STUDIES ON THE RESIDUE, DEGRADATION AND CHEMISTRY OF SOME SYNTHETIC AND NATURAL PESTICIDES

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IN
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BIDHAN CHANDRA KRISHI VISWAVIDYALAYA

BY

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BIDHAN CHANDRA KRISHI VISWAVIDYALAYA
WEST BENGAL, INDIA
MAY, 1988

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This is to certify that the work recorded in the thesis entitled "**STUDIES ON THE RESIDUE, DEGRADATION AND CHEMISTRY OF SOME SYNTHETIC AND NATURAL PESTICIDES**" submitted by Sri Pratip Kumar Haldar for the award of the Degree of Doctor of Philosophy in Agricultural Chemistry of the Bidhan Chandra Krishi Viswavidyalaya, is the faithful and bonafide research work carried out under our supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

(A. Chowdhury)
Co-Supervisor

(N. Adityachaudhury)
Supervisor

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Dated : Kalyani,
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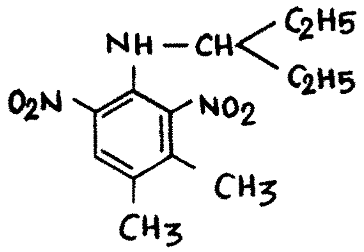
PREFACE

The widespread use of pesticides has now become an established practice in the control of pests attacking our cultivated plants and domestic animals. It is difficult to deny that serious consequences have followed due to the extended use of several pesticides. Pesticides, by their very nature, are toxic not only to pest species but to predaceous and parasitic insects and mites and to higher animals, including man. An even more complicated problem has now appeared and this concerns with the highly persistent pesticides. Many of these, notably the chlorinated hydrocarbons (DDT, BHC, etc.), are notorious for their persistence in the environment. The chlorinated hydrocarbons can, therefore, contaminate a substantial part of the biosphere, viz., air, water, soil, plants, animals, etc., by being transported from one system to another. These persistent pesticides occurring in biotic and abiotic systems could again be transferred to the diet of wild animals, feed stuff and fodder meant for farm animals, human food, etc., and finally to the bodies of human beings. Similarly, some herbicides may persist in soil, plant or in both after application and their prolonged persistence may cause serious injury to rotational crops. Besides these, the occurrence of pesticides in soil is also hazardous from the point of view of microbial flora and fauna.

Pendimethalin (I), a recent member of dinitroaniline herbicides, is a new introduction in the Indian Sub-continent and is reported to possess selective weed control properties against a number of crops (cotton, soybeans, maize, wheat, rice, groundnut). No detailed systematic research work on the persistence of pendimethalin in soil has been carried out under Indian climatic conditions. It has therefore been thought worthwhile to undertake a systematic study on the dissipation pattern



(I)

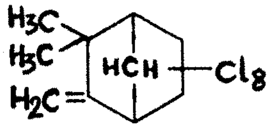
of pendimethalin under both laboratory and field conditions. However, it must be well known to all of us that residue data alone are not enough as it does not give the total picture necessary for judging the environmental toxicity of a pesticide. Thus, our knowledge will remain incomplete without a more detailed information about the metabolites and degradation products formed from the pesticides under both abiotic and biotic conditions.

In this investigation,

(An attempt has) therefore (been made to study the photodegradation behaviour of pendimethalin under laboratory condition by exposure to sunlight for identifying the photodegraded products as well as to isolate the photometabolites found in soil after application of pendimethalin to soil and subjecting it to sunlight exposure.)

Water, as we know, is the main means of transporting pesticides in the environment. In India, the Ganga river serves as one of the richest sources of water. Among the various polluting sources, pesticide run-off from agricultural and municipal uses through river linked water bodies plays a significant role in the accumulation of pesticide residues in river water and sediments. It is now well documented that the chlorinated insecticides like BHC, DDT, Aldrin, Dieldrin, Endosulfan are very persistent in nature and these may, therefore,pose potential hazards to the ecosystem as well as affect seriously the water quality.

It has been reported that dieldrin and DDT at a level of 10^{-5} mg/l in water is undersirable for long time consumption by wild life[1]. DDT and Endosulfan are also toxic to fresh water fish. The ability of toxaphene (II) to accumulate in water-food chains is especially great and the degradation of toxaphene occurs very slowly. As for example, its content in water deminished insignificantly during six years. In fact, the current level of chlorinated pesticides like BHC, DDT,Endosulfan, etc. in the Ganga waters has not been assessed in West Bengal. (It)was, therefore,(has also been thought worthwhile to study the residue levels of organochlorines like BHC, DDT, Endosulfan and its isomers/metabolites occurring in Ganga waters.)



(II)

A brief résumé of the work embodied in Part-I of this thesis is presented below.

Studies on the dissipation and degradation of pendimethalin in soil

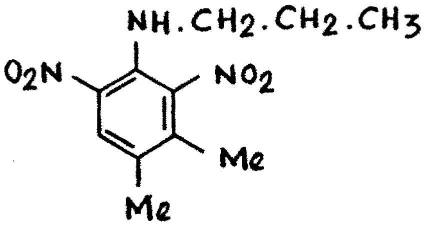
Dissipation study of pendimethalin was carried out with Kalyani soil under laboratory and field conditions following application of pendimethalin in two treatment doses (T_1 = 1 kg ai ha^{-1}; T_2 = 2 kg ai ha^{-1}). Both types of study revealed that no residue could be detected in soil 45 days after application of pendimethalin.

With a view to understanding the photodegradation behaviour, pendimethalin dissolved in different solvents was exposed to sunlight for 80 hours. After usual work up, the solutions of pendimethalin were subjected to thin layer as well as gas chromatography for detection of photodegradation products. n-Hexane solution of pendimethalin after 80 hours of irradiation in sunlight followed by preparative t.l.c. furnished an orange solid which was later proved to be a new photometabolite of pendimethalin. This compound is being designated as compound-A, the structure elucidation of which is outlined below.

Compound-A crystallised from hexane-benzene as pale orange crystals, m.p. 132°, and analysed for $C_{11}H_{15}O_4N_3$ (M^+ 253).

The IR spectrum revealed the presence of a secondary amino group at 3400 cm^{-1} along with other absorptions (3480, 1640, 1980, 1560 to 1310, 1260, 1190 and 900 to 750 cm^{-1}). The NMR spectrum disclosed the presence of a $-CH_3$ group (3H singlet at 1.5δ), a secondary aromatic amino proton of one proton intensity at 6.85δ (multiplet) and a sharp singlet (S) at 8.2δ confirming the presence of an aromatic proton. The appearance of a 10H-singlet at 2.2δ could be explained by the presence of two aromatic $-CH_3$ groups along with four $-CH_2-CH_2$ protons.

The mass spectrum of compound-A showed a molecular ion peak at m/z 253 (M^+) along with a number of other peaks. The fragment at m/z 211 could arise by loss of 42 m.u. ($M^+-CH-CH_2-CH_3$). The base peak at m/z 194 might arise by loss of 17 m.u. from the peak at m/z 211 which was then stabilized as the dinitromethyl cyclopenta dienyl radical (V). Based on the above spectral evidence, the structure of compound-A has been settled as (VI).



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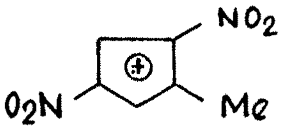


(V)

Another photometabolite could also be detected following irradiation of aqueous solution of pendimethalin. The latter crystallised from pet.ether-benzene (1:1) as red needles, m.p. 242° and is designated as compound-B. The spectral studies could not be done due to paucity of pure compound-B.

In order to see the photodegradation behaviour of pendimethalin in soil, a laboratory study was also made with Kalyani soil. Pendimethalin solutions were spotted uniformly over each soil plates and exposed to sunlight for 7, 15 and 30 days. After usual work up the concentrated solutions were subjected to thin layer chromatography and gas chromatography. Both compound-A (Rf 0.4), compound-B (Rf 0.15) along with an unknown compound (Rf 0.25) could be observed in the soil plates of 30 days exposure. The gas chromatography of soil extracts after exposure of soil plates for different days revealed that the photodegradation of pendimethalin was faster as time elapsed and compound-B was found to be the major product followed by compound-A. A plausible pathway of photodegradation of pendimethalin has also been suggested.

Studies on the residues of some pesticides occurring in Ganga waters

(With a view to monitoring the level of organochlorine pesticides in Ganga water, samples were collected from the eastern bank starting from Kalyani (Dist. Nadia) to Jagaddal (Dist. 24-Parganas) at a stretch of about 25 km. Again water samples from the western bank were collected from Kunti-Ghat

(Mogra-Chinsurah block) to Chandannagar (Dist. Hooghly) at a stretch of about 25 km. In addition to these, water samples were also collected from canals linking the Ganga river from both eastern and western banks.

The water samples thus collected from different pockets of the Ganga basin were analysed for BHC, endosulfan and DDT. n-Hexane (distilled) was used as partitioning solvent for extraction of these pesticides from water samples,) The extracts thus obtained after usual clean up were analysed by gas chromatography. Recovery study of BHC, DDT and endosulfan in water was quite satisfactory and the method could be adopted for the determination of BHC, DDT and endosulfan in water.

The concentration of BHC-isomers (α-, β-, γ- and δ-) occurring in linked canals as determined by gas chromatography revealed that the concentration of total BHC (0.0097 ppm) was found in the Gosai canal in Chandannagar block. The highest concentration of γ-isomer (0.004 ppm) was found in Benipur and Chandrahati canal in Mogra-Chinsurah block. In Ganga water, the highest amount of α-isomer as well as the total BHC could be found in water samples collected from Kankinara Jute Mill pocket. The β-isomer was found at the highest level in water samples collected from Khasbati, the eastern bank of Ganga. Water samples collected from the eastern bank i.e., 200 mts away and opposite to Kunti Ghat showed the highest concentration of γ-isomer. The highest concentration of δ-isomer could be detected in the water samples collected from Ramprasad Ghat, Halisahar.

The residues of DDT isomers/metabolites occurring in linked canals were found in two out of 12 samples. The highest amount of op', op' as well as total DDT could be detected in the Ganga water samples near Bandel brick fields. This study further indicated that the highest amount op'-DDT was found in water samples near Hooghly Imam Bara.

The endosulfan residues could be detected in 5 samples out of the 36 collected from selected pockets of the Ganga river. α-Endosulfan could be found in all five samples whereas β-endosulfan was only found in one sample. Endosulfan cyclic sulphate was not found in any one of the analysed samples.

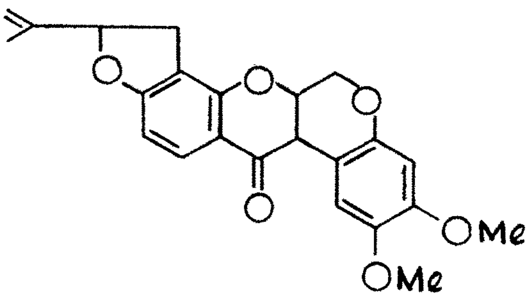
Thus, (this study revealed that a systematic monitoring of the organochlorinated residues (BHC, DDT, Endosulfan) is very much needed throughout the year with the prime objective of assessing the level of such persistent organochlorinated pesticides in Ganga water.)

The complicated problem of pest and pest control is causing a lot of re-thinking among scientists all over. The potential hazards of synthetic insecticides to men, plants and wild life, toxicity in the form of residue, resistance of pests, have all compelled the scientists to find out new chemicals for pest control which are effective, lasting and without hazards

It is well known that insecticides and fungitoxic substances derived from plant sources are practically free from the harmful effects of the synthetics. A number of higher plants belonging to different plant families are known to possess insecticidal, fungicidal, piscicidal, insect-repellent, insect-attactant and medicinal properties.

Among the botanical insecticides, rotenone (III) has been found to be one of the reputed insecticides. Over the years, synthetic studies on rotenoids and their derivatives have been intensively carried out by a number of chemists all over the world.

Recently a compound possessing modified isoflavanoid system could be isolated from the plant Tephrosia candida (Fam : Leguminosae) and its structure was established as dehydrodihydrorotenone (IV) from spectral evidence[2]. Finally, its structure was confirmed by its partial synthesis from rotenone. The synthesis of dehydrodihydrorotenone (IV) involved catalytic hydrogenation of rotenone as one of the major steps and dihydrorotenone (VI) could only be obtained as the hydrogenated product



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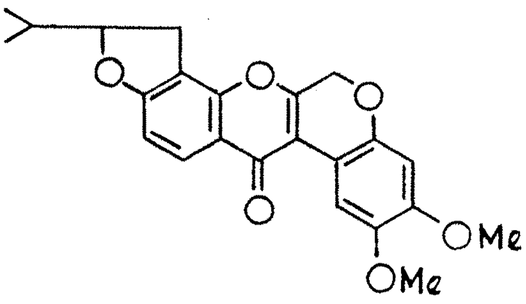


(IV)

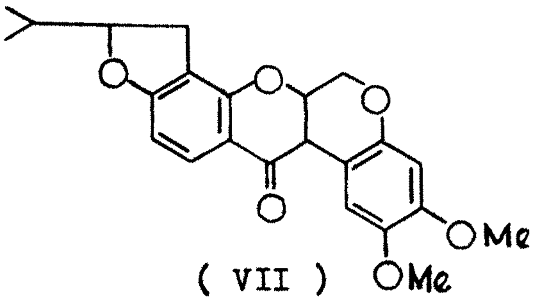
of rotenone. A survey of literature on the hydrogenation of rotenone using different catalysts and solvents prompted the present investigator to undertake further hydrogenation with rotenone for isolation and characterisation of the minor hydrogenated products.

Under a research programme on naturally occurring oxygen heterocycles, we became interested to undertake phytochemical investigation on some flavonoid-bearing plants. A close scrutiny of literature survey revealed that a limited number of chalcones substituted at various positions in A-rings are found in some *Didymocarpus* species belonging to *Gesneriaceae* family. Interestingly, *Didymocarpus pedicellata* and *Didymocarpus aurentica* elaborate phloroglucinol derived chalcones and flavanones. Very recently phytochemical investigation on yet another *Didymocarpus* species *viz.*, *D.podocarpa* furnished 7,8-epoxy-5, 6-dehydro kawain[3] from the petrol ether extract of this plant. These information prompted the present investigator to undertake further phytochemical investigation on *D.podocarpa* (Fam : *Gesneriacea*) for isolating oxygen heterocycles possessing biological properties.

A brief résumé of the work embodied (in Part-II of this thesis) is presented below.

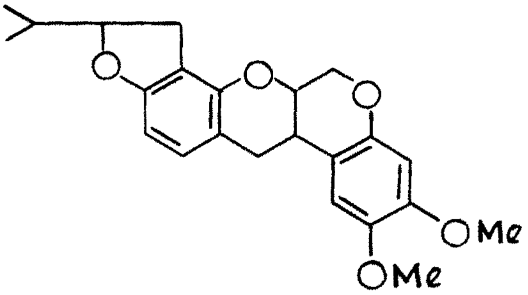
Studies on the catalytic hydrogenation of rotenone in different solvent systems

(Catalytic hydrogenation of rotenone with different catalysts using various organic solvents was carried out with the idea of identifying the major and minor products. Hydrogenation of rotenone in ethyl alcohol in presence of Pd/C catalyst followed by chromatography furnished dihydrorotenone (VII) and two minor products which were designated as compound-A and compound-B. These two minor products, to our knowledge, have not been previously reported from rotenone. The structure elucidation of these two new hydrogenated products of rotenone is outlined below.


(VII)

(a) Compound-A crystallised from ethanol as colorless needles, m.p. 157°. Mass spectrometrically derived molecular weight (M^+ 382) established its molecular formula as $C_{23}H_{26}O_5$. The UV spectrum $[\lambda_{max}^{EtOH}$ 227, 288 $]$ spoke in favour of a rotenoid system. The IR spectrum of compound-A (2980, 1610, 1510, 1470, 1315, 1230 to 880) revealed the absence of a $>C = O$ group when compared with the IR spectra of other rotenoids. The NMR spectrum disclosed the presence of two methoxyl groups (3.76δ S, 6H). The appearance of two doublets at δ6.36 (J = 7Hz) and 6.76(J=7Hz) provided ample evidence for the presence of two *ortho*-protons in compound-A. The signals at 4.26 δ (2*H*, J = 7Hz) could be readily assigned to the C-12 methylene protons of a rotenoid system which additionally proved the absence of a keto functionality at C-12. The peaks at δ 6.3 (*S*, 1*H*) and δ 6.64 (*S*, 1*H*) were assignable to the protons at C_4 and C_1 (aromatic protons) of the A-ring of rotenoid skeleton. A multiplet centred at 4.62 δ could be attributed to the C_6-methylene protons of B-ring. The signals at δ 0.92 (*S*, 3*H*), δ 0.98 (*S*, 3H) for two *gem* $-C\left\langle\begin{matrix}CH_3\\CH_3\end{matrix}\right.$, and δ 2.62 (*t*, 2*H*), δ 3.14 (*m*, 2*H*) for four methylene protons provided evidence for the presence a γ-, γ -dimethyl pyran system.

The mass spectrum of compound-A showed, in addition to the molecular ion peak at m/z 382 (M^+), some diagnostic peaks. The appearance of two peaks at m/z 192 (base peak) and at m/z 191 could be accounted for the RDA fragments. These facts further provided evidence that compound-A was devoid of a keto group. The peak at m/z 177 could arise due to loss of a methyl group from the peak at m/z 192. The loss of CO from the ion fragment at m/z 177 might give rise to a peak at m/z 149. Based on these facts, it was quite logical to represent the structure of compound-A as either (VIII) or (IX).



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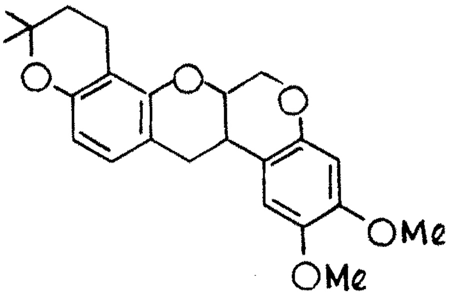
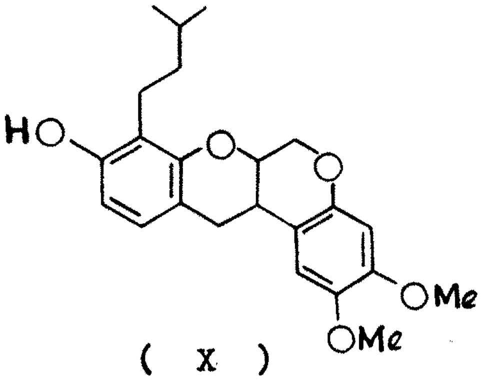


(IX)

Compound-A gave no characteristic signals of methine proton in the NMR spectrum which confirmed that compound-A possessed the proposed structure (IX). A plausible mechanism for the formation of compound-A from rotenone has also been suggested.

