν (CH=CH) (trans)

^1H nmr spectra

Further evidence for the tautomeric nature of the compounds is provided by the ^1H nmr spectra. The ^1H nmr spectra of all the compounds displayed a singlet (1H) downfield at δ ~16 ppm and another singlet at δ~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 **2d** are brought out in figures 2.1 - 2.3. The phenolic protons on the aryl rings of the compounds **2b, 2d** and methyl group on aryl ring of the compound **2a** showed signals as expected. The position of the aryl proton signals are also as expected. The assignments of various proton signals observed in the ^1H nmr spectra of the compounds are assembled in table 2.4. The assignments are in agreement with the earlier data on related compounds discussed in chapter 1.

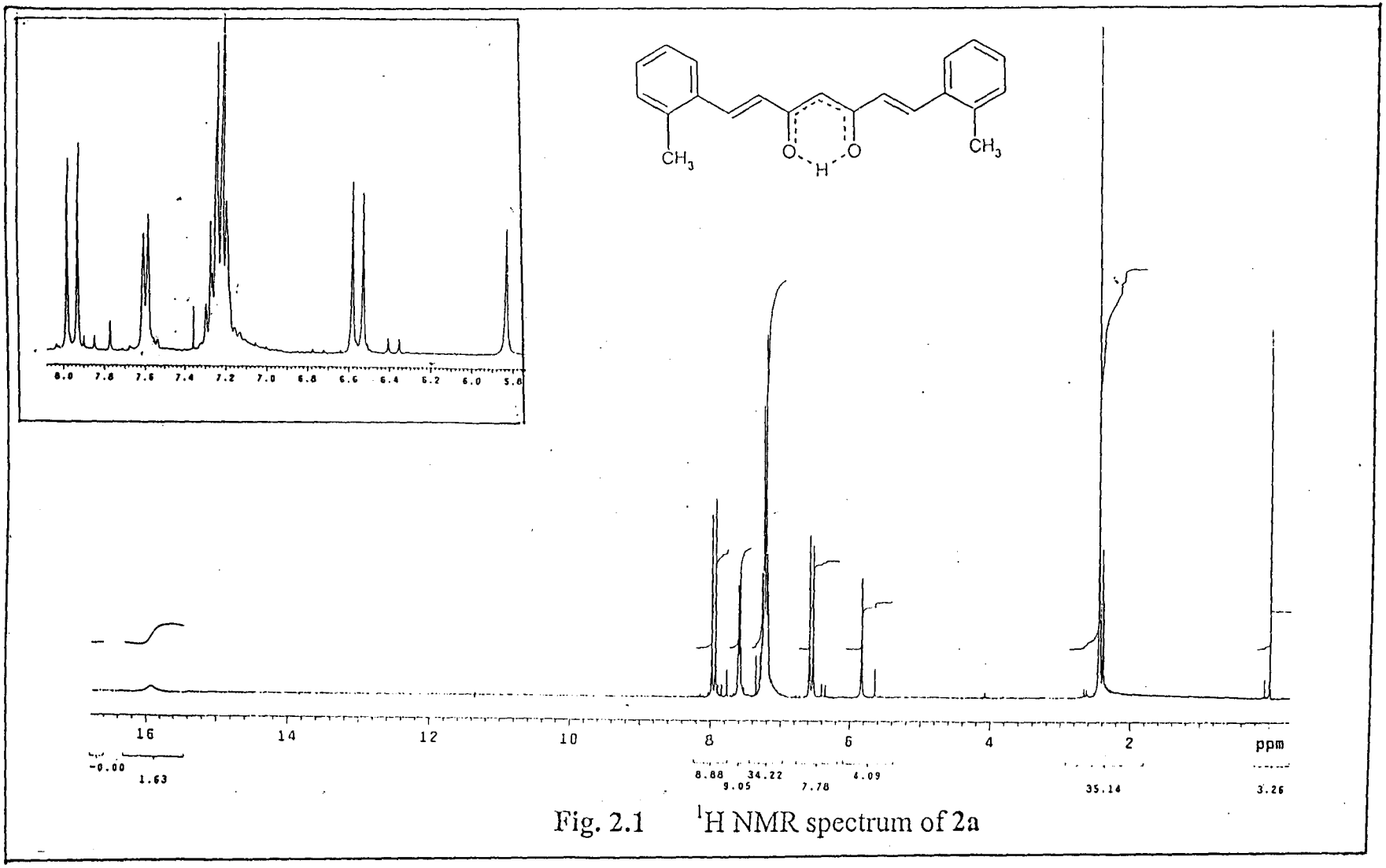


Fig. 2.1 ^{1}H NMR spectrum of 2a

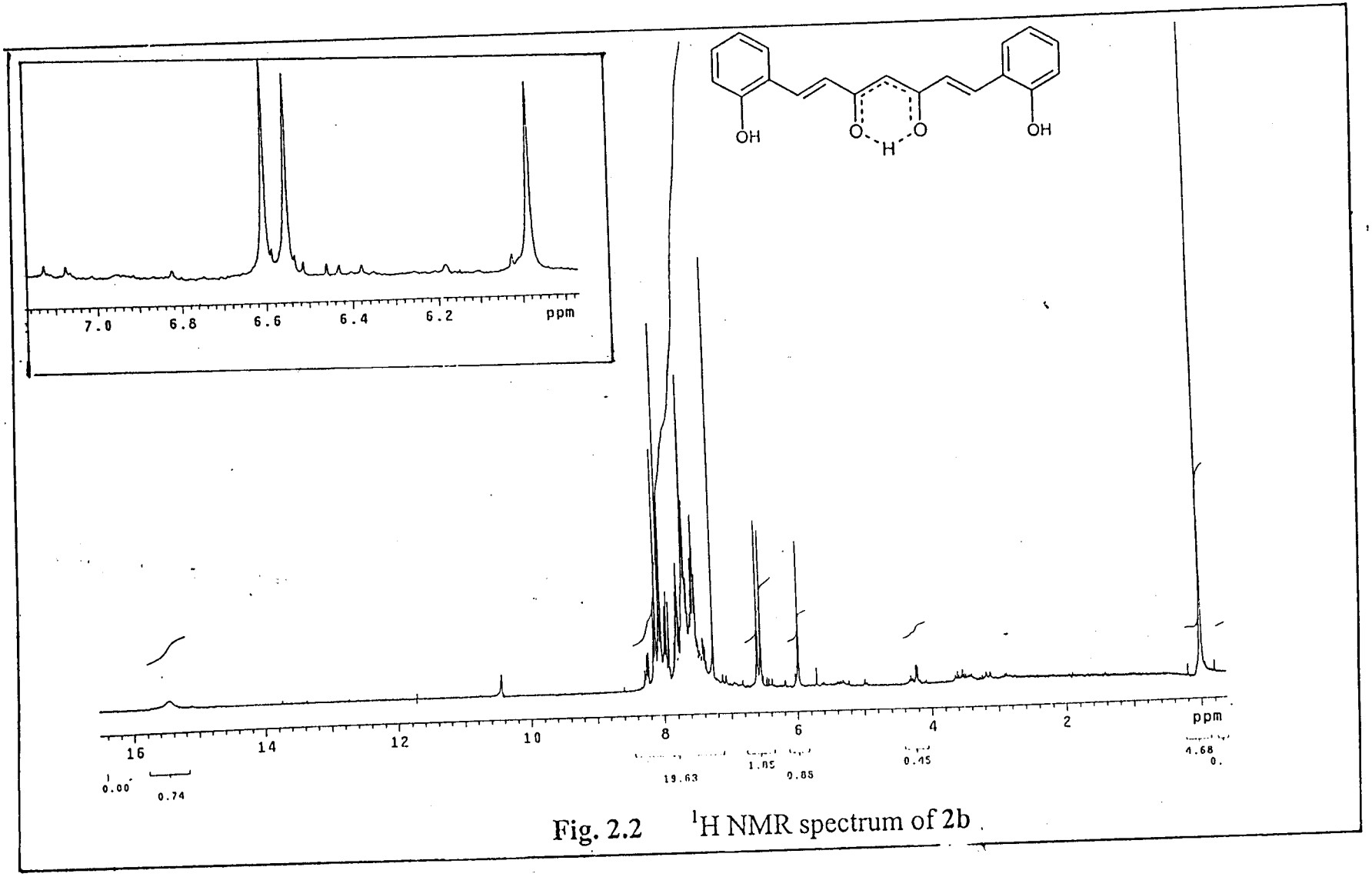


Fig. 2.2 ^{1}H NMR spectrum of 2b .

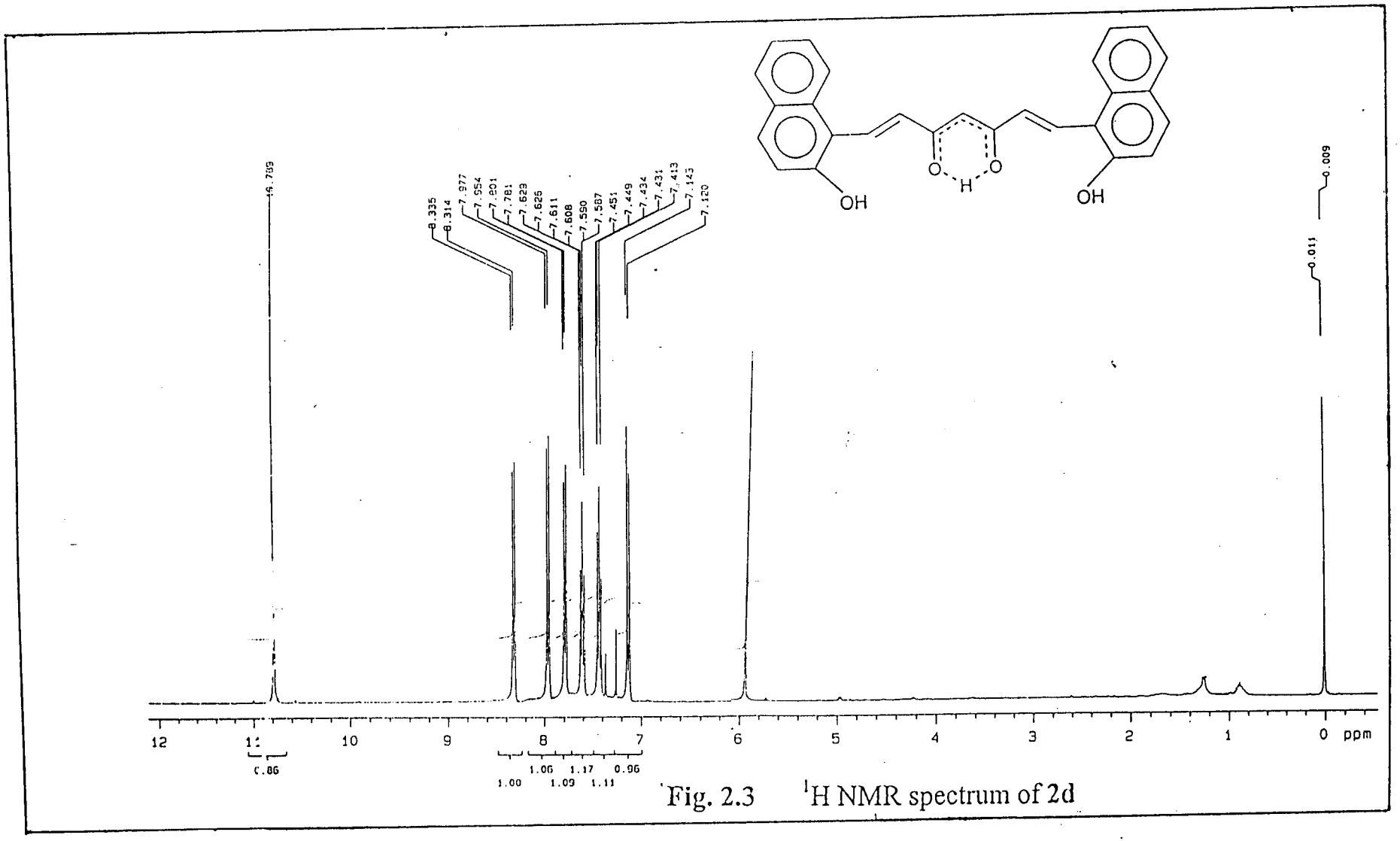


Fig. 2.3 ^{1}H NMR spectrum of 2d

Table 2.4

Characteristic ^1H nmr spectral data of ortho substituted 1,7- diarylheptanoids

Compounds	Chemical shifts (δ ppm)					
	Enolic	Methine	Alkenyl	Aryl	*Ortho*-substituents Hydroxy	Methyl
2a	15.945	5.836	6.533-7.986	7.198-7.609	-	2.465
2b	15.559	6.083	6.552-7.580	7.400-8.220	10.439	-
2c	15.920	5.898	6.640-8.079	7.164-7.789	-	-
2d	16.041	5.909	7.1420-7.977	7.413-8.335	10.789	-

Mass spectra

Mass spectra of all the *ortho*-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 (2.4–2.7). Important peaks appeared in the spectra of all the ortho substituted 1,7-diarylheptanoids can be conveniently accounted by the fragmentation pattern given in **scheme 2.2**.

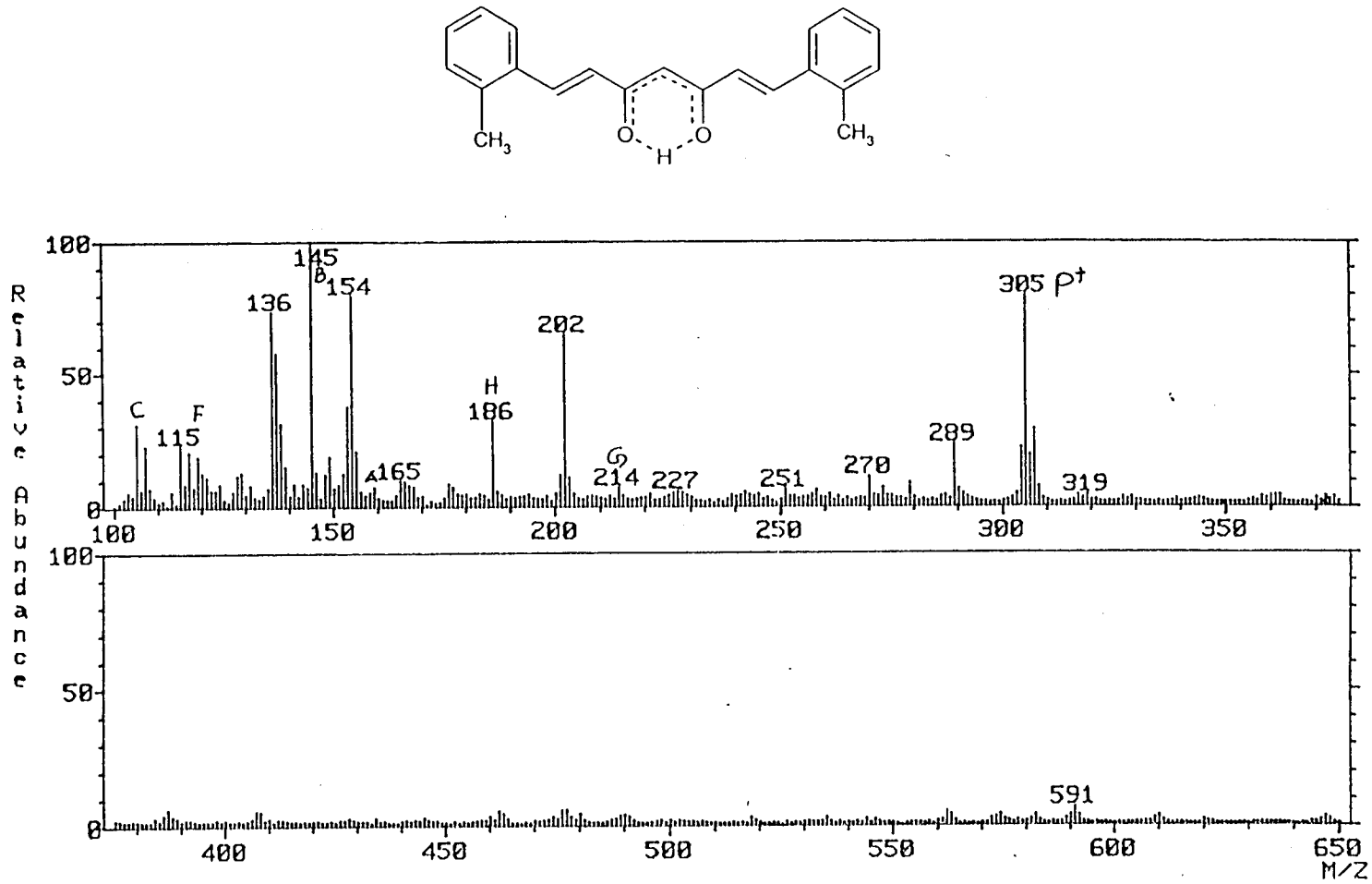


Fig. 2.4 Mass spectrum of 2a

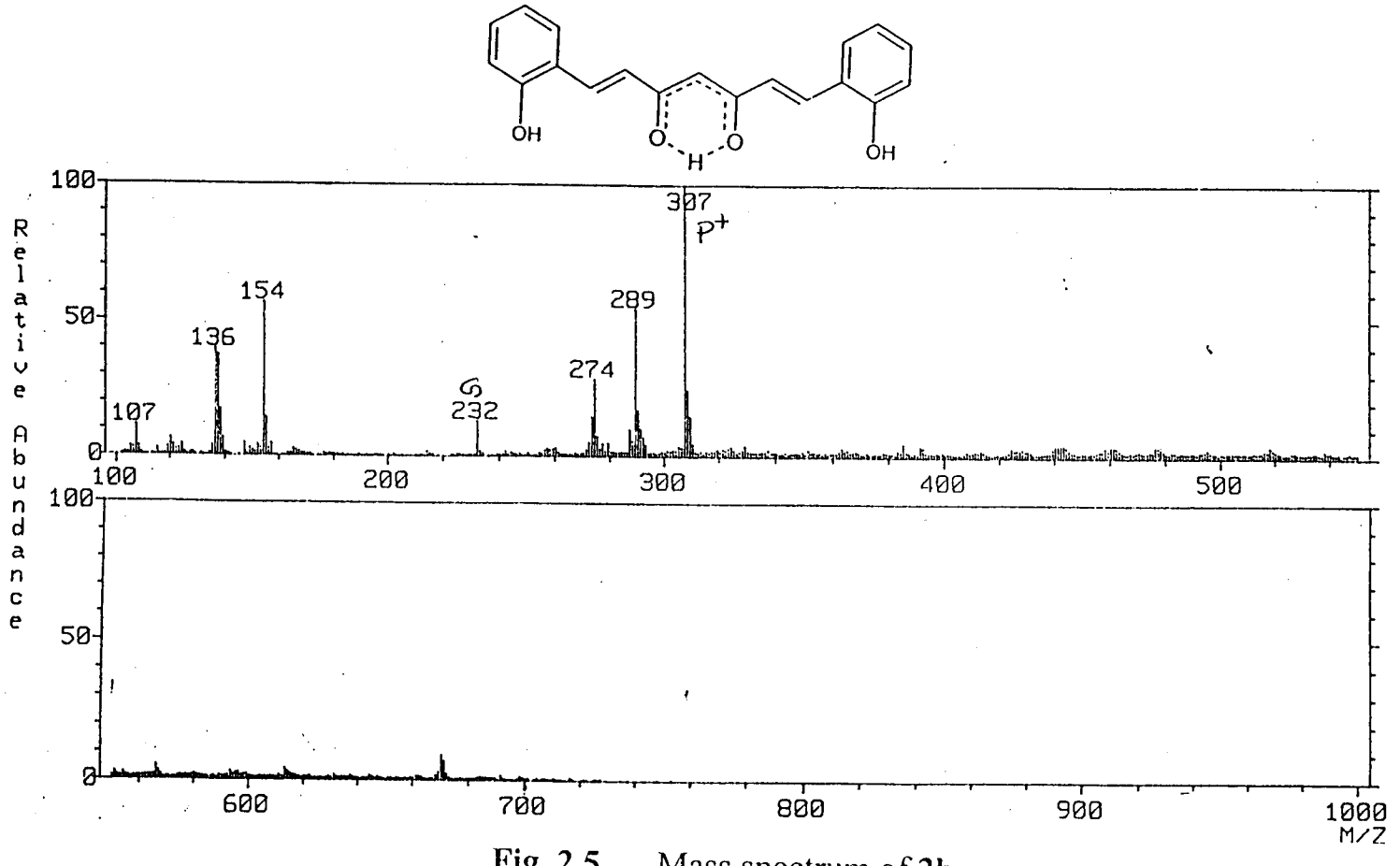


Fig. 2.5 Mass spectrum of **2b**

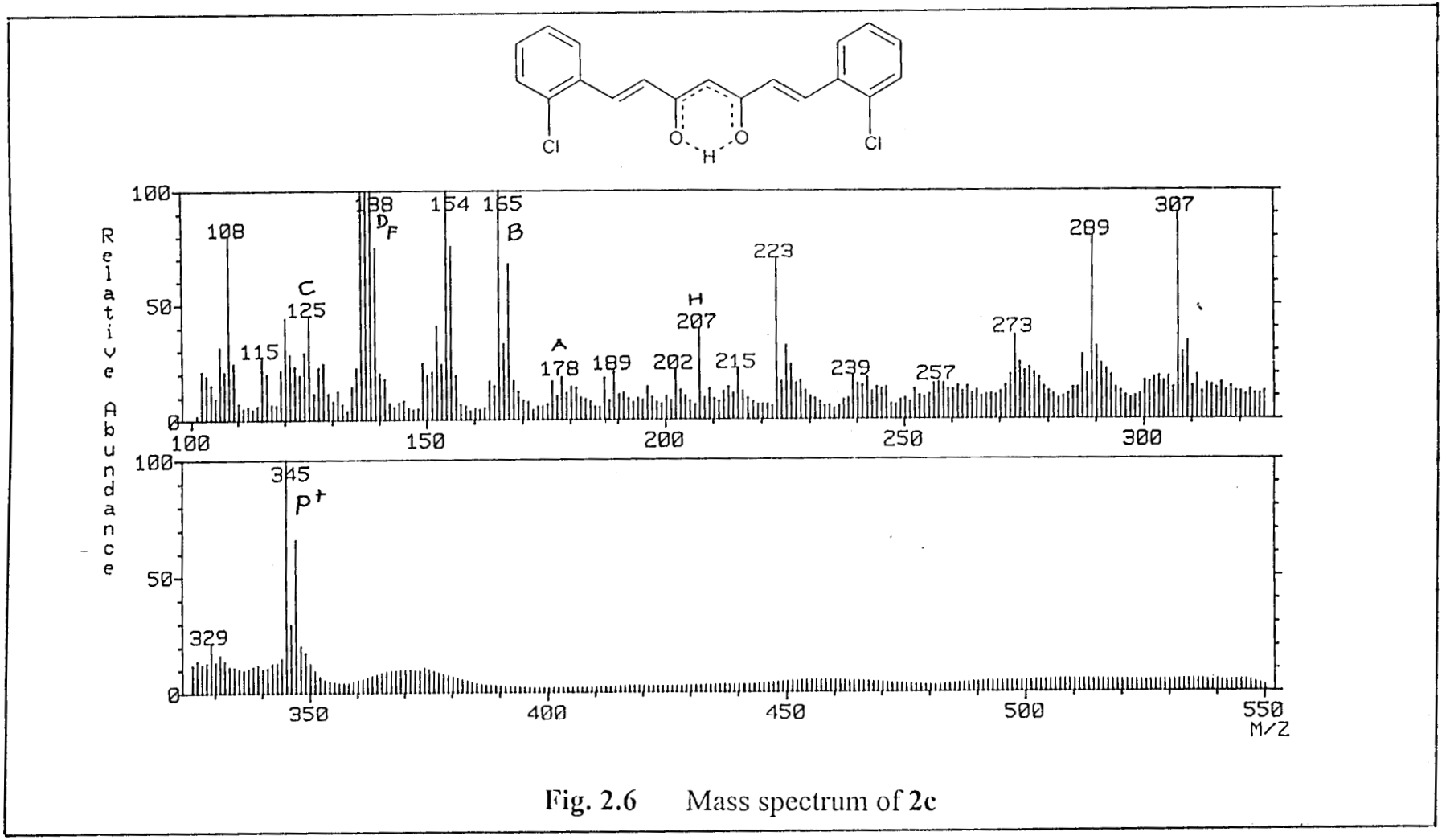


Fig. 2.6 Mass spectrum of **2c**

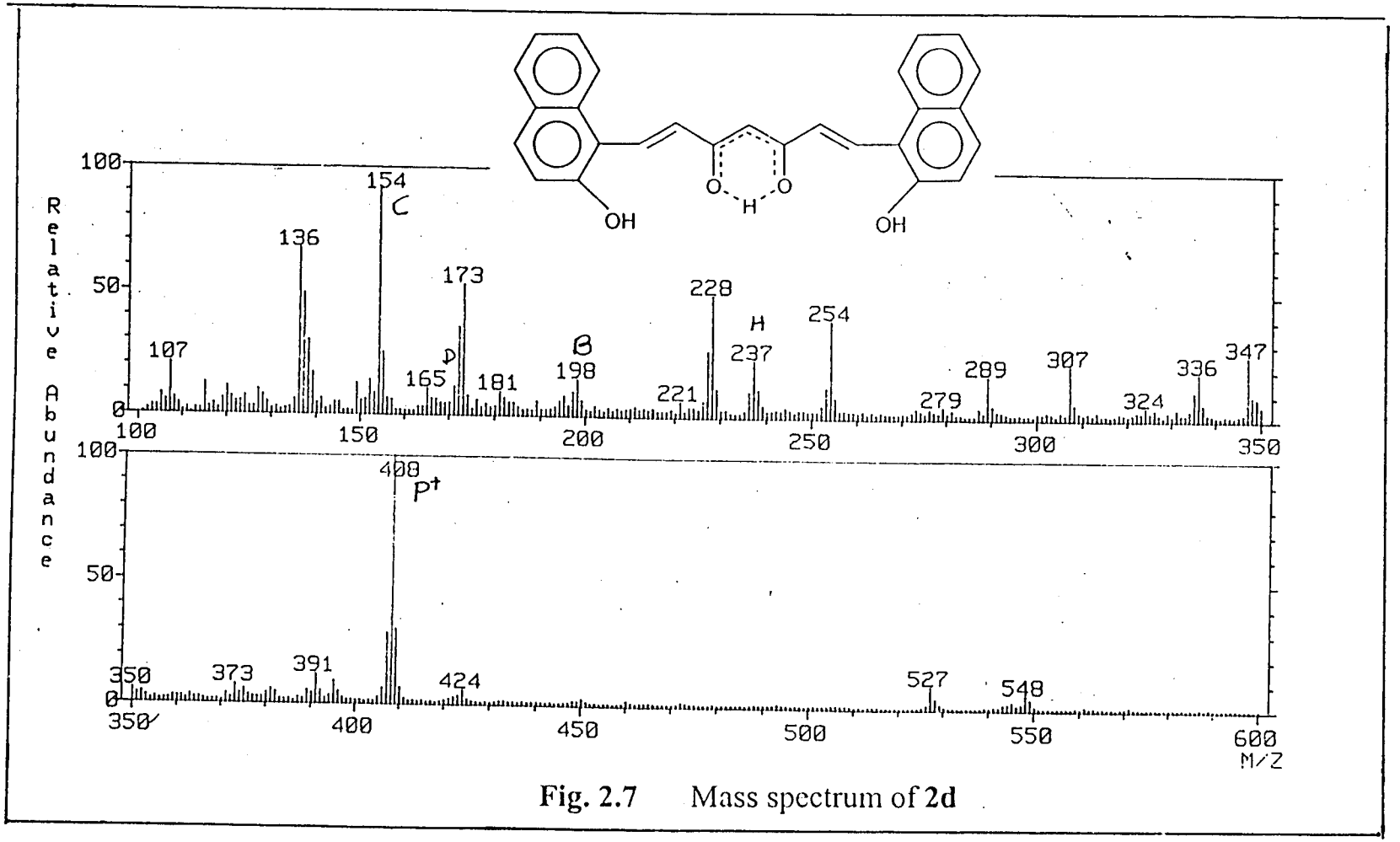
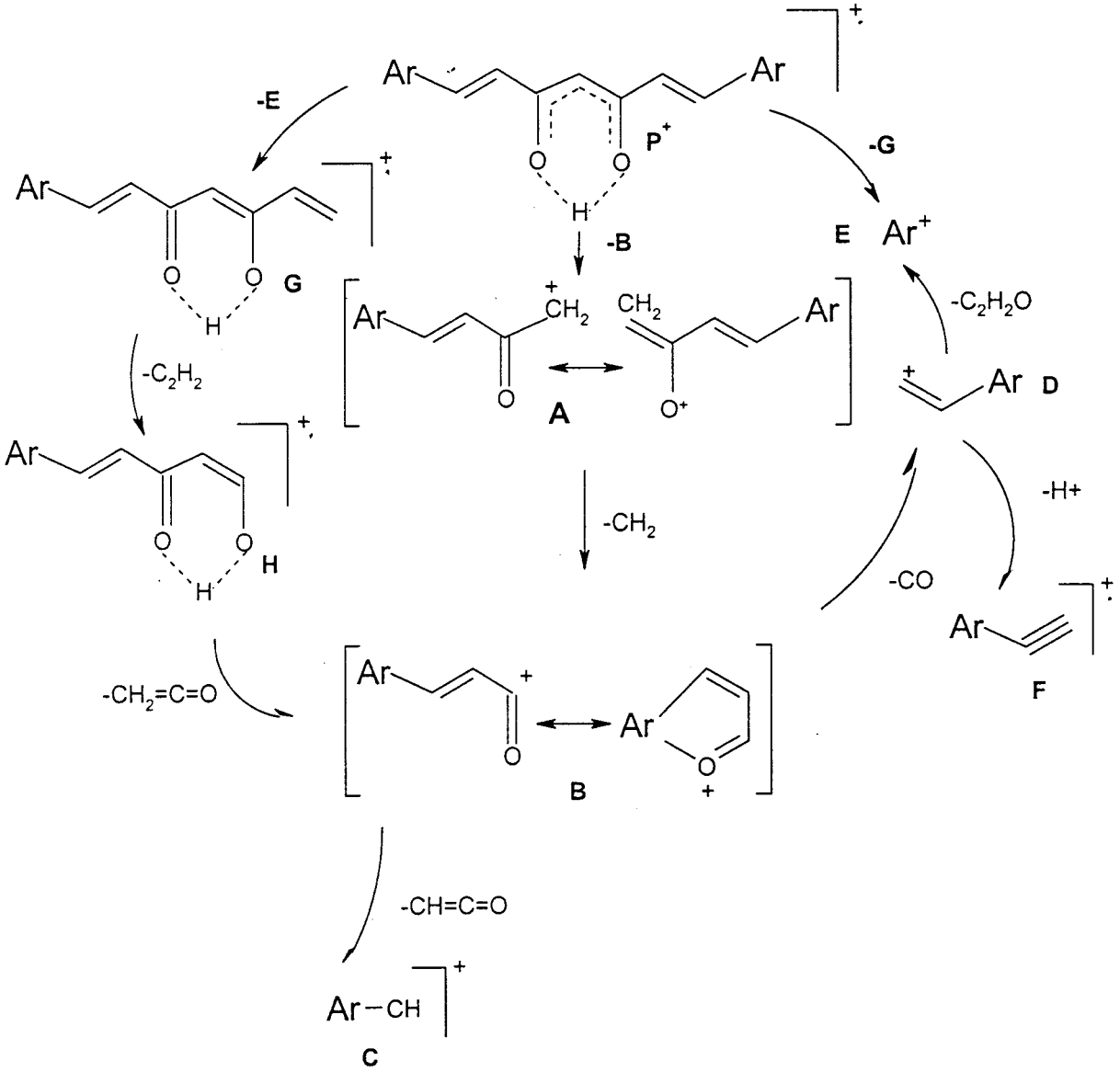


Fig. 2.7 Mass spectrum of **2d**

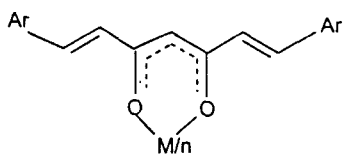


Scheme 2.2

Characterisation of metal chelates

Elemental analytical data of the complexes of VO(IV), Co(II), Ni(II) and Cu(II) suggest their $[ML_2]$ stoichiometry and $[ML_3]$ for Fe(III) complexes. Analytical and physical data of the metal complexes are given in tables 2.5-2.9.

Conductometric studies show that all the complexes behave as non electrolytes in dmf (specific conductance <10 Ω^1 cm^{-1} in 1 0^{-3} M solution) and do not contain the anion of the metal salt used for the preparation. Magnetic moment measurements show that nickel(II) complexes are diamagnetic, Cu^{2+}, VO^{2+}, Co^{2+} and Fe^{3+} chelates show normal paramagnetic moment. The uv, ir, nmr and mass spectral data suggest structure **2.3** of the complexes. The data are discussed below.



n = 2 for VO(IV), Co(II), Ni(II) & Cu(II)
n = 3 for Fe(III)

2.3

Table 2.5

Physical, analytical and ir spectral data of the oxovanadium (IV) chelates of ortho substituted 1,7-diarylheptanoids (HL)

Oxovanadium (IV) Chelates of	MP ^{0}C	μ_{eff} BM	Elemental analysis (%) Found/(Calcd)*			Characterisitc ir stretching bands (cm^{-1})	
			C	H	V	C-O	V-O
2a	167	1.78	73.15 (74.88)	5.01 (5.65)	7.95 (7.49)	1585	456 428
2b	151	1.76	67.55	4.65	7.95 (7.49)	1590	469 426
2c	130	1.73	61.51 (60.39)	3.55 (3.45)	7.01 (6.75)	1599	465 419
2d	150	1.76	75.85 (74.91)	4.90 (4.39)	6.31 (5.89)	1590	468 419

** The calculated value corresponds to the [VOL_2] composition where L stands for the deprotonated ligand.*

Table 2.6

Physical, analytical and characteristic ir spectral data of iron(III) chelates of ortho substituted 1,7-diaryl heptanoids (HL)

Iron (III) chelates of	MP (C^0)	μ_{eff} BM	Elemental analysis (%) Found/(Calcd)*			Characteristic ir stretching bands(cm^{-1})	
			C	H	Fe	C-O	Fe-O
2a	174	5.92	77.78 (78.34)	6.32 (5.91)	6.01 (5.80)	1582	452 419
2b	160	5.89	70.55 (70.01)	5.01 (4.60)	5.95 (5.73)	1595	459 417
2c	142	5.90	63.44 (62.87)	3.72 (3.58)	4.95 (5.14)	1585	455 419
2d	167	5.87	75.67 (76.11)	4.83 (4.46)	3.93 (4.39)	1582	465 422

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

Table 2.7

Physical, analytical and characteristic ir spectral data of the Cobalt(II) chelates of ortho substituted 1,7-diarylheptanoids (HL)

Cobalt (II) chelates of	MP (C^0)	μ_{eff} BM	Elemental analysis (%) Found/(Calcd)*			Characteristic ir stretching bands (cm^{-1})	
			C	H	Co	C-O	M-O
2a	160	4.90	70.60 (71.91)	5.98 (5.71)	8.81 (8.42)	1592	465 430
2b	148	4.79	62.89 (64.32)	4.93 (4.51)	8.99 (8.32)	1595	465 419
2c	128	4.89	59.25 (58.09)	3.97 (3.82)	7.84 (7.52)	1589	453 419
2d	131	4.77	72.03 (71.28)	4.49 (4.40)	6.22 (6.49)	1580	455 419

** The calculated value corresponds to the $[CoL_2 (H_2O)_2]$ composition where L stands for the deprotenated ligand.*

Table 2.8

Physical, analytical and characteristic ir spectral data of the nickel(II) chelates of ortho substituted 1,7-diarylheptanoids (HL)

Nickel (II) chelates of	MP C^0	Elemental analysis (%) Found/(Calcd)*			Characteristic ir stretching bands(cm^{-1})	
		C	H	Ni	νC-O	νNi-O
2a	165	76.33 (75.71)	6.39 (5.68)	9.04 (8.80)	1595	465 426
2b	149	68.27 (67.70)	4.37 (4.38)	9.11 (8.70)	1598	469 419
2c	130	61.29 (60.96)	3.76 (3.41)	7.50 (7.82)	1593	460 424
2d	139	73.90 (74.15)	4.57 (4.28)	7.15 (6.69)	1585	496 427

** The calculated value corresponds to the [Ni L_2] composition where L stands for the deprotenated ligand.*

Table 2.9

Physical, analytical and characteristic ir spectral data of the copper(II) chelates of ortho substituted 1,7- diarylheptanoids (HL)

Copper (II) chelates of	MP (C^0)	μ_{eff} BM	Elemental analysis (%) Found/(Calcd)*			Characteristic Ir absorption bands (cm^{-1})	
			C	H	Cu	νC-O	νCu-O
2a	161	1.76	76.43 (75.28)	5.81 (5.67)	10.01 (9.48)	1598	464 415
2b	149	1.75	66.59 (67.30)	4.01 (4.42)	9.04 (8.71)	1590	455 419
2c	125	1.78	61.23 (60.67)	3.78 (3.46)	8.11 (7.85)	1599	465 424
2d	133	1.80	74.27 (73.85)	4.96 (4.33)	6.49 (6.72)	1595	467 409

** The calculated value corresponds to the [Cu L_2] composition where L stands for the deprotenated ligand.*

Uv spectra

The characteristic uv absorption maxima of the diketones due to π→π* and n→π* transitions show only slight bathochromic shifts in the spectra of the metal complexes. It is, therefore, evident that no structural alteration 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 ~1595cm^{-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 ~418 cm^{-1} assignable to ν(M-O) vibrations. Spectra of the cobalt(II) complexes show characteristic bands at ~3400 cm^{-1} and 3800 cm^{-1} due to coordinated water molecules. Important ir bands of the complexes are included in tables (2.5-2.9).

^1H nmr spectra

In the ^1H nmr spectra of the dimagnetic nickel(II) complexes, the enolic proton singlet (δ~16ppm) of the free ligand is absent. 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. The spectra of the nickel(II) complexes of **2a, 2b** and **2d** are given as figures 2.8, 2.9 and 2.10 respectively. Integrated intensities of the various proton signals agree well with the $[NiL_2]$ stoichiometry of the complexes.

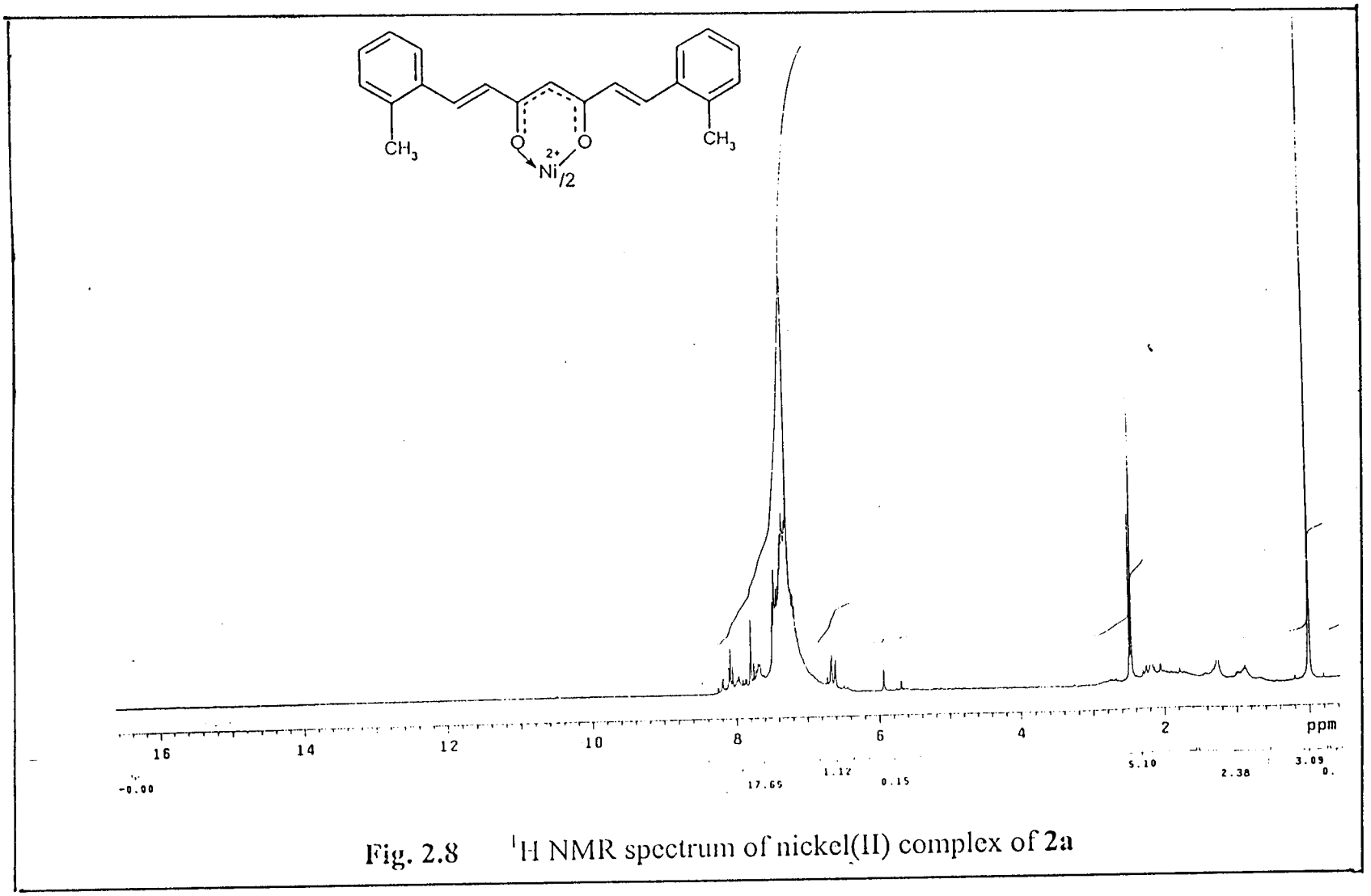


Fig. 2.8 ^{1}H NMR spectrum of nickel(II) complex of **2a**

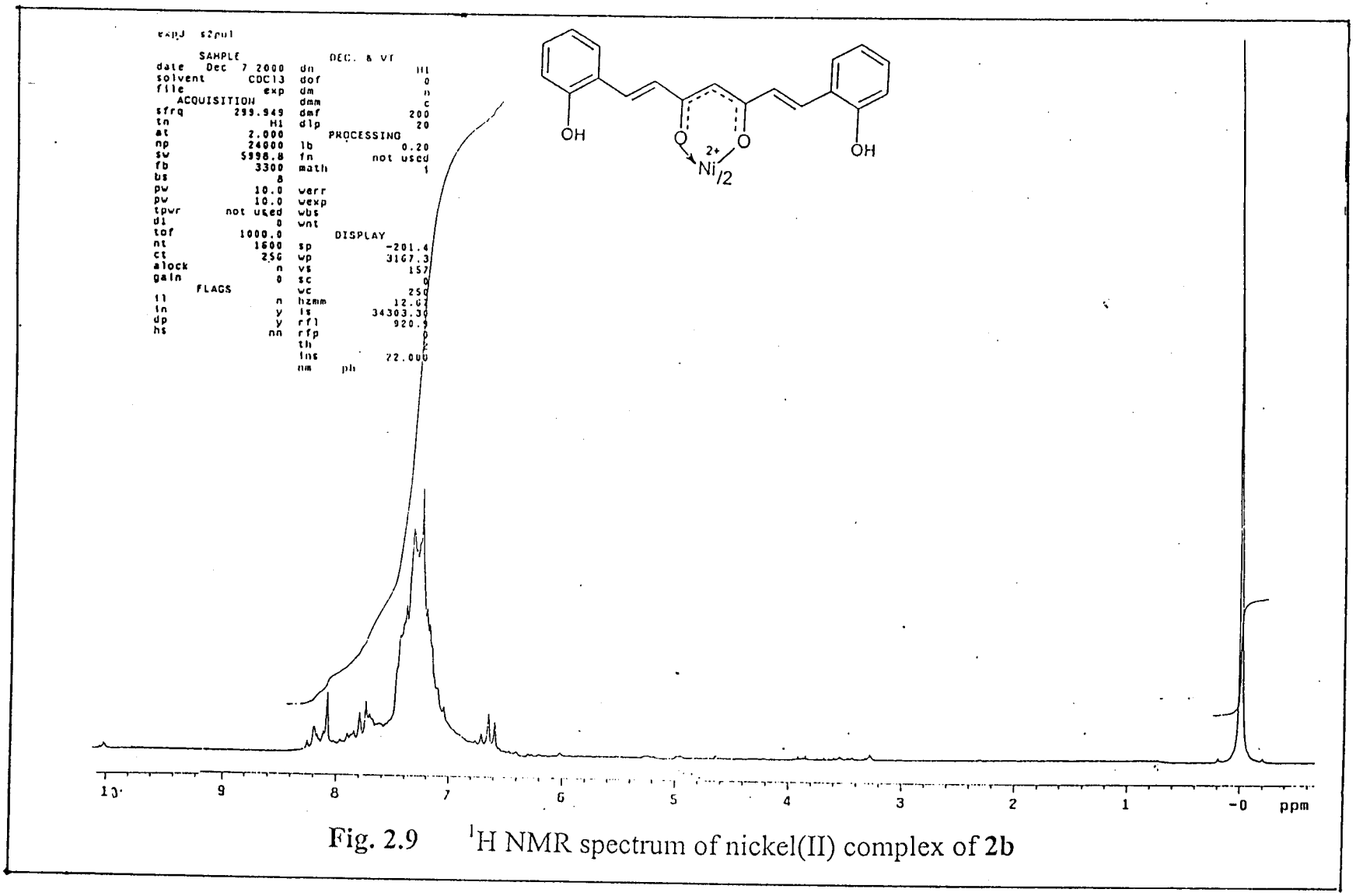


Fig. 2.9 ^{1}H NMR spectrum of nickel(II) complex of **2b**

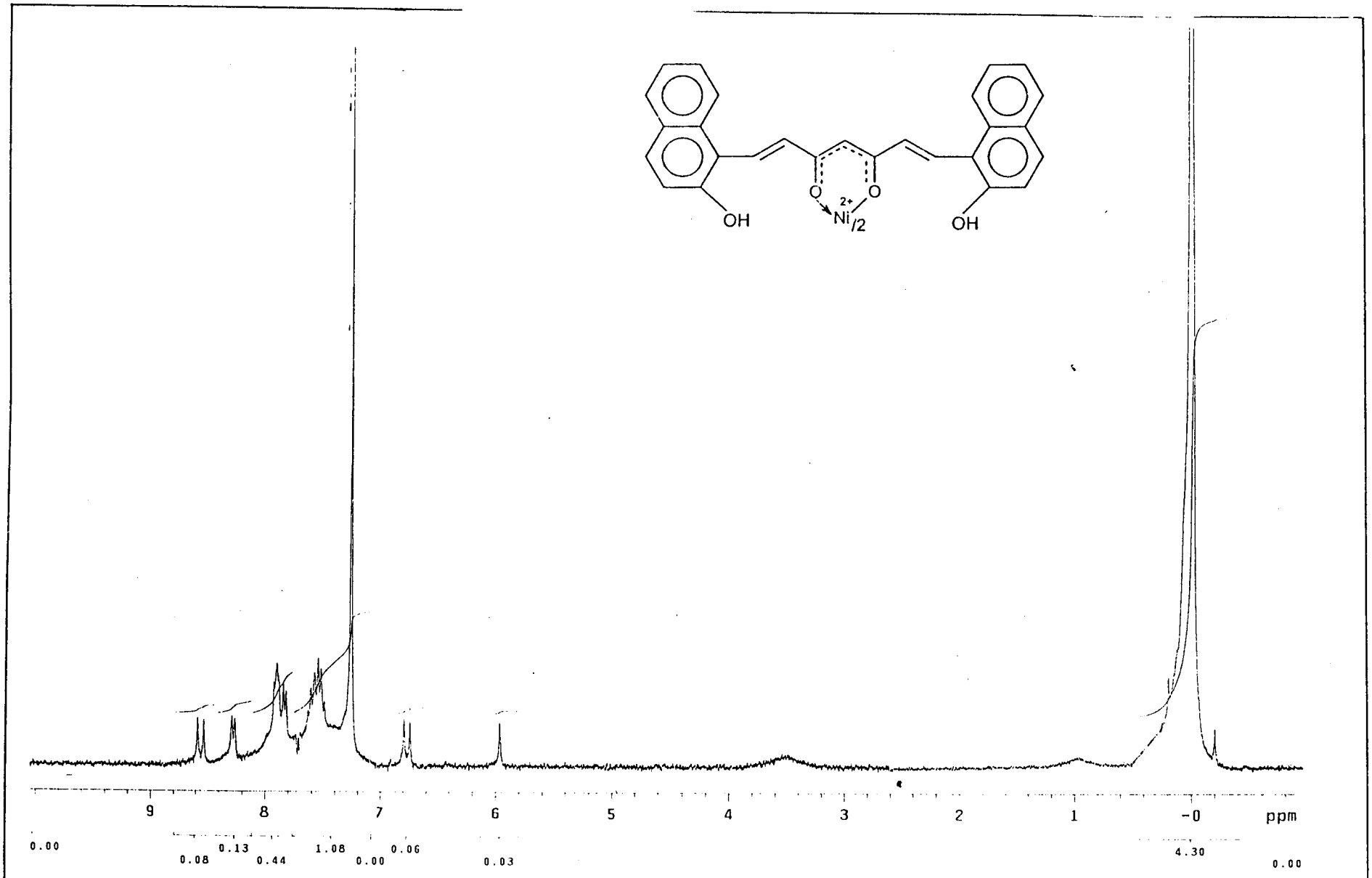


Fig. 2.10 ^{1}H NMR spectrum of nickel(II) complex of **2d**

Mass spectra

The FAB mass spectra of copper chelates of *ortho*-substituted 1,7-diarylheptanoids show that stepwise removal of aryl groups is a characterstic feature. All the metal chelates show a relatively intense P^+ ion peaks in confiormity with their $[CuL_2]$ stoichiometry. Peaks due to $[CuL]^+$, L^+ and fragments of L^+ are sometimes more intense. Peaks at m/z corresponding to $[P\text{-}nAr]^+$ where n=1 to 4 are also present in the mass spectra of metal chelates. Typical spectra of the copper(II) complexes are given in figures (2.11-2.14). The probable fragmentation pattern of the complexes based on the observed peaks and the stabilisation of the fragments through cyclisation are illustrated in **scheme 2.3**.