(b) The light brown crystals (compound-B), m.p. 143°(pet.ether: chloroform) analysed for the molecular formula $C_{23}H_{28}O_5$ (M^+ 384). The UV spectrum of this compound [λ_{max}^{EtOH} 237, 287, 300]. spoke in favour of a rotenoid system[4]. The IR spectrum (3400, 2960, 1610, 1520, 1410, 1360 to 1050, 870 to 810 and 800 to 550 cm^{-1}) revealed the presence of a phenolic -OH group and the absence of keto group from the rotenoid system. From a careful analysis of the NMR spectrum of compound-B, it was evident that compound-B contained two methoxyl groups (3.8 δ ,S, 6H). The peaks at 6.36δ(J = 7 Hz) and 6.8 δ (J = 7 Hz) provided evidence for the presence of two ortho-protons. The appearance of a peak at 4.2 δ (J = 7 Hz) could be attributed to the two methylene protons at C-12 position of the rotenoid system. The signals at 6.6 δ (S, 1H) and 6.4 δ (S, 1H) could be assigned respectively to C_1-H and C_4-H of the A-ring of a rotenoid system. The appearance of a double-doublet at 4.68 δ suggested the presence of two methylene protons attributable to the C-6 position of the B-ring. The signals at 0.92 δ (S, 2H) and 1.06 δ (s, 3H) for two methyl groups, 3.24 δ (m, 2H) for two methylene protons, 1.92 δ (sextet, 2H) for two central methylene protons and 2.76 δ (t, 1H) for a methine proton provided evidence for presence of an isopentyl side chain in ring-D.

The mass spectrum of compound-B showed, in addition to the molecular ion peak at m/z 384 (M^+), some more diagnostic peaks. The appearance of two prominent peaks at m/z 192 (base peak) and m/z 191 could be accounted for the RDA fragments. The peak at m/z 177 arose due to loss of a methyl group from the ion peak at m/z 192. The peak at m/z 149 might arise due to loss of CO from the ion fragment at m/z 177. The peak at m/z 136 resulted by the expulsion of an isobutyl group from the peak at m/z 193. Based on these facts, the structure of compound-B was settled as (X). A plausible mechanism for the formation of compound-B has also been suggested.



(X)

Chemical investigation of *Didymocarpus podocarpa*

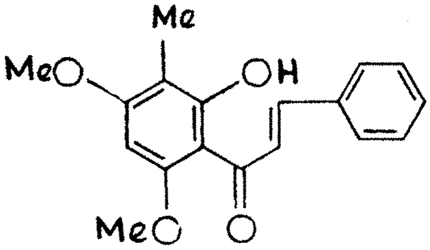
Extraction of the whole plant of *D.podocarpa* with petroleum ether (b.p. 60–80°) followed by chromatography of the concentrated petrol extract furnished an orange coloured crystalline compound, designated as compound-X.

Compound-X, m.p. 141°, crystallising from petroleum ether-benzene as orange plates analysed for $C_{18}H_{18}O_4$ (M^+ 298). It gave a brown colour with ferric chloride. Functional group analysis revealed the presence of two $-OCH_3$ (two 3*H*-singlets at 3.95δ and 4.0 δ), one aromatic $-CH_3$ (3*H*-singlet at 2.06 δ) a conjugated $>C=O$ (ν_{max}^{KBr} 1615 cm^{-1}), a chelated OH (ν_{max}^{KBr} 3200 cm^{-1}, 1-*H* singlet at 14.06δ , exchangeable with D_2O) and a complex aromatic substitution pattern (ν_{max}^{KBr} 1600, 1550, 1125, 790, 745 cm^{-1}) with a monosubstituted benzene ring (ν_{max}^{KBr} 700 cm^{-1}).

The NMR spectrum of compound-X showed two *trans*-olefinic protons at 7.7δ as a 2H-singlet providing evidence for a chalcone system[5].

The appearance of a sharp 1*H*-singlet and a 5*H*-multiplet at 6.03δ and 7.43δ respectively revealed that the A-ring of compound-X possessed one aromatic proton and the B-ring was unsubstituted. The mass spectrum of compound-X showed peaks characteristics of chalcones . The unsubstituted nature of the B-ring of compound-X was confirmed by the appearance of two prominent peaks at m/z 221 (M^+-77; M^+-C_6H_5) and m/z 195 (M^+-103,

$M^{+}-C_6H_5-CH{=}\overset{+}{C}H$) in the mass spectrum. It could also be understood that all the substituents were present in the A-ring. The UV spectrum [λ_{max}^{EtOH} 343 nm (log ϵ 3.8), $\lambda_{max}^{NaOAc + EtOH}$ 344 nm] favoured a phloroglucinol substitution pattern in the A-ring and an unsubstituted B-ring. Thus all these spectral evidence indicated that compound-X might be 4',6'-dimethoxy-3'-methyl-2'-hydroxy-chalcone (XI). It was then found to be identical with aurentiacin (X) in all respects[6] [m.p., m.m.p., Co.TLC and superimposable IR].



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The thesis entitled "Studies on the residue, degradation and chemistry of some synthetic and natural pesticides" consists of the following parts.

Part - I	:	Studies on the residue, degradation and chemistry of some synthetic pesticides.
Part - II	:	Studies on the chemistry of some natural pesticides.

CONTENTS

LIST OF TABLES

TABLE PARTICULARS PAGE

Part - I

Part - II

LIST OF FIGURES

Part - I

Part - II

LIST OF SCHEMES

Part -I

Part - II

LIST OF CHARTS

Part - I

Studies on the residue, degradation and chemistry of some synthetic pesticides

CHAPTER - I

Studies on the dissipation and degradation of Pendimethalin in soil

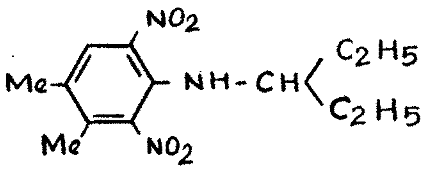
SECTION - A

INTRODUCTION

Among the numerous plant protection chemicals, herbicides are the fastest growing class employed to control the competitive interference of weeds in crop productivity. India had a modest beginning in herbicides and the use of herbicides is becoming popular day by day. Herbicide consumption in India has been increased from 15 M.T. in 1970 to 1520 M.T. in 1984. Among the major agricultural crops about 10% of wheat are lost due to weeds in the world. Reduction in yield due to weeds has been roughly estimated as 32-35% in cereals, pulses and oil seeds in our country[1].

Herbicides whether applied to soil or in plant are ultimately distributed in desirable plants, soil, water and other components of the environment and may persist as toxic residues. Since herbicides are toxic by their very nature, the widespread and indiscriminate uses may contribute a potential hazard to our environment. Research recommendations on chemical control of weeds are considered as incomplete if data on toxic residues in herbicides are not provided.

There are many classes of herbicides in the market to-day amongst which the class of dinitroanilines is of recent origin[2]. The dinitroanilines are used as soil applied, pre-emergent herbicide in selective weed control in various crops, mainly against grasses. Pendimethalin (I), a recent member of dinitroaniline herbicide is a new introduction in the Indian subcontinent. The herbicidal property of this compound was first evaluated by P.L.Sprankle (1974) and reported to have the selective weed control properties against a number of crops, viz., cotton, soybeans, maize, wheat, rice, groundnut and also to control suckers in tobacco. Although pendimethalin is stable under alkaline and acidic conditions[3] but so far no detailed systematic research work on the persistence of pendimethalin in soil has been carried out pertaining to Indian climatic conditions. Therefore, it was thought worthwhile to undertake a systematic study on the dissipation pattern of pendimethalin under laboratory and field conditions following application of pendimethalin at the recommended (1 kg ai/hec) and twice (2 kg ai/hec) the recommended doses. An attempt has further been made to correlate the dissipation patterns of pendimethalin under both laboratory and field conditions.



(I)

Although studies on the persistence of applied pesticides are of primary importance, our knowledge will remain incomplete until the environmental transformation products of pesticides are identified. A quick decrease of the residues of active principle itself is not yet a gurantee for the safety of herbicide. Knowledge about the dynamic of the active compound is also necessary for hygenic and toxicological evaluation of metabolites can not be made without knowledge of their origin and their metabolic degradation. It is very well known that some of these transformation products are more toxic than the original pesticides. For example, urea herbicides give rise to azo compounds on soil surface by the action of light which are more harmful than the residues of the original pesticides[4].

It is now recognized that amongst the physical forces, sunlight is the most powerful agency, especially in tropical countries such as ours in degrading and transforming pesticides. Information on the various transformations suffered by pesticides by the agency of light after being released in the environment is of vital importance to those seriously concerned with questions of environmental quality and public health as well as important for understanding the optimum conditions of pesticide use.

It is now well documented that dinitroaniline class of herbicides has been shown to undergo drastic changes upon exposure to ultraviolet light and artificial or natural sunlight[5] through the processes of N-dealkylation, reduction of nitro groups, etc. Therefore it is quite resonable to assume that pendimethalin, a representative member of dinitroaniline class of herbicides, is expected to undergo photochemical changes. With these objectives in view an attempt has been made in the present investigation to study the photodegradation behaviour of pendimethalin under laboratory condition by exposure to sunlight with a view to identifying the photodegraded products formed. An attempt has further been made to isolate the photometabolites found in soil after application of pendimethalin to soil and subjecting it to sunlight exposure.

Since very little work has so far been carried out on the dissipation, persistence, residual analysis and degradation studies of pendimethalin in soil systems with field crops, therefore an attempt has been made to present a review of literature pertaining to the types of dinitroaniline herbicides so far reported.

SECTION - B

REVIEW OF LITERATURE

B.1 Types of dinitroaniline herbicides

Table-1.1. List of dinitroaniline herbicides

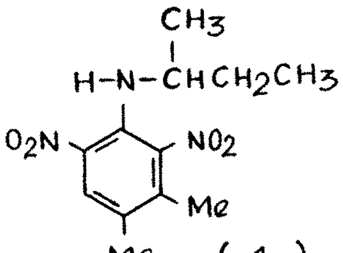
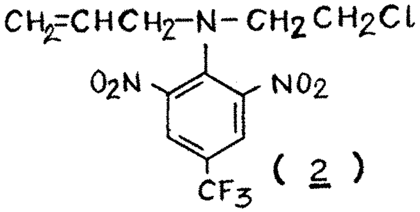
Common, code or trade name(s)	Chemical name	Structure	Water solubility (ppm)	Vapour pressure mm Hg (at °C)
AC-92390	N-sec butyl-2,6-dinitro-3,4-xylidine	 (1)	0.5	-
BAS 3870-H	N-allyl-N-(2-chloroethyl)-2,6-dinitro-4-(trifluoromethyl) aniline	 (2)	0.003	4.0×10^{-5} (30 C)

Table-1.1 contd.

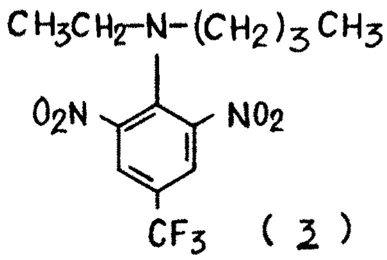
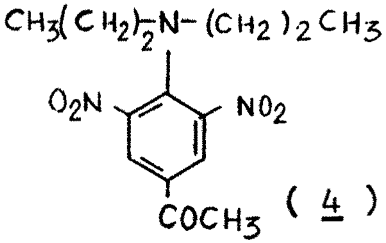
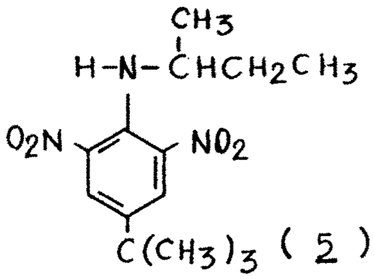
Common, code or trade name(s)	Chemical name	Structure	Water solubility (ppm)	Vapour pressure mm H_g (at °C)
Benefin (Balan)	N-butyl-N-ethyl-α, α-, α -trifluro-2,6-dinitro-p-toluidine	 (3)	0.1	3.81×10^{-5}
Bulab-37 (Buban 37)	3',5'-dinitro-4'-(di-propyl-amino) acetophenone)	 (4)	-	-
Butralin (Amex, A-820)	4-(1,1-dimethylethyl)-N-(1-methyl-propyl)-2, 6-dinitrobenzonamine	 (5)	1.0	Essentially non volatile (50°C)

Table-1.1 contd.

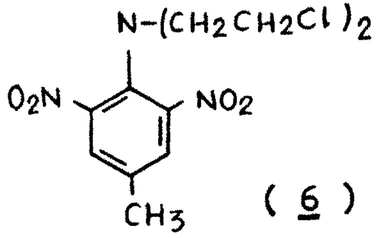
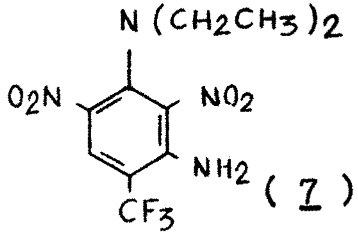
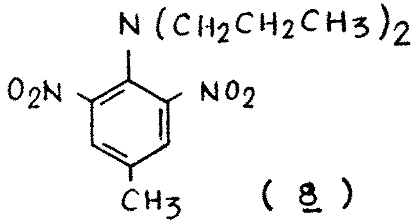
Common, code or trade name(s)	Chemical name	Structure	Water solubility (ppm)	Vapour pressure mm Hg (at °C)
Chlornidine (Torpedo, AN-56477)	N,N-bis(2-chloroethyl)-4-methyl 2,6-dinitroamine	 (6)	0.08	4.3×10^{-6} (25 °C)
Dinitramine (Cobex)	N^4,N^4-diethyl-α-, α-, α-trifluro-3,5-dinitrotoluene-2,4-diamine	 (7)	1	3.6×10^{-6} (25 °C)
Dipropalin	2,6-dinitro-N,N-dipropyl-p-toluidine	 (8)	-	-

Table-1.1 contd.

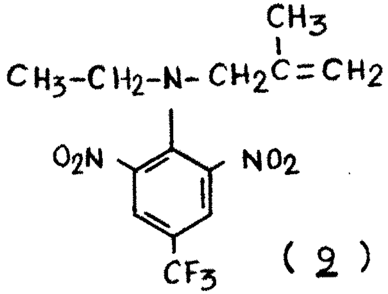
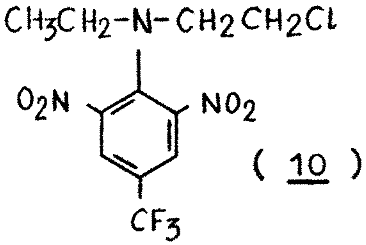
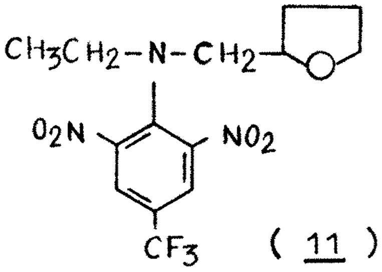
Common,code or trade name(s)	Chemical name	Structure	Water solubility(ppm)	Vapour pressure mm H_9 (at °C)
Ethalfluralin (Sonalan (EL-161)	N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluromethyl)-benzenamine	 (9)	0.21 (pH 7)	8.2×10^{-5} (25 °C)
Fluchlordin Basalin, (BAS 392-H, BAS 3921-H, BAS 3924-H)	N-(2-chlorethyl)-2,6-dinitro-N-propyl-4-(trifluromethyl) aniline	 (10)	0.001	2.5×10^{-5} (25 °C)
GS-38946 (CGA-11607)	N-ethyl-N-tetrahydrofurfuryl-4-trifluoromethyl-2,6-dinitroaniline	 (11)	$<$ 0.1	(Somewhat more volatile than profluralin)

Table-1.1 contd.

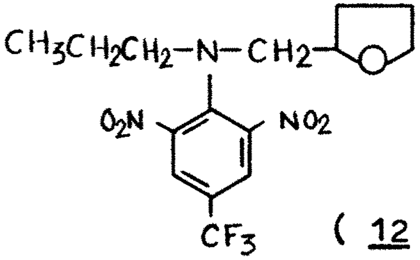
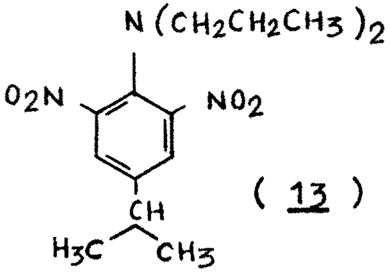
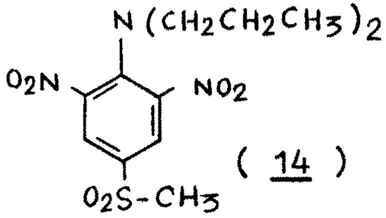
Common, code or trade name(s)	Chemical name	Structure	Water solubility (ppm)	Vapour pressure mm Hg (at °C)
GS-39985 (CGA-14397)	N-propyl-N-tetrahydro-furfuryl-4-trifluoro-methyl-2,6-dinitro-aniline	 (12)	<1.0	-
Isopropalin (Paarlan)	2,6-dinitro-N,N-dipropyl-cumidine	 (13)	0.11	1.45×10^{-5} (30 C)
Nitralin (Planavin)	4-(methylsulfonyl)-2,6-dinitro-N,N-dipropyl-aniline	 (14)	0.6	1.8×10^{-8} (25 C)

Table-1.1 contd.