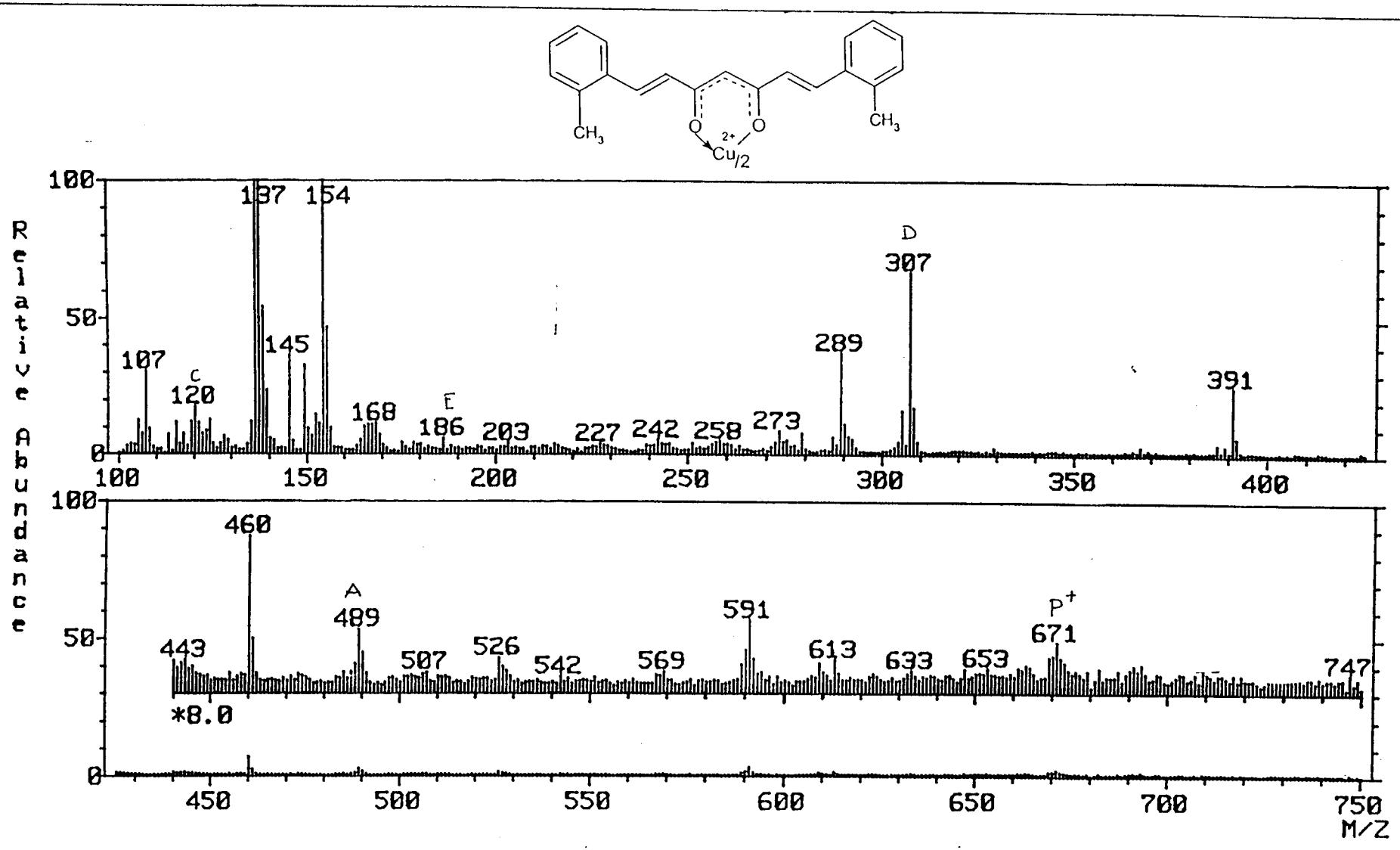


Fig. 2.11 Mass spectrum of copper(II) complex of 2a

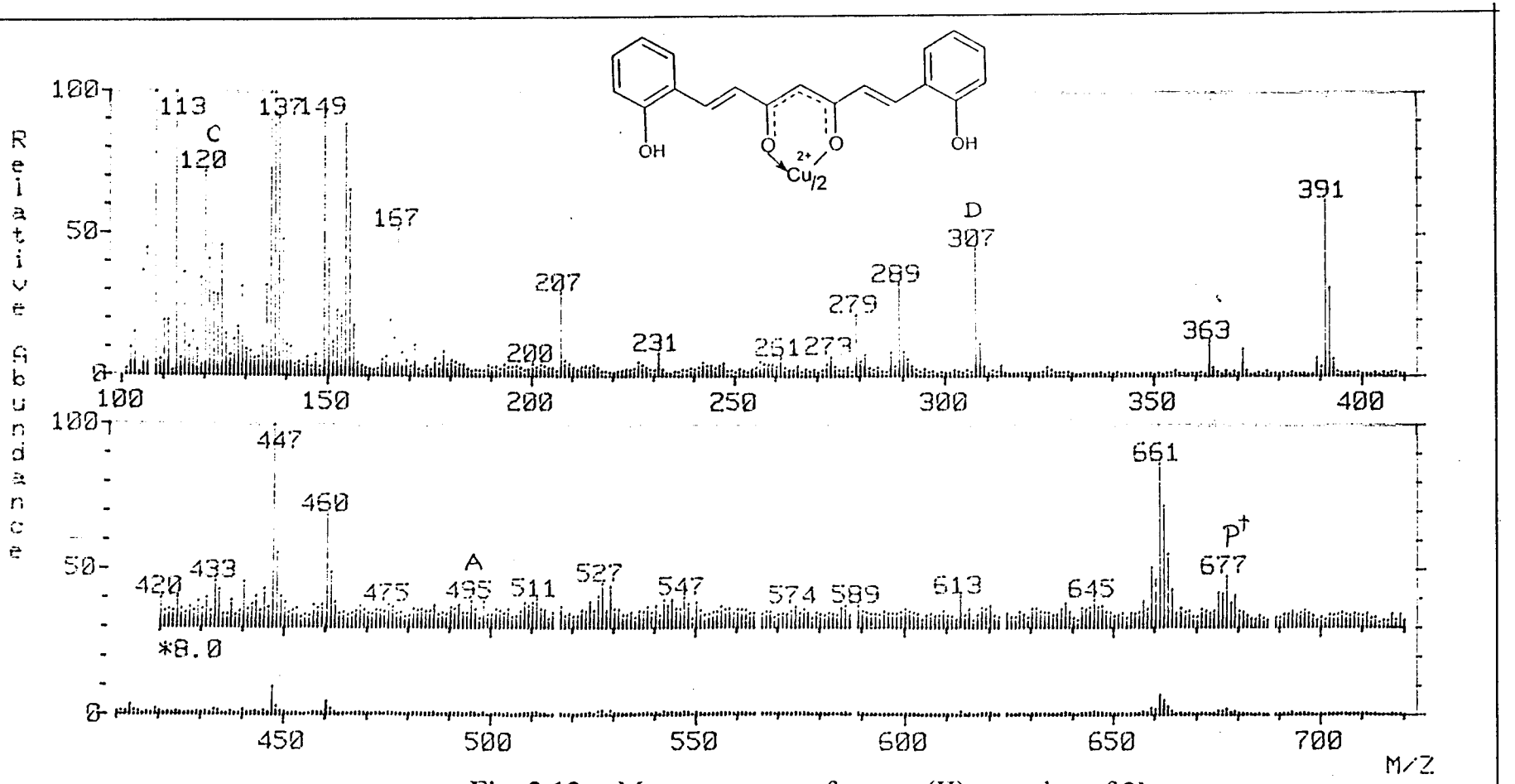


Fig. 2.12 Mass spectrum of copper(II) complex of **2b**

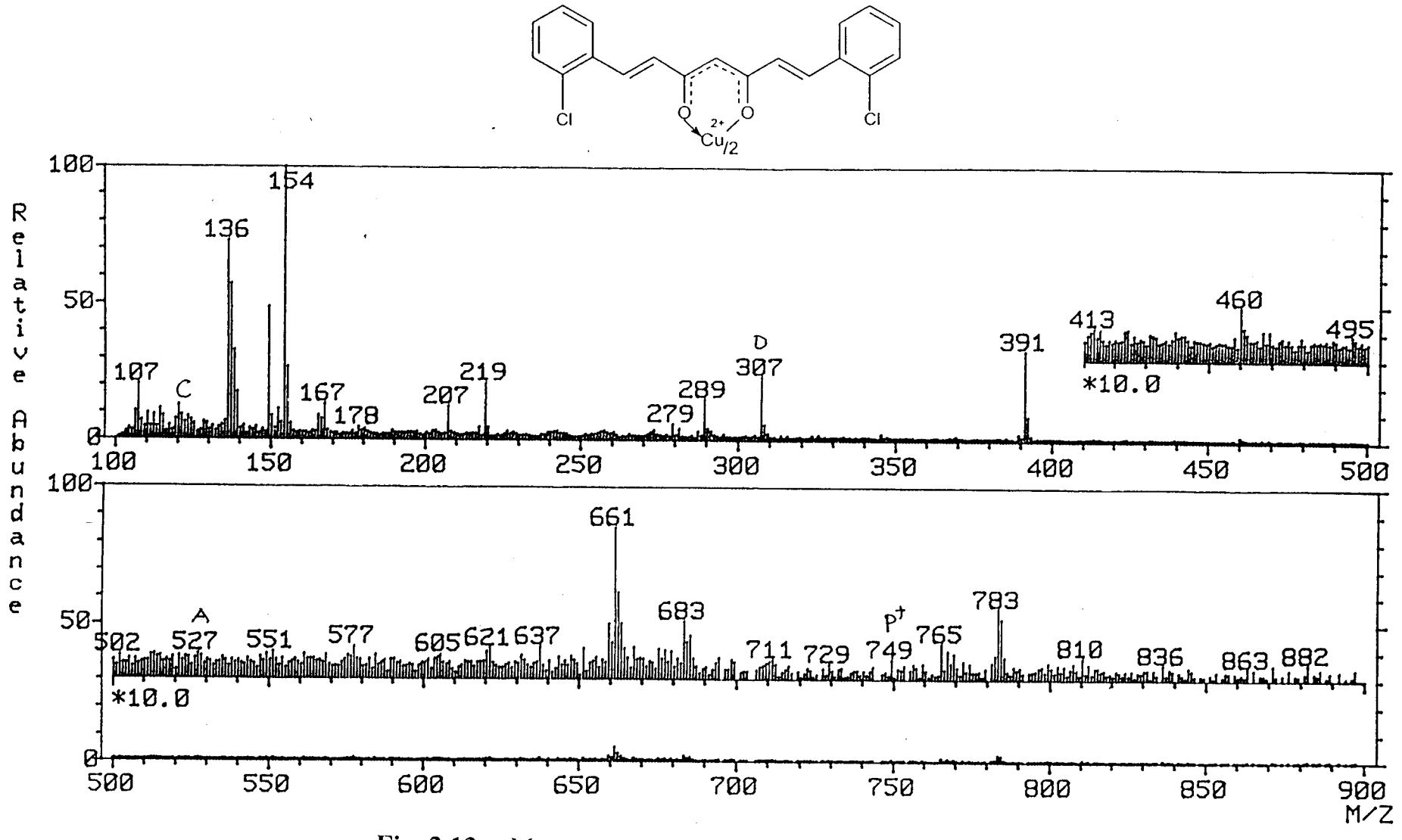


Fig. 2.13 Mass spectrum of copper(II) complex of **2c**

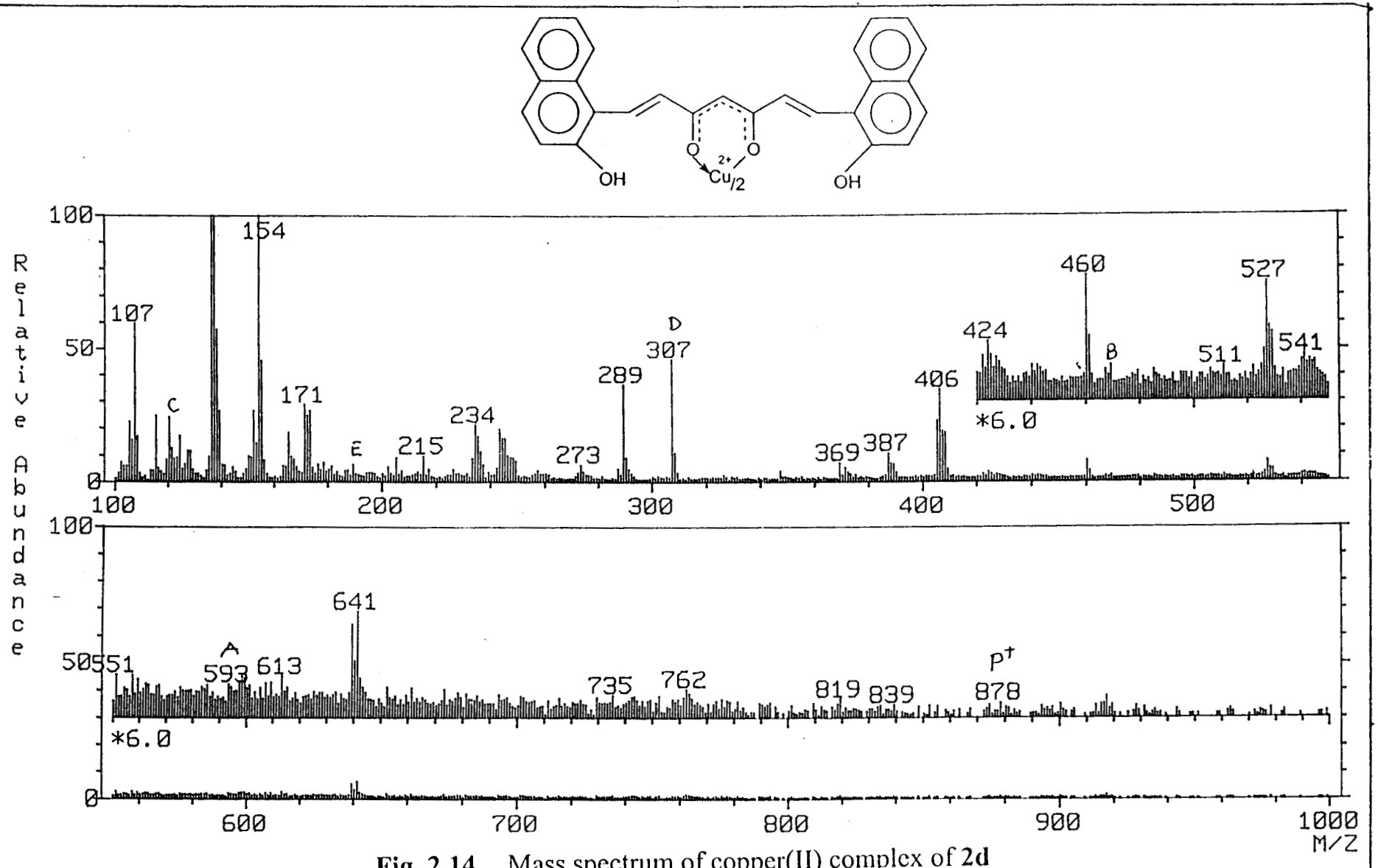
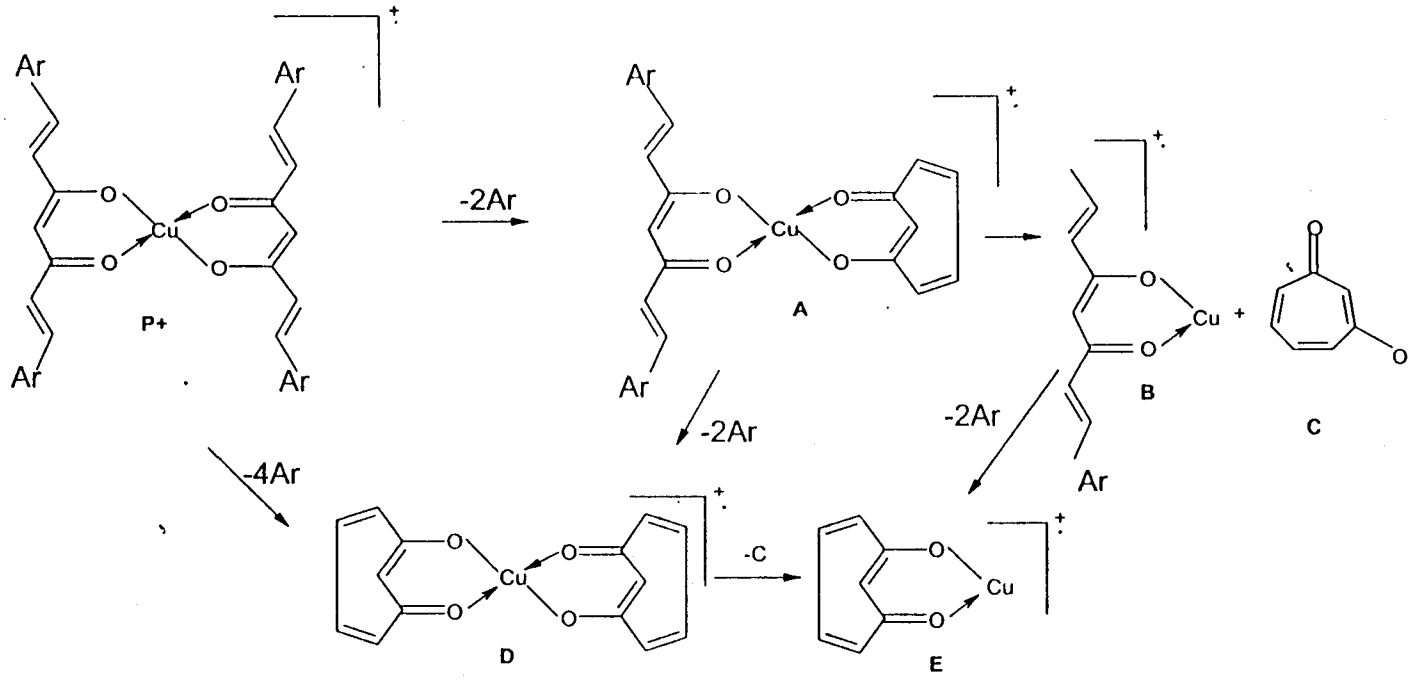


Fig. 2.14 Mass spectrum of copper(II) complex of **2d**



Scheme 2.3

Esr spectral studies of copper(II) complexes

The esr spectral parameters of copper(II) complexes of **2a** and **2d** obtained from DMF solution at 77^0K are given in table 2.10.

Table 2.10

*Esr spectral data of copper(II) complexes of **2a** and **2d***

Copper(II) complex of	$g_{\|}$	$g_{\perp}$	$A_{\|}$	$A_{\perp}$
2a	2.2658	2.07156	31.2	168
2d	2.2794	2.0650	30.6	153

Compared to the esr spectral data of **1b** (chapter I) the obeserved g values of **2d** suggest more covalency for metal-ligand bond.

SECTION 2

ANTITUMOUR ACTIVITY OF *ORTHO*- SUBSTITUTED 1,7-DIARYLHEPTANOIDS AND THEIR COPPER(II) COMPLEXES

Results of the antitumour activity of the *ortho*-substituted 1,7-diarylheptanoids and their copper chelates are discussed below. The experimental details and the methods adopted for various measurements are same as discussed in **Chapter 1, Section 2**.

Short term *in vitro* cytotoxicity

In vitro cytotoxicity studies were carried out using Ehrlich ascites carcinoma cells. Different concentrations of the compounds (1-50 μg/mL) dissolved in minimum quantity of DMSO and diluted with PBS, were incubated at 37^0C together with tumour cells ($1x10^6$cells) suspended in phosphated buffer solution. Cytotoxicity was determined after 3h by the trypan blue exclusion method as described earlier.

Determination of cytotoxicity of compounds towards tissue cultured L929 cells

The effect of compounds on growth inhibition of tissue cultured cells were studied using L929 cells. The cells ($5x10^4$cells/well) were plated in trazon's 96 well flat bottomed titre plates and incubated for 24h at 37^0C in 5% CO_2 atmosphere. After incubation, different concentrations (1-10 μg/mL) of test compounds (**2a-d** and their copper complexes) were added to the wells and incubated for a further period of 48h. The cells were then detached by trypzinisation (0.2%) and stained with crystal violet. The cytotoxicity was calculated by measuring optical density at 570 nm after eluting the dye from the cells.

Determination of the effect of compounds in reducing ascites tumour

Swiss albino mice (6 per group) were injected intraperitonialy with Ehrlich ascites tumour cells ($1x10^6$ cells/animal). Simultaneously the animals were injected (ip) with the test compounds (200 μmoles/Kg body weight) and the injections were continued for 10 days. The death pattern of animals due to tumour burden was noted. The percentage increase in life span were calculated as described in Section 2, Chapter 1.

Determination of the effect of compounds on solid tumour development

Solid tumours were induced in nine groups of swiss albino mice (6 per group) by subcutaneous injection of dalton's lymphoma ascites cells ($1x10^6$ cells/animal) on the right hind limbs. One group was kept as control. Administration of compounds (200 μmol/Kg body wt) by ip were started simultaneously for other groups and continued for 10 days. The diameter of the tumour was measured twice weakly. Tumour volume was calculated as given in Chapter 1 Section 2.

Results and Discussion

Short term in vitro cytotoxicity

The short-term *in vitro* cytotoxicity of the curcuminoid analogues (**2a-d**) and their copper(II) complexes towards Ehrlich ascites cells are given in table 2.11. The results indicate that metal complexation enhances the cytotoxicity considerably. Concentration required to produce 50% cell death in Ehrlich ascites tumour cells were found to be 5.2 μg/mL, 6.5 μg/mL, 10 μg/mL and 17.0 μg/mL respectively for **2d**, **2b**, **2c** and **2a**. The copper chelates produced the same effect at much lower concentrations of 4.0 μg/mL 4.2 μg/mL 4.7 μg/mL and 6.0 μg/mL respectively.

Table 2.11

Short-term in vitro cytotoxicity of compounds towards EAC cells

Compounds	Percentage cell-death at different concentrations				
	1 μg/mL	5 μg/mL	10 μg/mL	25 μg/mL	50 μg/mL
2a (HL^1)	22	35	42	59	85
2b (HL^2)	26	43	64	75	94
2c (HL^3)	21	36	50	62	81
2d (HL^4)	27	48	61	81	98
$[Cu(L^1)_2]$	26	46	61	89	100
$[Cu(L^2)_2]$	32	54	74	100	100
$[Cu(L^3)_2]$	27	51	69	94	100
$[Cu(L^4)_2]$	30	56	78	100	100

Ehrlich ascites carcinoma cells (1 million cells/mL) in presence of various concentrations of compounds (1-50 μg/mL) were incubated for 3h at 37^0C. The short-term cytotoxicity was determined by trypan blue dye exclusion method.

Cytotoxicity towards L929 cultured cells

The results of cytotoxic activity of the compounds in tissue culture are given in figure 2.15 and 2.16 which also indicate that the copper chelates are more cytotoxic than the respective curcuminoid analogues. The percentage cell deaths at 1 μg/mL concentration were 34.2 ± 1.8, 32.9 ± 3.0, 28.2 ± 2.1 and 17.7 ± 3.1 for **2d, 2b, 2c** and **2a** respectively. The respective copper chelates showed 60.5 ± 2.1, 56.4 ± 1.6, 38.9 ± 2.9 and 30.2 ± 2.8 percentage cell death at the same concentration.

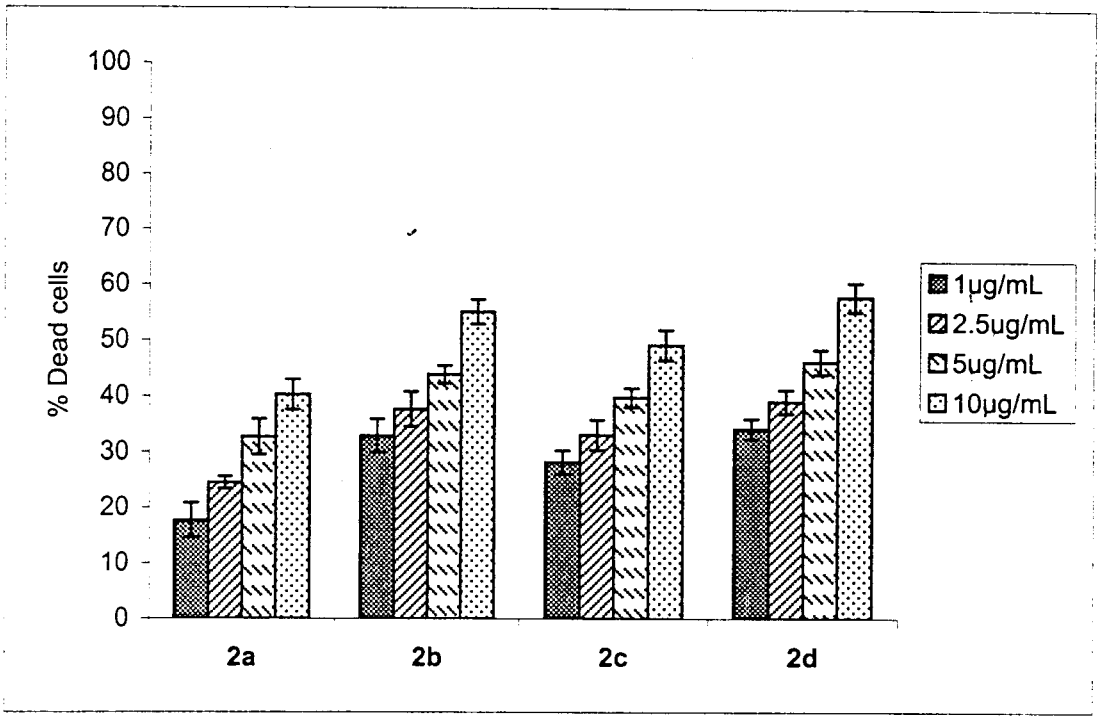


Fig. 2.15 Cytotoxicity (2a-2d) in culture towards L929 cells

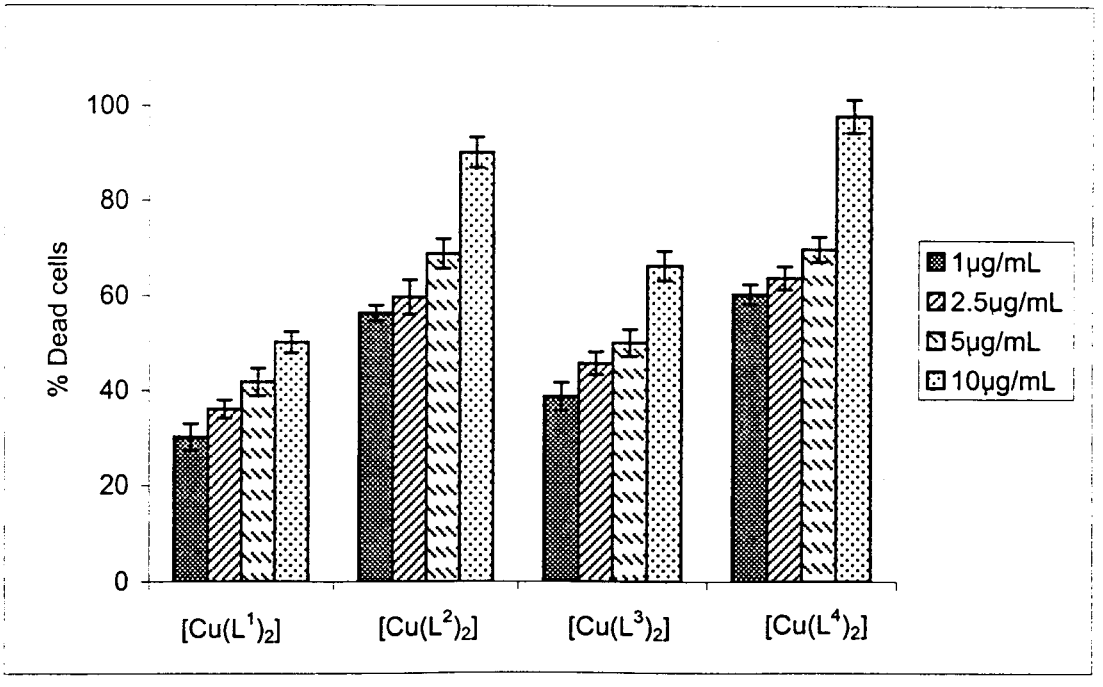


Fig. 2.16 Cytotoxicity of copper complexes (2a-2d) in culture towards L929 cells

Effect of compounds on ascites tumour reduction

All the compounds when administered (ip) could produce a significant increase ($P < 0.001$ from normal) in the life span of mice bearing ascites tumour (Table 2.12). Copper complexation increased considerably the life span of tumour bearing mices. Among the compounds screened for ascites tumour reduction studies, **2a** is having the lowest percentage (52.2) increase in life span. However, the copper complexes of **2d** is with maximum activity which increased the life span of tumour bearing mice by 101.7 %.

Table 2.12

Effect of compounds on ascites tumor reduction

Compound	No. of animals with tumour	No. of days survived	Increase in Life Span (%)
Control	6/6	17.3±1.1	
2a (HL^1)	6/6	26.4±3.6	52.21*
2b (HL^2)	6/6	30.1±2.9	73.98*
2c (HL^3)	6/6	26.8±2.9	54.90*
2d (HL^4)	6/6	30.2±2.9	74.61*
$[Cu(L^1)_2]$	6/6	28.8±3.1	66.50*
$[Cu(L^2)_2]$	6/6	34.7±3.3	100.59*
$[Cu(L^3)_2]$	6/6	30.6±3.1	76.88*
$[Cu(L^4)_2]$	6/6	34.9±2.9	101.69*

* $P < 0.001$

Values are means of ±SD of six determinations. Animals were injected (ip) with Ehrlich ascites tumour cells ($1x10^6$ cells/animal), compounds (200 μmoles/Kg body wt) were injected (ip) for 10 days.

Effect of compounds on solid tumour development

The reduction in solid tumour volume in mices by the intraperitonial administration of compounds are given in figure 2.17 and 2.18. The data show that compared to free curcuminoids their respective copper complexes are found more active in reducing tumour volume. Tumour volumes were 5.04 cm^3, 3.40 cm^3, 3.45 cm^3, 4.01 cm^3 and 4.41 cm^3 on day-31 for control, curcumin analogues **2d, 2b, 2c** and **2a** respectively. The tumour volumes on day 31 for their copper complexes were respectively 1.67 cm^3, 1.76 cm^3, 3.45 cm^3 and 4.01 cm^3, for **2d, 2b, 2c** and **2a**.

The results clearly revealed that **2d** and **2b** with hydroxyl group on the aromatic rings show maximum activity towards *in vitro* and *in vivo* antitumour studies. The copper complexes dramatically enhanced the activity as is evident from the data given. Among the *ortho*-substituted curcuminoids **2d** and its copper chelate are highly active. This can be attributed to the presence of an hydroxyl group at the *ortho* position of the naphthyl rings and increase in unsaturation compared to the phenyl rings of natural curcuminoids. Thus it can be seen that increase in conjugation also contribute to the cytotoxicity of curcuminoids.

Fig. 2.17 Effect of compounds (2a-2d) on solid tumour volume

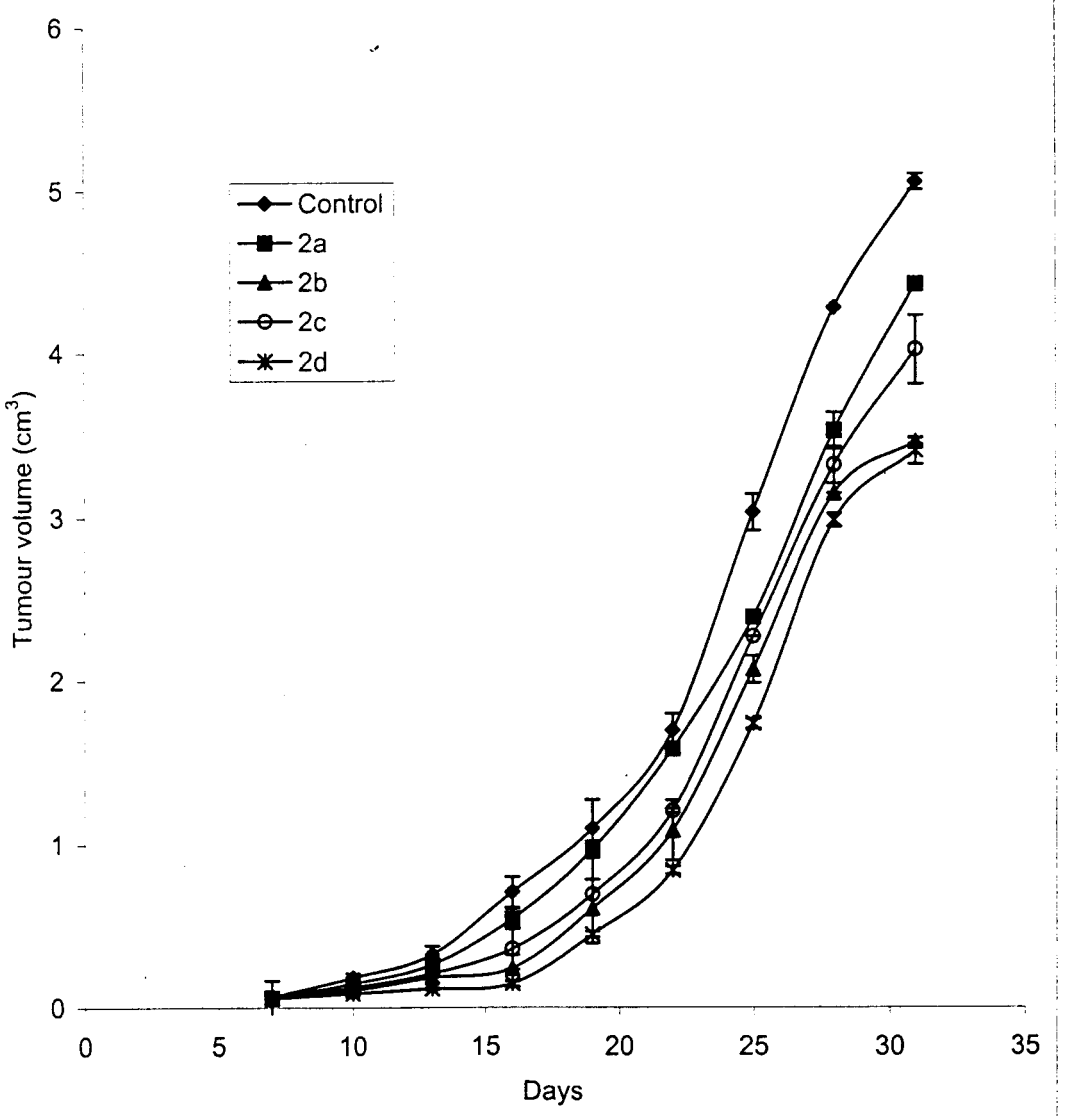
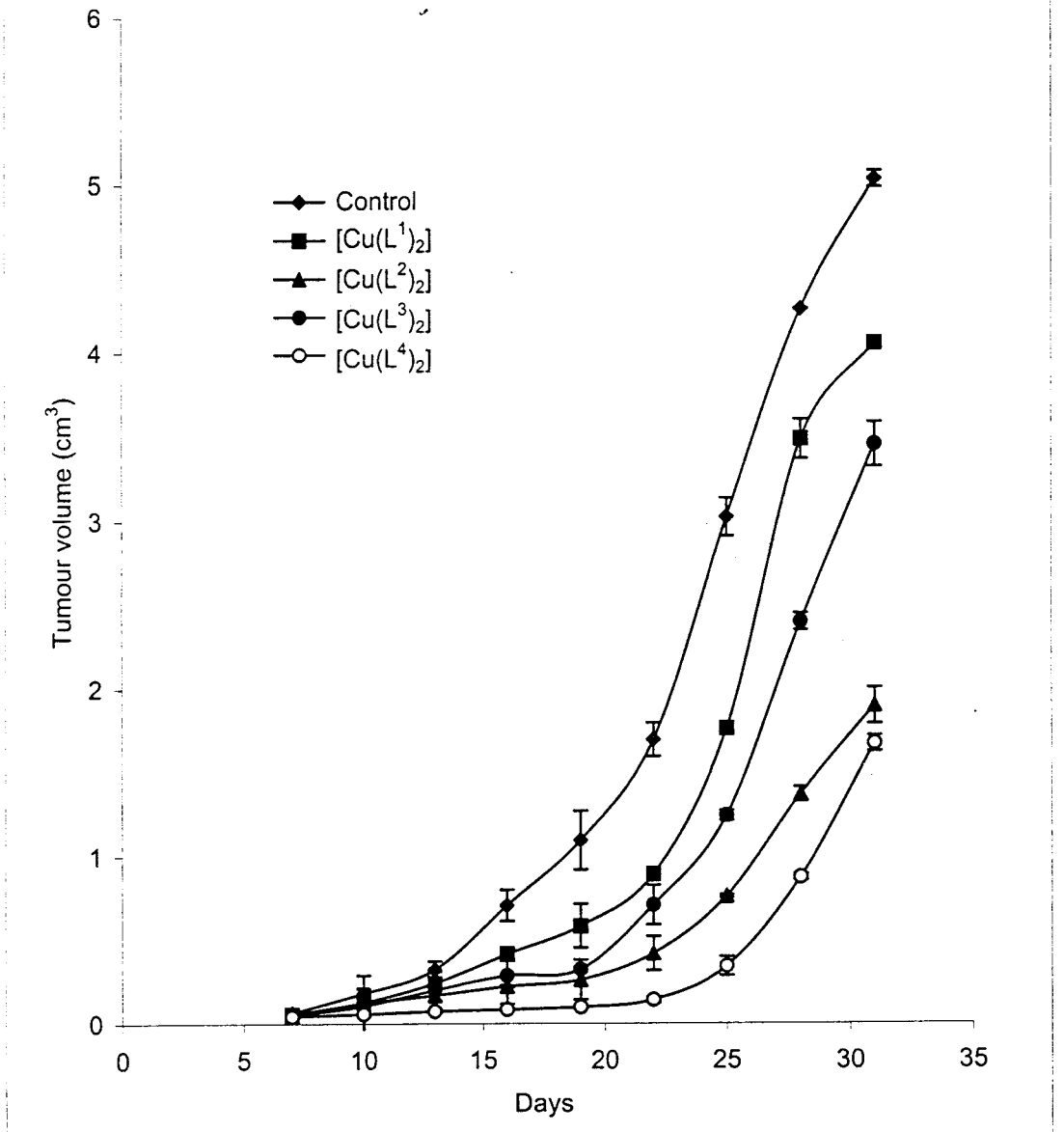


Fig. 2.18 Effect of copper complexes of (2a-2d) on solid tumour volume



CHAPTER III

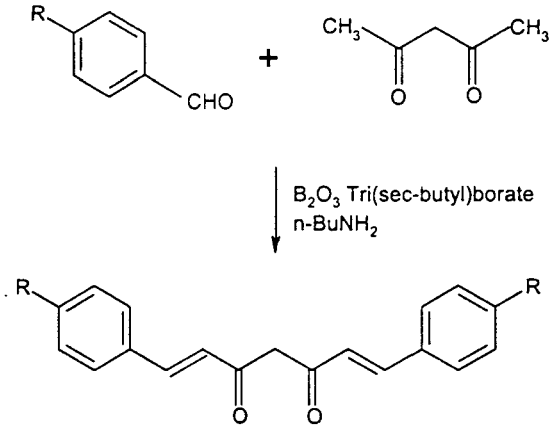
SYNTHESIS, CHARACTERISATION AND ANTITUMOUR ACTIVITY OF 1,7-BIS (*PARA*-SUBSTITUTED PHENYL) HEPTA-1,6-DIENE -3,5-DIONES AND THEIR METAL CHELATES

SECTION 1

SYNTHESIS AND CHARACTERISATION OF *PARA*-SUBSTITUTED 1, 7-DIPHENYLHEPTANOIDS AND THEIR METAL CHELATES

Synthesis of *para*-substituted 1,7-diphenylheptanoids

These diphenylheptanoids were prepared according to the reaction **scheme 3.1**. The synthetic details and purification methods are similar to those given for the 1,7-diarylheptanoids discussed in chapter 1.



Scheme 3.1

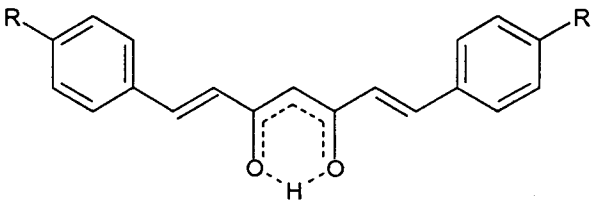
Synthesis of metal chelates

Oxovanadium(IV), iron(III), cobalt(II), nickel(II) and copper(II) complexes of the compounds were prepared by the following general procedure. An ethanolic solution of the metal salt (0.001 mol, 10 mL) was added to a solution of the 1,7-diarylheptanoid (0.002 mol) in 50% ($^{v}/_{v}$) ethanol-methanol mixture with stirring and the mixed solution was

refluxed for ~1h and the volume was reduced to half, cooled to room temperature and the precipitated complex was filtered, washed with ethanol and recrystallised from hot methanol.

Results and Discussion

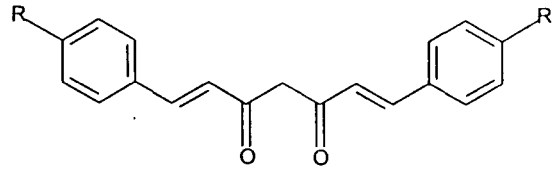
The *para*-substituted benzaldehydes used for the synthesis of the 1,7-diarylheptanoids (**3a-d**) considered in the investigation along with their systematic name, yield, etc, are brought out in table 3.1. Maximum yield was obtained for **3d** where phenyl ring of the aldehyde used contain N,N-dimethylamine, an electron donating substituent. All the compounds (**3a-d**) are crystalline in nature and are freely soluble in common organic solvents. The elemental analytical data and observed molecular weight (Table 3.2) of the compounds fully agree with their formulation as in structure **3.1**.



3.1

Table 3.1

*Synthetic details of the para substituted 1,7-diarylheptanoids (**3a-d**)*



Compound	Aldehyde used for synthesis	R	Systematic name	Trivial name	Colour	Yield %
3a	4-methoxybenzaldehyde	$-OCH_3$	1,7-bis(4-methoxyphenyl) -1,6-heptadiene-3,5-dione	Bis(4-methoxy cinnamoyl) methane	Orange	68
3b	4-ethoxybenzaldehyde	$-OCH_2CH_3$	1,7-bis(4-ethoxyphenyl)-1,6-heptadiene-3,5-dione	Bis(4-ethoxy cinnamoyl) methane	Yellow	70
3c	4-hydroxybenzaldehyde	-OH	1,7-bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione	Bis(4-hydroxy cinnamoyl)methane	Red	70
3d	4-dimethylamino benzaldehyde	$-N(CH_3)_2$	1,7-bis[4-N,N-dimethyl aminophenyl]-1,6-heptadiene -3,5-dione	Bis(4-dimethyl amino cinnamoyl)-methane	Red	75

Table 3.2

*Physical, analytical and uv spectral data of the para-substituted 1,7-diarylheptanoids, (**3a-d**)*

Compounds	MP (C^0)	Elemental analysis (%) Found/(Calcd)			Mol. Weight	λ_{max} (nm)	logϵ
		C	H	N			
3a	108	74.98 (75.10)	5.83 (5.95)	-	330 (336)	261 398	4.09 4.59
3b	138	76.21 (75.64)	7.10 (6.59)	-	367 (364)	265 402	4.08 4.70
3c	132	73.86 (74.03)	5.10 (5.19)	-	305 (308)	263 408	4.08 4.67
3d	142	78.10 (76.24)	7.91 (7.18)	7.60 (7.73)	364 (362)	256 420	4.12 4.71

Characterisation of the para-substituted 1,7-diphenylheptanoids

Uv spectra

The uv spectra of the compounds in methanol(10^{-3}M) show two bands with λ_{max} at ~380 nm and ~256 nm (Table 3.2). The λ_{max} of low energy n→π* transitions show a bathochromic shift when the aryl substituents exert an electron releasing effect.

Infrared spectra

The ir spectra of all the compounds show a strong band in the region 1600-1635 cm^{-1} and a broad band in the range 3000–3500 cm^{-1} for chelated carbonyl group as expected for structure **3.1**. The observed position and intensity of these bands indicate that the compounds exist almost completely in the enol form. A medium intensity band observed at ~975 cm^{-1} is possibly arising from the trans –CH=CH- double bond absorption. The important ir absorption bands and their probable assignments are given in table 3.3.

Table 3.3

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

Compounds				
3a	**3b**	**3c**	**3d**	Probable assignments
1618	1632	1616	1631	ν(C=O) chelated
1587	1597	1576	1599	ν(C-C) phenyl
1561	1570	1563	1560	
1548	1550	1541	1545	ν(C=C) alkenyl
1522	1518	1528	1529	ν_{as}(C-C-C) chelate ring
1458	1479	1450	1458	ν_{s}(C-C-C) chelate ring
1096	1088	1101	1101	β(C-H) chelate ring
1063	1043	1068	1065	
966	970	981	980	ν(CH=CH) (trans)

^{1}H nmr spectra

The ^{1}H nmr spectra of the compounds **3b** and **3a** are reproduced in figures 3.1 and 3.2. The positions of the enolic proton signal at ~16 ppm (table 3.4) suggest the strong intramolecular hydrogen bonding of the enolic proton. The phenolic proton signal of **3c** is at 10.043 ppm. The chemical shift values and integrated intensities of the methine, alkenyl, and aryl substituents all agree well with their formulation as in **3.1**

Table 3.4

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

Compound	Chemical shift (δppm)				
	enolic	Methine	Alkenyl	Aryl	Phenyl substituent
3a	16.43	6.83	7.98-7.90	6.833-7.09	3.822 (OCH_3)
3b	16.08	5.78	6.49-7.61	6.88-7.511	1.4319 4.068 (OC_2H_5)
3c	17.21	6.84	8.18-8.20	6.82-7.36	10.043 (OH)
3d	15.68	5.59	6.27-7.55	6.65-7.75	3.089 [$N(CH_3)_2$]

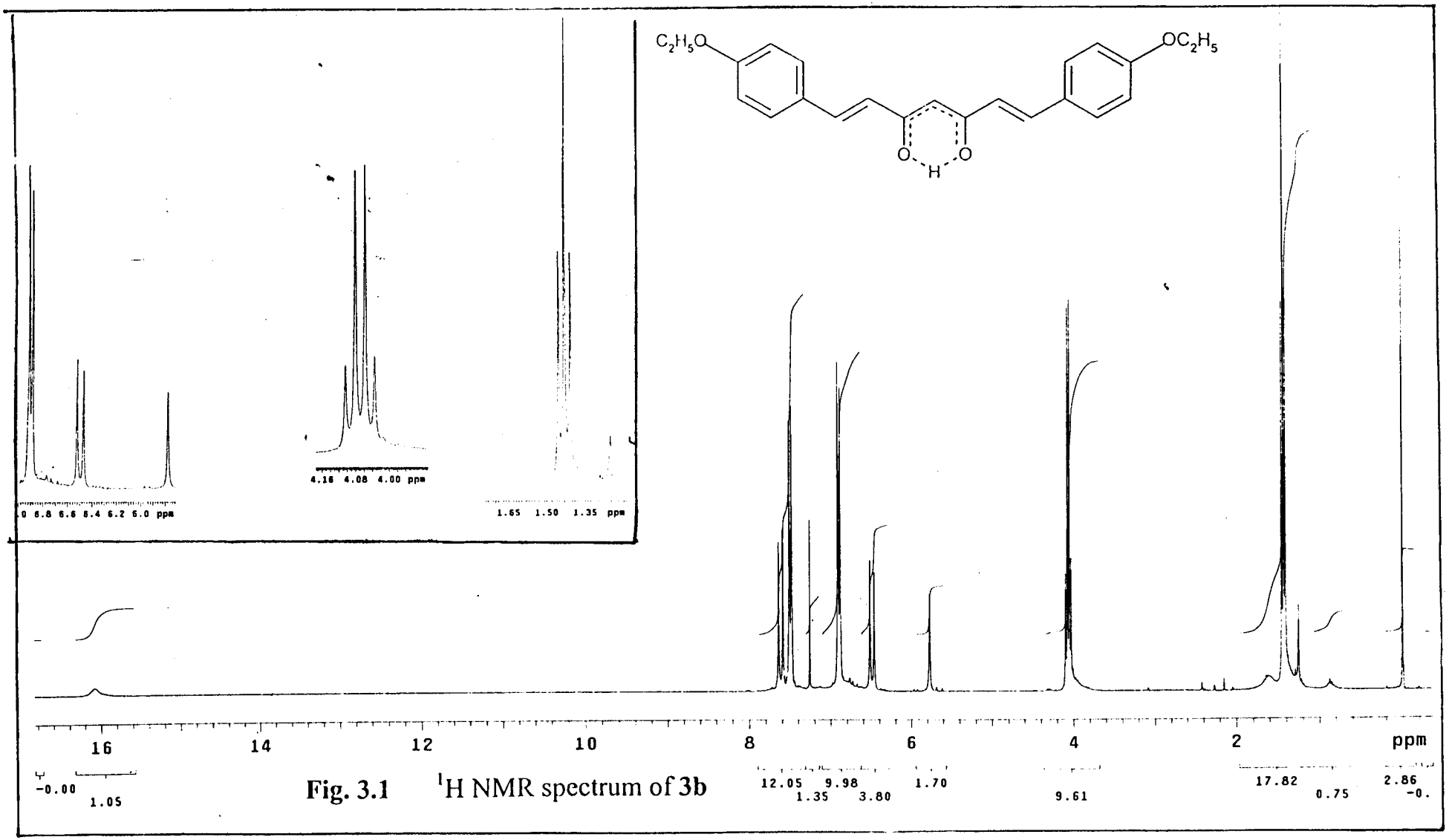


Fig. 3.1 ^{1}H NMR spectrum of **3b**

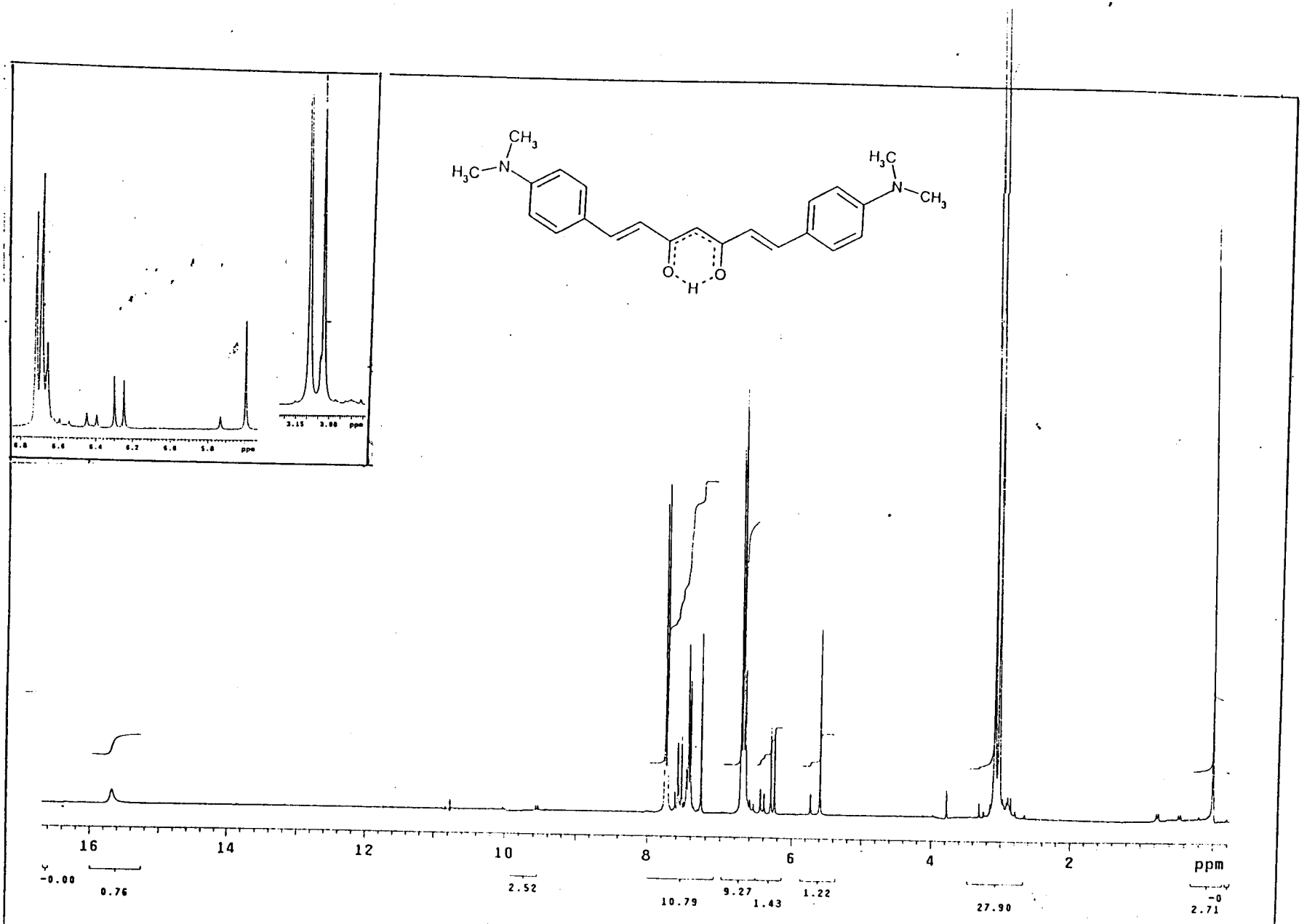


Fig. 3.2 ^{1}H NMR spectrum of **3d**

Mass Spectra

In addition to intense molecular ion peak (P^+), peaks due to the elimination of O, OH and $C_3HO_2^+$ species from the molecular ion is the most common feature of the mass spectra of all the compounds. Mass spectra of compounds **3b** and **3d** are reproduced in figures 3.3 and 3.4. Important peaks appeared in the spectra of the compounds can be conveniently accounted by the fragmentation pattern given in **scheme 3.2** and fragments in table 3.5.

Table 3.5

*Important mass spectral fragments of (**3b** and **3d**)*

Fragments*	**3b**	**3d**
P^+	364	362
A	189	188
B	175	174
C	134	133
D	147	146
E	121	120
F	146	147
G	243	242
H	217	216

*The alphabets corresponds to the fragments given in **scheme 3.2**

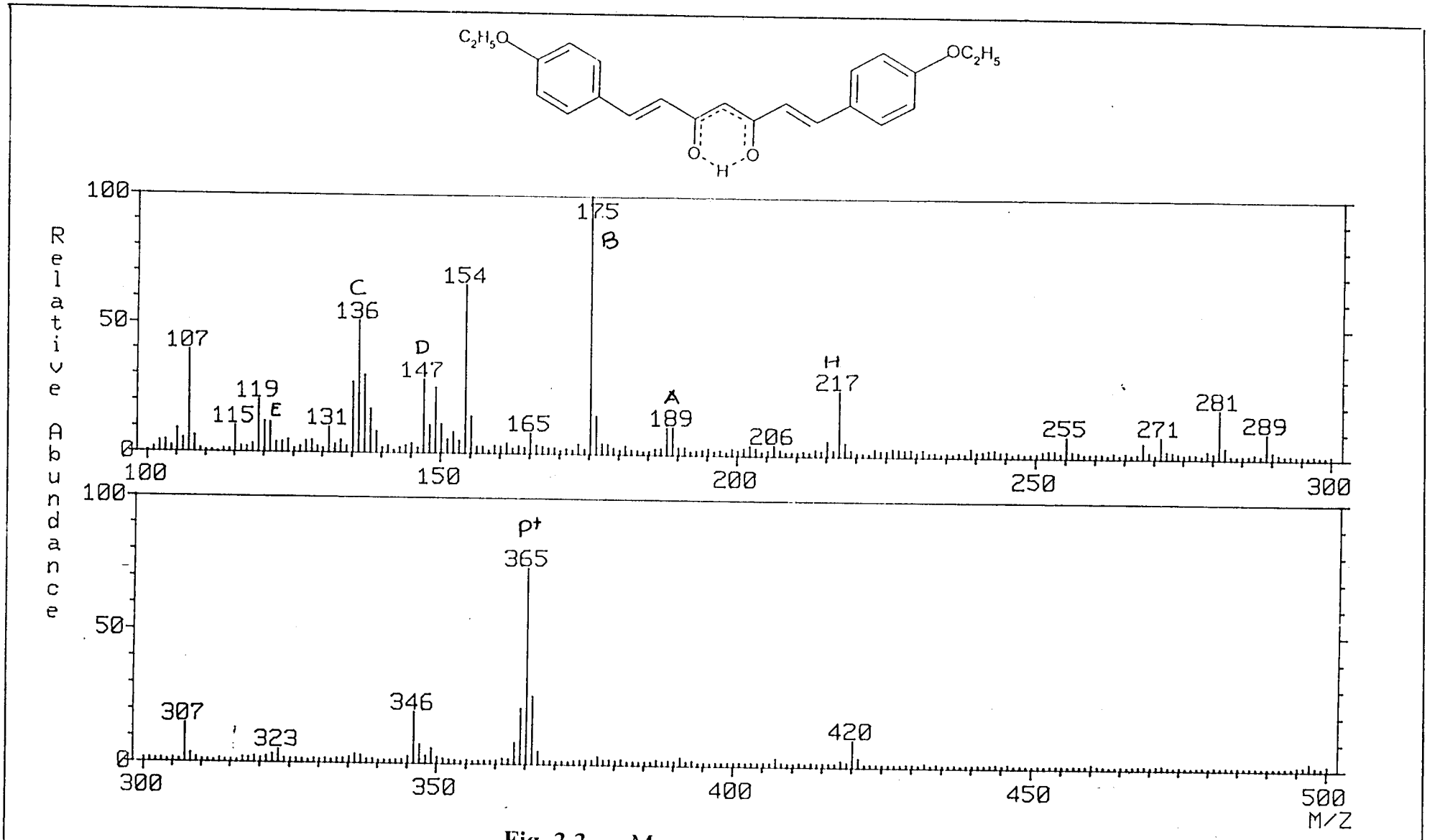


Fig. 3.3 Mass spectrum of **3b**

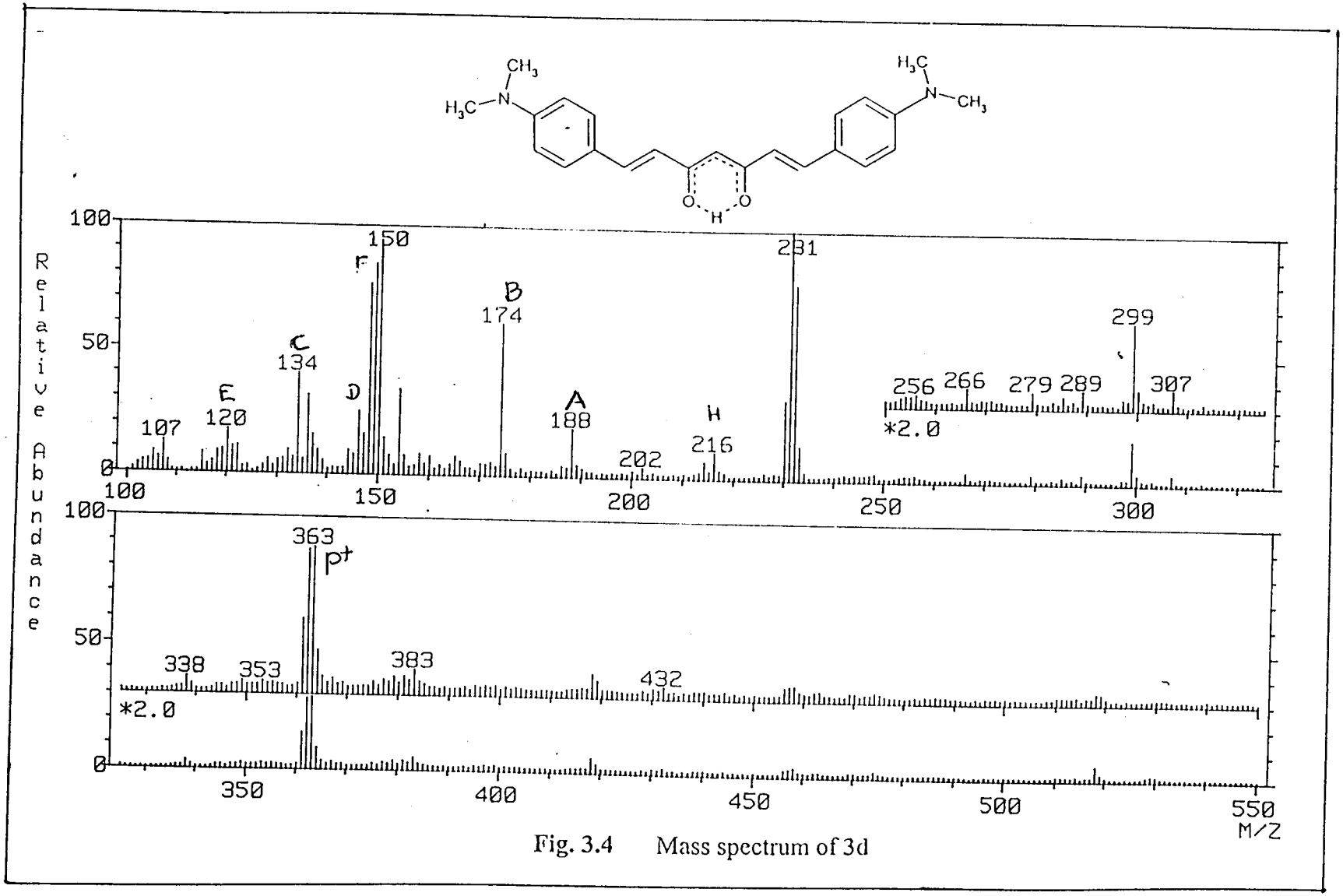
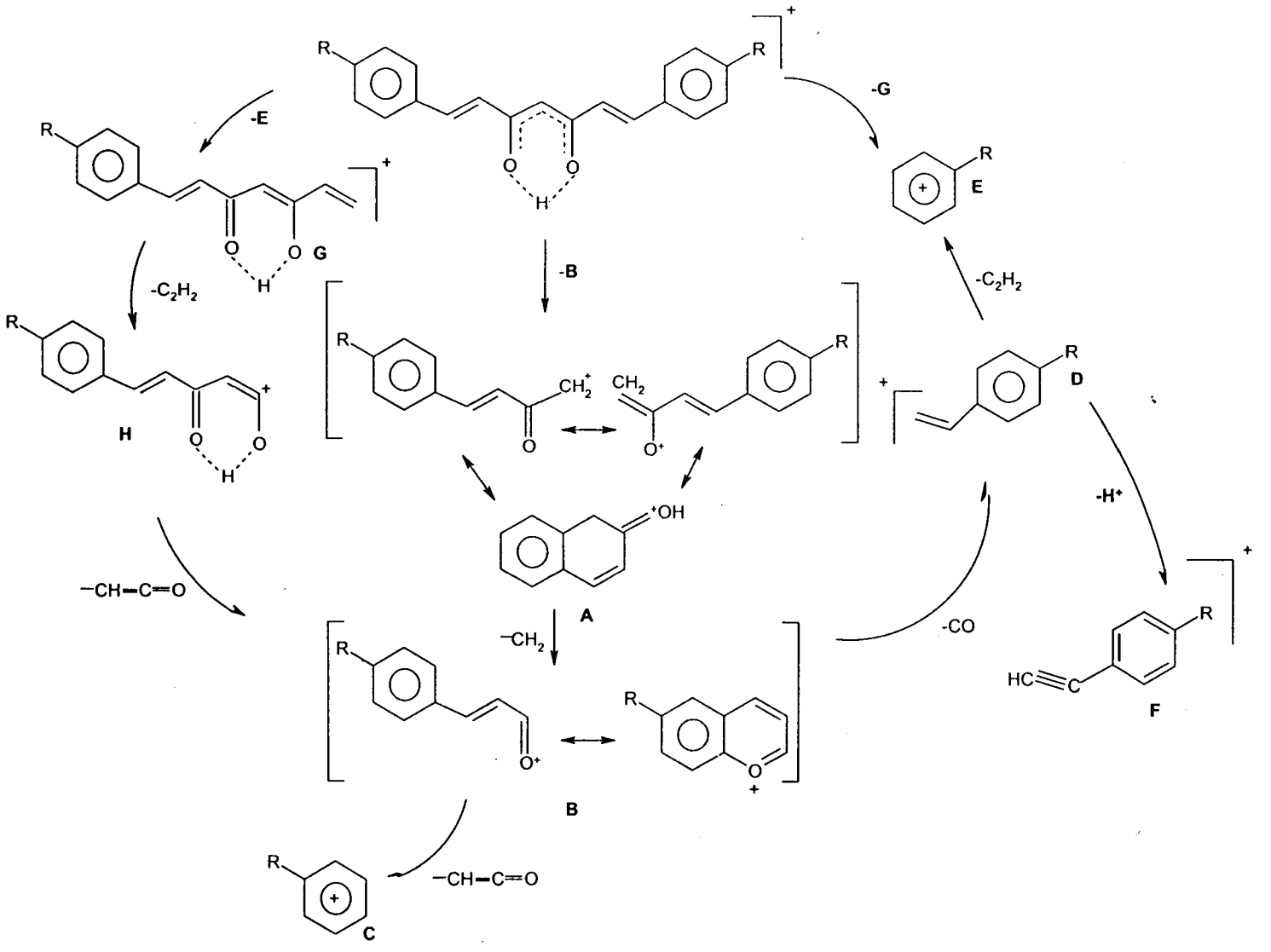


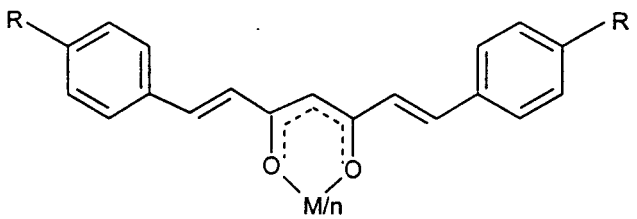
Fig. 3.4 Mass spectrum of 3d



Scheme 3.2

Characterisation of metal chelates

Analytical and physical data of the metal complexes given in table 3.6-3.10 suggest a 1:2 metal-ligand stoichiometry except for iron(III) complexes which are of $[FeL_3]$ nature. All the metal complexes are non-hygroscopic and stable in air. The conductivity measurements from 10^{-3}M solution in DMSO indicated their nonionic nature (the measured specific conductance <10 $\Omega^{-1}cm^{-1}$). Nickel(II) complexes are diamagnetic and all others are paramagnetic. The electronic, ir, ^{1}H nmr, esr and mass spectral data of the complexes are in agreement with the structure **3.3**.



3.3

n = 2 for VO(IV), Co(II), Ni(II) and Cu(II)
n = 3 for Fe(III)

Table 3.6

*Physical and analytical data of the oxovanadium(IV) chelates of the para-substituted 1,7-diarylheptanoids, **(3a-d)***

Vanadyl(IV) Chelate of	MP °C	μ_{eff} BM	Elemental analysis (%) Found/(Calcd)*					
			C		H		V	
3a	169	1.72	67.64	(68.39)	5.01	(5.42)	7.23	(6.92)
3b	173	1.74	80.12	(79.65)	7.17	(6.93)	7.85	(7.36)
3c	166	1.73	67.19	(66.96)	4.96	(4.70)	7.66	(7.49)
3d	180	1.72	70.22	(69.96)	6.83	(6.59)	6.87	(6.46)

* The calculated value correspond to the $[VOL_2]$ composition where L stands for the deprotonated ligands

Table 3.7

*Physical and analytical data of iron(III) chelates of para-substituted 1,7-diarylheptanoids **(3a-d)***

Iron (III) Chelates of	μ_{eff} BM	MP (^{0}C)	Elemental analysis (%) (Found/calcd)*		
			C	H	Fe
3a	5.78	189	69.87 (71.25)	5.90 (5.65)	5.01 (5.27)
3b	5.82	187	70.73 (72.31)	6.90 (6.29)	4.31 (4.89)
3c	5.86	179	71.87 (70.01)	5.02 (4.91)	5.33 (5.73)
3d	5.85	201	73.01 (72.69)	7.08 (6.85)	5.11 (4.92)

* The calculated value correspond to the $[FeL_3]$ composition where L stands for the deprotonated ligands

Table 3.8

*Physical and analytical data of cobalt(II) chelates of para-substituted 1,7-diarylheptanoids (**3a-3d**)*

Cobalt (II) Chelates of	MP (^{0}C)	μ_{eff} BM	Elemental analysis (%) (Found/calcd)*			
			C		H	Co
3a	170	4.88	72.49	(71.38)	6.21 (5.94)	8.73 (8.35)
3b	172	4.91	73.44	(72.25)	6.95 (6.80)	8.01 (7.72)
3c	169	4.93	70.4	(69.9)	5.84 (5.52)	9.71 (9.05)
3d	183	4.86	76.29	(75.61)	8.01 (7.39)	8.41 (8.08)

* The calculated value correspond to the $[CoL_2(H_2O)_2]$ composition where L stands for the deprotonated ligands

Table 3.9

*Physical and analytical data of nickel(II) chelates of para-substituted 1,7-diarylheptanoids (**3a-3d**)*

Nickel (II) Chelates of	MP (^{0}C)	Elemental analysis (%) Found/(Calcd)*					
		C		H		Ni	
3a	161	68.32	(69.09)	5.09	(5.41)	8.51	(8.01)
3b	166	71.34	(70.25)	5.39	(6.02)	7.77	(7.44)
3c	164	67.03	(67.67)	5.02	(4.67)	9.31	(8.70)
3d	173	71.04	(70.60)	6.96	(6.53)	6.95	(7.47)

* The calculated value correspond to the $[NiL_2]$ composition where L stands for the deprotonated ligands

Table 3.10

*Physical and analytical data of copper(II) chelates of para-substituted 1,7-diarylheptanoids (**3a-3d**)*

Copper (II) Chelates of	MP (^{0}C)	μ_{eff} BM	Elemental analysis (%) Found/(Calcd)*		
			C	H	Cu
3a	191	1.74	68.13 (68.71)	5.08 (5.40)	8.52 (8.66)
3b	193	1.77	70.69 (69.92)	6.12 (6.02)	7.93 (8.04)
3c	187	1.76	67.08 (67.31)	4.25 (4.72)	9.18 (9.37)
3d	201	1.75	69.88 (70.27)	6.94 (6.62)	7.76 (7.51)

* The calculated value correspond to the [CuL_2] composition where L stands for the deprotonated ligands

Uv Spectra

The uv absorption bands of the ligands remain almost unaltered in the spectra of the metal complexes indicating that no structural alteration has occurred during complexation. The n→π* transition of the carbonyl chromophore of the free ligands showed a slight bathochromic shift (Table 3.11-3.15) indicating the involvement of the dicarbonyl moiety in the chelate formation.

Infrared spectra

In the ir spectra of all the complexes, the hydrogen bonded carbonyl band at ~1620 cm^{-1} disappeared and instead a new band appeared at ~1580 cm^{-1}. The broad free ligand band in the region 3000-3500cm^{-1} also cleared up in the spectra of complexes. This indicate replacement of the chelated proton by the metal ion during complexation. In the case of the metal complexes of **3c** the presence of a broad medium intensity band at ~3300 cm^{-1} suggest that the phenolic OH group remains free and not involved in bonding with the metal ion. That the carbonyl groups are involved in metal chelate formation is further evident from the appearance of two medium intensity bands in the region 400-470 cm^{1} due to νM-O vibrations. In cobalt(II) complexes, additional bands due to coordinated water molecules observed at ~3500 cm^{-1} and ~910 cm^{-1}. Characteristic ir absorption bands of complexes are brought out in tables 3.11-3.15.

Table 3.11

*Characteristic ir and uv spectral data of oxovanadium(IV) complexes of **3a- 3d***

Vanadyl (IV) chelates of	Characteristic ir stretching bands (cm^{-1})			λ_{max}
	νC=O	ν_{as} C-C-C	νFe-O	(nm)
3a	1585	1515	471 423	262 403
3b	1588	1518	479 426	265 407
3c	1590	1516	480 419	263 414
3d	1580	1518	469 418	260 428

Table 3.12

*Characteristic ir and uv spectral data of Fe(III) complexes of **(3a-3d)***

Iron(III) chelates of	Characteristic ir absorption bands (cm^{-1})			λ_{max}
	νC-O	ν_{as}C-C-C	νFe-O	(nm)
3a	1588	1510	465 419	262 403
3b	1582	1512	459 419	265 407
3c	1596	1512	465 419	263 414
3d	1591	1514	473 421	260 428

Table 3.13

*Characteristic ir and uv spectral data of Co(II) complexes of (**3a-3d**)*

Cobalt(II) chelates of	Characteristic ir absorption bands (cm^{-1})			λ_{max} (nm)
	νC-O	ν_{as} C-C-C	νCo-O	
3a	1598	1514	483 419	264 403
3b	1592	1514	469 419	262 406
3c	1591	1516	474 423	263 412
3d	1600	1518	484 427	261 427

Table 3.14

*Characteristic ir and uv spectral data of Ni(II) complexes of (**3a-3d**)*

Nickel (II) chelates of	Characteristic ir absorption bands (cm^{-1})			λ_{max} (nm)
	νC-O	ν_{as} C-C-C	νNi-O	
3a	1586	1516	460 425	264 404
3b	1585	1514	469 421	263 406
3c	1585	1520	464 428	265 411
3d	1600	1522	473 419	263 426

Table 3.15

*Characteristic ir and uv spectral data of Cu(II) complexes of (**3a-3d**)*

Copper(II) chelates of	Characteristic ir absorption bands (cm^{-1})			λ_{max} (nm)
	νC-O	ν_{as} C-C-C	νCu-O	
3a	1588	1517	465 424	265 406
3b	1588	1516	465 419	264 407
3c	1582	1521	460 428	265 413
3d	1600	1522	473 424	264 428

^{1}H nmr spectra

The replacement of enolic proton of the ligands by metal ion is further confirmed by the disappearence of the signal at δ~16 ppm in the ^{1}H nmr spectra of the nickel(II) complexes. The methine signals shifted towards the downfield of the spectra indicating the decreased electron density around the central carbon atom of the pseudo aromatic metal chelate ring system. The presence of trans alkenyl proton in complexes is also confirmed from their J values (~16Hz). The ^{1}H nmr spectra of nickel complexes of **3b** and **3d** are reproduced in figures 3.5 and 3.6. The integrated intensities of all protons matches the suggested structure **3.3** of the compounds.