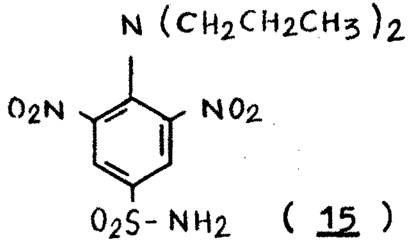
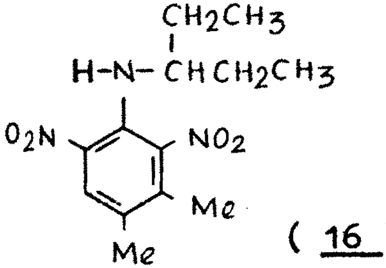
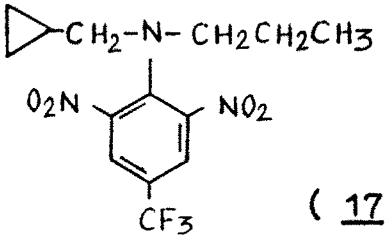
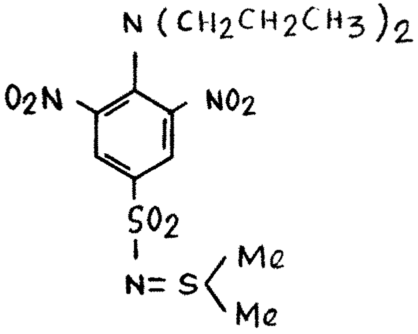
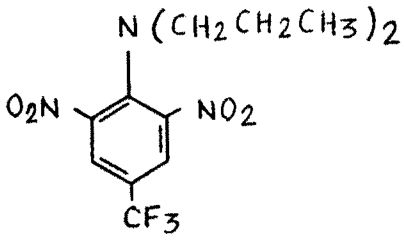
Common,code or trade name(s)	Chemical name	Structure	Water solubility(ppm)	Vapour pressure mm H_9 (at °C)
Oryzalin (Surflan Ryzelan)	3,5-dinitro-N^4,N^4-dipropyl sulfanilamide	 (15)	2.5	1×10^{-7} (30 C)
Penoxalin (Prowl, AC-92553 Pendimethalin)	N-(1-ethylpropyl)-2, 6-dinitro-3,4-xylidine	 (16)	<0.5	3×10^{-5} (25 C)
Profluralin (Tolban, CGA-10832)	N-(cyclopropylmethyl)- α-, α-, α -trifluoro-2, 6-nitro-N-propyl-p-toluidine	 (17)	0.1	6.9×10^{-5} (20 C)

Table-1.1 contd.

Common, code or trade name(s)	Chemical name	Structure	Water solubility (ppm)	Vapour pressure mm H$_9$ (at °C)
Prosulfalin (EL-131)	N-[4-(dipropylamino)-3,5-dinitro-phenyl] sulfonyl)-S,S-dimethylsulfulimine	 (18)	5.6	$< 1 \times 10^{-7}$
Trifluralin (Treflan)	α-, α-, α -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine	 (19)	0.3	1.99×10^{-4} (29.5 C)

B.2 Analytical methods for identification and estimation of dinitroaniline herbicides and related compounds :

The analytical methods applied so far for detection and estimation of pendimethalin involve methanol extraction, partitioning by n-hexane, clean up by florisil in column chromatography, detection by TLC and estimation by Gas Liquid Chromatography.

Probst et al[6] (1967) analysed the trifluralin residues in plant tissues and soil samples, employing methanol extraction. The methanol extracts were partitioned first by methylene chloride and then by n-hexane, benzene and benzene-methanol mixtures. The eluates were identified by TLC and quantified by gas chromatography using an electron capture detector.

Golab et al[7] (1967) have determined the surface residue of trifluralin and its 26 metabolic products in carrot grown in trifluralin treated soil by methanol extraction and partitioning with n-hexane. The various cleaned up extracts were examined by two dimensional TLC systems, coated with silica gel GF, employing the following solvent combinations :

(i) n-hexane-ethylene dichloride (1:1)
(ii) n-hexane-methanol (97:3) and
(iii) benzene-ethyl acetate-acetic acid (50:30:20)

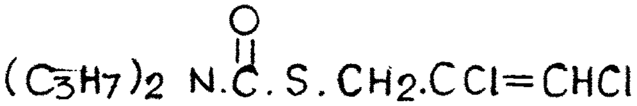
The identified materials were quantified by gas chromatography utilising an electron capture detector.

Crossby and Bowers[8] (1968) described that substituted anilines readily undergo gas chromatography and may be detected quantitatively in amounts down to 50 pg by using an electron capture detector.

Li and Zhang[9] (1980) described a method for determining the rate of dissipation and the mobility of trifluralin in soil. The simple rapid method was developed for GLC determination of trifluralin residues in soil, using an electron capture detector and obviating interference from other pesticides such as BHC; recoveries of 89-106% were obtained from 3 soil types.

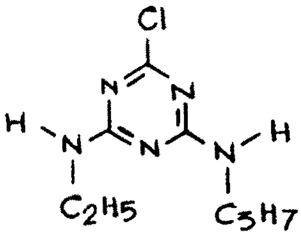
Duseja[10] (1981) studied the dissipation of three dinitroaniline herbicides in soil. The soils were extracted with methanol and to the direct extract of 20 ml methanol/10 g soil 5 ml, <u>iso</u>-octane were added. A Varianaerograph gas chromatograph, model 2440 equipped with electron capture detector was used for analysis.

Deleu and Copin[11] (1982) described a method of separation of dinitroaniline herbicides by capillary gas chromatography. Benfluralin (**3**), butralin (**5**), ethalfluralin (**9**), isopropalin (**13**), nitralin (**14**), oryzalin (**15**), pendimethalin (**16**) were analysed on glass column coated with and 18 µm film of SE-52 using H_2 as a carrior gas, N/P thermoionic detection and temperature programming from 80 to 100°C at 0.7°/min then from 110 to 240° at 3.5/min. The detection limit observed as 50 pg for each herbicide except for oryzalin it was found to be 350 pg.



$(C_3H_7)_2\ N.\overset{O}{\overset{\|}{C}}.S.CH_2.CCl{=}CHCl$

(20)



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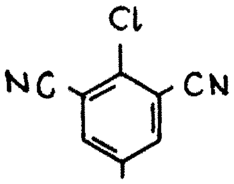

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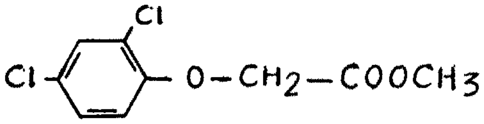

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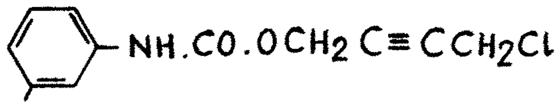

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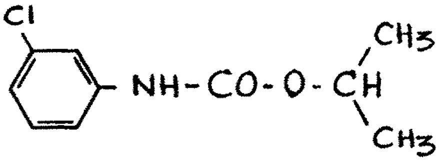

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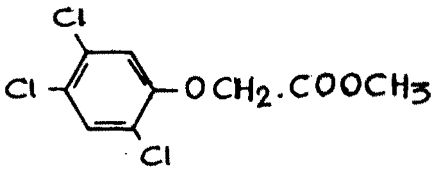

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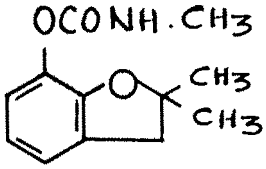

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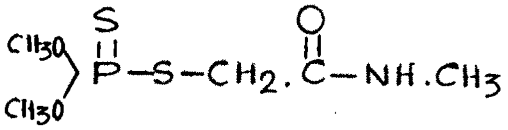

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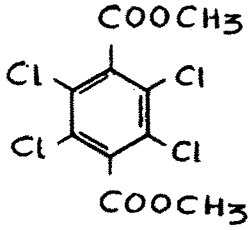

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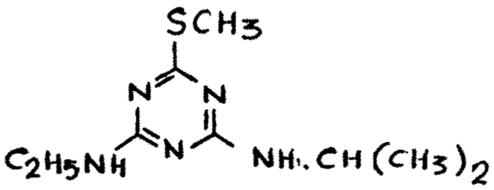

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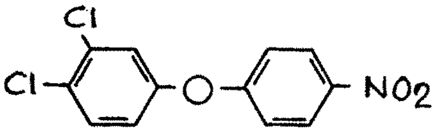

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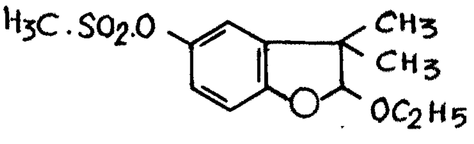

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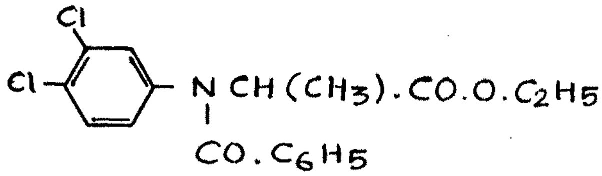

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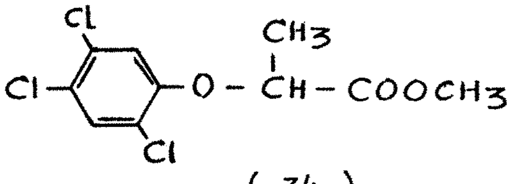


(34)

Fig. 1.1. Structures of some pesticides.

Lee and Chau[12] (1983) have determined the residue of trifluralin (19), di-allate (20), atrazines (21), barban (22), diclofop-methyl and benzoyl-prop.ethyl (31) in natural waters at the level of parts pertrillion. The method involved acidification of sample upto pH $\leqslant 1$ and extraction with CH_2Cl_2. The extract was washed with aq 2% $KHCO_3$, dried and cleaned on a deactivated florisil column and further purified on an activated florisil column. The herbicides were determined by GLC on OV-1, OV-225 or OV-17/OV-210. The detection limits varied from 5 to 100 ng/lt of the herbicide in water.

Mangani and Bruner[13] (1983) have described a method of sample enrichment and G.C.-M.S. determination of herbicides in water. Water containing herbicides were passed through a column of carbotrap and eluted with light petroleum : toluene (2:1) to reach an enrichment factor of C 1000. The herbicides were determined by GLC on a glass column packed with 1.5% SP-2250 + 1.95% SP-2401 on supelcoport (100-120 mesh) and operated at 200°C with N or He as carrior gas at 40 ml/min. for gas chromatography, mass spectrometry (MS) or electron capture detection (ECD). Dichlobenil (23), trifluralin (19), chloropropham (24), 2,4-D methyl (25), 2,4,5-T methyl (26), chlorthal-dimethyl (33) and fenopropa methyl (34) were all separated and determined by E.C.D.

Deleu and Copin[14] (1984) have described a capillary gas chromatographic method, based on Carbo Erba Fractovap 2900 chromatograph, equipped with an electron capture detector and a N-specific detector. A column of CPSIL 19CB (0.9 µm) operated with temp. programming from 60 to 130°C at 40°/min. and then 220° at 6°/min. with H_2as carrior gas was used to analyse a mixture of trifluralin (19), carbofuran (27), and its 3-hydroxy metabolite, dimethoate (28), ametryne (29), ethofumesate (32), pendimethalin (16) and nitrofen (30). The method was applied to drainage water containing 1-80 ng/l of each pesticide.

Stout and Steller[15] (1984) described a GC/MS method involving negative ion chemical ionization for determination of pendimethalin and fostheitan in maize tissue and sulphadimidine in animal tissues. A Finnigan 4023 GC/MS instrument with a column of 3% OV-225 on chromosorb WHP operated at 220° was used to determine pendimethalin. Methane was the carrior gas (15 ml/min.). The MS used CH_4 as reagent gas; negative ions monitored for pendimethalin were at m/e 281 (dwell time 200 ms).

B.3. Persistence and dissipation of dinitroaniline herbicides

The persistence of a pesticidal chemical in crop or in soil may be affected by a large number of factors (Gunther and Blinn, 1955). Among these, the most important factors are : chemical nature of the pesticidal molecule; its physical and chemical organic properties; the crop on which it is used; the soil in which it is applied; climatic condition, rate and method of application, interval between application of the pesticide and harvest, management practices, etc.

Parka and Tepe[16] (1969) analysed the soil samples from 107 locations which were treated with trifluralin (19) for 1, 2, 3 or 4 consecutive years. Analysis of soil samples indicated that there was a steady and continuous decline in the level of trifluralin present in the soil which occurred with time.

Savage and Barrantine[17] (1969) reported that the persistence of α- , α- , α -trifluro-2,6-dinitro-N, N-diproproyl-P-toluidine (trifluralin) was increased by deep incorporation as compared to shallow incorporation of surface application from bioassay and chemical analysis of residual trifluralin.

Harrison and Anderson[18] (1970) developed a simplified soil extraction procedure for the recovery of trifluralin. Recoveries of approximately 90-93% and 64% of the soil incorporated trifluralin were obtained over 7 days and 14 days periods respectively when treated soils were exposed to the atmosphere or maintained under airtight conditions, whereas under UV light trifluralin in methanol or n-hexane degraded into 7 products which were observed in GLC. No degradative product was obtained in dark condition after 30 days.

Hein and Parochetti[19] (1970) have reported the residual herbicidal activity of trifluralin (19), benefin (3) and nitralin (14) as affected by volatility and ultraviolet light. Trifluralin, benfluralin and nitralin were applied to the surface of soil samples at field capacity moisture. The samples were irradiated by fluorescent lamps. Bio assays using sudan grass showed no significant differences between irradiated and non irradiated samples when the containers were covered with quartz glass to prevent volatilisation. Volatilization played major role to significant losses of benfluralin and trifluralin but was not significant for nitralin.

Savage[20] (1973) reported that 4-methyl sulfonyl-2, 6-dinitro-N, N-dipropyl aniline (nitralin) and α-, α-, α -trifluoro 2, 6-dinitro-N, N-dipropyl-p-toluidine (trifluralin) were dissipated to a low residual level within 3-4 months following each application at the rate of 0.84 or 1.68 kg ai/ha. Soil samples were extracted with acetone, filtered and aliquot of the filtrates was analysed by GLC.

Kennedy and Talbert[21] (1974) compared the persistence of dinitroanillines on soil surface by analysisisng spectrophotometrically of a silt loam soil, after 24 hours of surface treatment with microdroplets of dinitroaniline herbicides. Recoveries showed 93-103% for fluchloralin (10) and oryzalin (15) 80-94% for benfluralin (3), dibutalin, nitralin (14) and dinitramine (7) 66 and 51% for trifluralin (19) and profluralin (17) respectively.

Spender and Clirth[22] (1974) studied the volatilisation rate of trifluralin in soil. Trifluralin vaporised more rapidly when surface applied i.e., in wet surface soil than when incorporated into the soil, i.e., into 10 cm deep soil.

Application to a dry soil surface resulted essentially in no volatilisation.

Parochetti et al[23] (1975) studied the volatility of 11 dinitro-aniline herbicides. Herbicides studied were nitralin, oryzalin,benefin, dinitramin, fluchloralin, iso-propalin, trifluralin, butralin, pendimethalin, and profluralin. Vapours of herbicides from soil were trapped in florisil column under controlled laboratory condition for a 3 hours period. No vapour losses of nitralin and oryzalin were detected at highest temperature, whereas under the same condition vapour losses approached 25% of benefin, profluralin and trifluralin. Vapour losses for the remaining herbicides studied were moderate with values ranging between 2 and 13%.

Gingerich and Zimdahl[24] (1976) compared the persistence of 2,6-dinitro-N, N-dipropyl cumidine (isopropalin) and 3,5-dinitro-N^4, N^4-dipropyl sulfanilamide (oryzalin) in soil. The aerobic rate of disappearance of isopropalin was slower than that of oryzalin.

Walker and Bond[25] (1977) investigated that the herbicide AC 92, 553 or Stomp was lost rapidly from an inert surface and 97% loss was recorded after 28 days at 25°. Losses from soil surface occurred more slowly and were greater from wet compared with dry soils. The time for 50% disappearance in a sandy loam soil at 75% of field capacity was inversely related with temperature.

Savage, K.[26] (1978) studied the dissipation rates of 7 substituted dinitro-aniline herbicides in soil using first order kinetics. Flooding the soil significantly increased the dissipation rate of trifluralin, fluchoralin, profluralin and pendimethalin and the dissipation rate was less affected in case of dinitramine and butralin. Volatilisation of trifluralin, fluchoralin and ethalfluralin from Bosket sandy loam was reduced by flooding when compared with volatilisation from the same soil with a moisture content equivalent to field capacity. Pendimethalin showed low volatility. The flooding effect on dissipation rate was apparently not due to increased volatilisation.

Smith and Oliver and Lusby[27] (1979) studied the degradation of pendimethalin and its N-nitroso and N-nitro derivatives in flooded anaerobic soil by mass spectrometry, TLC and autoradiography. Pendimethalin was found to be more stable than N-nitroso and N-nitro derivatives. But the degradation of both these groups of derivatives was found to be much more rapid in anaerobic soil than aerobic conditions.

Savage and Jordan[28] (1980) studied the persistence of trifluralin, fluchloralin and pendimethalin applied to soil surface in a series of field experiments. Trifluralin and fluchoralin were lost more rapidly than pendimethalin. Half life values for the first 3 to 5 days for trifluralin, fluchoralin and pendimethalin respectively were 2, 1.5 and 4 days on silt loam and 2, 4 and 6 days on clay. Rates of loss were much lower for the remainder of the 7 or 12 day sampling periods. Shading from direct sunlight greatly reduced the loss of fluchoralin and pendimethalin as compared to exposure to full sunlight but loss in case of trifluralin remained fairly rapid under shaded conditions. Rainfall on the first day insignificantly increased dissipation of fluchoralin and pendimethalin.

Barett et al[29] (1981) described the dissipation of pendimethalin in flooded and non-flooded soil. GLC and sorghum bio assays showed that pendimethalin dissipation under various cropping and irrigation systems approximated to first order kinetics. The dissipation rate was found more rapid than predicted by the model for the first 10-14 days for non-flooded and flooded rice. The half-life of pendimethalin ranged from 19 to 27 days in soil sown to soybeans or rice with flush irrigation, compared to only 8-16 days in flooded rice plots.

Duseja[30] (1981) studied the soil dissipation of trifluralin, profluralin and dinitramin. At pH 6.4 the dissipation of all the herbicides was found most rapid and the dissipation rate increased with increasing temperature, moisture content and time.

Fetvadjieva and Nikolova[31] (1983) carried out several test in laboratory and growth chamber conditions showed that soil moisture content of 20-60% influenced the degradation

1.98 kg pendimethalin, 1.4 kg ethofumesate and 2 kg metachlor/ ha is sandy loam soil at temperatures of 10-13° and 28-30°C. The compounds were degraded in the order pendimethalin $<$ ethofumesate $<$ metotachlor.

Barrett and Lavy[32] (1983) have determined the effects of soil water content on pendimethalin dissipation. Pendimethalin persistence was studied in the field for 2 years with low land rice, upland rice and soybean. Soil moisture content greatly influenced the dissipation of pendimethalin which was very rapid for the 1st, 2nd week after application. In all treatments except soybeans during the 1st year, more than 50% of the pendimethalin disappeared in 1 week.

Nelson *et al*[33] (1983) developed a sequential extraction procedure for pendimethalin, trifluralin and oryzalin residues in soil using acidic-methanol followed by 95:4:1 (v/v/v) acetone : water : HCl solution and then extraction with 0.5 N NaOH with further fractionation of the NaOH extract by partial precipitation with mineral acids.