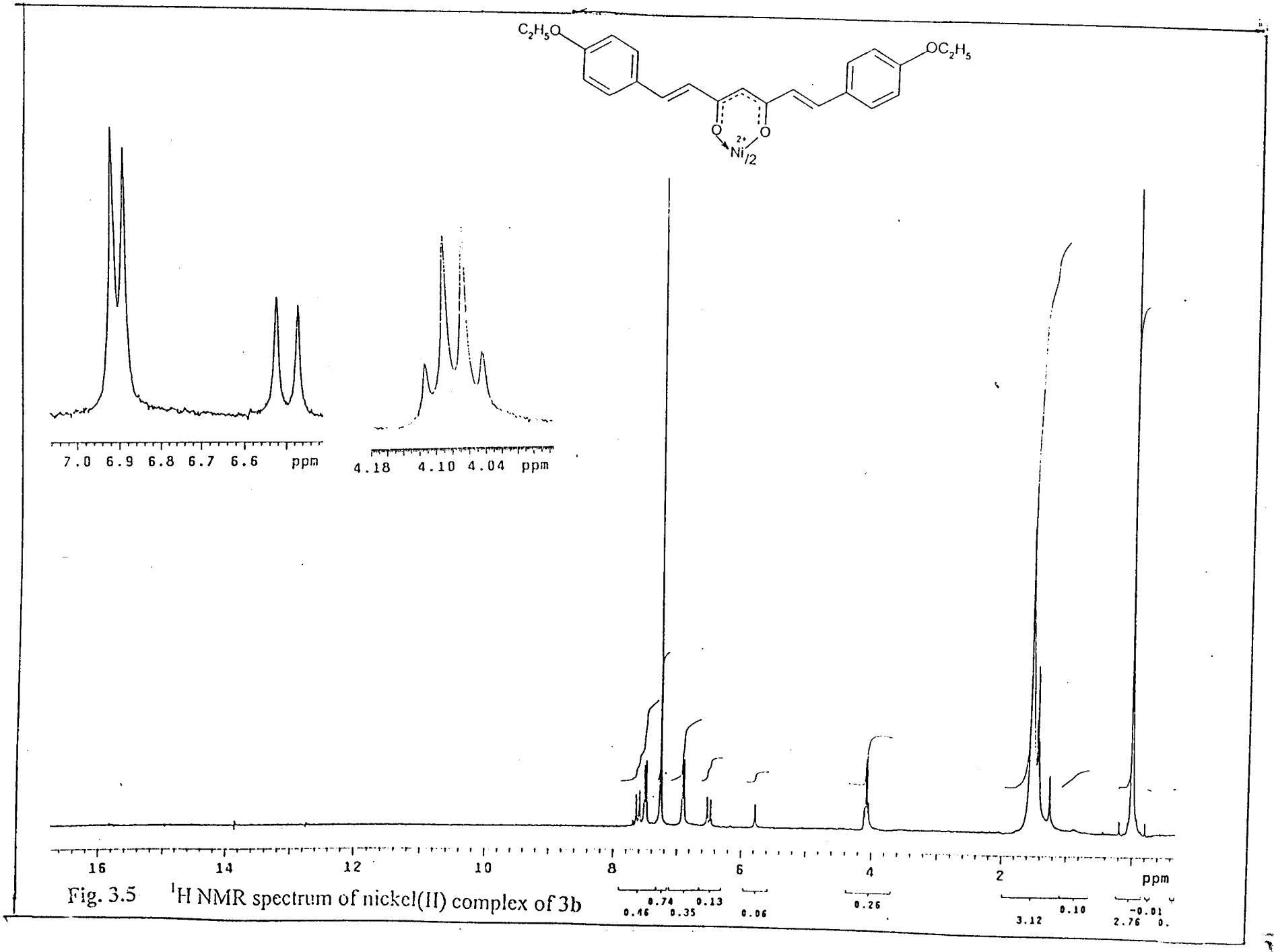


Fig. 3.5 ^{1}H NMR spectrum of nickel(II) complex of 3b

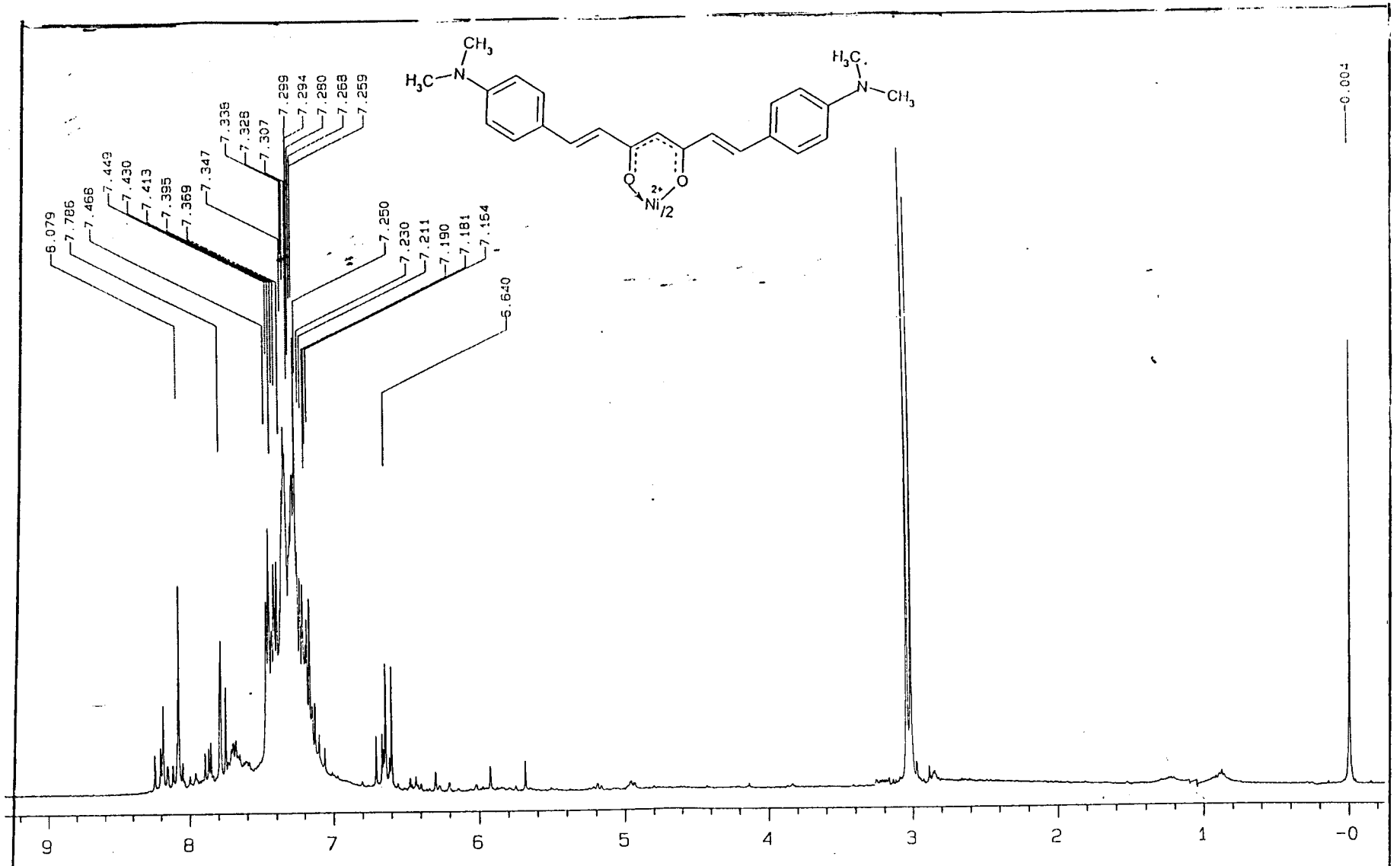


Fig. 3.6 ^{1}H NMR spectrum of nickel(II) complex of **3d**

Mass spectra

Irrespective of the nature of aryl substituents, all the copper(II) chelates of para-substituted 1,7-diarylheptanoids show relatively intense peaks at m/z corresponding to $[CuL_2]$ in agreement with their formulation. However peaks due to $[CuL]^+$, L^+ and fragments of L^+ are sometimes more intense. Peaks corresponding to stepwise elimination of aryl groups are also present in the mass spectra of metal chelates. Copper containing peaks can be easily identified by the natural abundance of ^{63}Cu and ^{65}Cu in the ratio 2:1. Mass spectra of compounds **3a, 3b** and **3d** are reproduced in figures 3.7-3.9. The possible fragmentation pattern of the complexes are given in **scheme 3.3**. The mass spectrum of the vanadyl complex of **3a** (Fig. 3.7) shows an intense molecular ion peak corresponding to the stoichiometry $[VO(L)_2]$.

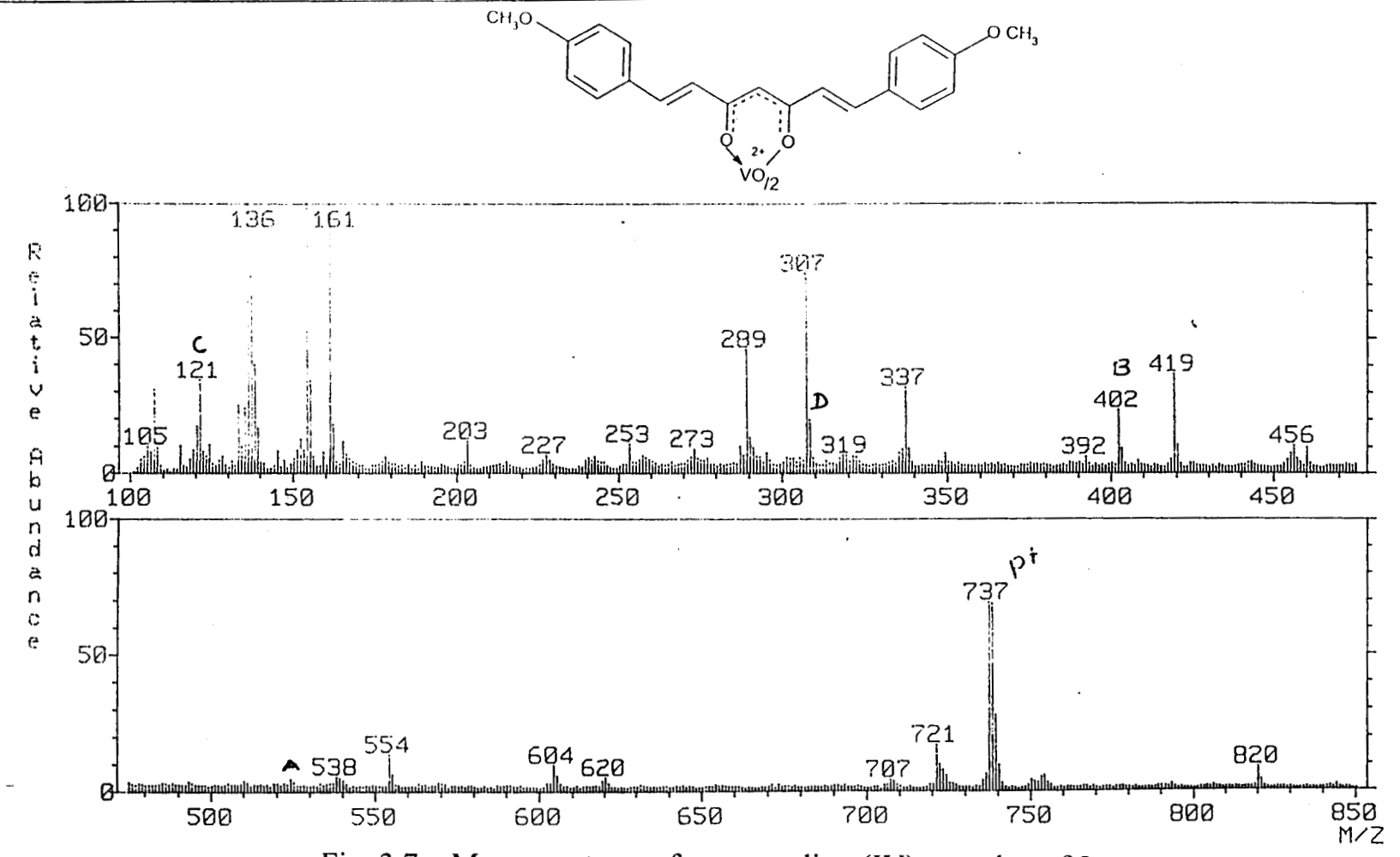


Fig. 3.7 Mass spectrum of oxovanadium(IV) complex of **3a**

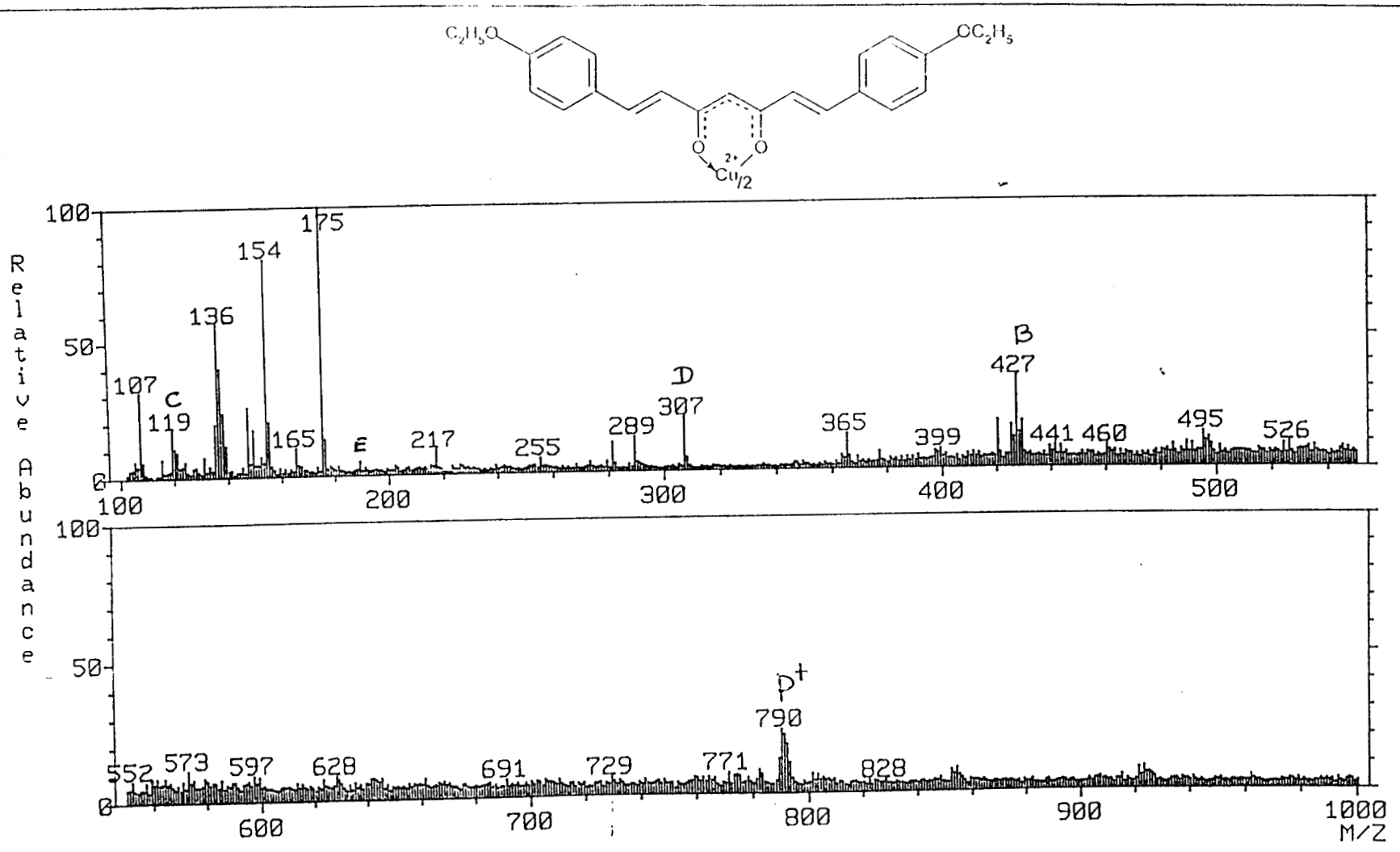


Fig. 3.8 Mass spectrum of copper(II) complex of **3b**

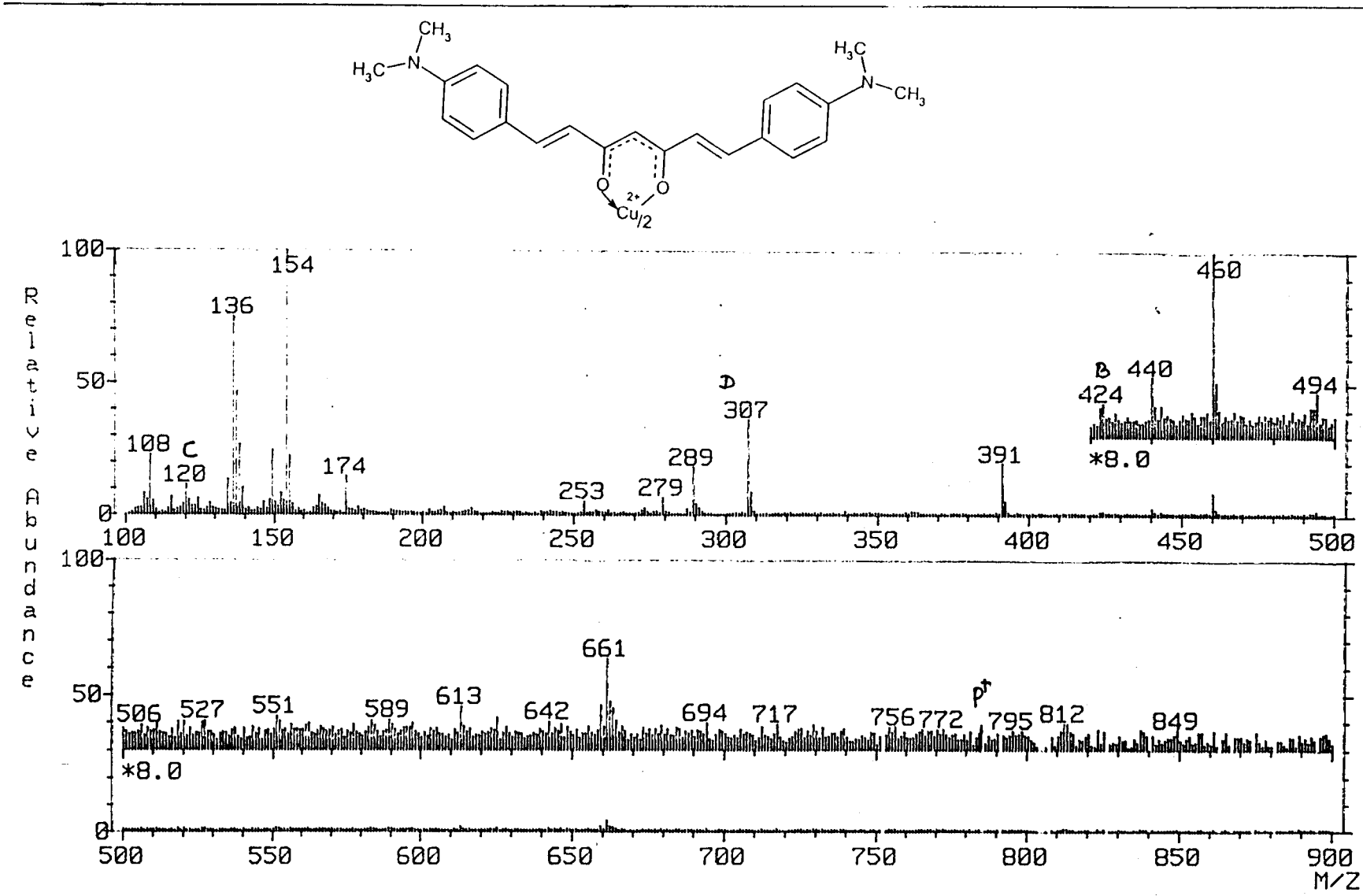
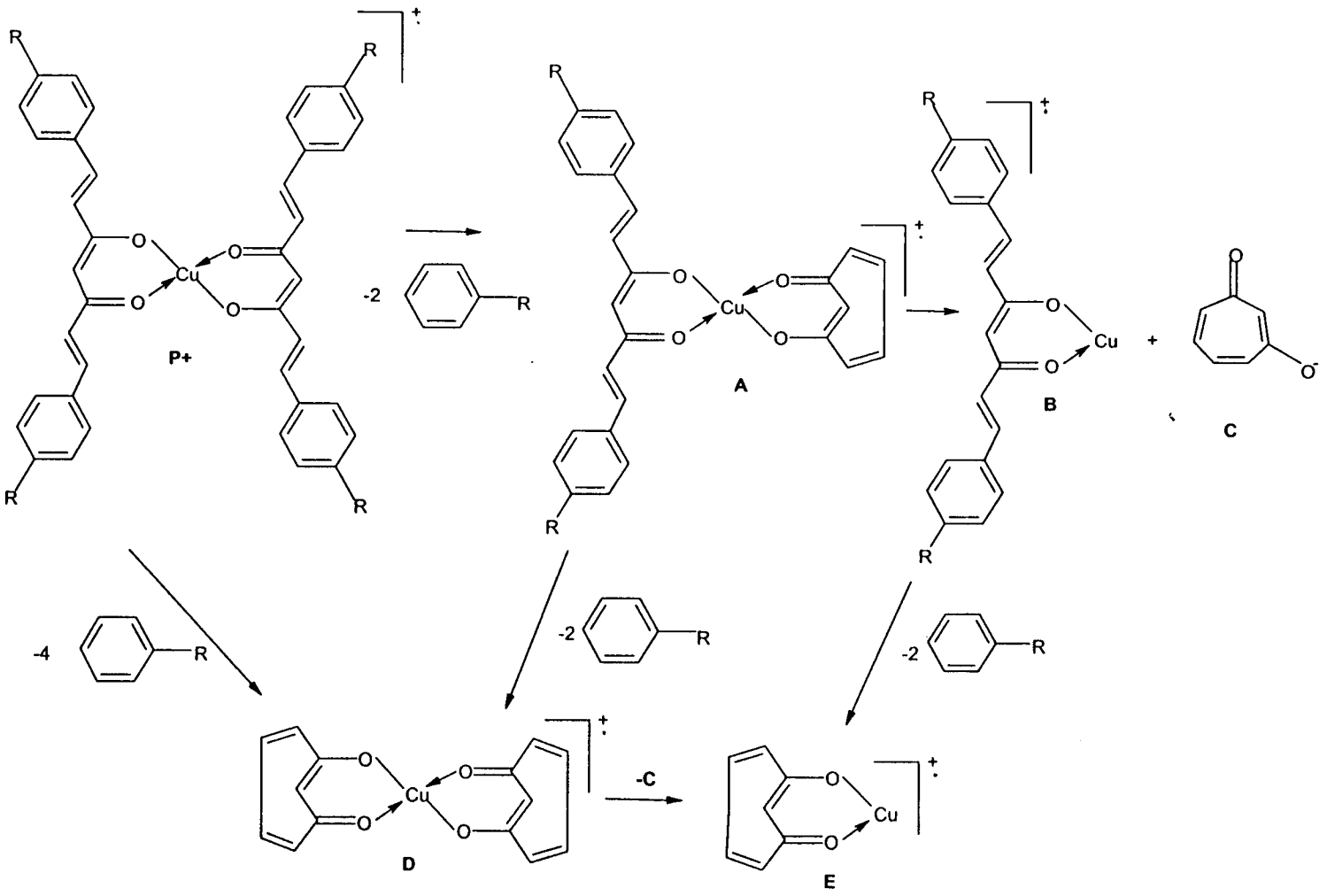
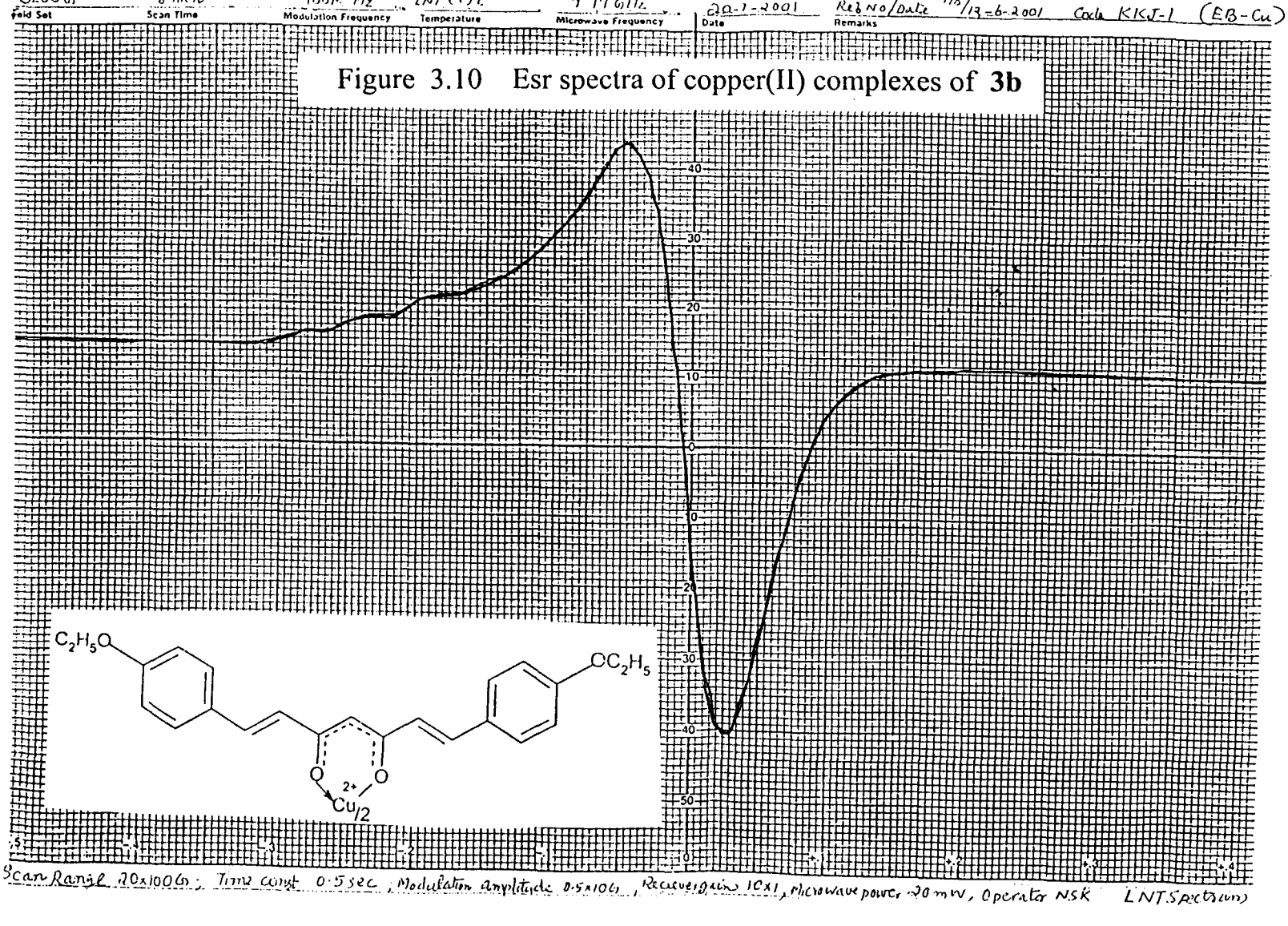


Fig. 3.9 Mass spectrum of copper(II) complex of **3d**



Scheme 3.3


3200 G
Field Set
8 min
Scan Time
100k Hz
Modulation Frequency
LNT (R)°c
Temperature
9.17 GHz
Microwave Frequency
20-1-2001
Date
Ref No/Date 178/13-6-2001
Remarks
Code KKJ-1 (EB-Cu)
Figure 3.10 Esr spectra of copper(II) complexes of 3b
40
30
20
10
0
10
20
30
40
50
C_2H_5O
OC_2H_5
O
O
2+
Cu/2
Scan Range 20x100G; Time const 0.5sec, Modulation amplitude 0.5x10G, Receiver gain 10x1, Microwave power 20 mW, Operator NSK LNT Spectrum

Esr spectral studies of copper(II) complexes

The esr spectrum of copper(II) complex of **3b** was measured at 77^0 K in DMF solution. The observed $g_{||}$, $g_{\perp}$ $A_{||}$ and $A_{\perp}$ values are given in table 3.16. The g values are comparable to that reported for copper acetylacetonates[260] for which $g_{||}$ = 2.264 and $g_{\perp}$ = 2.036. This suggests extensive delocalisation in the chelate ring and significant covalent character for the metal ligand bonds. The esr spectra of **3b** is reproduced in figure 3.10

Table 3.16

*Esr parameters of copper(II) complex of **3b** in DMF at 77^0K*

Cu(II) complex of	$g_{||}$	$g_{\perp}$	$A_{||}x10^{-4}cm^{-1}$	$A_{\perp}$ $x10^{-4}cm^{-1}$
3b	2.2791	2.0715	166.9	46.3

SECTION 2

ANTITUMOUR ACTIVITY OF *PARA*-SUBSTITUTED 1,7-DIPHENYLHEPTANOIDS AND THEIR COPPER(II) COMPLEXES

The four *para* substituted curcuminoid analogues (**3a-d**) and their copper(II) complexes included in this chapter were examined for their cytotoxic and antitumour properties. The experimental procedures for the *in vitro* and *in vivo* studies are similar to that in Section 2, Chapter1. The results are discussed below.

Results and Discussion

Short term in vitro cytotoxic activity

All compounds showed *in vitro* cytotoxicity in the short term assay (Table 3.17). Compounds **3a, 3b, 3c** and **3d** showed 50% inhibition respectively at 25 μg/mL, 11 μg/mL, 7.5 μg/mL and 23 μg/mL concentration. Copper complexes of these compounds are found to be more active. Concentration needed for 50% cell death were ~5 μg/mL for copper complexes of **3c** and **3b** however it is ~7 μg/mL for copper complexes of **3a** and **3d**.

Table 3.17

Short-term in vitro cytotoxicity of compounds towards EAC cells

Compounds	Percentage cell-death at different concentrations				
	1μg/mL	5 μg/mL	10 μg/mL	25 μg/mL	50 μg/mL
3a (HL^1)	16	25	35	50	69
3b (HL^2)	23	40	46	63	89
3c (HL^3)	25	42	60	75	90
3d (HL^4)	17	29	38	52	72
$[Cu(L^1)_2]$	20	40	65	82	92
$[Cu(L^2)_2]$	27	49	70	100	100
$[Cu(L^3)_2]$	29	52	71	100	100
$[Cu(L^4)_2]$	21	39	67	86	97

Ehrlich ascites carcinoma cells (1 million cells/mL) in presence of various concentrations of compounds (1-50 μg/mL) were incubated for 3h at 37^0C. The short term cytotoxicity was determined by trypan blue dye exclusion method.

Cytotoxicity of compound in tissue culture experiments

The cytotoxicity of the compounds (**3a-d**) and their copper complexes towards L929 cells are shown in figures 3.11 and 3.12.

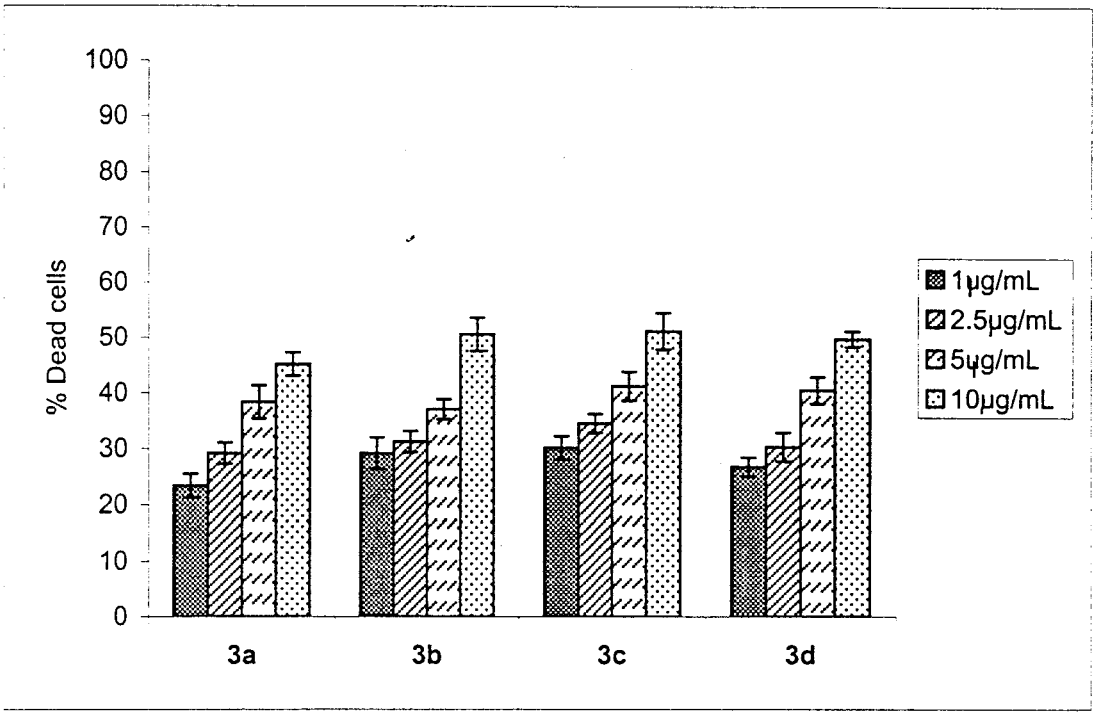


Fig. 3.11 Cytotoxicity (3a-3d) in culture towards L929 cells

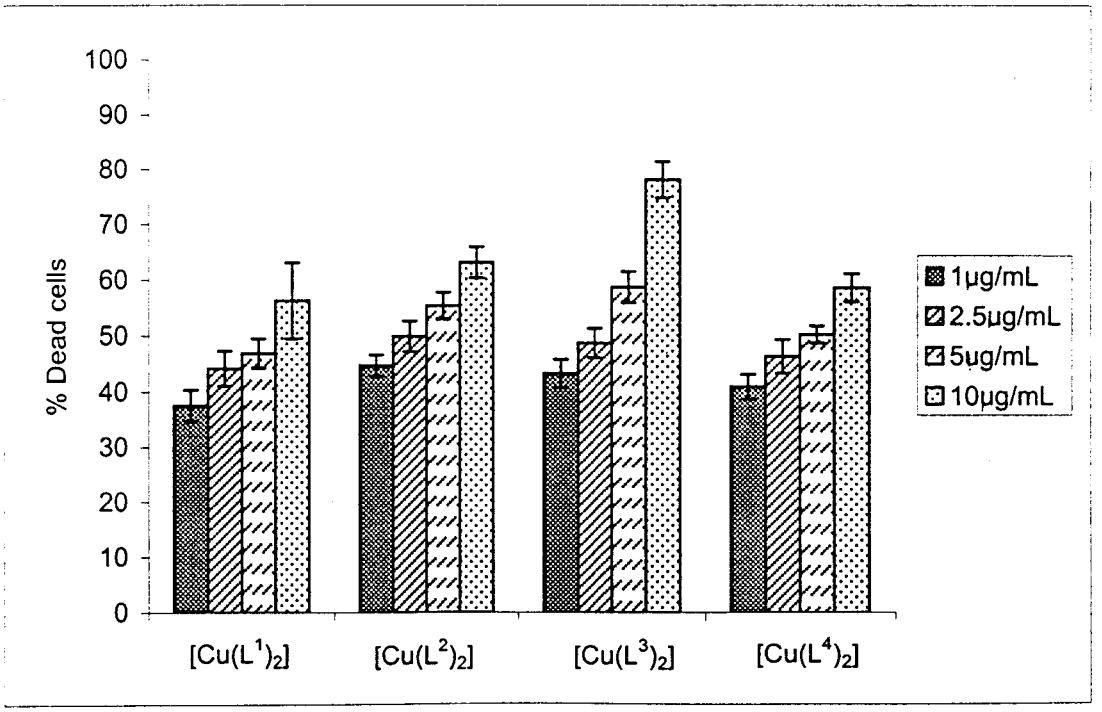


Fig. 3.12 Cytotoxicity of copper complexes (3a-3d) in culture towards L929 cells

Compound **3c** which is found to be the most active one, produced 50% cell death at ~5 μg/mL concentration and copper complex of **3c** produced the same effect ~2.3 μg/mL concentration. Compound **3a** which is the least active one in this group produced 50% cell death at 13 μg/mL concentration.

Effect of compounds on ascites tumour development

All the compounds produced a significant increase ($P < 0.001$ from normal) in the life span of ascites tumour bearing mice. Table 3.18 indicate that copper(II) complexes are highly effective in increasing the life span of tumour bearing mice compared to the curcumin analogues. Percentage increase in life span were 51.4, 56.6, 68.8 and 64.7 for curcuminoid analogues **3a, 3d, 3b** and **3c** respectively. However the percentage increase in life span were 58.9, 71.7, 74.6 and 86.1 respectively for their copper complexes.

Table 3.18

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±1.1	
3a (HL^1)	6/6	26.2±2.1	51.42*
3b (HL^2)	6/6	29.2±3.1	68.81*
3c (HL^3)	6/6	28.5±2.7	64.71*
3d (HL^4)	6/6	27.1±2.8	56.59*
$[Cu(L^1)_2]$	6/6	27.5±2.8	58.89*
$[Cu(L^2)_2]$	6/6	30.2±3.4	74.58*
$[Cu(L^3)_2]$	6/6	32.2±2.1	86.13*
$[Cu(L^4)_2]$	6/6	29.0±3.0	71.70*

*$P < 0.001$

Values are means of ±SD of six determinations. Animals were injected (ip) with Ehrlich ascites tumour cells ($1x10^6$ cells/animal), compounds (200 μmol/Kg body wt) were injected (ip) for 10 days.

Effect of compounds on solid tumour development

The compounds (**3a-d**) and their copper chelates showed a significant reduction of solid tumour volume in mice as evident from figures 3.13 and 3.14.

Fig. 3.13 Effect of compounds (3a-3d) on solid tumour volume

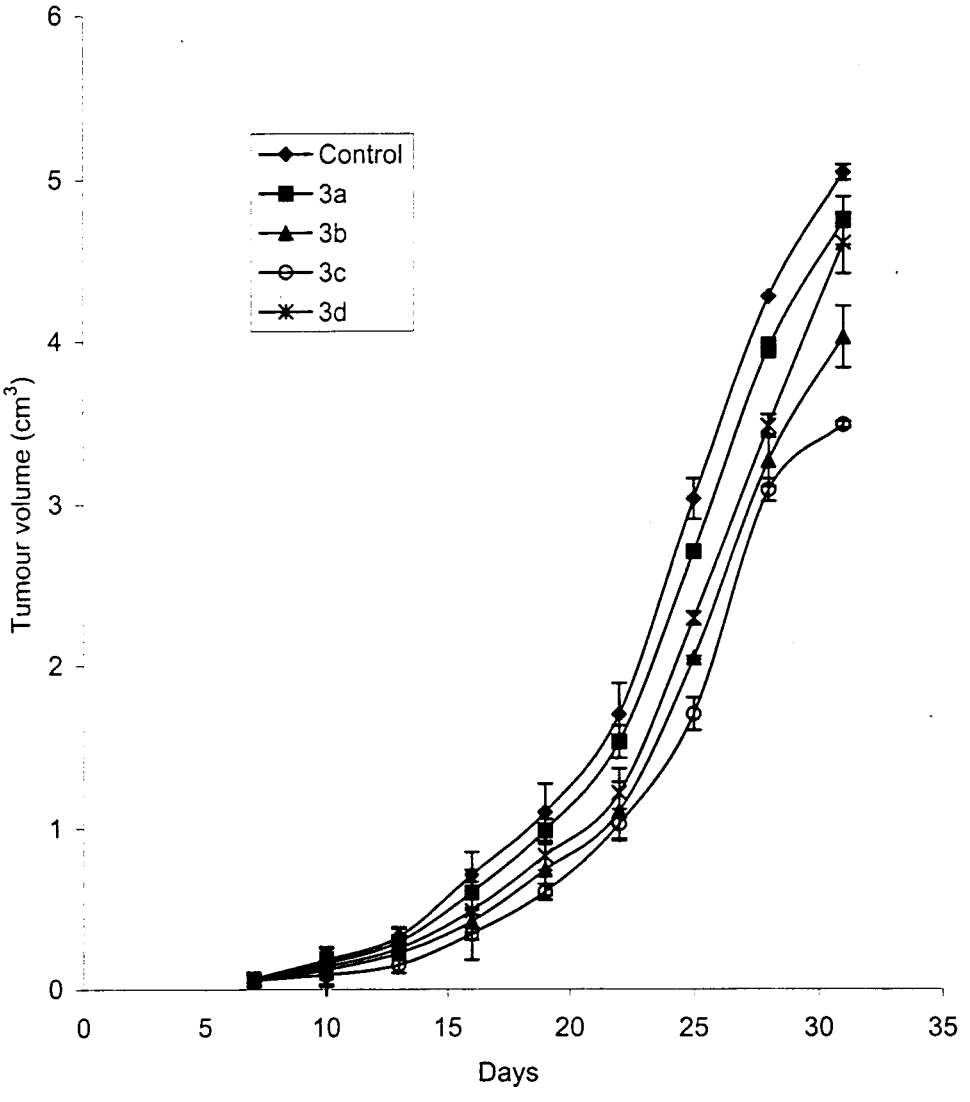
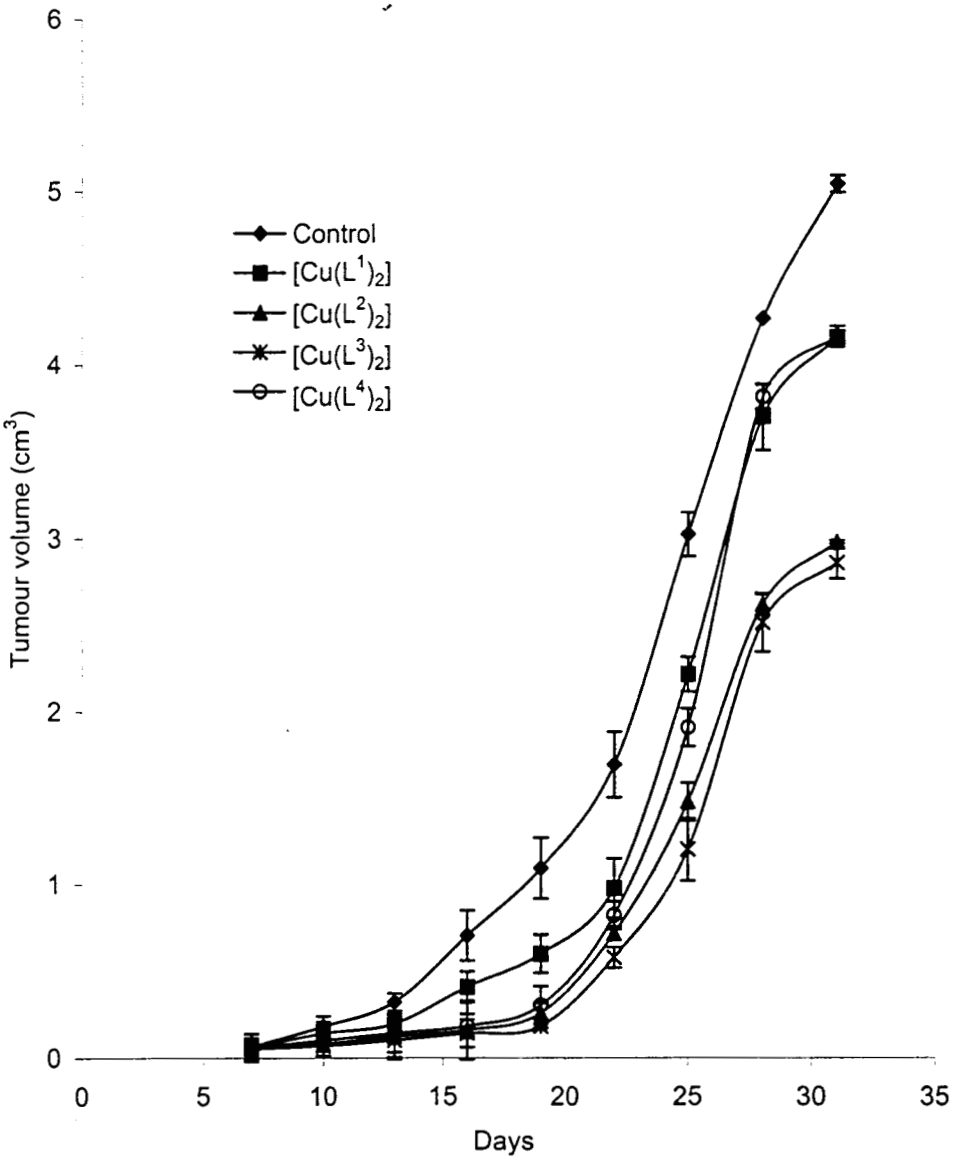


Fig. 3.14 Effect of copper complexes of (3a-3d) on solid tumour volume



The tumour volumes were 5.04 cm^3, 3.48 cm^3, 4.01 cm^3, 4.60 cm^3 and 4.74 cm^3 on day-31 for control, **3c, 3b, 3d** and **3a** respectively. However the tumour volumes were 2.86 cm^3, 4.16 cm^3, and 4.21 cm^3, on day-31 for their respective copper chelates.

It has been reported that among the natural curcuminoids isolated from turmeric, curcumin III which is a para substituted derivative (same as **3c**) is more active than the other two as a cytotoxic and antitumour agent[209]. Hence it is reasonable to believe that the hydroxyl group on the *para* position of phenyl ring enhances cytotoxic and antitumour activities. The present study confirms this argument from the experimental results of compound **3c** which is the most active compound in the group. It also reveals that copper complexation significantly increases the antitumour activities compared to free curcuminoid analogues.

CHAPTER IV

SYNTHESIS, CHARACTERISATION AND ANTITUMOUR ACTIVITY OF 1,7-BIS (DISUBSTITUTED PHENYL)–1,6 HEPTADIENE -3,5-DIONES AND THEIR METAL CHELATES

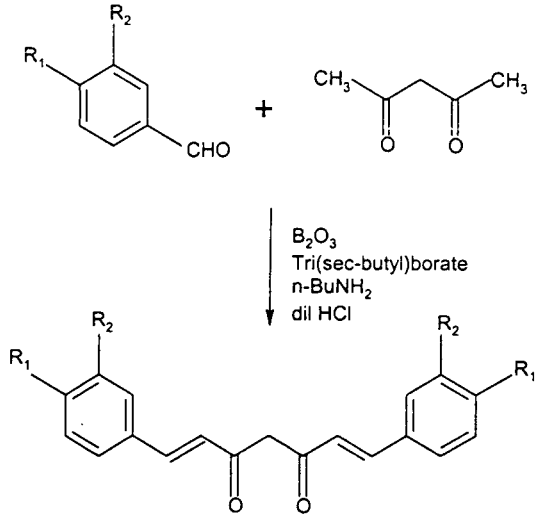
SECTION 1

SYNTHESIS AND CHARACTERISATION OF DISUBSTITUTED 1,7-DIPHENYLHEPTANOIDS AND THEIR METAL COMPLEXES

The curcuminoids extracted from turmeric contain mainly three 1,7-diarylheptanoids (structures **1, 2** and **3** in Part II of the thesis) popularly known as curcumin I, curcumin II and curcumin III. Among these nearly 40% is curcumin I which is a symmetrical disubstituted compound. The synthesis and spectral characterisation of the compound is well documented[141-143,147,149]. Metal chelates of Cu^{2+}, Ni^{2+}, Zn^{2+} and Pd^{2+} of the compound are also known[142]. Similarly synthesis and characterisation of **4c** and its Cu^{2+}, Ni^{2+} and Zn^{2+} complex are also reported recently[142]. However no report exist on synthesis and characterisation of metal complexes of **4b**. Therefore metal complexes of **4b** are discussed in this chapter. Also included in this chapter are the coordination aspects of VO^{2+}, Co^{2+} and Fe^{3+} complexes of all the three compounds **4a-c**.

Synthesis of disubstituted 1,7-diphenylheptanoids

These ∝,β-unsaturated diketones were synthesised by condensing disubstituted aldehydes (vanillin, piperonal, veretraldehyde) with acetyl acetone-boron complex in presence of tri(secbutyl)borate using *n*-butylamine as the condensing agent (**Scheme 4.1**).



Scheme 4.1

Synthesis of the metal chelates

Oxovanadium(IV), iron(III), cobalt(II), nickel(II) and copper(II) complexes were systhesised by the following general method.

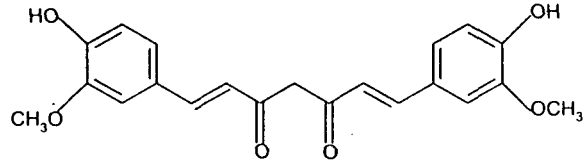
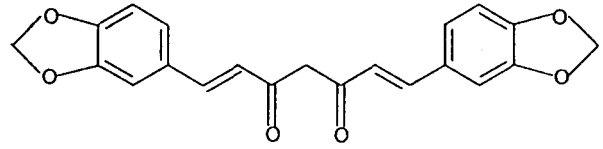
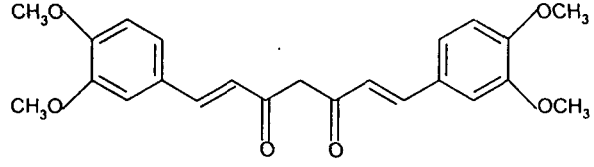
A solution of metal salt (.001 mol) in ethanol (25 mL) was added to a methanolic solution of the diketone (0.002 mol, 25 mL) with stirring. The reaction mixture was refluxed for ~1h and the volume was reduced to half. On cooling to room temperature the complex gets precipitated, which was filtered, washed with cold ethanol and recrystallised from hot methanol.

Results and Discussion

These *meta, para*-substituted 1,7-diarylheptanoids are intensely coloured, crystalline and freely soluble in common organic solvents but insoluble in water. Synthetic details, systematic name, etc. of the compound are given in table 4.1. The observed melting points and uv absorption maxima of the compounds are almost identical to reported values[142].

Table 4.1

*Synthetic details of the disubstituted 1,7-diarylheptanoids (**4a-c**)*

Comp-ounds	Aldehydes used for synthesis	Structure	Systematic name	Colour	Yield (%)
4a	4-hydroxy-3-methoxy benzaldehyde (vanillin)		1,7-Bis(4-hydroxy-3-methoxy phenyl) -1,6-heptadiene -3,5-dione	orange red	76
4b	3,4-methylene dioxy benzaldehyde (piperonaldehyde)		1,7-Bis(3,4-methylene dioxyphenyl) -1,6-heptadiene -3,5-dione	red	70
4c	3,4-dimethoxy benzaldehyde (veretraldehyde)		1,7-Bis(3,4-dimethoxy phenyl) -1,6-heptadiene-3,5-dione	brownish red	72

Characterisation of the disubstituted 1,7-diphenylheptanoids

The disubstituted 1,7-diarylheptanoids were characterised on the basis of their uv, ir, ^{1}H nmr and mass spectral data. The observed spectral data of the compounds agree well with similar data reported earlier. The spectral data obtained in the present study are briefly mentioned below inorder to facilitate the discussion of the spectral data of metal complexes.

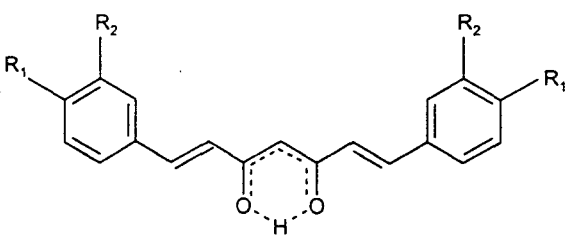
The uv spectra of the compounds showed λ_{max} at ~ 440 nm and at ~ 268 nm (Table 4.2), assignable to the n→π* transition of the carbonyl group and π→π* of the conjugated system. The λ_{max} of low energy n→π* transition shows a bathochromic shift when electron releasing substituents are present in the aryl ring.

Table 4.2

*Physical and analytical data of disubstitued 1,7-diarylheptanoids (**4a-c**)*

Compound	M.P. (°C)	Elemental analysis (%) Found/ (Calcd)		Mol. Weight Found/ (Calcd)	λmax (nm)	log ∈
		C	H			
4a	183	69.10	5.85	372	265	4.09
		(68.48)	(5.43)	(368)	431	4.74
4b	161	68.79	4.85	367	270	4.19
		(69.23)	(4.40)	(364)	442	4.76
4c	218	70.11	5.97	399	268	4.12
		(69.69)	(6.06)	(396)	445	4.79

The ir spectra of all the compounds are characterised by the presence of a strong band at ~1615 cm^{-1} and no other band is observed in the region 1600-1800 cm^{-1}. This band can be assigned to the stretching of intramolecularly hydrogen bonded carbonyl function. Since no other band is present in the region 1650 – 1750 cm^{-1} suggest that the compound exist entirely in the enolic form as in structure **4.2**. A broad band in the region 3200-3700 cm^{-1} observed in the spectra is as expected from the strong intramolecular hydrogen bonding of the structure. Characteristic ir data of the compounds and their probable assignments are given in table 4.3.



4.2

Table 4.3

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

Compounds			Probable assignments
4a	**4b**	**4c**	
1619	1603	1617	ν(C=O) chelated
1583	1585	1568	ν(C=C) phenyl
1566	1570	1568	
1540	1550	1542	ν (C=C) alkenyl
1526	1525	1529	ν_{as}(C-C-C) chelate ring
1448	1443	1453	ν_{s}(C-C-C) chelate ring
1093	1099	1096	β(C-H) chelate ring
1065	1055	1062	
988	970	979	ν(CH=CH) (trans)

The ^{1}H nmr spectra of all the compounds displayed a one proton singlet in the lowfield at δ~ 16 ppm and another singlet at δ~ 6.8 ppm assignable respectively to the strong intramolecularly hydrogen bonded enolic proton and to the methine hydrogen. The methoxy protons and phenolic protons on the aryl rings of the compounds showed signals as expected. The *trans* orientation of alkenyl protons is evident from its observed J values (~16 Hz). The ^{1}H nmr spectra of compound **4b** is reproduced in fig.4.1. The ^{1}H nmr spectral data of all the compounds are given in table 4.4.

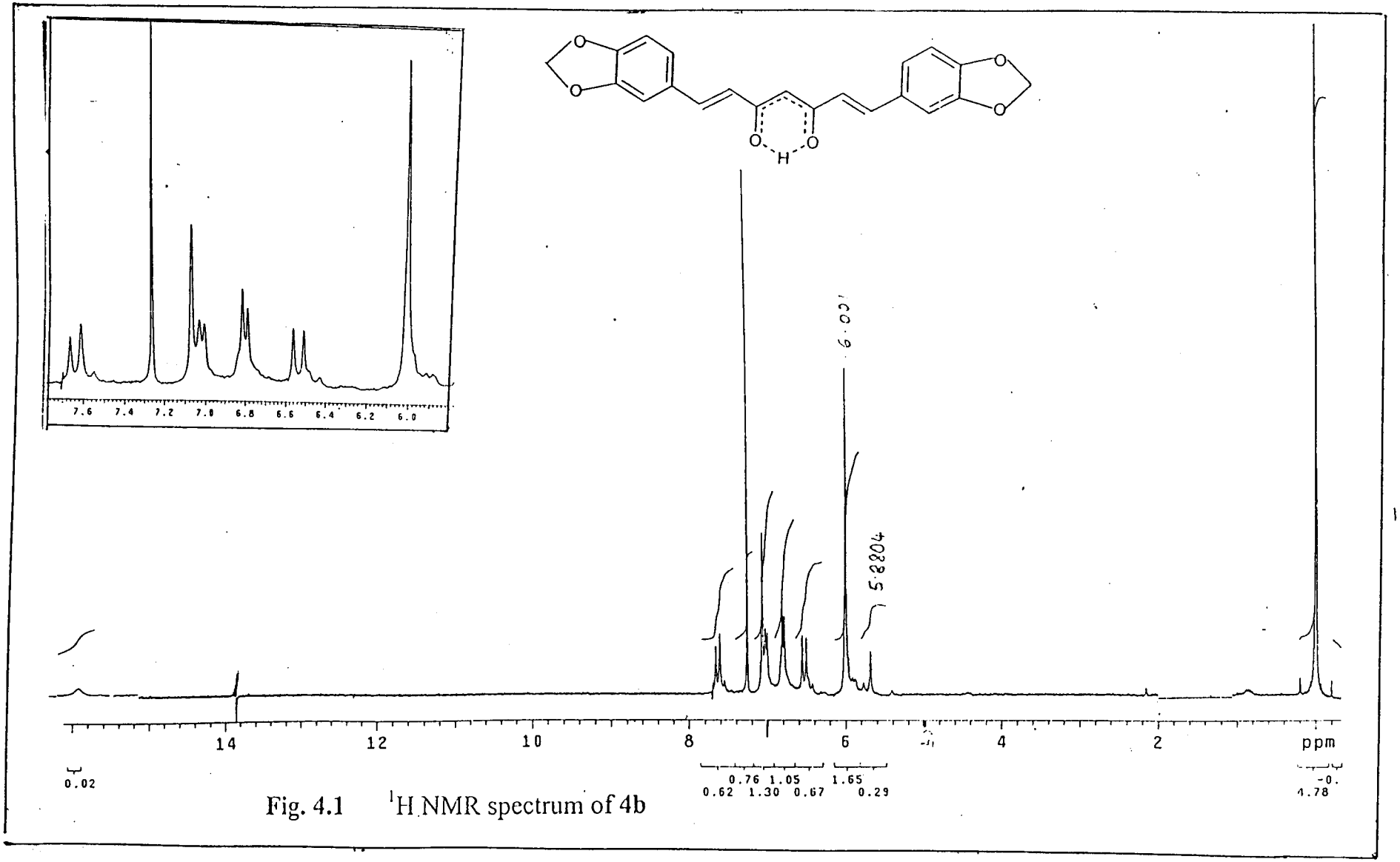


Fig. 4.1 ^{1}H NMR spectrum of 4b

Table 4.4

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

Compound	Chemical shift (δ ppm)				
	Enolic	Methine	Alkenyl	Aryl	Aryl substitution
4a	17.0763	6.8413	8.1632 (2H) 8.1183 (2H)	6.8427- 7.3640 (6H)	3.9208 (methoxy) 10.042 (phenolic)
4b	15.9870	5.8804	6.5615 (2H) 7.6638 (2H)	6.7870- 7.6126 (6H)	6.001 (dioxymethylene)
4c	16.3468	6.7725	7.9563 (2H) 8.0478 (2H)	6.8995- 7.0473 (6H)	3.8607 (methoxy) 3.8302 (methoxy)

Mass spectra of the compounds show intense peaks due to $P^{\cdot}/(P+1)^{+}$. Peaks due to elimination of O, OH, etc are also common. Mass spectra of compound **4b** is reproduced in figure 4.2. The observed fragmentation pattern of the compounds can be conveniently explained as given in **scheme 4.2**, and fragments in table 4.5.

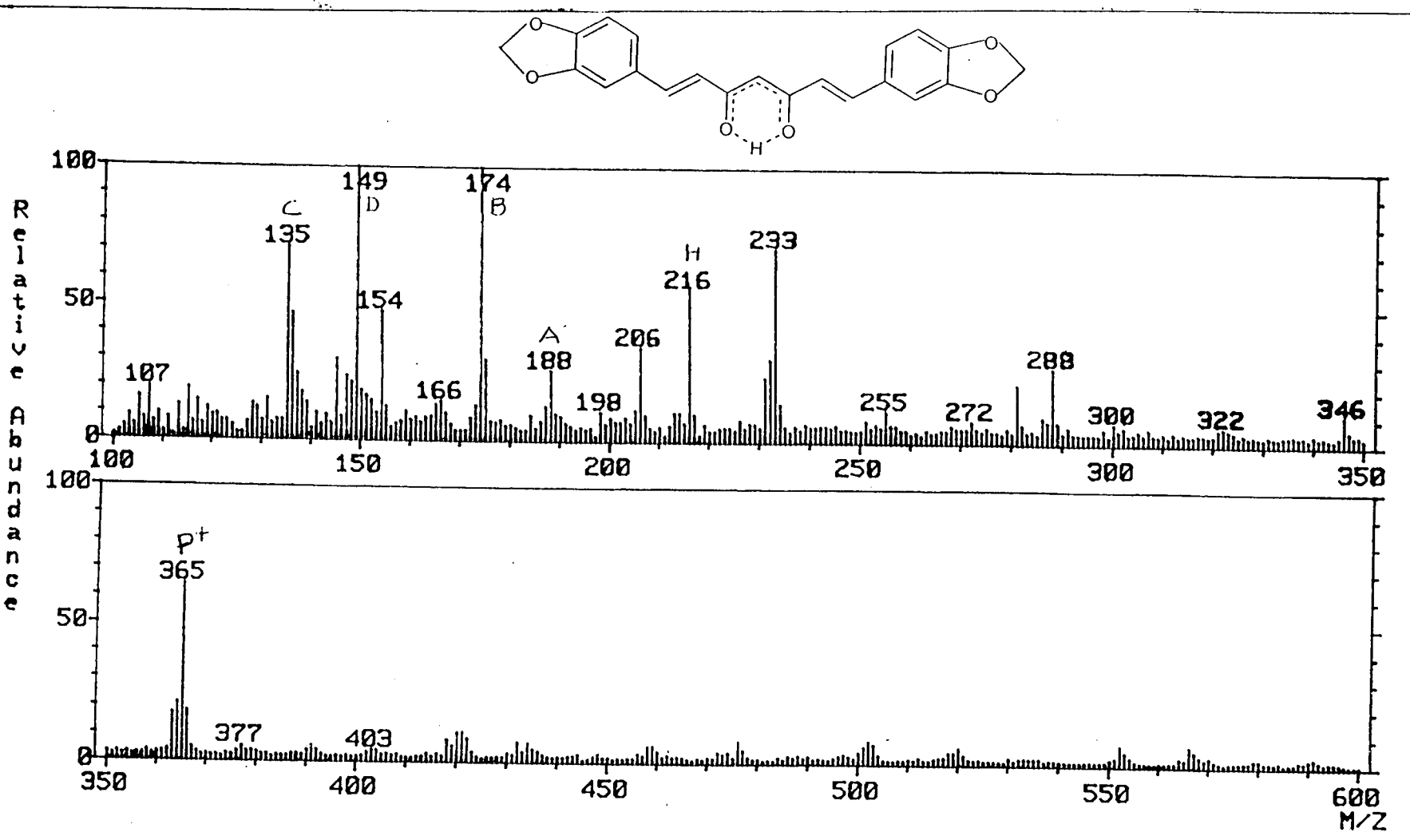
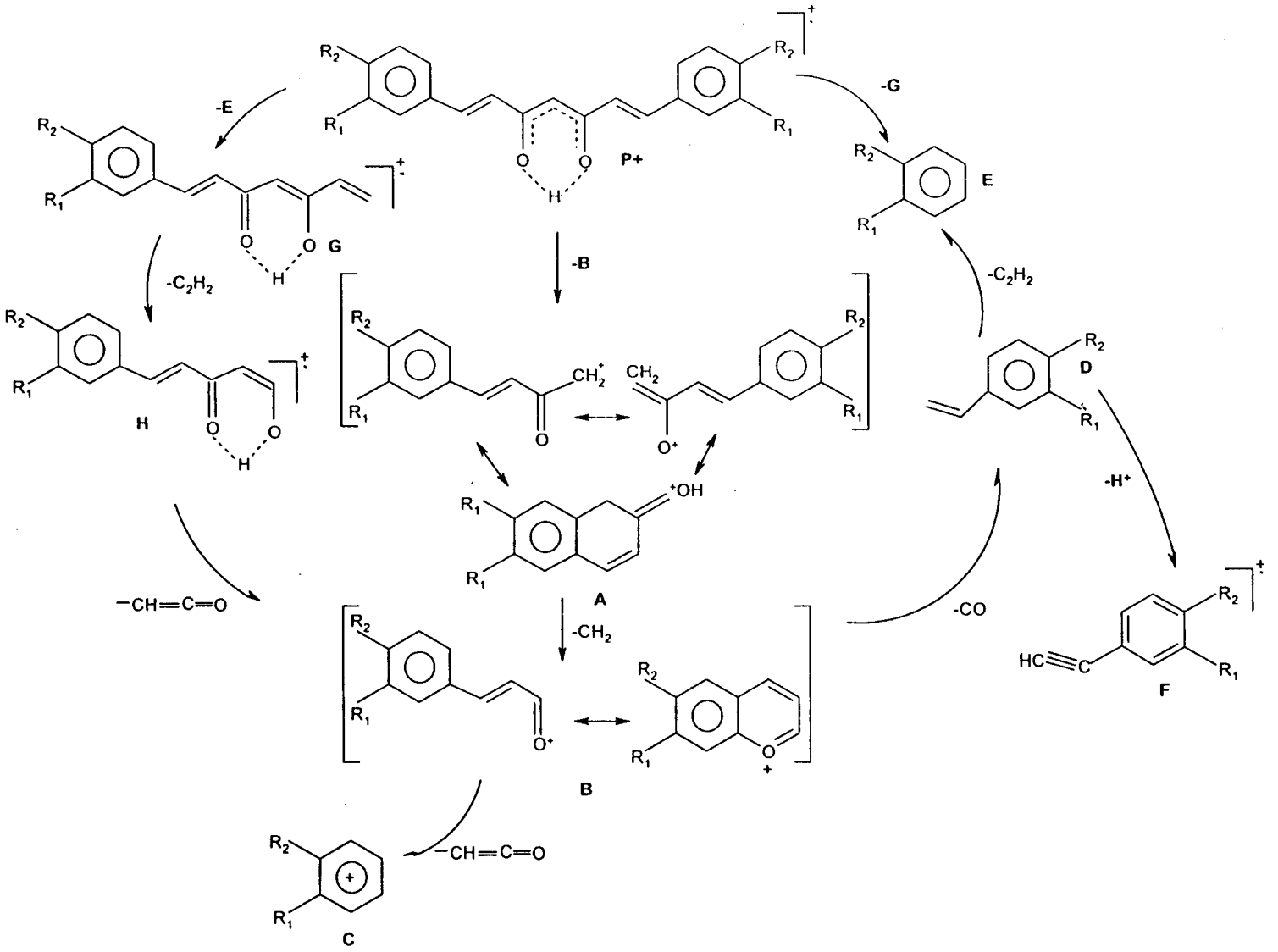


Fig. 4.2 Mass spectrum of **4b**



Scheme 4.2

Table 4.5

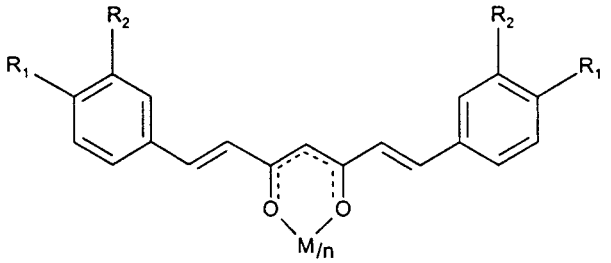
*Fragments present in the mass spectra of compounds **(4a-c)***

Compound	Fragments*								
	P^+	A	B	C	D	E	F	G	H
4a	368	191	177	136	149	123	148	245	219
4b	364	189	175	134	147	121	146	243	217
4c	396	205	191	150	163	137	162	259	233

* The letters corresponds to the fragments given in the scheme **4.2**

Characterisation of the metal chelates

The analytical and physical data of the metal complexes given in table 4.6-4.10 support the $[ML_3]$ composition of Fe(III) complexes and $[ML_2]$ stoichiometry for all other metal complexes. All the metal complexes behaved as nonelectrolytes [specific conductance < 15 Ω^{-1} cm^{-1} in dmso (10^{-3}M) solution]. Cu(II), Co(II), Fe(III) and VO(IV) showed a normal magnetic moment and the nickel(II) complexes are diamagnetic. The observed ir, nmr and mass spectral data of the metal complexes discussed below, conform to the replacement of enolic proton of the ligand by metal ion with the formation of a stable six–membered metal chelate ring in which both oxygens of the dicarbonyl function are involved in bonding with the metal ion as in structure **4.3**.