Berayon and Mercado[34] (1983) reported that the persistence of pendimethalin in Guadalupe clay, Lipa clay loam and Taal fine sandy loam soils was evaluated in green house grown plants of sorghum cv. UPL Sg 5 and by GLC of soil samples treated with 1.5, 3.0 or 6.0 kg pendimethalin/ha. Herbicidal activity was rapidly lost from the soil 20-50 days after application and the rate was slow thereafter. Higher residual phytotoxicity was found in clay loam soil than in clay or fine sandy loam at 50 and 110 days. After 3 months residues were found minimal in all the three soils. The herbicide was found in the upper 5 cm soil layer irrespective of the level of irrigation water applied.

Chowdhury et al[35] (1986) reported that the post harvest samples of rice and wheat did not contain any pendimethalin residues.

B.4. Degradation of dinitroaniline herbicides and related compounds

Herbicide transformations in soil can be caused by three main types of processes; photochemical, chemical and microbial[36].

Substituted dinitroanilines undergo photodecomposition in presence of water in sunlight or UV wave length after a considerable time of exposure. The photodegradation products of dinitroanilines in water and on dry soil after exposing to sunlight are often polar and include dealkylated and cyclic intermediates. Dinitroanilines degrade more rapidly in anaerobic than aerobic soils and the metabolites often resemble those products formed by the photolytic mechanism of dealkylation and cyclization[37].

The degradation sequence of trifluralin (19) and benifin (3) in soils, plants and animals almost follows dealkylation and reduction reactions[38]. Thus most of these dinitroaniline herbicides are converted to numerous metabolites including dealkylated and reduced products through chemical and bio-chemical transformations.

Probst *et al*[39] (1967) carried out various experiments with carbon-labelled trifluralin to reveal the different degradative path ways of trifluralin in soils and plants. Aerobic degradation proceeded through a dealkylation step followed by progressive reduction, whereas anaerobic degradation occurred with a preliminary reduction prior to dealkylation. Extensive residue analysis of tolerent crops showed that trifluralin was not readily absorbed from soil. However carrots absorbed small quantities as determined by residue analysis and radioactive tracer studies. The major portion of incorporated radioactivity was identified as unchanged trifluralin and major metabolite found was the dealkylated product (Fig.1.2 and 1.3).

Probst *et al*[43] (1975) suggested that benifin degraded in soil by dealkylation, then nitro reduction and produced many increasingly polar metabolites. One of these α-, α-, α -trifluro-2, 6-dinitro-p-cresol(x) was found in soil treated with benefin or trifluralin (Fig.1.4).

Laanio *et al*[40] (1973) showed that $^{14}CF_3$ labelled Dinitramine (1) was degraded to four identified metabolites by a soil fungus, Paecilomyces sp. and suggested the path ways as shown in Fig. 1.5. Benzimidazoles (2) and(3) were also products of dinitramine photolysis.

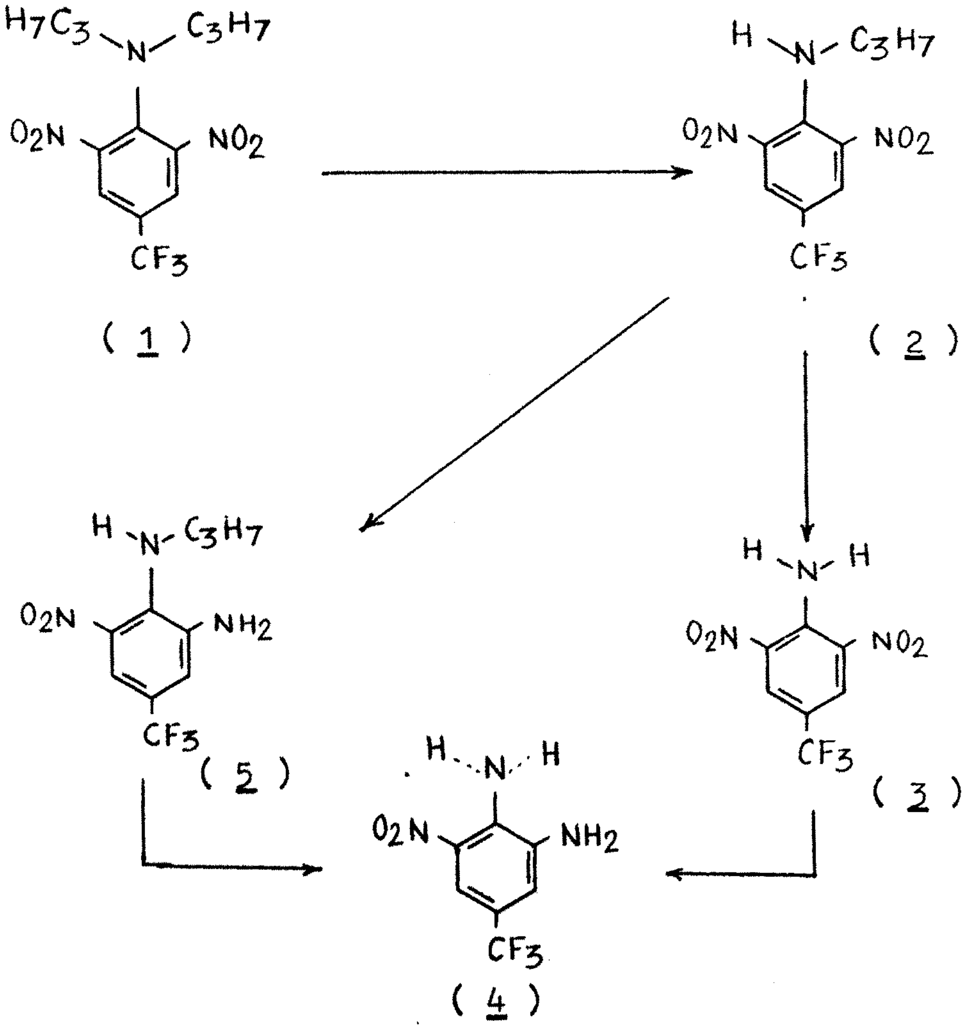


Figure-1.2 Postulated pathway of aerobic trifluralin degradation (Probst *et al.*, 1967).

Compound-1	Trifluralin
Compound-2	α-, α-, α-trifluro-2, 6-dinitro-N-propyl-p-toluidine
Compound-3	α- , α-, α-trifluro-2,6-dinitro-p-toluidine
Compound-4	α- , α-, α-trifluro-5-nitrotoluene-3,4-diamine
Compound-5	α- , α-, α-trifluro-5-nitro-N^4-propyl-toluene-3, 4-diamine.

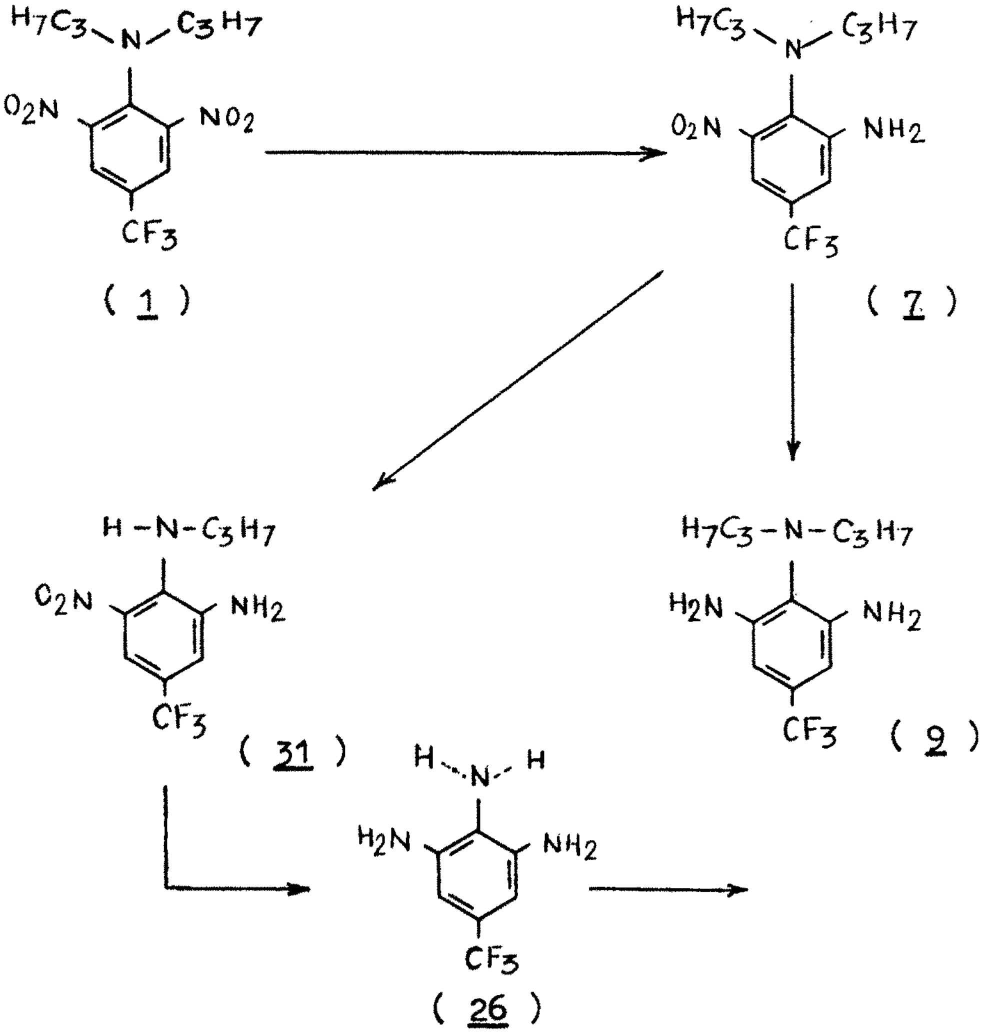


Figure-1.3 Postulated pathway of anaerobic trifluralin degradation (Probst et al., 1967).

Compound- 1	Trifluralin
Compound- 7	α-, α-, α-trifluro-N^4, N^4-dipropyl-5-nitro toluene-3, 4-diamine
Compound- 9	α-, α-, α-trifluro-N^4, N^4-dipropyl-toluene-3, 4, 5-triamine
Compound-26	α-, α-, α-triflurotoluene-3, 4, 5-triamine
Compound-31	α-, α-, α-trifluro-N^4-propyl toluene 3, 4, 5-triamine.

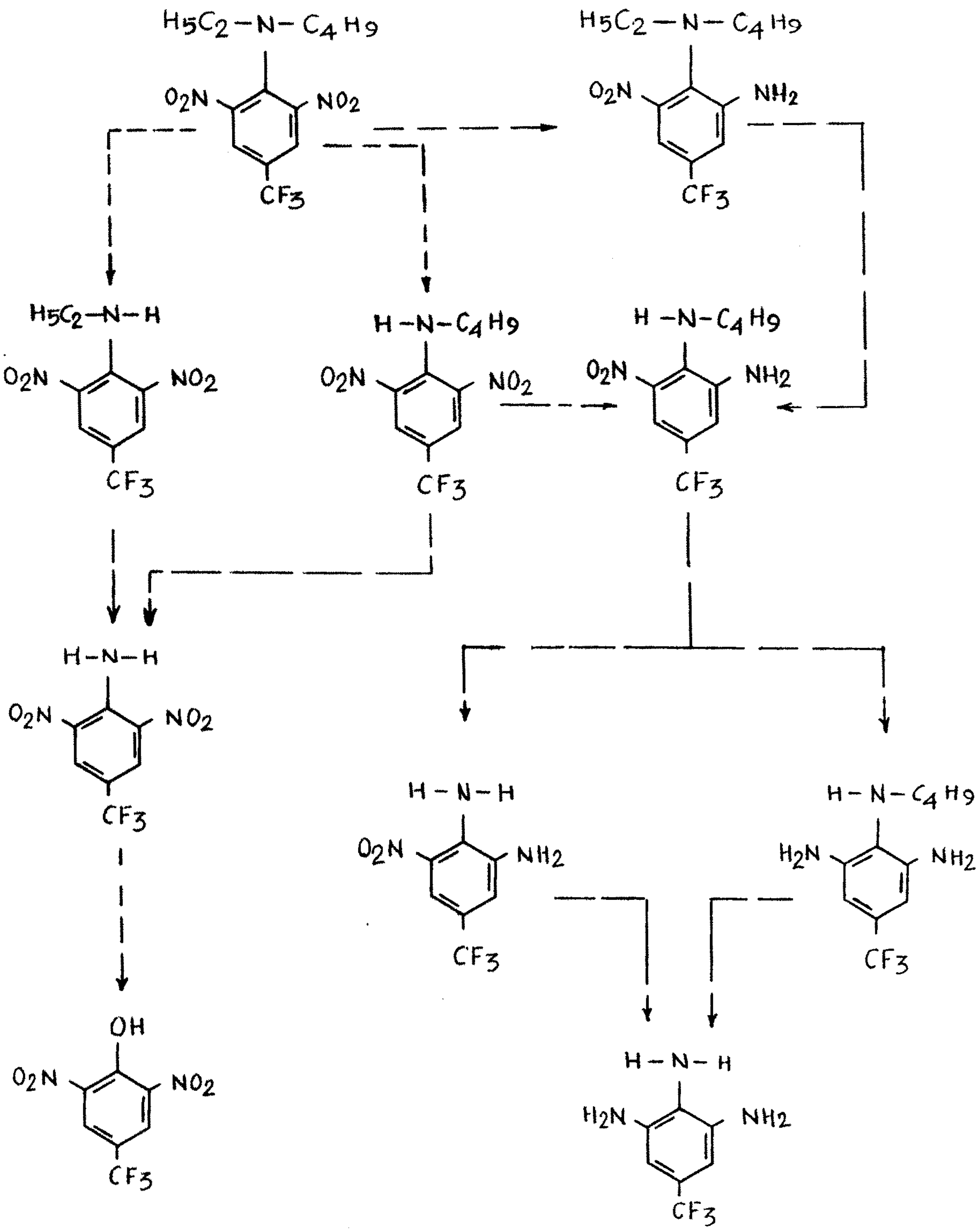


Figure-1.4 Postulated pathway of aerobic benifin degradation in soil (Probst and Tepe).

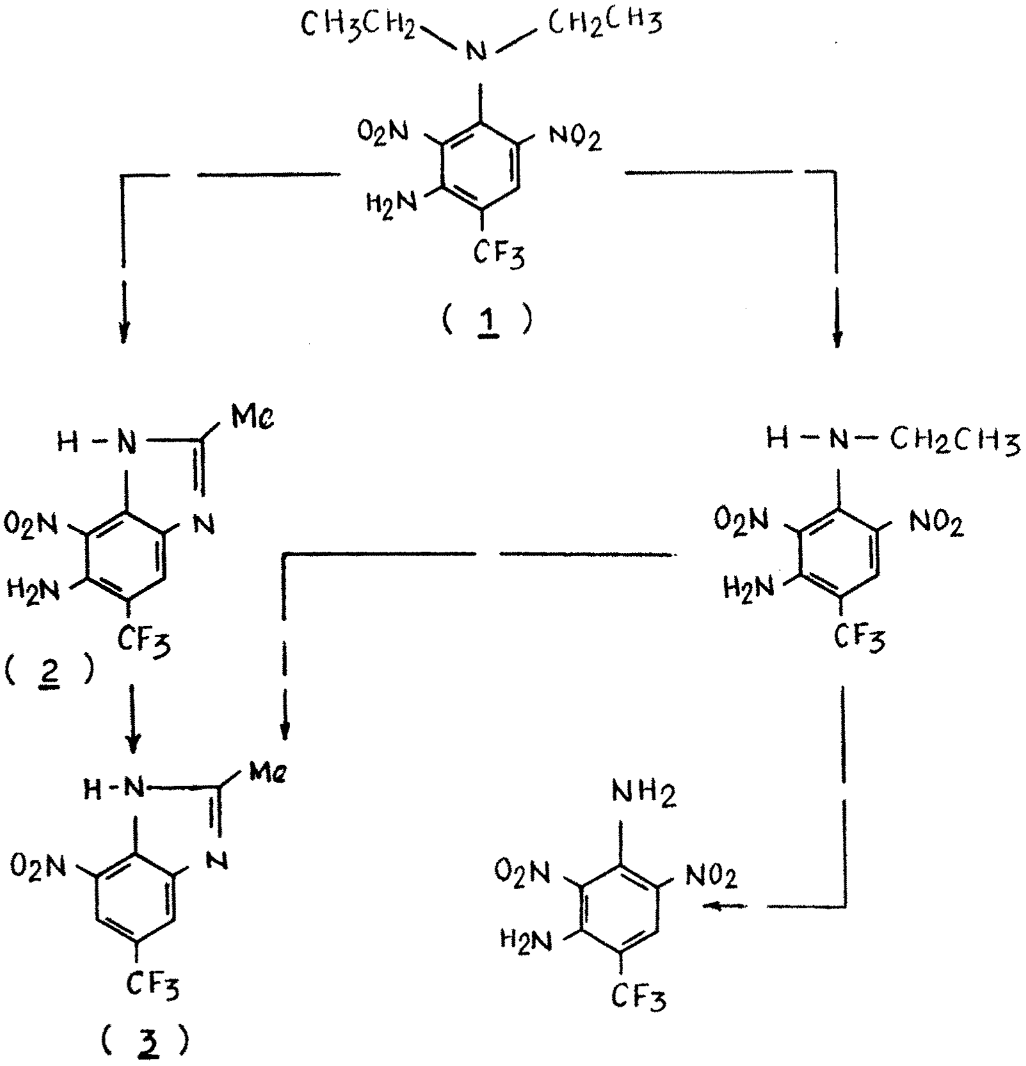
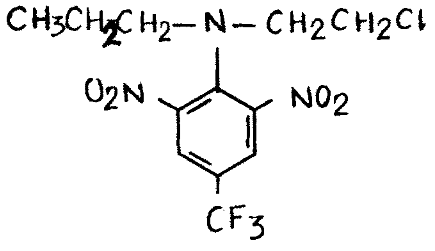


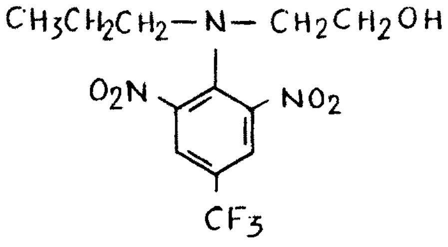
Figure-1.5 Metabolism of dinitramine by a soil fungus.

Kearney <u>et al</u>[42] (1974) isolated a major product from Chillum silt loam, incubated with ^{14}C butralin which was the fully dealkylated 4-t-butyl-2, 6-dinitroaniline and the major product from <u>Paecilomyces sp</u>. cultures enriched with butralin was the oxidised analog 3- (4-t-butyl-2, 6-dinitroanilino)-2-butanol.