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

n = 3 for Fe(III)

4.3

Infrared Spectra

The strong free ligand band at ~ 1615 cm^{-1} disappeared in the spectra of all the metal complexes. Instead, a new band appeared at ~1580 cm^{-1} in addition to bands due to various C=C stretching vibrations. This strongly suggest the replacement of the enolic proton by metal ion and the band at ~1580 cm^{-1} can confidently be assigned to metal bonded carbonyl stretching vibration. This is further supported by the appearance of medium intensity bands at ~470 and ~420 cm^{-1} arising from νM-O vibrations. (Table 4.6-4.10).

The broad free ligand band in the region 3200 – 2700cm^{-1} is absent in the spectra of all the complexes indicating the replacement of enolic proton by metal ion. Thus the observed ir data are in agreement with the formation of stable chelates through the 1,3-diketonyl function and the

aryl substituents are not involved in metal chelation. That the non involvement of aryl substituents in the complex formation is evidenced from the ir spectra of the metal chelates of **4a** where relatively broad band in the region 3600-3200 cm^{-1} appeared assignable to free phenolic OH group as in free ligand. In the ir spectra of cobalt(II) complexes, the appearance of bands at ~3500 cm^{-1} and ~890 cm^{-1} suggest the presence of coordinated water molecule.

Table 4.6

*Analytical and characteristic ir spectral data of oxovanadium(IV) chelates of **4a-c***

Oxovanadium (IV) chelates of	M.P. (C^0)	μ_{eff} BM	Elemental analysis (%) Found/(Calcd)*			Characteristic ir stretching bands (cm^{-1})		
			C	H	V	C-O	C-C-C	V-O
4a	214	1.71	64.51 (62.92)	4.21 (4.74)	7.02 (6.37)	1589	1513	470 423
4b	187	1.74	64.87 (63.56)	4.01 (3.78)	6.58 (6.43)	1599	1520	485 430
4c	239	1.73	65.21 (64.41)	5.81 (5.37)	6.46 (5.95)	1596	1515	470 420

* The calculated value correspond to the $[VOL_2]$ composition where L stands for the deprotonated ligands

Table 4.7

*Analytical and characteristic ir spectral data of iron(III) chelates of **4a-c***

Iron(III) chelates of	M.P. (^{0}C)	μ_{eff} BM	Elemental analysis (%) Found/(Calcd)*			Characteristic ir stretching bands (cm^{-1})		
			C	H	Fe	C-O	C-C-C	Fe-O
4a	216	5.89	66.23 (65.34)	4.21 (4.94)	4.82 (4.84)	1600	1514	1514
4b	189	5.91	67.53 (66.02)	5.22 (4.98)	5.01 (4.89)	1591	1516	469 426
4c	239	5.90	61.27 (60.92)	4.93 (4.59)	5.00 (4.51)	1609	1519	480 419

* The calculated value correspond to the [FeL_3] composition where L stands for the deprotonated ligands

Table 4.8

*Analytical and characteristic ir spectral data of cobalt(II) chelates of **4a-c***

Cobalt(II) chelates of	M.P. (^{0}C)	μ_{eff} BM	Elemental analysis (%) Found/(Calcd)*			Characteristic ir stretching bands (cm^{-1})		
			C	H	Co	C-O	C-C-C	Co-O
4a	197	4.91	61.24 (60.79)	5.88 (5.07)	6.95 (7.11)	1589	1506	469 426
4b	201	4.89	61.52 (61.39)	4.89 (4.14)	6.97 (7.19)	1609	1504	471 428
4c	214	4.79	62.01 (62.37)	6.08 (5.65)	6.25 (6.66)	1585	1514	471 419

* The calculated value correspond to the [$CoL_2(H_2O)_2$] composition where L stands for the deprotonated ligands

Table 4.9

Analytical and characteristic ir spectral data of nickel(II) chelates of ***4a-c***

Nickel (II) chelates of	M.P. (^{0}C)	Elemental analysis (%) Found/(Calcd)*			Characteristic ir stretching bands (cm^{-1})		
		C	H	Ni	C-O	C-C-C	Ni-O
4a	203	74.56 (74.91)	4.80 (4.93)	9.55 (9.64)	1582	1512	455 423
4b	210	65.11 (64.23)	5.07 (4.84)	7.96 (7.48)	1595	1506	472 420
4c	230	64.87 (65.04)	5.28 (5.42)	6.78 (6.92)	1590	1523	458 421

* The calculated value correspond to the [NiL_2] composition where L stands for the deprotonated ligands

Table 4.10

Analytical and characteristic ir spectral data of copper(II) chelates of ***4a-c***

Copper(II) chelates of	M.P. (^{0}C)	μeff (BM)	Elemental analysis (%) Found/(calcd)*			Characteristic ir stretching bands (cm^{-1})		
			C	H	Cu	C-O	C-C-C	Cu-O
4a	209	1.75	74.11 (74.32)	4.78 (4.89)	10.1 (10.35)	1580	1518	416 425
4b	212	1.77	64.22 (63.84)	5.09 (4.81)	8.32 (7.98)	1582	1512	479 434
4c	243	1.81	64.27 (64.67)	5.18 (5.39)	7.25 (7.44)	1583	1523	465 423

* The calculated value correspond to the [CuL_2] composition where L stands for the deprotonated ligands

^{1}H nmr Spectra

The most characteristic feature of the ^{1}H nmr spectra of the diamagnetic nickel(II) chelates is the absence of proton signals above δ~ 12 ppm. This strongly suggest the replacement of enolic proton by the metal ion in the complexes. A striking feature of the ^{1}H nmr spectra of the nickel(II) chelate of (**4a**), is the presence of the phenolic proton signal at δ~ 10.06 ppm. This undoubtedly establishes that the phenolic proton is not replaced by the metal ion during complexation. The integrated intensities of the aryl and alkenyl signals are in agreement with the 1:2 metal ligand stoichiometry of the chelates. ^{1}H nmr spectra of nickel (II) complex of **4b** is reproduced in figure 4.3.

Mass spectra

The FAB mass spectra of iron(III) chelate of **4a** and copper(II) chelate of **4b** show relatively intense peaks at m/z corresponding to $[FeL_3]$ and $[CuL_2]$ in agreement with their formulation. Peaks due to the elimination of phenyl, styryl, cinnamoyl, etc groups from the molcular ion are also observed in the spectra. In addition to many fragments of ligands, all the spectra are characterised by the presence of a large number of fragments containing metal ions. The mass spectra of iron(III) chelate of **4a** and the spectra of copper(II) chelate of **4b** are reproduced in figures 4.4 and 4.5.

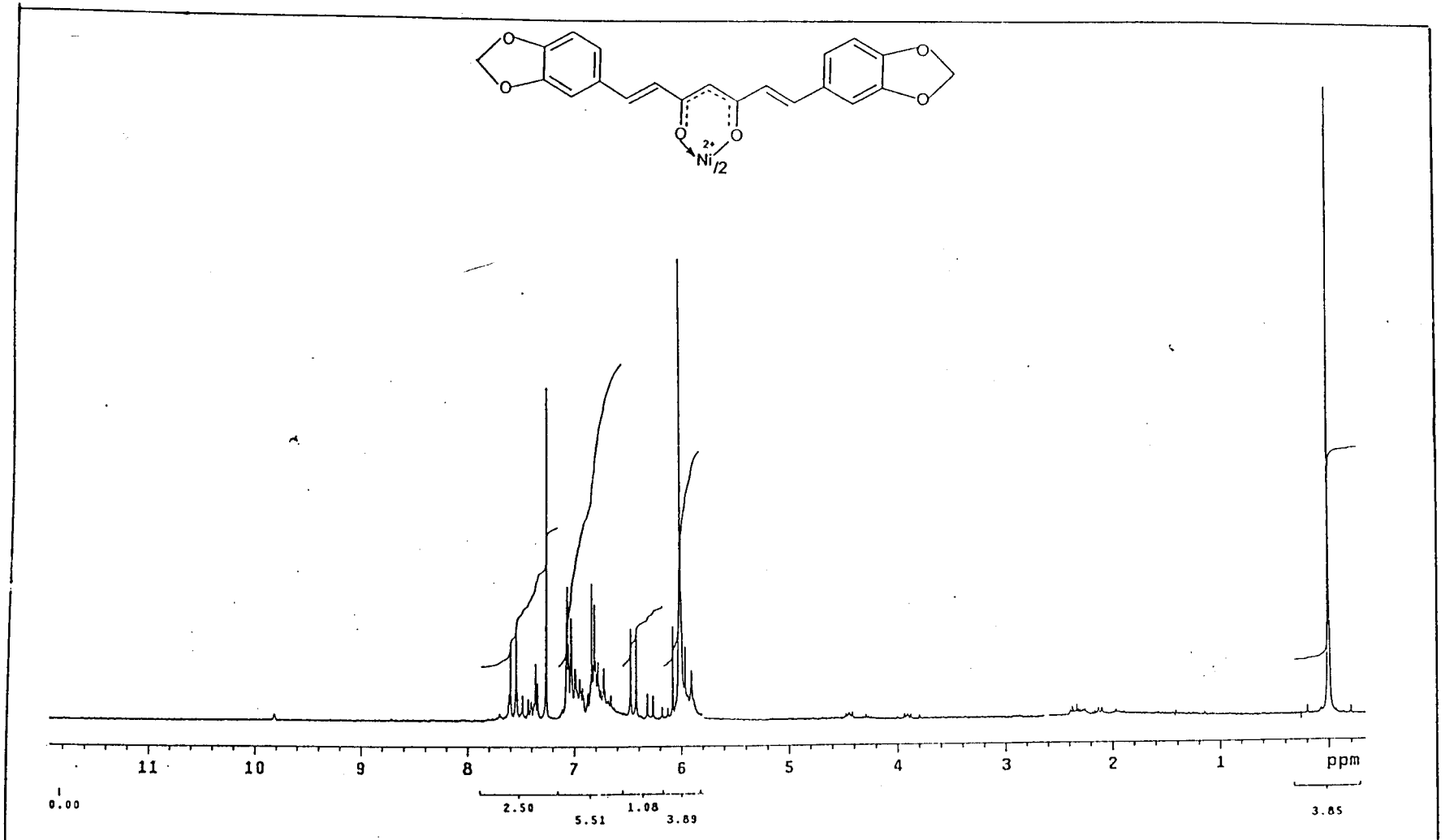


Fig. 4.3 ^{1}H NMR spectrum of nickel(II) complex of 4b

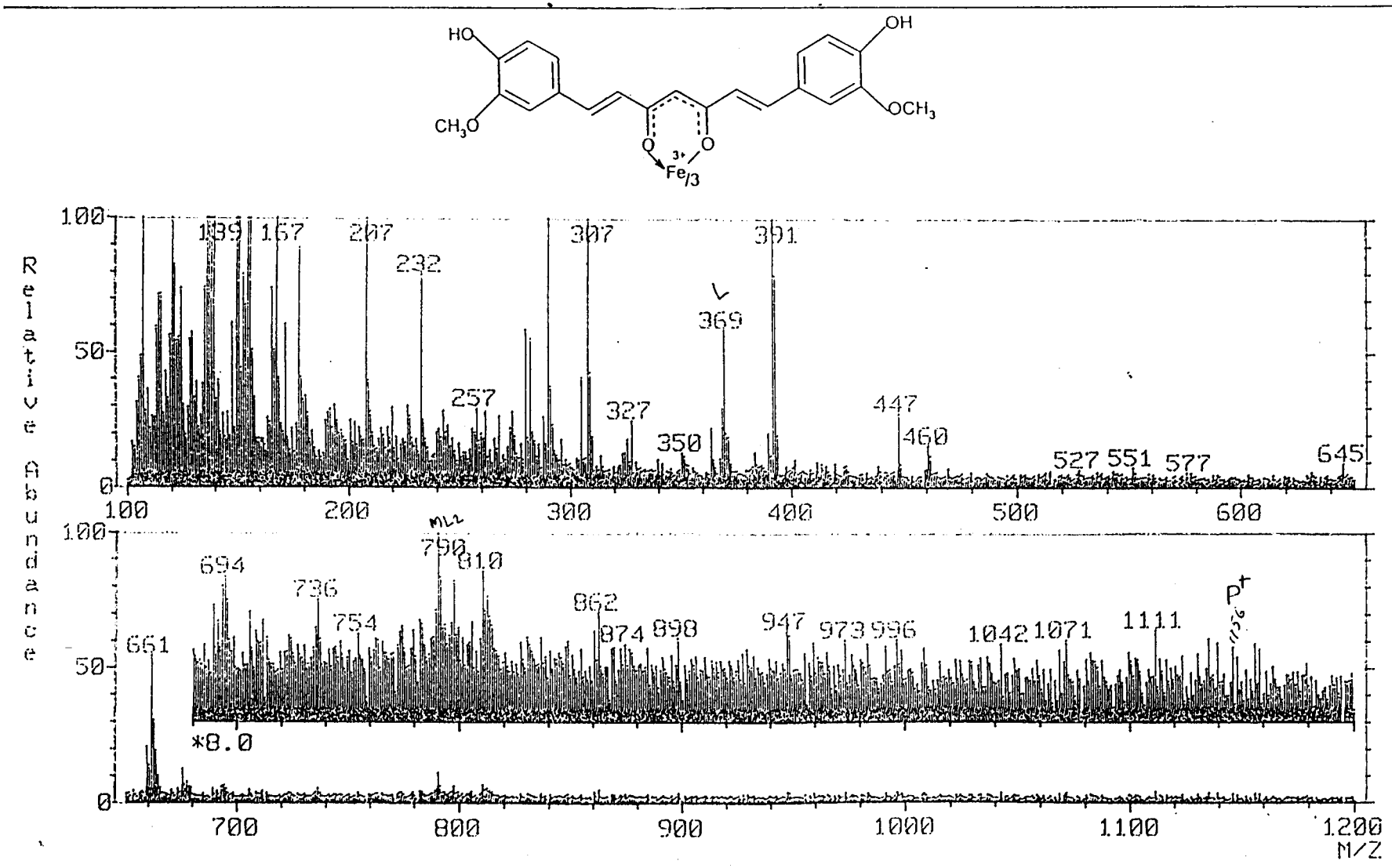


Fig. 4.4 Mass spectrum of iron(III) complex of **4a**

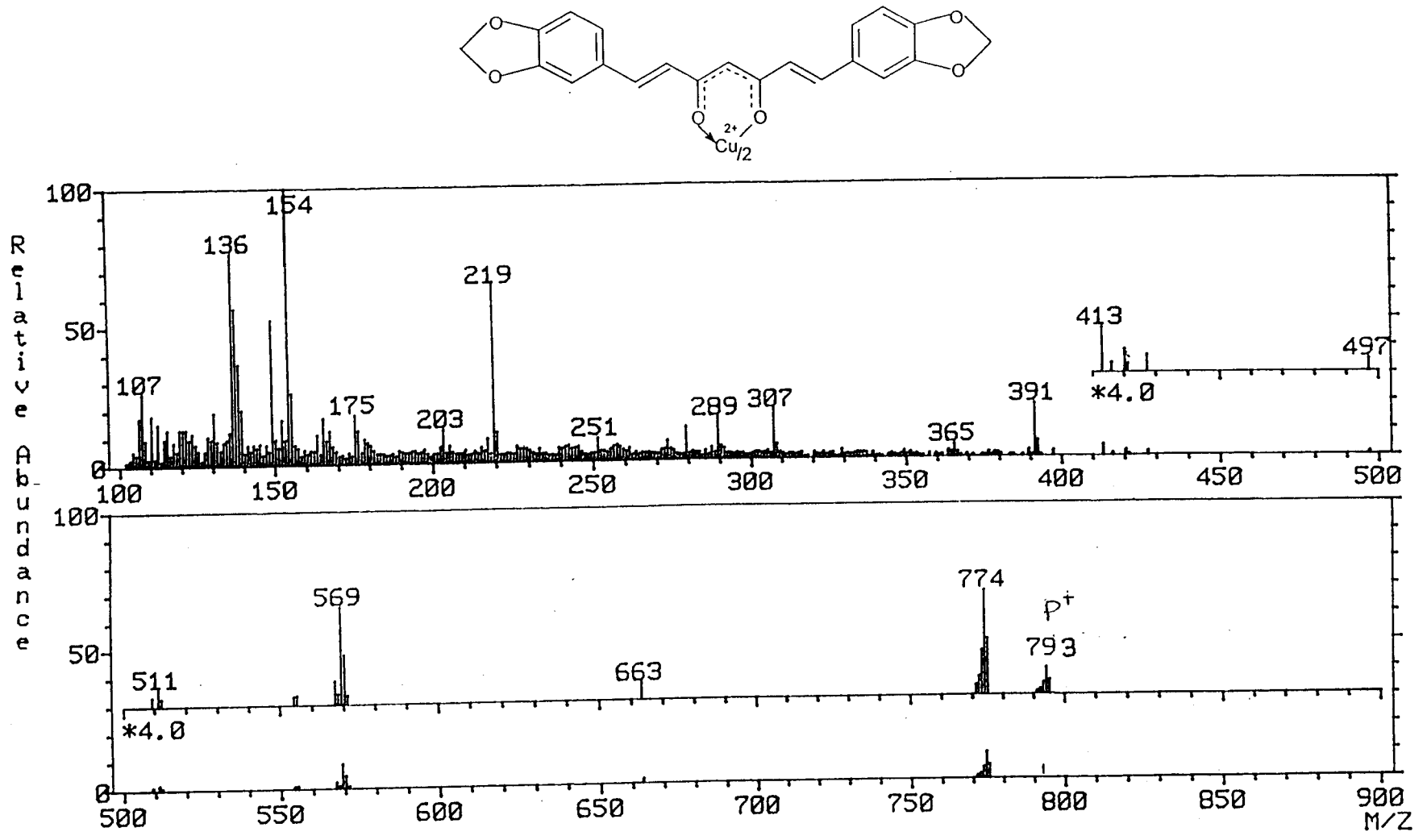


Fig. 4.5 Mass spectrum of copper(II) complex of **4b**

SECTION 2

ANTITUMOUR ACTIVITY OF DISUBSTITUTED 1,7-DIPHENYLHEPTANOIDS AND THEIR COPPER(II) COMPLEXES

The curcuminoid analogues (**4a-c**) and their copper complexes were subjected to various cytotoxic as well as antitumour studies. The experimental procedures and other methods were similar to that described in Section 2, Chapter 1. The results obtained are discussed below.

Results and Discussion

In vitro cytotoxic activity of compounds

All the compounds showed *in vitro* cytotoxicity in the short term assay (Table 4.11). The concentrations needed for producing 50% cell death were ~11.0 μg/mL, ~18.5 μg/mL and 26.5 μg/mL for **4a, 4b** and **4c** respectively. Their copper complexes produced 50% cell death at ~5.1 μg/mL, ~6.3 μg/mL and 9.0 μg/mL respectively.

Table 4.11

Short term in vitro cytotoxicity of compounds towards EAC cells

Compounds	Percentage cell-death at different concentrations				
	1 μg/mL	5 μg/mL	10 μg/mL	25 μg/mL	50 μg/mL
4a (HL^1)	19	35	46	64	84
4b (HL^2)	18	31	40	57	78
4c (HL^3)	16	27	33	46	69
[Cu $(L^1)_2$]	19	35	46	64	84
[Cu $(L^2)_2$]	18	31	40	57	78
[Cu $(L^3)_2$]	16	27	33	46	69

Ehrlich ascites carcinoma cells ($1x10^6$cells/mL) in presence of various concentrations of compounds (1-50 μg/mL) were incubated for 3h at 37^0C. The short-term cytotoxicity was determined by trypan blue dye exclusion method.

Cytotoxicity of the compounds towards tissue culture cells

The compounds were found to be cytotoxic to L929 cultured cells at much lower concentration (Figure 4.6 & 4.7). Concentrations needed for 50% cell death were considerably lowered upon complexation with copper. $\mathbf{IC_{50}}$ values for **4a, 4b** and **4c** were 10 μg/mL, 13.5μg/mL and 23 μg/mL respectively while for their copper complexes the values were 5.0 μg/mL, 6.0 μg/mL and 10.5 μg/mL respectively.

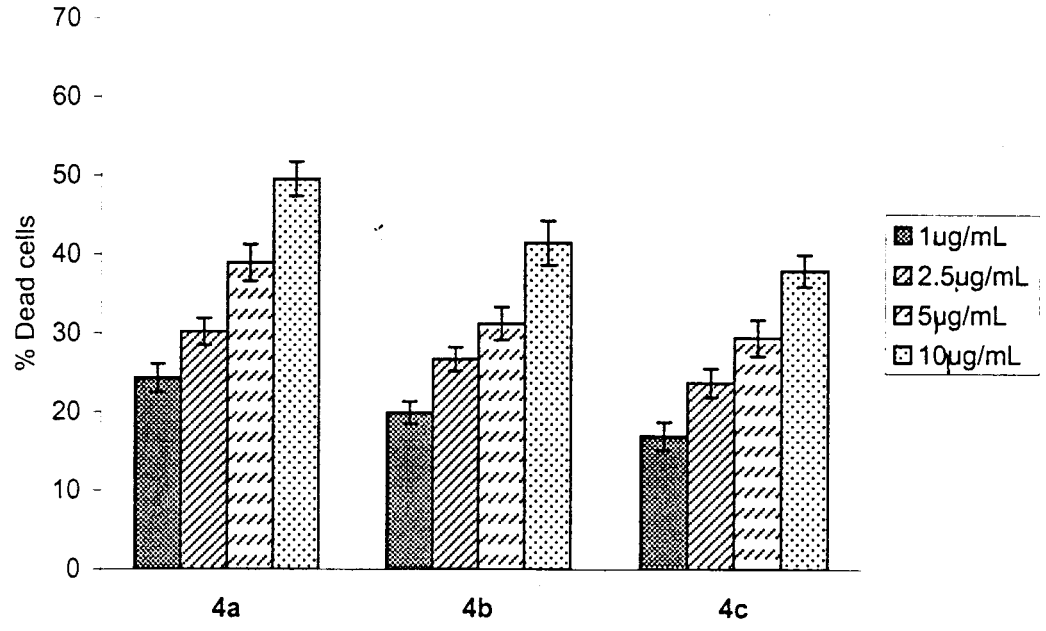


Fig. 4.6 Cytotoxicity of (4a-c) in culture towards L929 cells

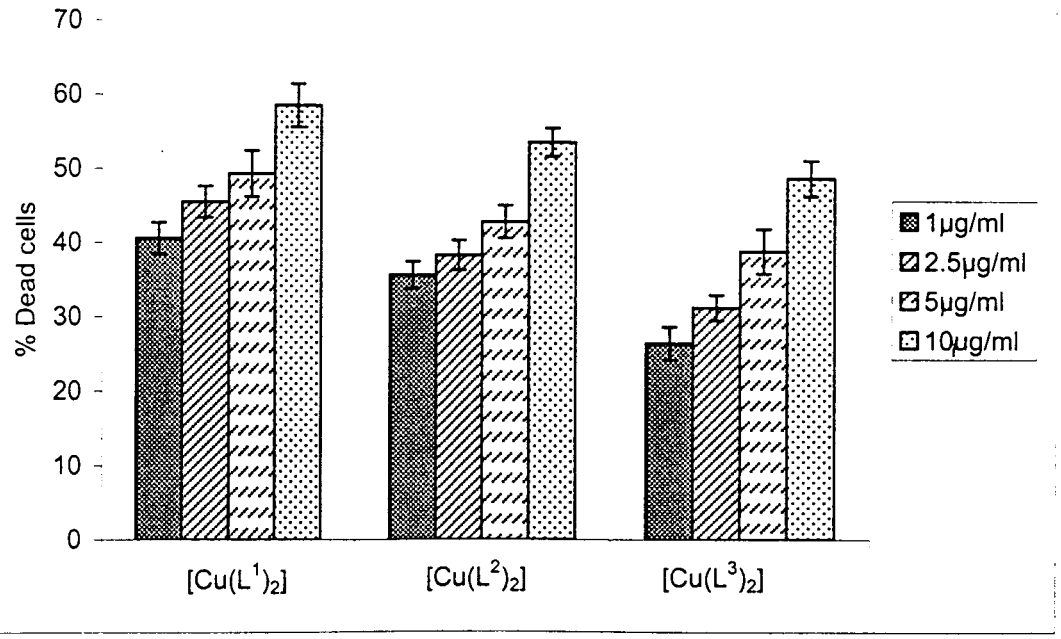


Fig. 4.7 Cytotoxicity of copper complexes (4a-c) in culture towards L929 cells

Effect of compounds on ascites tumour development

All the test compounds produced an increase in life span of ascites tumour bearing mice (Table 4.12). The most active compound in this series was found to be the copper complex of **4a** which produced 89.02% increase in life span of tumour bearing mice. Copper complexes are generally found to be more active than their counterparts.

Table 4.12

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±1.1	
4a (HL^1)	6/6	29.4±2.8	69.94*
4b (HL^2)	6/6	29.2±3.6	68.78*
4c (HL^3)	6/6	28.3±2.1	63.58*
[Cu $(L^1)_2$]	6/6	31.1±3.0	79.76*
[Cu $(L^2)_2$]	6/6	30.3±3.7	75.14*
[Cu $(L^3)_2$]	6/6	30.8±2.8	78.03*

*$P < 0.001$

Values are means of ±SD of six determinations. Animals were injected (ip) with Ehrlich ascites tumour cells ($1x10^6$ cells/mL), compounds (200 μmoles/Kg body wt) were injected (ip) for 10 days.

Effect of compounds on solid tumour development

All the compounds when injected intraperitonially in mice showed a significant reduction of solid tumour volumes (Fig. 4.8 & 4.9).