Otto and Drescher[41] (1973) studied the fluchoralin (1) transformation in Bosket and very sandy loam soils contained in greenhouse pots cropped with cotton, soybeans or peanuts. At 3 months, about 52% of the total residues was methanolextractable; by 5 months only 24% was extractable and 75% remained as bound residue. Of the extractable (benzene, benzene-methanol, methanol) residues in one experiment, the following products (2, 3, 4) and unidentified polar products were obtained.



(1)



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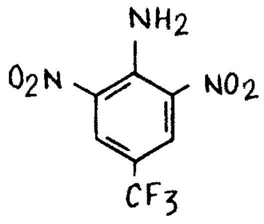

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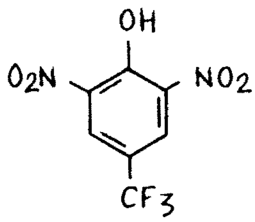
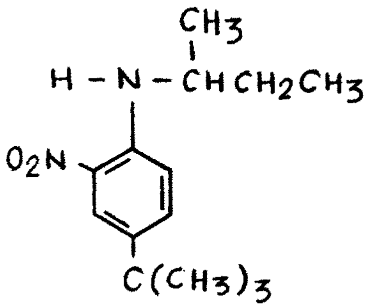
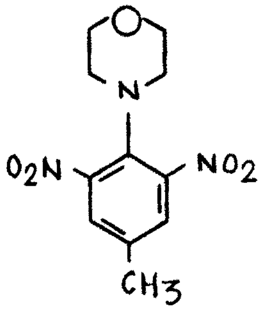


(4)

Kearney *et al*[44] (1975) reported that when six (ring-^{14}C) dinitroanilines were aerobically incubated upto 7 months in Matapeake silt loam, the rate of degradation was dinitramine > chlornidine > butralin > profluralin > trifluralin > fluchoralin. The major isolated component was always the parent herbicide. Typical transformations observed included N-dealkylation and benzimidazole formation. Three unusual matabolites were also reported, a denitrified form (I) of butralin, a morpholine (II) from chlornidine, and quinoxaline (III) from fluchloralin.



(I)



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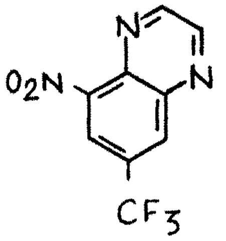


(III)

Golab et al[45] (1979) isolated 28 transformation products and none of them exceeded 3% of the initially applied trifluralin over a period of 3 years. After 3 years less than 1.5% of the applied trifluralin could be detected in soil. About 4% was distributed among numerous transformation products and 38% remained as soil bound residue.

Oliver and Lusby[46] (1979) studied the anaerobic soil metabolism of ^{14}C-pendimethalin and its nitroso and N-nitro derivatives in a glass chamber, equipped with electrodes and containing soil and ground hay. Then it was flooded with water, flushed with N_2 and allowed to stand until the reduction potential measured (-300 mv) and then pendimethalin, N-nitrosopendimethalin and N-nitro-pendimethalin were added. After few days, the soil was extracted with methanol and the metabolites were isolated by sequential dry column and high pressure liquid chromatography. The major metabolites of N-nitroso pendimethalin and N-nitro pendimethalin identified were N- (1-ethyl propyl)-N-nitroso-(6-amino-3, 4-dimethyl-2-nitro) aniline and 1-(1-ethyl propyl)-5, 6-dimethyl-7-nitro benzimidazole .

Lustry *et al*[47] (1980) isolated the products obtained from metabolism of 2, 6-dinitro-4-(trifluoromethyl) [ring ^{14}C] benzamine by a *Streptomyces* sp. Metabolites were separated by dry column chromatography, purified by HPLC and identified by chemical ionization and electron impact mass spectral analysis and IR. Products identified were 3-nitro-5-(trifluoromethyl)-1, 2-benzenediamine, N [amino-5-(trifluoromethyl) phenyl] acetamide, N-[2-amino-3-nitro-5 (triflueoromethyl) phenyl] methane sulfinamide, a diphenyl diazene and a diphenyl diazene oxide.

William *et al*[48] (1981) reported that [^{14}C] N-nitroso-pendimethalin, a contaminant of the dinitroaniline herbicide pendimethalin was metabolised to several products by a *Streotomyces* sp. isolated from soil. Reduction of nitro group was characteristic of all metabolites. Metabolism of [^{14}C]-N-nitrosopendimethalin yielded two hydroxy methyl metabolites. Identifications were made by chemical ionisation and electron impact mass spectrometry, chromatographic and spectral comparisons and synthesis.

Kearney *et al*[49] (1982) examined the cleaned up extracts of $[^{14}C]$ labelled N-nitrosopropyl-amine (NDPA) and N-nitrosopendimethalin (NP) from soybean group in NDPA and NP treated silt lcam soil. Residues of NDPA in beans and other plant parts could not be detected by $[^{14}C]$ or thermal energy analyser. Low levels of $[^{14}C]$ were found from plants grown in NP amended soil which were not identified. The residues were quantified by GLC using a column packed with 15% carbowax 30-M-TPA on 60-80 mesh Gas-Chrom-P with argon carrier flow rate, 84 cm^3/min.

Isencee and Dubey[50] (1983) studied the distribution of pendimethalin in an aquatic microecosystem. Liquid scientillation analysis and TLC showed that where pendimethalin entered water absorbed to soil or sediment, little desorbed and any which reached the water degraded rapidly. Pendimethalin accumulated by snails was degraded much more rapidly than that accumulated by fish. Accumulation patterns indicated a complex relationship between the metabolites in the water and aquatic organisms. With its rapid degradation pendimethalin poses little danger to the aquatic environment.

Leitis and Crosby[52] (1974) reported that trifluralin(19) decomposed readily in water or aqueous methanol at sunlight wave lengths to form various products and photo decomposition of trifluralin involves oxidative N-dealkylation, nitro reduction and cyclization. Dealkylation may due to free radical

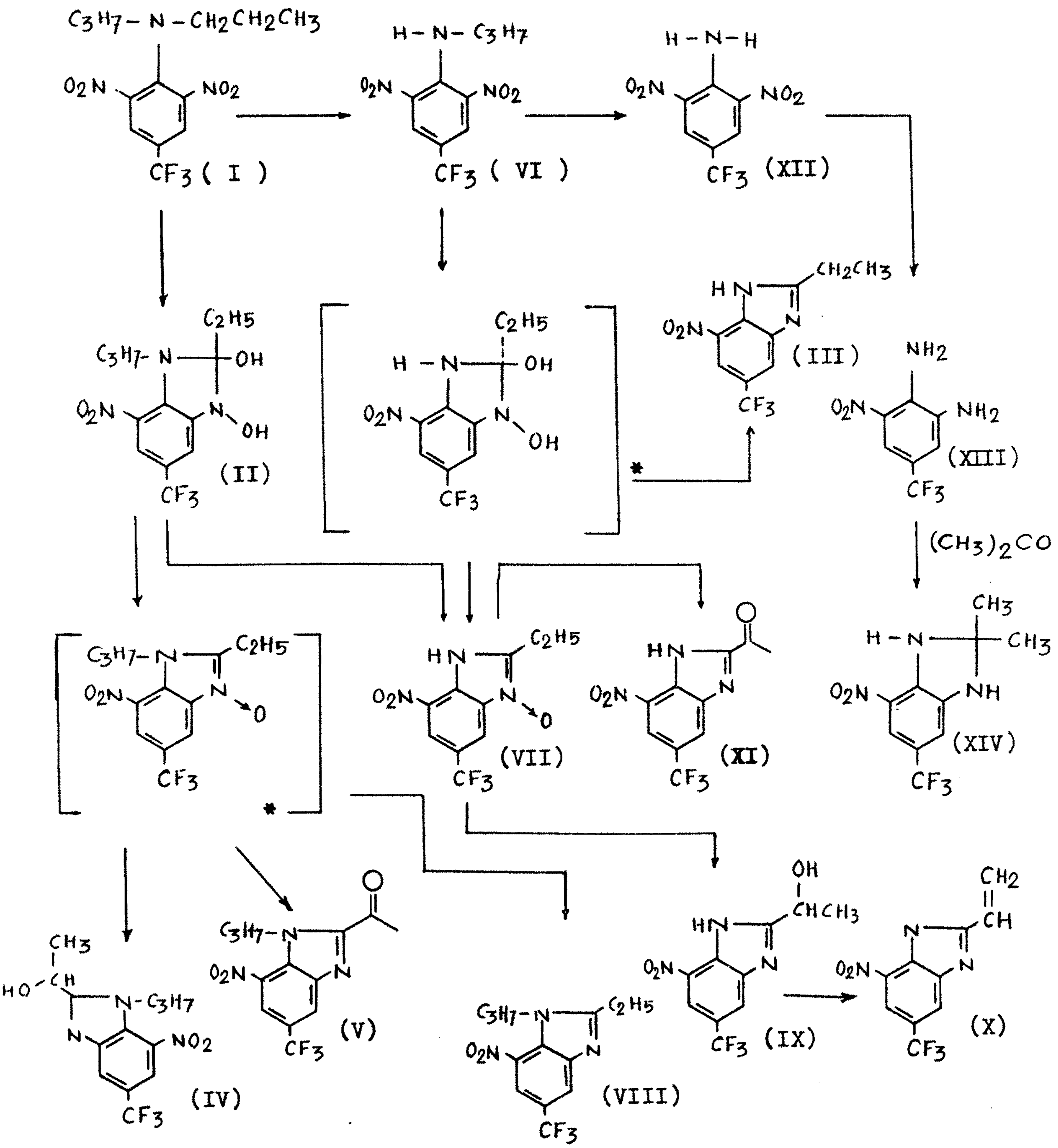


Fig. 1.6. Photodecomposition of trifluralin in water (after Leitis and Crosby, 1974).

oxidation by atmospheric oxygen. Trifluralin was very unstable to sunlight, especially in the presence of an organic solvent. The reaction sequence is shown in Fig.1.6. Dealkylated product VI was detected in water after 1 hour. The major products were the phenylene diamine (XIII) in acidic conditions and 2-ethyl-7-nitro-5 (trifluoromethyl) benzimidazole (VIII) in alkaline condition. Compound XIII reacted with acetone during extraction to form XIV. Under all conditions the highly polar 2, 3-dihydroxy-2-ethyl-7 nitro-1 propyl-5 trifluromethyl benzimidazoline 3-oxide II was present in significant amounts but was degraded by heat or further irradiation.

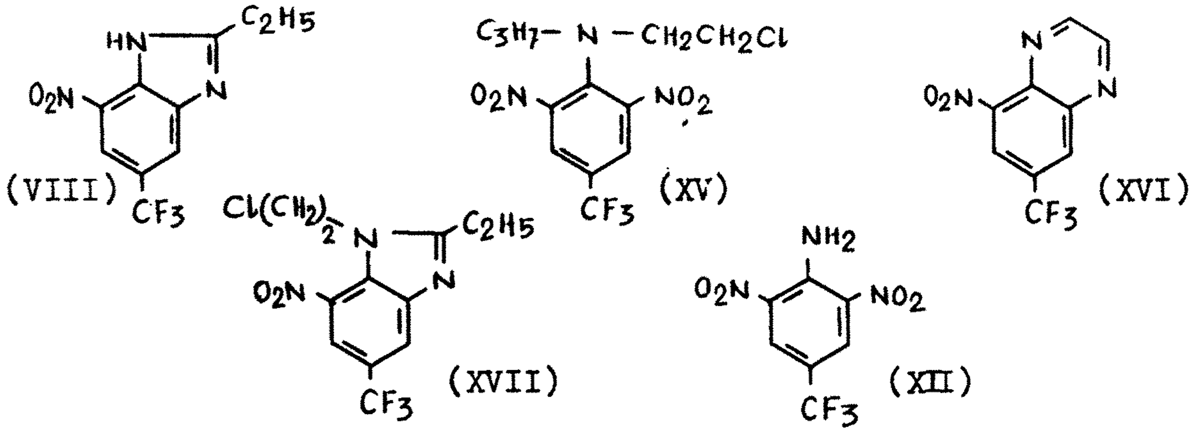


Fig.1.7. Fluchoralin XV and its photo degraded products (XVI and XVII)

Niles and Zabik[53] (1974) have examined photolysis of fluchoralin (XV) in aqueous methanolic solution, on soil and silica TLC plates and also as thin films of the solid herbicide. After 13 days of exposure in sunlight it was found that only 16% was the parent and 54% was quinoxaline XVI. And after 48 days 3% of XV and 4% of XVI were found. Photostable products VIII and XII were obtained which are breakdown products from trifluralin. The major degraded products of fluchoralin in soil (in sumulated sunlight, 48 hrs) were found as VIII, XVI and XVII (Fig. 1.7).

Newsom and Woods[51] (1973) studied the photolysis of the herbicide dinitramine (XVIII) in aqueous solution after exposure to sunlight. Products XIX, XX and XXI (Fig. 1.8) were formed in water.

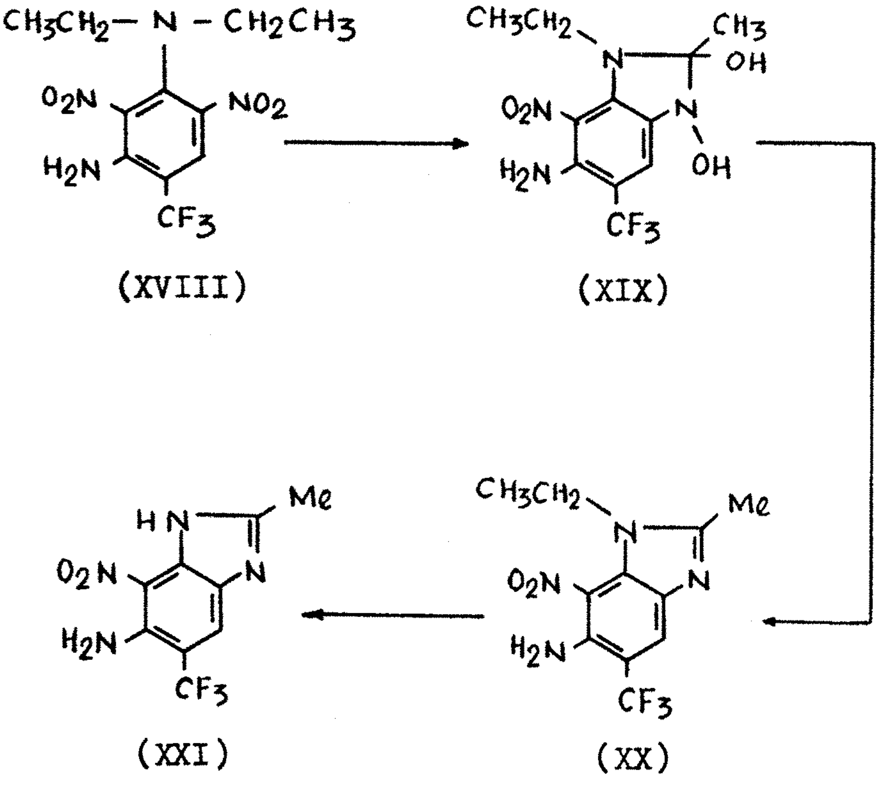


Fig. 1.8. Photodecomposition of dinitramine in water (Newsom and Woods, 1973).

Plimmer and Klingebiel[54] (1974) reported that Butralin (N-sec-butyl-4-tert-butyl-2, 6-dinitroaniline) photo degraded in water when irradiated in sunlight. The major product was dealkylated nitroso compound (4-t-butyl-2-nitro-6-nitrosoaniline) and minor products included 4-t-bytyl-2, 6-dinitroaniline.

Soderquist et al[55] (1975) examined vapour phase photodecomposition of trifluralin in the laboratory and the field. Photolysis on the soil surface was followed by volatilisation and thus photoproducts reached the atmosphere apparently through this route.

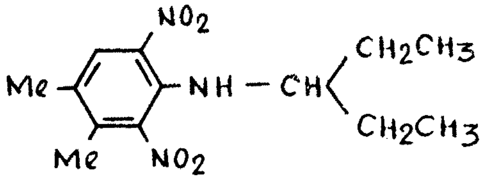
Parochetti and Dec[56] (1978) studied the photo decomposition of eleven dinitroaniline herbicides following 7 days exposure to unfiltered solar radiation in July and compared with that in the dark. Radio labelled herbicides were applied uniformly to dry soil thin layer plates. Decomposition of all dinitroanilines were found higher when exposed in sunlight than dark. Photo decomposition of each herbicide was 7.8% AC 92390, 9.0% butralin, 9.9% pendimethalin, 17.1% benifin, 18.4% trifluralin, 26.6% oryzalin, 30.4% fluchoralin, 40.6% nitralin, 47.6% profluralin, and 72.3% dinitramine.

Plimmer[57] (1978) carried out an experiment on photolysis of ^{14}C-labelled 2, 3, 7, 8-tetra chloro dibenzo-p-dioxin (TCDP) and ^{14}C-trifluralin on pre-coated silica plates on exposure to sunlight. After developing in hexane/chloroform and benzene respectively it was found that both compounds underwent considerable photodecomposition.

Saunders and Mosier[58] (1980) studied the photolysis of N-nitrosodi-n-propylamine (NDPA), a trace contaminant of trifluralin, in lake water and several other aqueous systems. The sunlight photolysis experiments in lake water showed that dissipation of NDPA was significant but variable. The laboratory study revealed also that NDPA photo degraded readily in neutral solution and the photodegradation rate was not pH dependent in the 3 to 9 range. The major photo product was found to be n-propylamine.

Sullivan <u>et al</u>[59] (1980) identified 3-azoxybenzene derivatives, N-propyl-2, 2'-azoxybis (α-, α-, α-trifluoro-6-nitro-p-toluidine), 2,2'-azoxy bis (α-, α-, α, -trifluoro-6-nitro-N-propyl-p-toluidine) and 2,2'-azoxy bis (α-, α-, α-trifluoro-6-nitro-p-toluidine with two azobenzene derivatives N-propyl-2,2'-azobis (α-, α-, α -trifluoro-6-nitro-p-toluedine) and 2,2'-azobis (α-, α-, α -trifluoro-6-nitro-N-propyl p-toluidine) as the products of UV photolysis of trifluralin in benzene solution.

The common name of pendimethalin[3] (I) was approved by BSI, ANSI, Newzealand and by WSSA. The IUPAC name is N-(1-ethyl propyl)-2,6-dinitro-3, 4-xylidine and Chemical Abstract it is designated as N-(1-ethyl-propyl-)-3, 4-dimethyl-2, 6-dinitrobenzenamine. It was introduced by American Cyanamid Co. under the code number 'AC 92553', the trade marks 'Prowl', 'Stomp' and 'Herbadox'. It is an orange-yellow crystalline solid, m.p. 56-57°, vapour pressure $3X10^{-5}$ mm of Hg at 25°C. Its solubility at 20° is 0.3 mg/1 water; soluble in chlorinated hydrocarbons and aromatic solvents. It is stable under alkaline and acidic conditions and is non-corrosive.



(I)

SECTION - C

EXPERIMENTAL

A laboratory incubation experiment and a field experiment were conducted in order to get an insight into the dissipation pattern of pendimethalin in soil. A short account of the experimental design and methods employed for the analysis of pendimethalin residues in soil has been summarised in this chapter.

C.1. Laboratory incubation experiment

The soil used for laboratory incubation study was collected from the surface layer (0-15 cm) of district seed farm, ABC block, Kalyani. The soil sample collected was air-dried, grounded and sieved through 2 mm nylon sieve. Some of the important characteristics of the soil under study are given in Table 1.2.

Table 1.2. Characteristics of the Kalyani soil

Soil colour	Initial pH	Soil organic matter	Cation exchange capacity	Initial moisture	Water holding capacity
Grey	7.19	1.68	6.60	2.35	32.90

20 gm portion of the soil was taken in a number of corning glass incubation tubes. The soil in each tube was treated with

pendimethalin in two treatment doses (T_1 = 1 kg ai/hectare, T_2 = 2 kg ai/hectare) and three replications of treatment were also taken. The acetone solution of pendimethalin after application in soil was stirred well with a glass rod for uniform mixing of pendimethalin in soil as well as to get the solvents evaporated off. The soil was then kept moist by applying water according to water holding capacity of the soil and incubated in the laboratory at room temperature (25 ± 2°C). Any loss of water through evaporation was compensated by periodic addition of water. The soil samples from each treatment replication were sampled 0, 7, 15, 30 and 45 days, after application of the treatment and analysed for stomp residue.

C.2. Field experiment

The field experiment on the dissipation of pendimethalin residues in soil was carried out with the same type of soil in the district seed farm, ABC block, Kalyani, during the boro season of 1985-86. The details of the field experiment adopted are given below.

Crop	:	Wheat; Variety : Sonalika
Design	:	Randomised block design
Treatment	:	T_1 = 1 kg ai/hectare
		T_2 = 2 kg ai/hectare
Replication	:	Three
Plot size	:	4 mt x 2 mt

Row to row distance : 20 cm
Plant to plant distance: 10 cm
Date of sowing : 27.12.85

C.2.1 Method and time of application

Pendimethalin, commercially available as Stomp 30 EC was applied as pre-emergent herbicide in two treatment doses. The amount of pendimethalin (Stomp 30% EC) required for each treatment replication was calculated on the basis of active ingradient of its commercial product and plot size. The required quantity was then diluted with water and these were applied separately at the rate of 500 litre/hectare.

C.2.2 Sampling

The soil samples were collected from six different locations at a depth of 0-15 cm with the help of a soil augur immediately (1 hr) after application. Further the soil samples were collected periodically 7, 15, 30 and 45 days after treatment application and analysed for stomp residue.

C.3 Residue analysis of pendimethalin

Residue analysis of pendimethalin involves three steps viz., extraction, clean-up and estimation.

C.3.1 Extraction of pendimethalin residues from soil

Soil samples in each tube (50 gm soil in case of field experiment) was transferred to a 250 ml conical flask using

50 ml methanol as washing solvent. Then 50 ml of acidified methanol (2% HCl v/v) was added per 10 gm of soil. The flask was covered tightly and kept over night. It was then shaken for 6 hours in a reciprocating shaker and kept aside for settling of soil particles after which the supernatant was filtered under vacuum using 50 ml of methanol as washing solvent. The extract was then concentrated on Kuderna-Danish evaporator using 1-2 drops of ethylene glycol in acetone.

C.3.2 Clean-up

The clean up process involves the following steps :

C.3.2.1 Solvent partioning

The concentrated extract was transferred to a 250 ml, dry and clean separatory funnel by washing with n-hexane and acidified with 20 ml of 0.1N HCl. The extract was then partitioned thrice with distilled n-hexane (100 ml each time) and the hexane layer was collected each time by passing over anhydrous sodium sulfate. The aqueous layers were discarded and the combined n-hexane layers were evaporated to dryness in a Kuderna-Danish evaporator using 1-2 drops of 10% ethylene glycol in acetone v/v. The solution thus made ready for final clean up by column chromatography.

C.3.2.2 Column Chromatography

In a clean and dry column a cotton pledget was introduced inside the bottom of the column and then n-hexane was poured into the column. Then a very little amount of anhydrous sodium sulphate was added with a view to making the base of the column and to make the eluates free of moisture. Previously weighed 8 g florisil were introduced with n-hexane. The solvent was run maintaining a constant flow for satisfactory column packing. The florisil settled uniformly and then a small quantity of anhydrous sodium sulphate was placed onto the top of the column. The sample was dissolved in 10 ml n-hexane and then transferred into the column and kept for a few minutes for adsorption. The column was then washed with 50 ml distilled n-hexane maintaining the effluent rate of 2-3 drops per second till the flow ceased. Then the column was eluted with 150 ml 10% benzene in hexane v/v. The eluate was collected and evaporated to dryness in a Kuderna-Danish evaporator. The residue was transferred for 10 ml volumetric flask with distilled n-hexane and made upto the volume. The solution was ready for Gas Chromatographic analysis.

C.4 Estimation of pendimethalin residue

C.4.1 Instrument

Gas liquid chromatograph (Hewlett Packard 5890A, U.S.A.), equipped with Ni^{63} electron capture detector and 6'x2 mm ID glass column packed with 3% OV-101 on chromosorb GHP (80-100 mesh).

C.4.2 Operating parameters

Oven temperature	:	190°C
Injection temperature	:	250°C
Detector temperature	:	250°C
Carrier gas	:	N_2
Flow rate	:	63 ml/minute.

C.4.3 Analysis of pendimethalin residues

A suitable aliquot of the cleaned up sample was taken in a test tube and 2.5 µl portion of each test solution was injected into the Gas Chromatograph with a 10 µl Hamilton syringe. The residues of pendimethalin were identified by identifying the retention time of sample peak with that of the pendimethalin standard and the amount of pendimethalin residue recorded in the integrated chart was calculated as the pendimethalin residues in µg/gm (ppm).

$$= \frac{X}{V_1} \cdot \frac{V}{M} \cdot \frac{1}{10^3}$$

X = Integrator reading in picogram

V_1 = µL of the sample injected

V = Total volume of cleaned up sample in ml

M = Weight of the sample taken for extraction.

C.5 Interpretation of residue data

C.5.1 Working out of the regression equations

Theoretically, the pesticide residue/deposit should fall logarithmically since the amount loss per unit time is proportional to the total amount present in a particular time, provided the entire quantity is equally exposed to the different agencies of dissipation such as weathering, metabolism, degradation reactions, etc. (Hoskins, 1961). It was established that when log of residues was plotted against time elapsed, a straight line was obtained that obeyed a general equation of a straight line.

$$Y = mx + C$$

where Y = log of residues in ppm

C = exposed initial deposite in ppm

x = time in days

m = slope of the regression time.

The experimental data (log residues versus time elapsed) were then subjected to simple regression analysis and the regression equations calculated.

C.5.2 Working out of residual half life (RL_{50})

RL_{50} is the time in days required to reduce to pesticidal activity to half.

$$RL_{50} \text{ or } t_{\frac{1}{2}} = \frac{\log 2}{\log K_1}$$

where $\log K_1$ = slope of the regression coefficient.

SECTION - D

RESULTS AND DISCUSSION

D.1 Dissipation of pendimethalin in soil

D.1.1 Recovery of pendimethalin in soil

Recovery studies were carried out in order to establish the reliability of the analytical methods and to know the efficiency of extraction and clean up steps employed for the present study, by fortifying the soil with 0.1, 0.3 and 0.5 ppm of pendimethalin. The analytical recovery data are presented in Table 1.3.

Table 1.3. Recovery of pendimethalin in soil samples

Sl. No.	Amount of pendimethalin added (ppm)	Amount of pendimethalin recorded (ppm)*	% recovery	Average %
1.	0.5	0.44	88.0	
2.	0.3	0.26	86.6	86.2
3.	0.1	0.084	84.0	

* Indicates average of three replications.

It is evident from Table-1.3 that the recovery of pendimethalin in soil varies from 84-88% with an average value of 86.2%. This was fairly a good recovery indicating thereby that the method was satisfactory and could be adopted for the determination of pendimethalin residue in soil.

D.1.2 Laboratory incubation experiment

The data of the initial deposits and per cent loss of pendimethalin at different time following application of pendimethalin in two treatment doses (T_1 = 1 kg ai ha^{-1}; T_2 = 2 kg ai ha^{-1}) in soil are summarised in Table-1.4. The rate of dissipation is shown in Fig. 1.9.

Table-1.4. Dissipation of pendimethalin in Kalyani soil under laboratory condition.

Days after treatment	T_1 (1 kg ai/ha)		T_2 (2 kg ai/ha)	
	*Residues of pendimethalin in ppm (µg/gm)	Progressive reduction (%)	*Residues of pendimethalin in ppm (µg/g)	Progressive reduction (%)
0	0.49		0.91	
7	0.47	4.08	0.88	3.29
15	0.35	28.57	0.70	23.00
30	0.05	89.79	0.12	86.80
45	ND	-	ND	-
	$Y = 2.83 - 0.034X$ Regression equation		$Y = 3.09 - 0.030X$ Regression equation	
	RL_{50} or $t_{\frac{1}{2}} = \frac{\log 2}{b} = 8.22$		RL_{50} or $t_{\frac{1}{2}} = \frac{\log 2}{b} = 10$	

* Indicates average of three replications

ND = Not detected.

The initial deposits of pendimethalin in soil were found to be 0.49 and 0.91 ppm in T_1 and T_2 respectively. Thereafter, the pendimethalin residue was found to decrease progressively with time from seventh day onwards, indicating a loss of 3.29-4.08% during the period of 7 days, while a loss of 23-28.57% in pendimethalin residue was observed during the period of 15 days irrespective of treatments T_1 and T_2. These data are in well accordance with the observations of Harrison and Anderson[18] (1970), who reported the loss of 7-10% and 36% during the period of 7 and 14 days respectively. Therefore, a slower rate of dissipation of pendimethalin was observed upto the period of 15 days, after which a faster dissipation of pendimethalin residue occurred in soil. After 30 days, the residues of pendimethalin decreased to 0.05 ppm in T_1 and 0.12 ppm in T_2, thus representing a loss of 89.79 and 86.8% during the period of 30 days in T_1 and T_2 respectively. However, no residue could be detected in soil 45 days after application of pendimethalin.

It has been reported that pendimethalin is stable under both acidic and alkaline conditions[3]. Further, Hein and parochetti[19] (1970) reported that residual herbicidal activity of dinitro aniline class of compounds is affected by volatility as well as ultraviolet light and volatilization loss is more rapid in wet than that of dry soil. However, pendimethalin showed low volatility as observed by Savage[26] (1978) who reported that flooding effect on dissipation rate of pendimethalin was apparently not due to increased volatilization. The slower rate of dissipation of pendimethalin during the period of 15 days could be attributed to low volatility and soil adsorption of pendimethalin. Thereafter, a sharp decrease in pendimethalin residue in soil could be due to its transformation through microbial or photochemical mechanisms.

D.1.3 Field experiment

The analytical results as obtained regarding the dissipation of pendimethalin in soil following application of it as pre-emergent herbicide at the rate of 1 kg ai ha^{-1} (T_1) and 2 kg ai ha^{-1} (T_2) are presented in Table-1.5 and the dissipation pattern in soil is shown in Fig.1.10.

It was found from Table-1.5 that the initial deposits of pendimethalin in soil after 1 hour of spray varied from 0.48-0.85 ppm irrespective of the treatments T_1 and T_2. Thereafter,pendimethalin residue declined progressively with time indicating a loss around 6.2-8.2% during the period of 7 days which is comparable with the results as observed in laboratory incubation experiment. However, progressive reduction in pendimethalin residue of 66.6% in T_1 and 51.7% in T_2 during the 15 days period was recorded in the field experiment while the corresponding figure in laboratory incubation experiment ranged from 23-28.57%. The faster rate of dissipation of pendimethalin in soil during the 15-days period might be attributed to its translocation in the wheat plant growing in soil. It was further evident from Table-1.5 that pendimethalin residue in soil decreased to 0.02 ppm in T_1 and 0.05 ppm in T_2 after 30 days of treatment application, thereby representing a loss of 94.1-95.8% irrespective of the treatments T_1 and T_2, which was much higher as observed in the laboratory incubation experiment. Furthermore, the dissipation of pendimethalin was found to be more or less parallel in both the treatments, T_1 and T_2. Finally, 45 days after application, no pendimethalin residue could be detected in the treatments, T_1 and T_2.

Table-1.5. Dissipation of pendimethalin (Stomp 30 EC) in soil under field condition.

Days after treatment	T_1 (1 kg ai/ha)		T_2 (2 kg ai/ha)	
	*Residues of pendimethalin (Stomp) in ppm(µg/g)	Progressive reduction (%)	*Residues of pendimethalin (Stomp) in ppm (µg/g)	Progressive reduction (%)
0	0.48		0.85	
7	0.45	6.2	0.78	8.2
15	0.16	66.6	0.41	51.7
30	0.02	95.8	0.05	94.1
45	ND		ND	

$Y = 2.8 - 0.048X$ Regression equation

$Y = 3.06 - 0.042X$ Regression equation

RL_{50} or $t_{\frac{1}{2}} = \frac{\log 2}{b} = 6.25$

RL_{50} or $t_{\frac{1}{2}} = \frac{\log 2}{b} = 7.14$

* Indicates average of three replications.

ND = Not Detected.

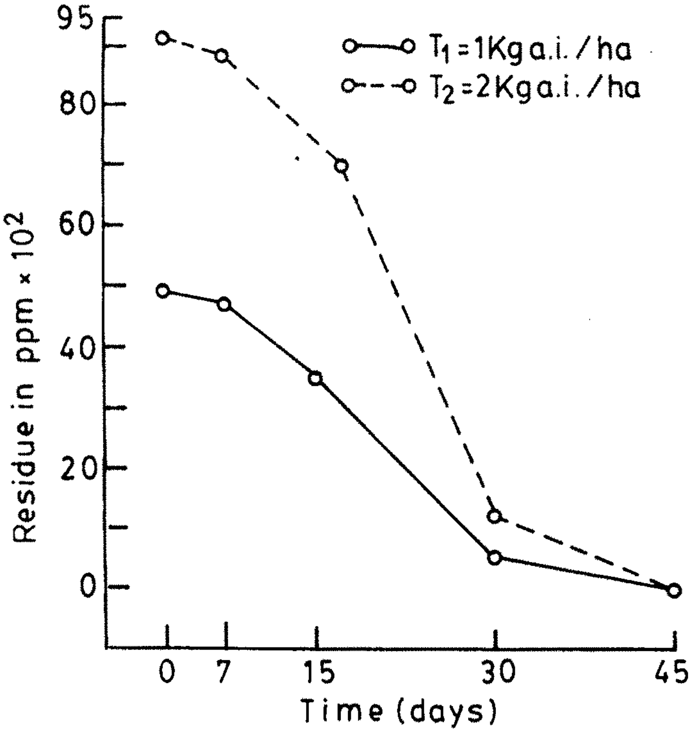


Fig.1.9: Dissipation pattern of pendimethalin in Kalyani soil. (Laboratory incubation experiment)

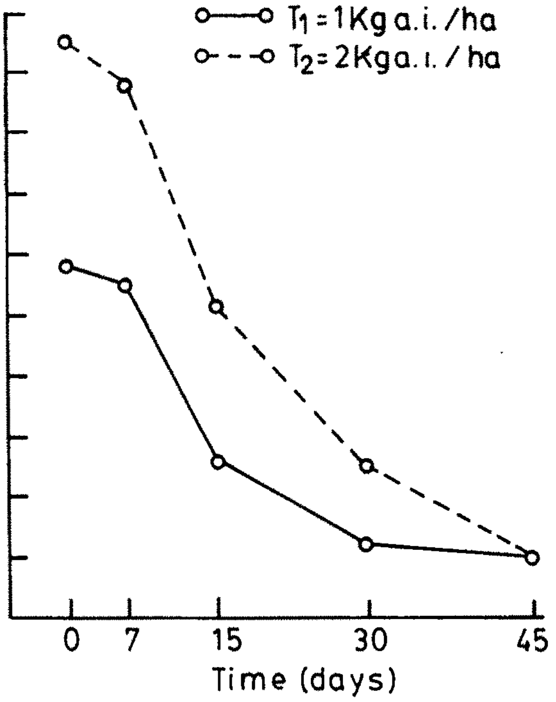


Fig.1.10: Dissipation pattern of pendimethalin in soil. (Field experiment)

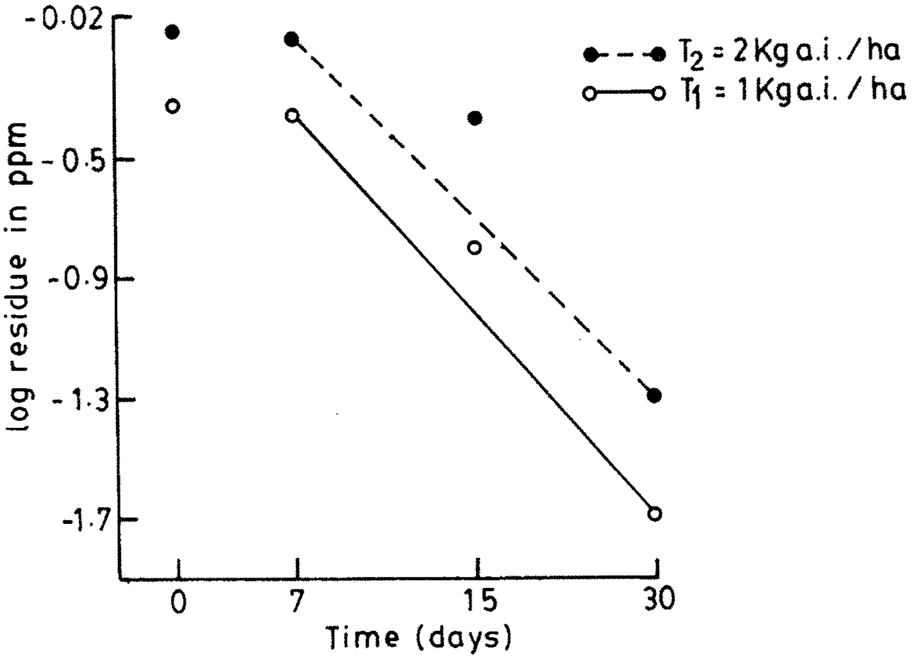


Fig.1.11: Linear plot for first order reaction of pendimethalin under field condition.