Fig. 4.8 Effect of compounds (4a-4c) on solid tumour volume

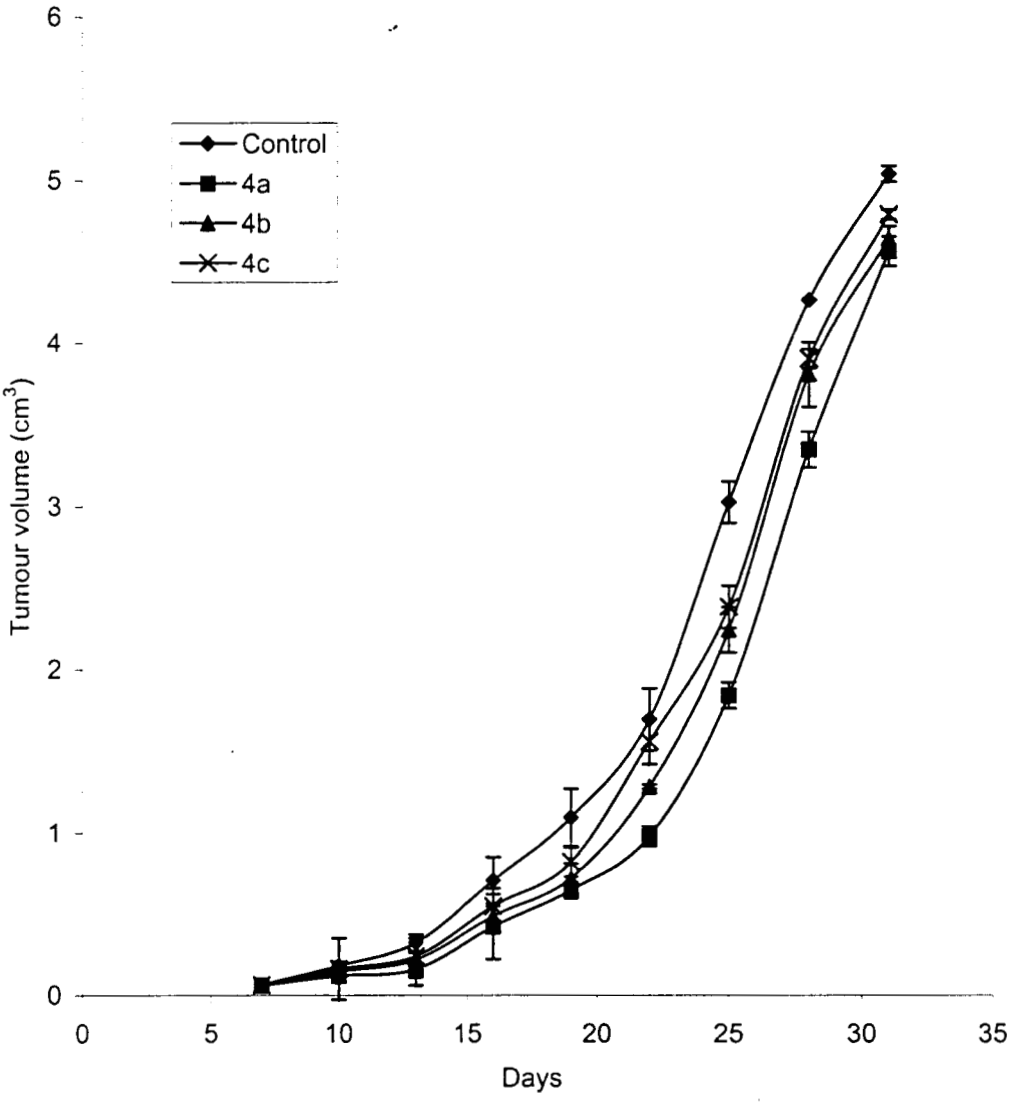
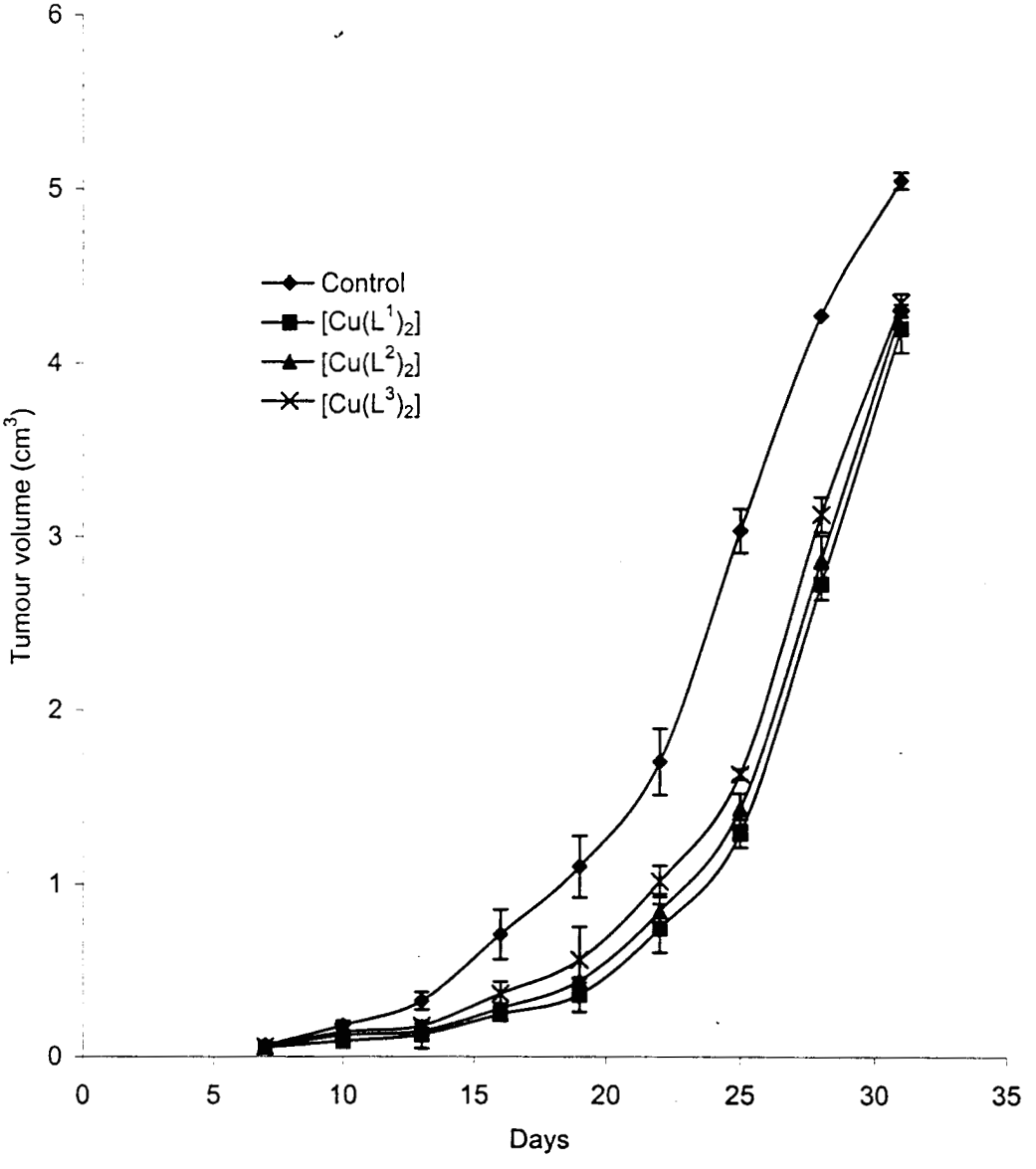


Fig. 4.9 Effect of copper complexes of (4a-c) on solid tumour volume



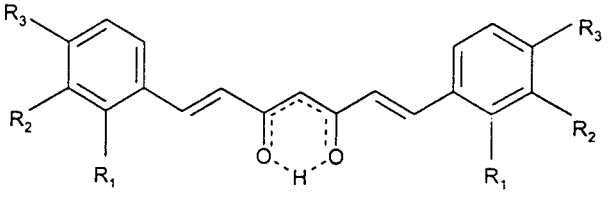
The effect was more pronounced when copper complexes were administrated. The tumour volumes on day-31 were 4.57 cm^3, 4.65 cm^3 and 4.79 cm^3 respectively for **4a, 4b** and **4c** compared to control (5.04 cm^3). The respective tumour volumes were 4.20 cm^3, 4.30 cm^3 and 4.32 cm^3 for copper complexes of **4a, 4b** and **4c** respectively.

The data suggest that the compounds having phenolic structure are having appreciable antitumour activity. Compounds like **4b** whose OH groups on the phenyl ring are bridged by a methylene group possess moderate activity while **4c** which is not having an OH group on phenyl ring is the least active one. The presence of a copper –oxygen bond may be the reason for the enhanced antitumour activity of the copper complexes.

SUMMARY

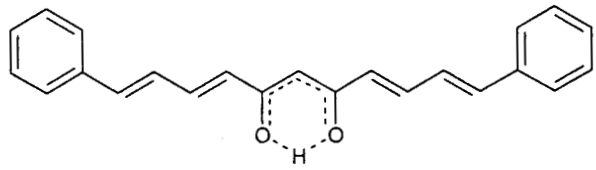
The fifteen 1,7-diaryltheptanoids (synthetic analogues of natural curcuminoids) considered in this investigation can be conveniently classified in to the following four structural types (**1, 2, 3** and **4**), based on the nature of the aryl groups.

Type 1



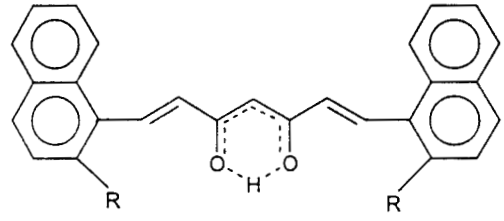
Compound	R_1	R_2	R_3
1a	H	H	H
2a	CH_3	H	H
2b	OH	H	H
2c	Cl	H	H
3a	H	H	OCH_3
3b	H	H	OC_2H5
3c	H	H	OH
3d	H	H	$N(CH_3)_2$
4a	H	$O–CH_2–O$	
4b	H	OCH_3	OH
4c	H	OCH_3	OCH_3

Type 2



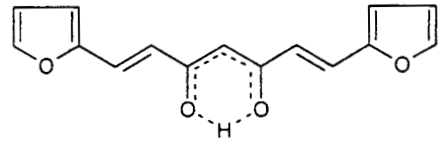
1b

Type 3



	R
1c	H
2d	OH

Type 4



1d

Spectral data (electronic, ir, nmr and mass) of all these 1,7-diarylheptanoids unequivocaly support the existence of the compounds entirely in the intramolecularly hydrogen bonded enol tautomeric form. All these compounds form stable metal chelates with VO^{2+}, Co^{2+}, Ni^{2+} and Cu^{2+} having $[ML_2]$ stoichimetry and Fe^{3+} with

[FeL_3] stoichometry. These unsaturated diketones behave as mono basic bidentate chelating agent. In all the metal chelates, only the diketo oxygens are involved in bonding with the formation of a stable six membered chelate ring invovling the metal ion. The spectral data clearly suggest that metal binding groups such as –OH, oxygen of furyl rings, etc present in certain diarylheptanoids are not involved in bonding with metal ions.

All the diarylheptanoids and their metal complexes exhibit significant antitumour activity. A comparative evaluation of the observed results are summarised below.

Short-term *in vitro* cytotocity

Concentrations of all the 1,7-diarylheptanoids required for 50% cell death (IC50) are shown in **figure 1**. The results show that in type 1 compounds, **2b** requires only 6.5 μg/mL for 50% cell death, which is lower than **3c**, the synthetic analogue of curcumin III of natural curcuminoids. Among the natural curcuminoids, curcumin III has been shown to be more active than curcumin I and II. The results in **figure 1** shows that the concentration requred for 50% cell death (IC_{50}) is in the order **2d< 2b < 3c < 2c <3b < 4a < 1b <1c < 2a < 4b <1d < 3d < 1a < 3a < 4c.**

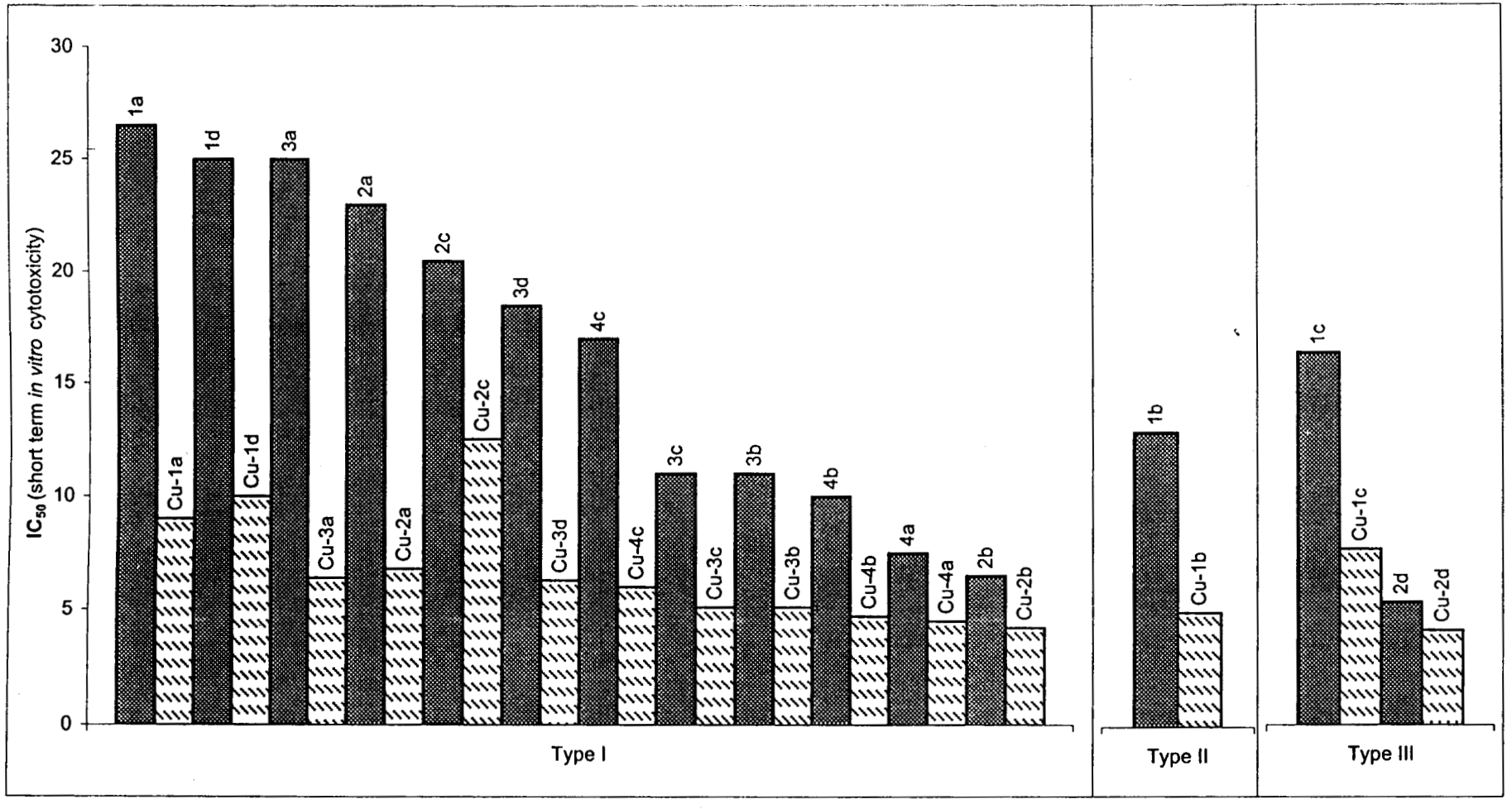


Fig. 1 Concentration needed for 50% cell death (short term *in vitro* cytotoxicity) of the compounds

A comparison of the data indicate that as conjugation increases the concentration needed for 50% cell death decreases. Thus compared to **1a** (type I), only about half the concentration is required in the case of **1b** (type II) and only one fourth concentration in the case of **2d** (type III). The IC_{50} value in the case of **2d** is significantly low compared to **2b**. In **2b** and **2d** a hydroxyl group is present adjacent to the olefinic linkage. Thus, it can be stated that, presence of at least one –OH group on the aryl rings and an increase in the olefinic linkage are favourable conditions for short-term *in vitro* cytotoxicity. Further, the substitution of aryl rings by heteroaryl rings such as furyl groups decreases the activity.

In order to study the effect of metal complexation on the cytotoxicity of the compounds, the VO^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} complexes of **2d** was screened for their possible cytotoxicity. The complexes of **2d** was choosen because among the 1,7-diarylheptanoids considered, the **2d** was the most active one. The results are brought out in **figure 2**.

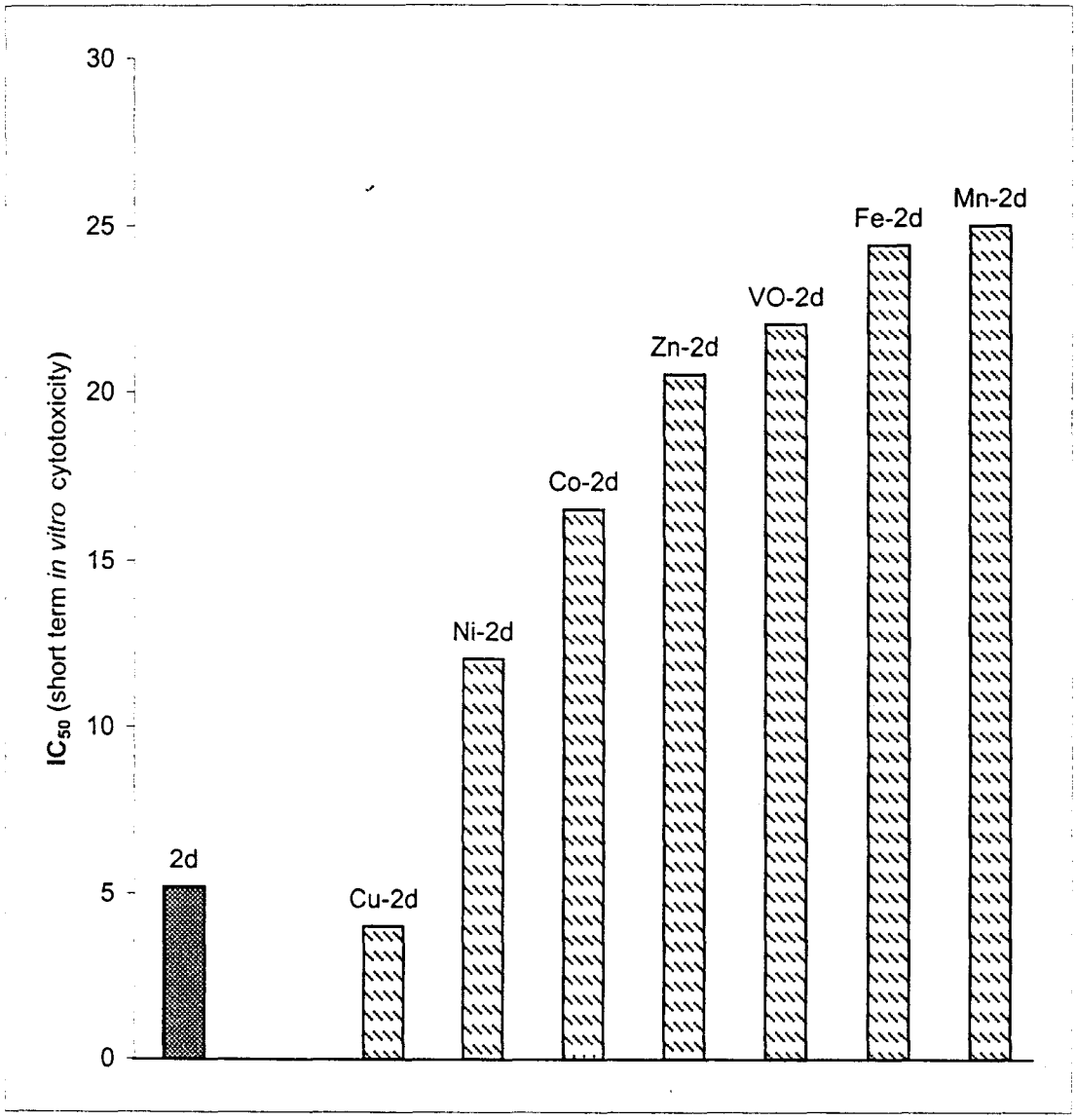


Fig. 2 Concentration needed for 50% cell death (short term *in vitro* cytotoxicity) of 2d and its various metal complexes

The diagram clearly shows that IC_{50} of all the complexes increased drastically except in the case of copper complex. The IC_{50} of copper complexes is less than half compared to the free 1,7-diarylheptanoids. Therefore, among the metal complexes, the antitumour and cytotoxicity of only copper complexes of the 1,7-diarylheptanoids were examined. The results obtained for all the copper complexes are included in **figure 1** along with the data of the free 1,7-diarylheptanoids. The results clearly indicate that the IC_{50} values are significantly less compared to the uncomplexed curcuminoids. The activity follow the same order as that of the curcuminoids. Thus, for the copper complexes of **2d**, **2b** and **3c**, the IC_{50} values are 4.0 μg/mL, 4.2 μg/mL and 4.5 μg/mL respectively which are the lowest values among the complexes studied. It is to be pointed out that, in these complxes, the OH group remain free and not involved in bonding with the metal ion.

Culture studies

For comparison, the IC_{50} values of all the curcuminoids towards L929 cultured cells are brought out in **figure 3**. The data clearly show that **2d** is the most active one followed by **2b** and **3c** and follows the same order as that observed in the case of short term *in vitro* cytotoxicity studies. In all the cases, copper complexation appreciably increased the activity of the compounds. In the case of **2b** and **2c,** its copper complexes have nearly ten fold activity.

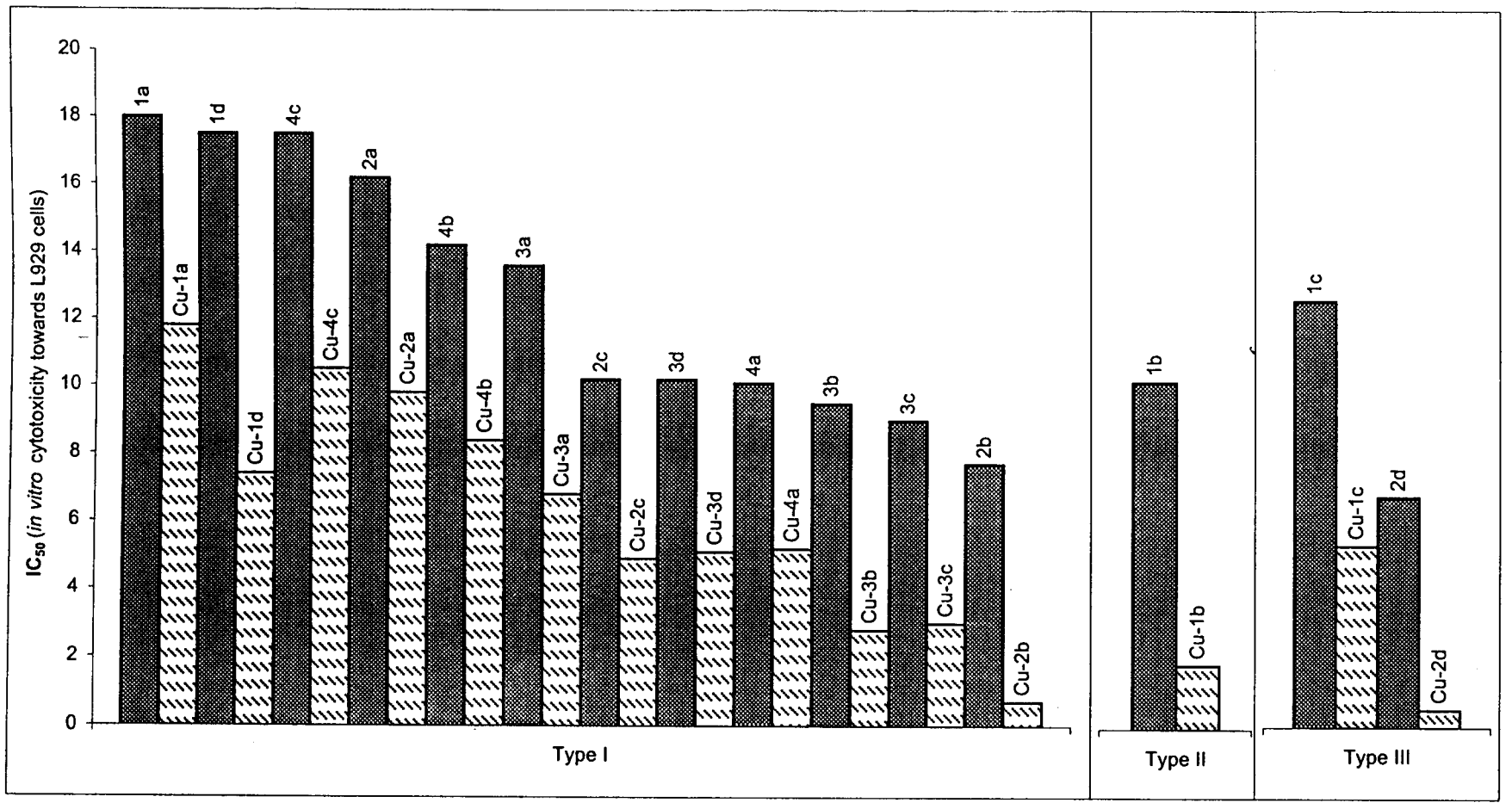


Fig. 3 Concentration needed for 50% cell death (*in vitro* cytotoxicity towards L929 cells) of the compounds

Increase in life span of tumour bearing mice by the administration of the compounds

Inorder to test the efficiency of these compounds as a successful drug against cancer, the percentage increase in life span of tumour bearing mice, administred with the curcuminoid analogues and their copper complexes were found out as discussed earlier (**figure 4**). The data clearly suggest that all the compounds were effective in increasing the life span of tumour bearing mices and copper complexes were many fold efficient in this regard. Among the curcuminoid analogues tested, **2d** and **2c** were more effective in increasing the life span of tumour bearing mice, compared to **3c** (synthetic analogue of natural curcumin III). Copper complexes of **2d**, **2b** and **3c** are highly efficient than their uncomplexed counterparts.

The present study confirm that 1,7-diarylheptanoids (curcuminoid analogues) are highly active in both *in vitro* and *in vivo* antitumour studies. The activity mainly depends on the degree of conjugation and on the presence of certain functional groups such as OH at suitable position on the aryl ring. Copper complexes of these compounds are found to increase significantly the life span of tumour bearing mices. Increase in life span of tumour bearing mice is in the order:

2d > 2b > 4a > 3b > 4b > 3c > 4c > 1b > 3d > 2c > 2a > 3a > 1c > 1d>1a

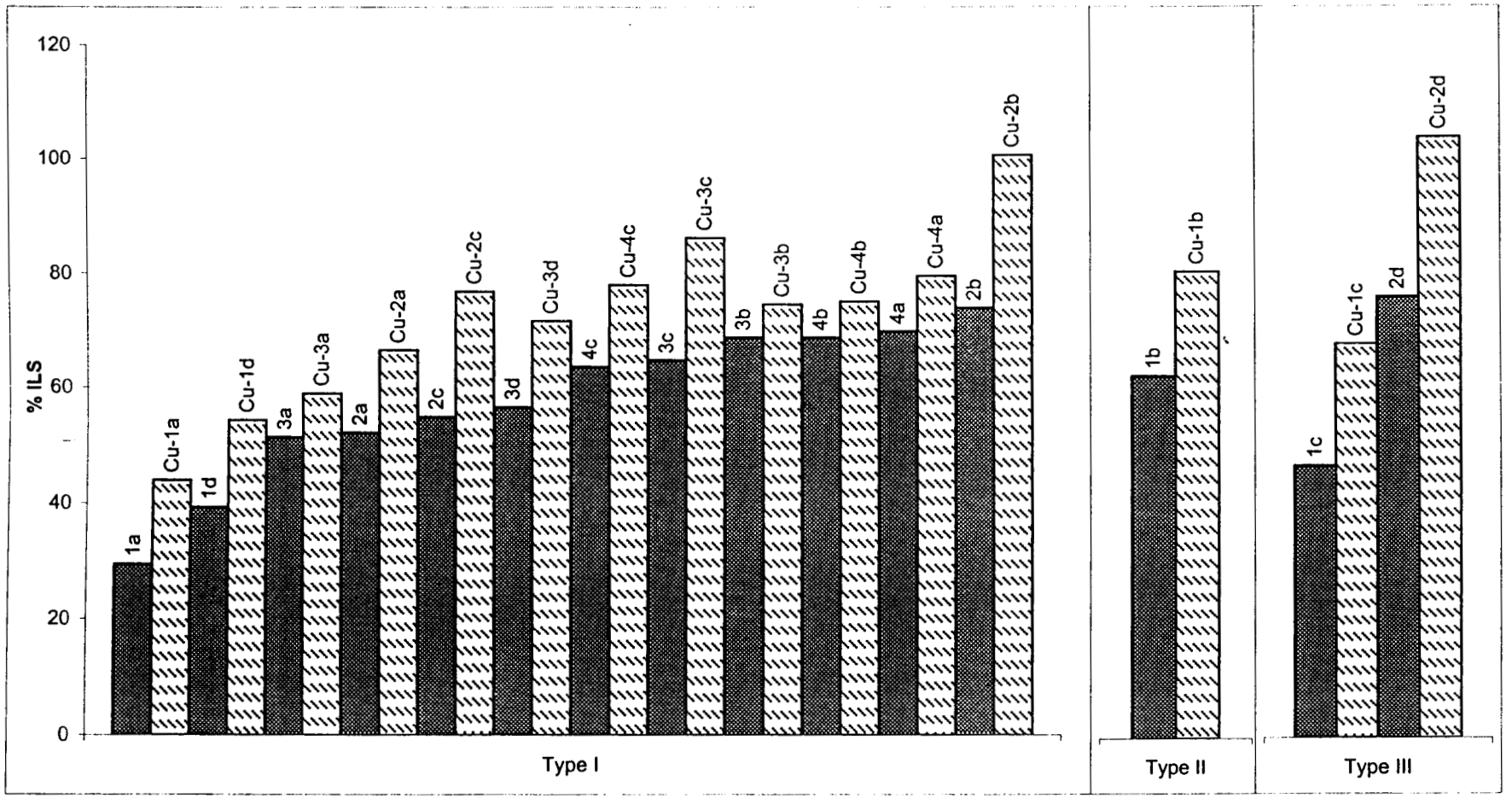


Fig. 4 Percentage increase in the life span of tumour bearing mice by the administration of the compounds

The same order follows in the case of copper complexes with much higher percentage increase in life span. Thus it can be seen that substitution on the aryl rings and the degree of conjugation are the major factors that determine the antitumour activity of the curcuminoids.

Effect of compounds on solid tumour development

The effects of administration of curcuminoid analogues and their copper complexes on solid tumour volume in mices (on day-31 compared to control) were studied. The results are given in **figure 5**. All the compounds produced significant reduction of solid tumour volume in mices ($P < 0.001$ from normal) on day-31. Tumour volumes (on day-31) were in the following order by the administration of curcuminoid analogues.

2d < 2b < 3c < 3b < 2c < 1b < 1c < 2a < 4a < 3d < 4b < 1d < 3a <4c <1a

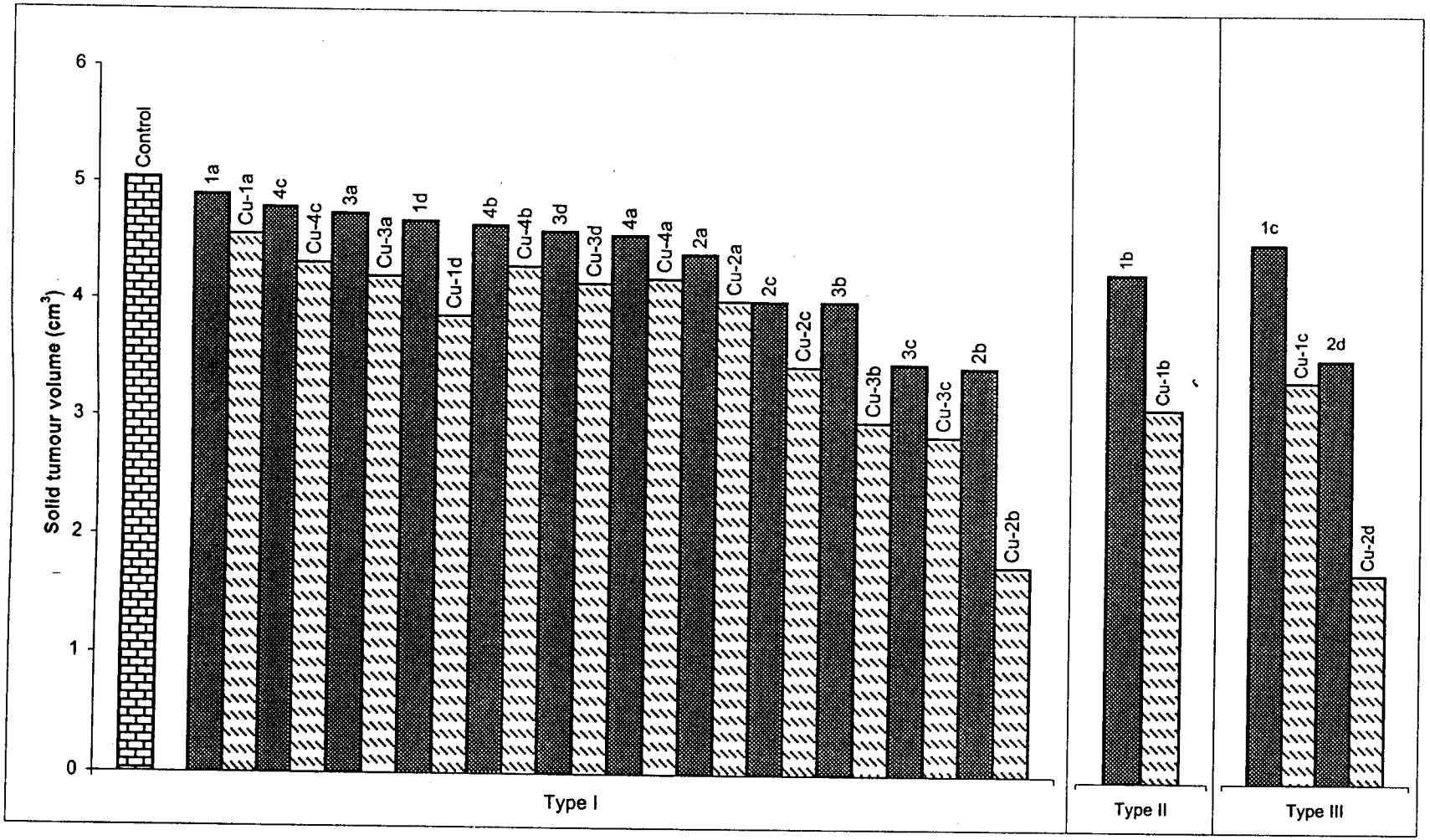


Fig. 5 Solid tumour volume of compounds on day - 31 with respect to control

Compared to curcuminoid analogues, their respective copper complexes are more efficient in reducing the tumour volume in mices. The tumour volume in mices by the administration of copper complex of **2d** (which is the most active compound) was found to be 1.67cm^3 which is nearly half that of the uncomplexed **2d**. The copper complexes follow almost the same order of activity as that of the curcuminoid analogues indicating that complexation with copper, aryl substitution and extented conjugation have profound effect on the activity of the compound.

The compounds **2d** and **2b** containing OH groups at *ortho* position with respect to the olefinic linkage and their copper complexes are more active. In metal complexes, the aryl OH group remain free and is not involved in bonding with the metal ion. From the foregoing, it can confidently be stated that free OH groups in the aryl rings is a structural requirement for the antitumour activity of 1,7-diarylheptanoids and related curcuminoid analogues.

PART IV

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