Again when the log of residues of pendimethalin in soil was plotted against the time elapsed, a straight line was obtained indicating that the dissipation of pendimethalin in soil followed the 1st order kinetics (Fig.1.11). The half life value of pendimethalin in soil was calculated from regression equation as 6.25 days in T_1 and 7.14 days in T_2 which was in well agreement with the results of laboratory incubation experiment. Therefore, the dissipation of pendimethalin in soil followed the same trend as observed in the laboratory incubation experiment indicating no significant difference between these two sets of experiments.

Furthermore, no pendimethalin residue could be detected in post harvest wheat grain and straw which was in well accordance with the results of Chowdhury *et al*[35] (1986).

D.2 Preliminary study on the photo-degradation behaviour of pendimethalin

With a view to finding out the photo degradation behaviour of pendimethalin, different distilled organic solvents *viz*., hexane, benzene, chloroform, acetone, methanol, ethanol and water were taken in wide mouth glass containers. Then 10 ml solution of pendimethalin (99.8%) at a concentration of 100 ppm were applied separately in respective solvents. Each of the glass container was covered tightly with celophane papers and 4-5 small pores were made on it. The glass container having pendimethalin solution was exposed to sunlight for 80 hrs with occasional monitoring of the irradiated solution by thin layer chromatography. After 80 hrs of exposure of pendimethalin solution in sunlight, the respective solvents were evaporated off to dryness and then extracted with distilled acetone. The acetone solution was then concentrated to appropriate volume which was then subjected to thin layer chromatography as well as gas chromatography. It may be mentioned that pendimethalin applied in water was extracted with chloroform in a separatory funnel and the chloroform solution after usual work up was concentrated and subjected to thin layer as well as gas chromatography for detection of photo-degradation products.

D.2.1 Thin layer chromatographic study of the photo-degradation behaviour of pendimethalin

Thin layer chromatography was carried out on 20 x 20 cm glass plates coated with 0.5 mm of silica gel G using hexane-benzene (7:3) as developing solvent and iodine as chromogenic reagent for visualization of the spots. The thin layer chromatographic behaviour of sunlight irradiated solutions of pendimethalin is shown in Fig.1.12.

The T.L.C. behaviour of sunlight irradiated pendimethalin in different solvent systems revealed that pendimethalin underwent photochemical transformation in all the solvent systems under study excepting methanol and ethanol solutions where only pendimethalin having Rf 0.7 could be visualized on thin layer plates. Again, the T.L.C. of irradiated hexane solution

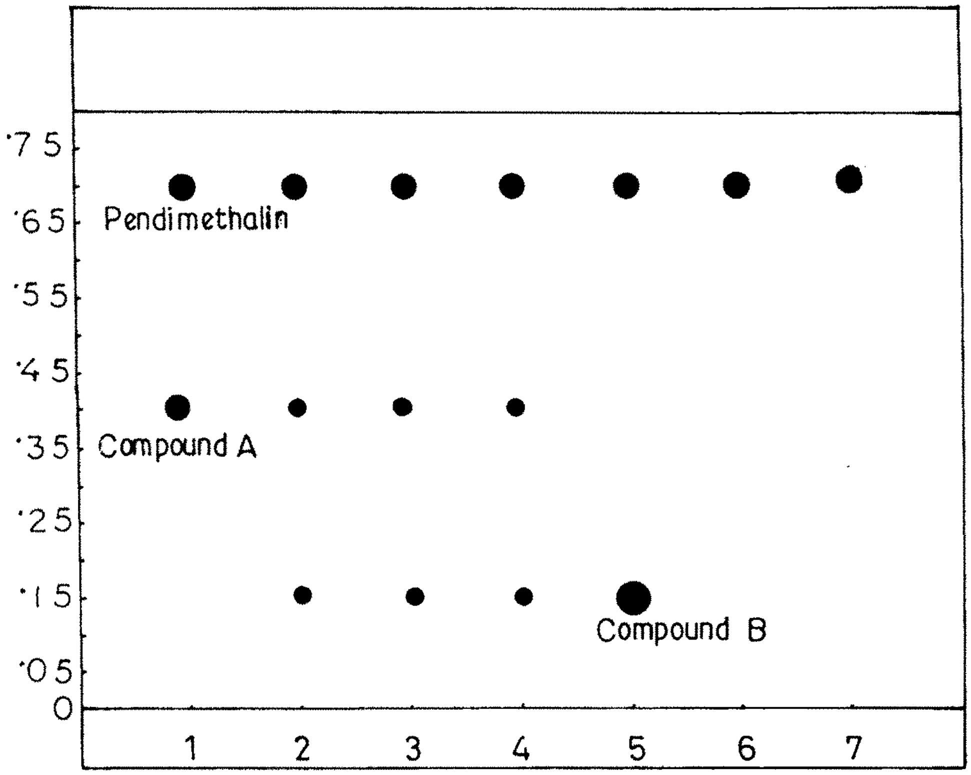


Fig 1.12 TLC behaviour of pendimethalin irradiated under sunlight in different solvent systems.

of pendimethalin showed another prominent spot at Rf 0.4 along with the spot of pendimethalin at Rf 0.7, while the benzene and acetone solutions of pendimethalin displayed yet another spot at Rf 0.15. The aqueous solution of pendimethalin, on the other hand, on exposure to sunlight showed only one prominent spot at Rf 0.15 along with the spot of pendimethalin.

D.2.2 Gas chromatographic study of the photo-degradation behaviour of pendimethalin

The sunlight irradiated solution of pendimethalin in different organic solvents after usual work up was subjected to Gas Chromatography with an H.P. Model 5890A Gas Chromatograph in order to find out the photodegradation behaviour of pendimethalin.

The gas chromatographic behaviour of pendimethalin as well as sunlight irradiated solutions of pendimethalin in different organic solvents has been summarized in Fig. 1.13. It was revealed from this figure that pendimethalin displayed a single peak at a retention time of 4.2 minutes. The hexane solution of pendimethalin after exposure to sunlight gave two peaks at the retention times of 2.2 and 4.2 minutes, while those of benzene and acetone solutions of pendimethalin showed three peaks at the retention times of 2.2, 3.17 and 4.2 minutes. Again, the gas chromatography of irradiated chloroform solution of pendimethalin showed 3 major peaks at the retention times of 2.2, 3.17 and 4.2 minutes along with a small peak, while in

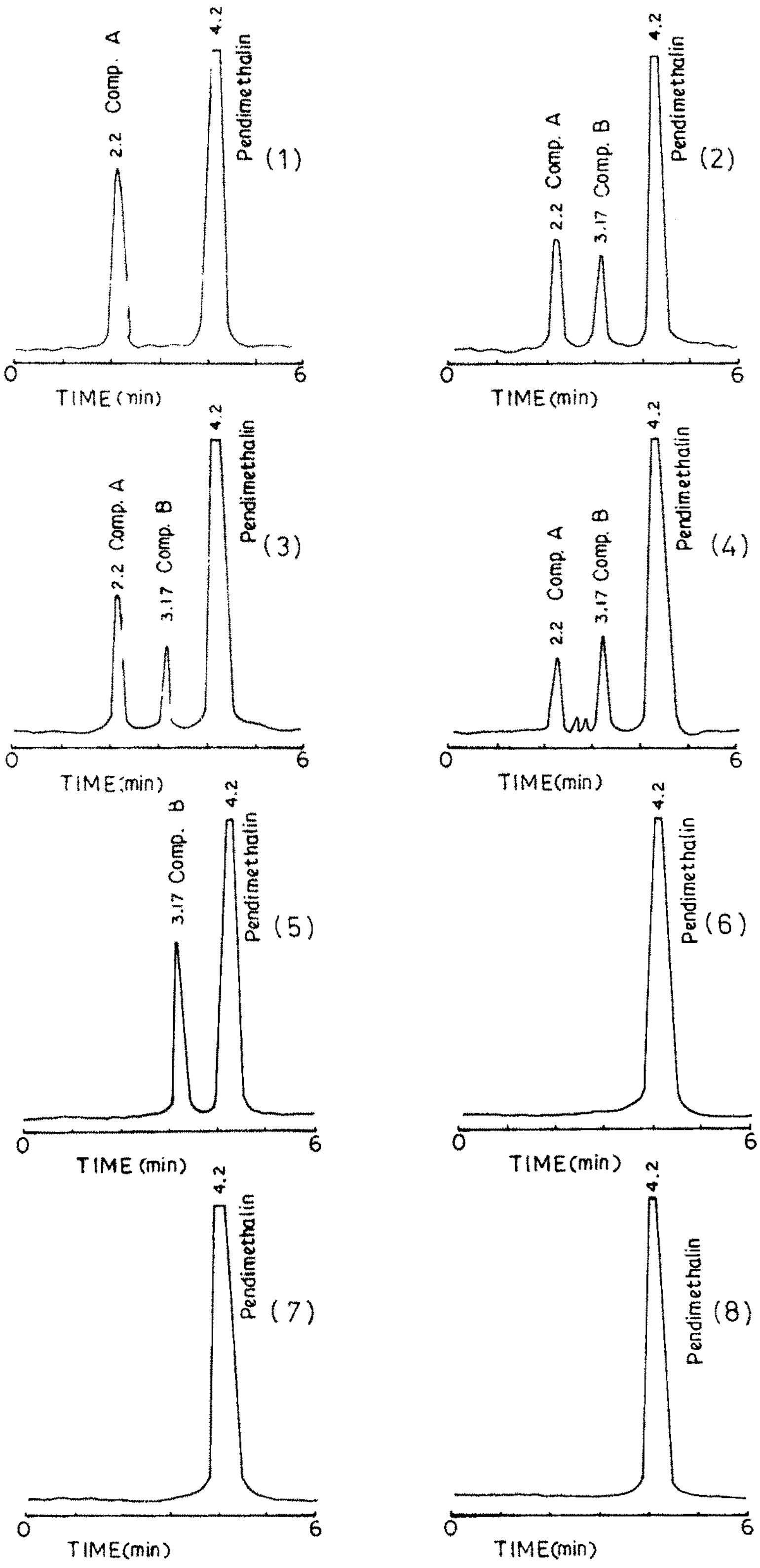


Fig 1.13 Gas chromatogram of pendimethalin irradiated under sunlight in different solvent systems:
1. Hexane 2. Benzene 3. Acetone 4. Chloroform
5. Water 6. Ethanol 7. Methanol.
8. GC of pendimethalin, RT- 4.2 min.

the case of aqueous solution of irradiated pendimethalin two peaks could be observed with the retention time of 3.17 and 4.2 minutes. However, the gas chromatography of methanolic and ethanolic solutions of pendimethalin after exposure to sunlight showed only the peak of pendimethalin at the retention time of 4.2 minutes. Thus, the gas chromatographic behaviour concerning the photodegradation of pendimethalin was found to be in well agreement with the observations made by thin layer chromatography.

Thus, from the foregoing discussion on the photodegradation behaviour of pendimethalin, it is evident that pendimethalin undergoes photochemical degradation in all the solvents except in methanol and ethanol. Again, the only photometabolite with Rf 0.4 and Rt 2.2 that has been identified in hexane, has further been detected in other solvents like benzene, acetone and chloroform. Another photometabolite with Rf 0.15 with Rt 3.17 minutes detected in water has also been found to occur in benzene, acetone and chloroform. Therefore, it is quite resonable to assume that the compounds having Rf 0.4, Rt 2.2 and Rf 0.15 and Rt 3.17 are the two major photometabolites of pendimethalin.

D.3 Chemistry of the photodegradation products of pendimethalin

D.3.1 Isolation

Analytical pendimethalin (500 mg, 99.8%) was taken in a wide mouth glass container and dissolved in 10 ml of distilled n-hexane. The hexane solution of pendimethalin was then exposed to sunlight fcr 80 hours. After 80 hours of exposure the only photo product formed was separated by preparative thin layer chromatography. The bands around 0.4 from preparative t.l.c. were scrapped out and extracted three times with benzene and filtered. The filtrate on concentration gave an orange solid which crystallized from hexane-benzene as pale orange crystals, m.p. 132°. The compound was designated as compound-A.

D.3.2 Homogeneity

Compound-A was proved to be homogenous as it developed a single iodine staining spot on t.l.c. plates using the following solvent systems (Table-1.6).

Table-1.6

Sl.No.	Solvent system	Rf
1.	Pet.ether	0.2
2.	Hexane-benzene (9:1)	0.3
3.	Hexane-benzene (7:3)	0.4

D.3.3 Physical properties

Compound-A crystallised from hexane-benzene (9:1) as pale orange needles, m.p. 132°. It was found to be soluble in hexane, petrol-ether, benzene, chloroform, acetone and insoluble in water.

D.3.4 Molecular formula of compound-A

The elemental analysis of compound-A gave the emperical formula as $(C_{11}H_{15}O_4N_3)_n$ and since the molecular weight of the compound-A was found to be 253 by Mass Spectrometry, the molecular formula of compound-A should be $C_{11}H_{15}O_4N_3$.

D.3.5 Infrared spectrum

With a view to determining the nature of the functional groups present in compound-A, its IR spectrum (Fig. 1.14) in KBr pellet was studied. The probable assignments of the important peaks are shown in Table-1.7.

Table-1.7

Position of the absorption peak (cm^{-1})	Intensity	Probable assignment
3480	low	Secondary amino group
3400	strong	
1640	medium	Aryl nitro group
1980	low	
1560	low	
1500	broad	
1415	low	
1310	low	

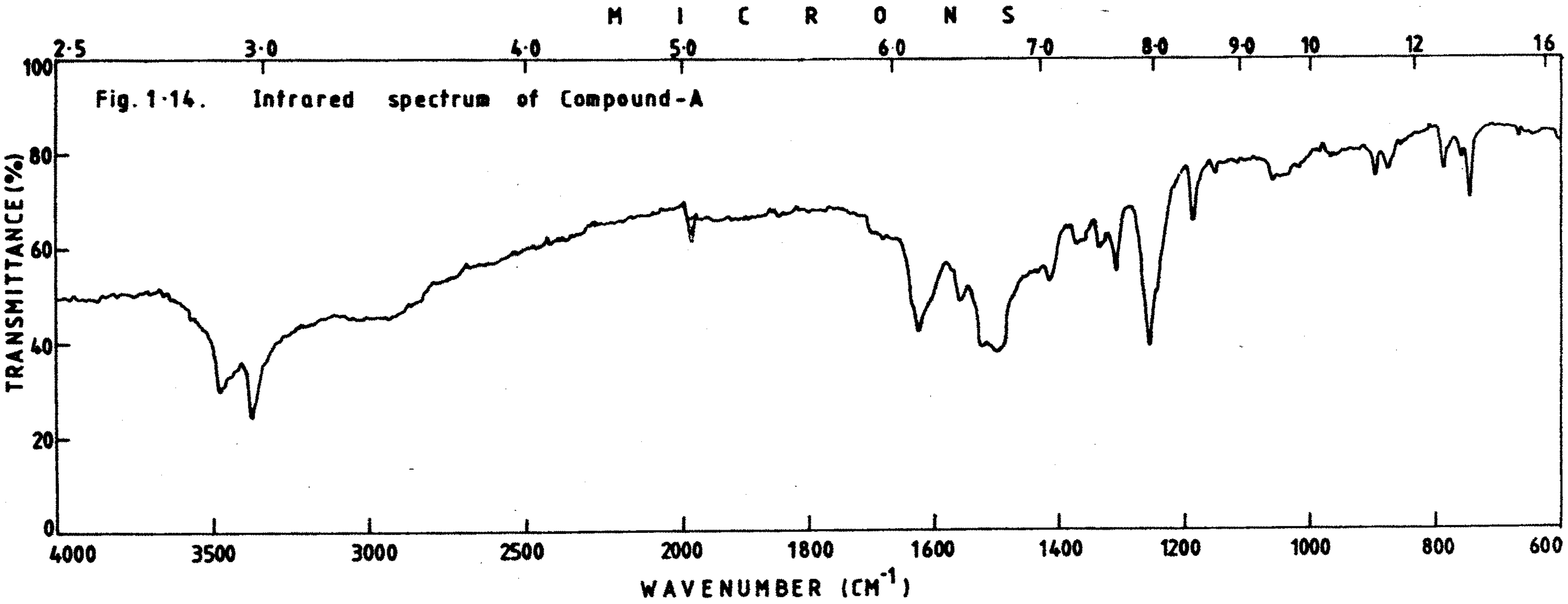


Fig. 1·14. Infrared spectrum of Compound-A

Table 1.7 contd.

Position of the absorption peak (cm^{-1})	Intensity	Probable assignment
1260	strong	Aromatic secondary amine(C-N)
1190	low	
900	low	
880	low	
780	low	Aromatic substituted ring
750	low	

D.3.6 Mass spectrum

The mass spectrum (Fig. 1.15) showed the molecular ion peak at m/z 253 (M^+, 95%) along with some diagonstic peaks at m/z 194 (100%), m/z 211 (87.5%) and a meta stable ion peak at m/z 30.

D.3.7 Nuclear magnetic resonance spectrum

The NMR spectrum (Fig. 1.16) was determined with a 60 MHz instrument using deutero chloroform as the solvent and TMS as internal standard. The chemical shift and their probable assignments are shown in Table-1.8.

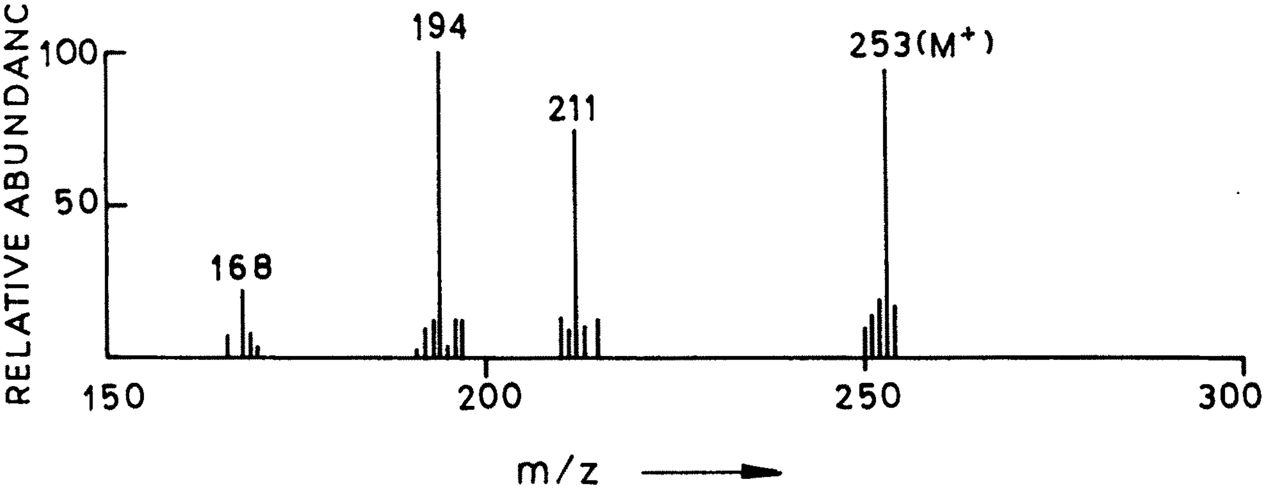


Fig. 1·15. Mass spectrum of Compound -A

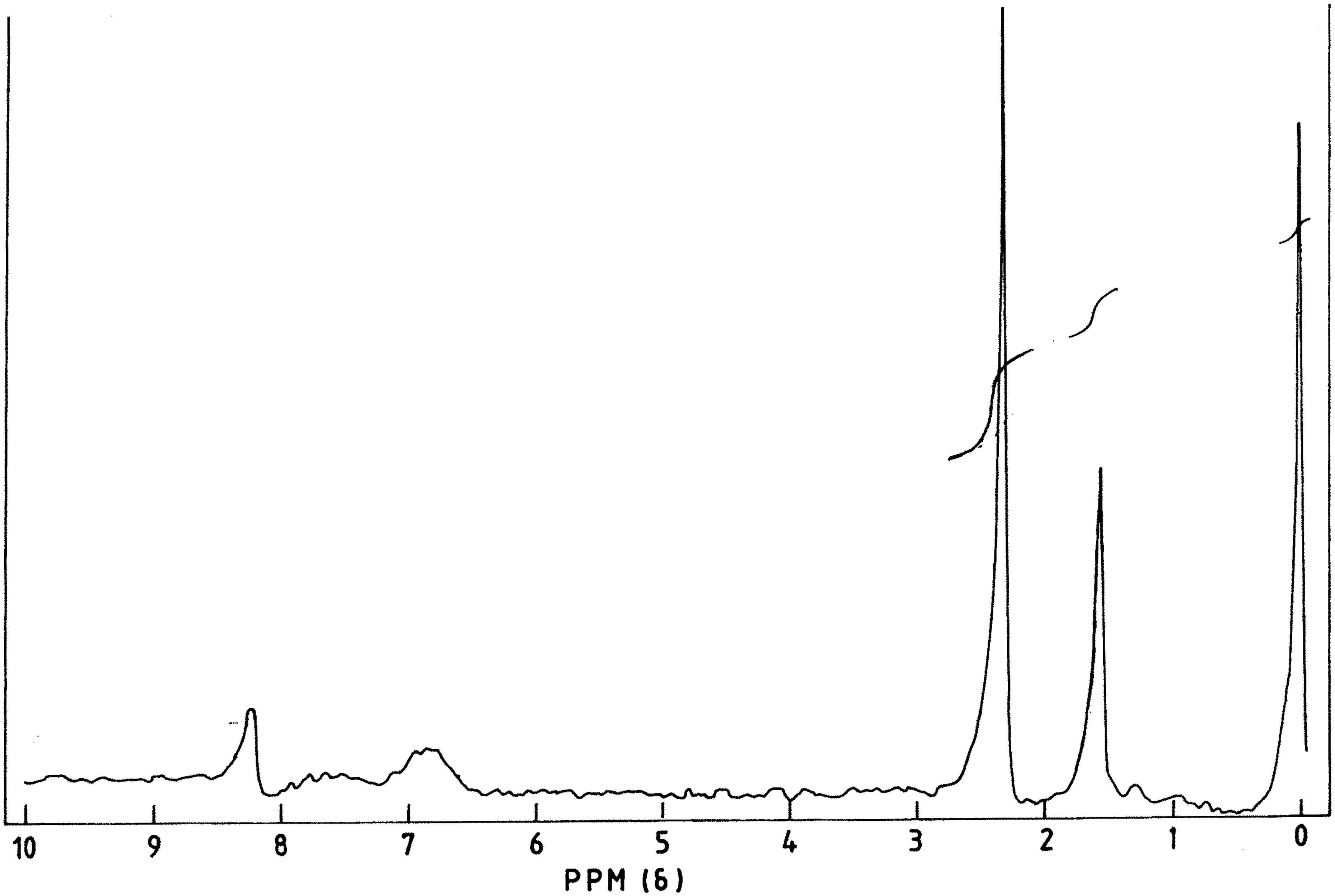


Fig. 1·16. NMR spectrum of Compound- A

Table - 1.8

δ in ppm	No. of protons	Multi-plicity	Probable assignment
1.5	3H	s	Methyl protons
6.85	1H	m	Aromatic amino proton
8.2	1H	m	Aromatic proton
2.2	10H	s	Aromatic methyl and methylene protons

D.3.8 Conclusions drawn from spectral evidence

The IR spectrum showed the presence of a secondary amino group at 3400 cm^{-1} along with other absorptions (1190 and 1980).

The NMR spectrum disclosed, besides, the presence of a $-CH_3$ group ($3\underline{H}$ singlet at 1.5δ), a secondary aromatic amino-proton of one proton intensity at 6.85δ as a multiplet and a sharp singlet (s) at 8.2δ , confirming the presence of an aromatic proton. A singlet (s) at 2.2δ of ten proton ($10\underline{H}$) intensity could be explained by the presence of two aromatic $-C\underline{H}_3$ groups along with four $-C\underline{H}_2-C\underline{H}_2$ protons. From the NMR data it could be confirmed that compound-A contained an N-alkyl side chain, viz., $-NH-CH_2-CH_2-CH_3$. The remaining groups were found to be the same as in the original pendimethalin.

The mass spectrum of compound-A showed a molecular ion peak at m/z 253 (M^+) along with a number of other peaks. The fragment at m/e 211 could be due to the loss of 42 m.u. (M^+-$CH.CH_2.CH_3$) from the molecular ion (M^+). The base peak at m/e 194 could arise due to the loss of 17 m.u. from the peak at m/e 211 which was stabilized as the dinitro methyl cyclopentadienyl radical**(X)**.The mass fragmentation pattern is depicted in Chart-1.1.

Thus, it could be ascertained from an analysis of the spectral data of compound-A (IR, NMR and Mass) that it differed from the original pendimethalin only in respect of one ethyl group attached to the secondary amino group, i.e., a-$NH-CH_2-CH_2-CH_3$ side chain was present in compound-A in place of $-\overset{|}{N}-CH(C_2H_5)_2$. On the basis of the above spectral evidence compound-A was identified as N-propyl,3, 4-dimethyl-2, 6-dinitro benzamine (Fig. 1.17). The latter is actually the partially N-dealkylated product of original pendimethalin (16).

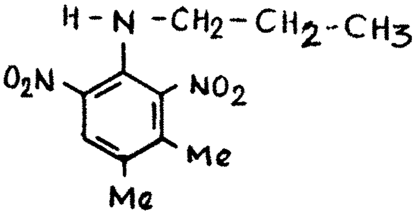


Fig. 1.17

In order to isolate as well as to identify another major product formed in water analytical pendimethalin (500 mg, 99.8%) was taken in water (10 ml) with a very little amount of ethanol and irradiated by the same procedure as adopted for the isolation of compound-A. The aqueous solution

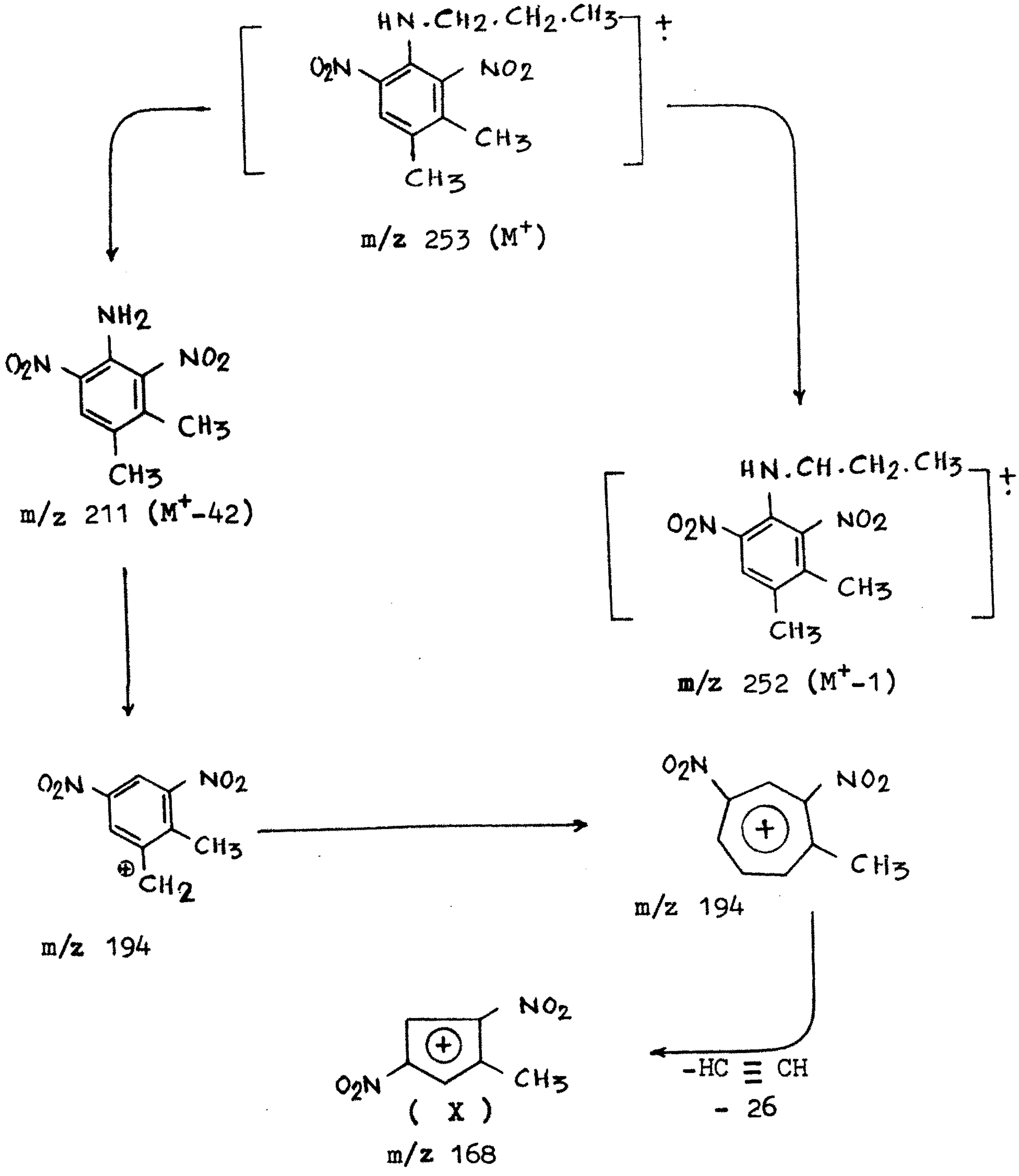


Chart-1.1. Plausible mass fragmentation pattern of compound-A was then partitioned with $CHCl_3$ (3 x 50 ml) and the whole $CHCl_3$ extract was concentrated to a small volume. Finally, the bands around Rf 0.15 were separated by preparative t.l.c. in the same way as adopted for the isolation of compound-A. The compound crystallised from pet.ether-benzene (1:1) as red needles, m.p. 242° and was designated as compound-B. Due to paucity of compound-B, the spectral analysis (IR, NMR and Mass) could not be carried out.

D.4 A laboratory study of the photodegradation of pendimethalin in soil

In order to see the photodegradation behaviour of pendimethalin in soil, a laboratory study was made with Kalyani soil, collected from the District Seed Farm, ABC Block, B.C.K.V., Kalyani, the characteristics of which are mentioned in Table-1.2 (SECTION-C). Soil thin layer plates were prepared from soil sieved to 2 mm. Each plate contained approximately 18 gm of soil applied at a thickness of 2 mm. Following application of the soil, all plates were air dried before use.

Thereafter, 2 ml of pendimethalin solution at a concentration of 250 ppm were spotted uniformly over each soil plates and each of these plates was then exposed to sunlight for 7, 15 and 30 days. Two replications along with one control were taken for each of the sampling days.

After the stipulated period of exposure, soils from each of the plates were scraped out and transferred separately to a blender for extraction. The soil was blended with 200 ml methanol for 3 minutes. The slurry thus obtained was vacuum filtered through Whatman No.1 filter paper. The extract thus obtained was evaporated to dryness under vacuum at 50°C. The concentrate was transferred with distilled hexane to a 25 ml volumetric flask which was then subjected to thin layer chromatography and Gas Chromatography.

A comparison of the thin layer chromatographic behaviour of compound-A, compound-B and the photoproducts formed in soil following exposure of soil plates for different periods is shown in Fig.1.18.

It was evident from Fig.1.18 that the soil plates irradiated for 7 days did not exhibit any significant spot other than pendimethalin (Rf 0.7). This observation suggested the low photo susceptibility of pendimethalin, which was in well accordance with the observation of Parochetti _et al_ (1978) who reported that dinitroaniline herbicides that are mono-substituted on the amino group would photodegrade less rapidly than those having disubstitution. However, compound-B (Rf 0.15) and an unknown metabolite (Rf 0.25) could be visualised on the chromatograms after exposure of soil plates for 15 days period. Both compound-A (Rf 0.4), compound-B (Rf 0.15) along with the unknown compound (Rf 0.25) could be observed in the soil plates of 30 days exposure.

The gas chromatography (Fig.1.19) of soil extracts after exposure of soil plates for different days suggested that

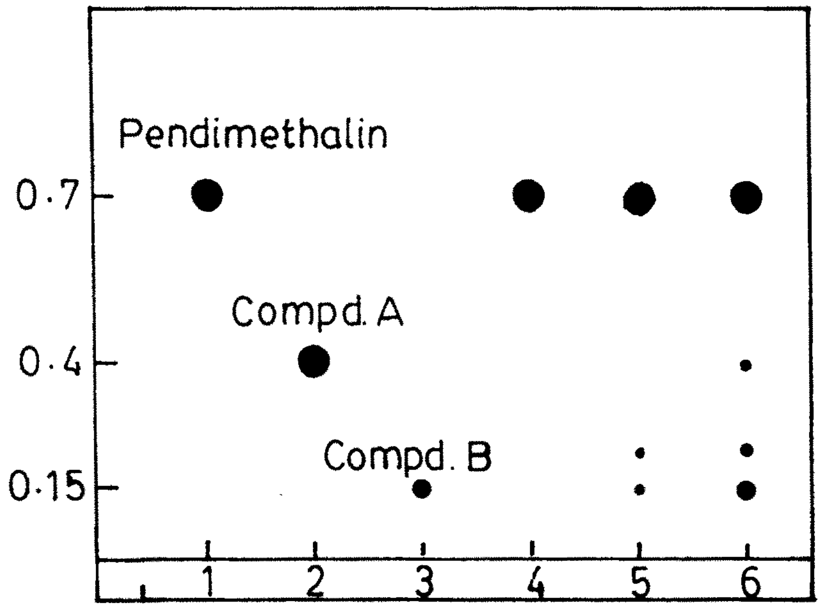


Fig 1.18 TLC behaviour of pendimethalin in soil exposed under sunlight for 7, 15 and 30 days.

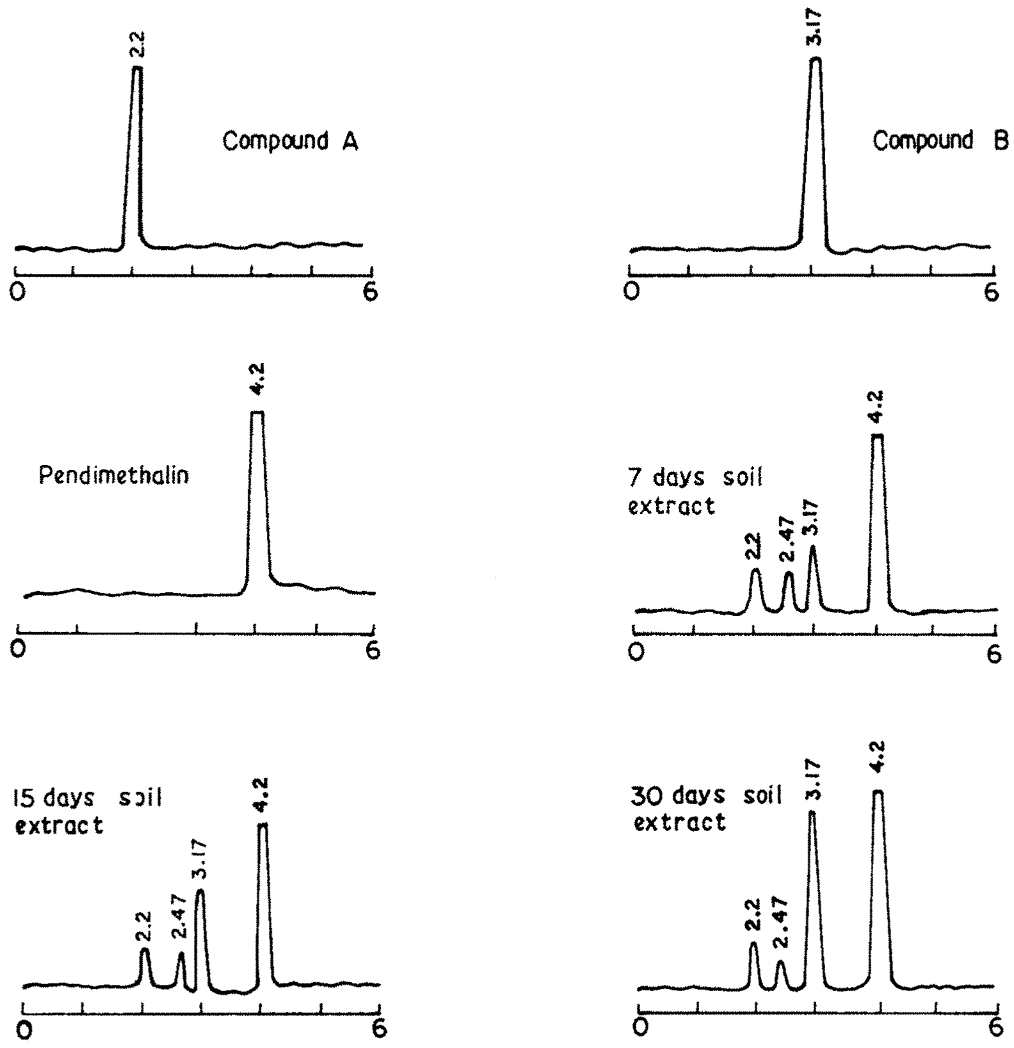
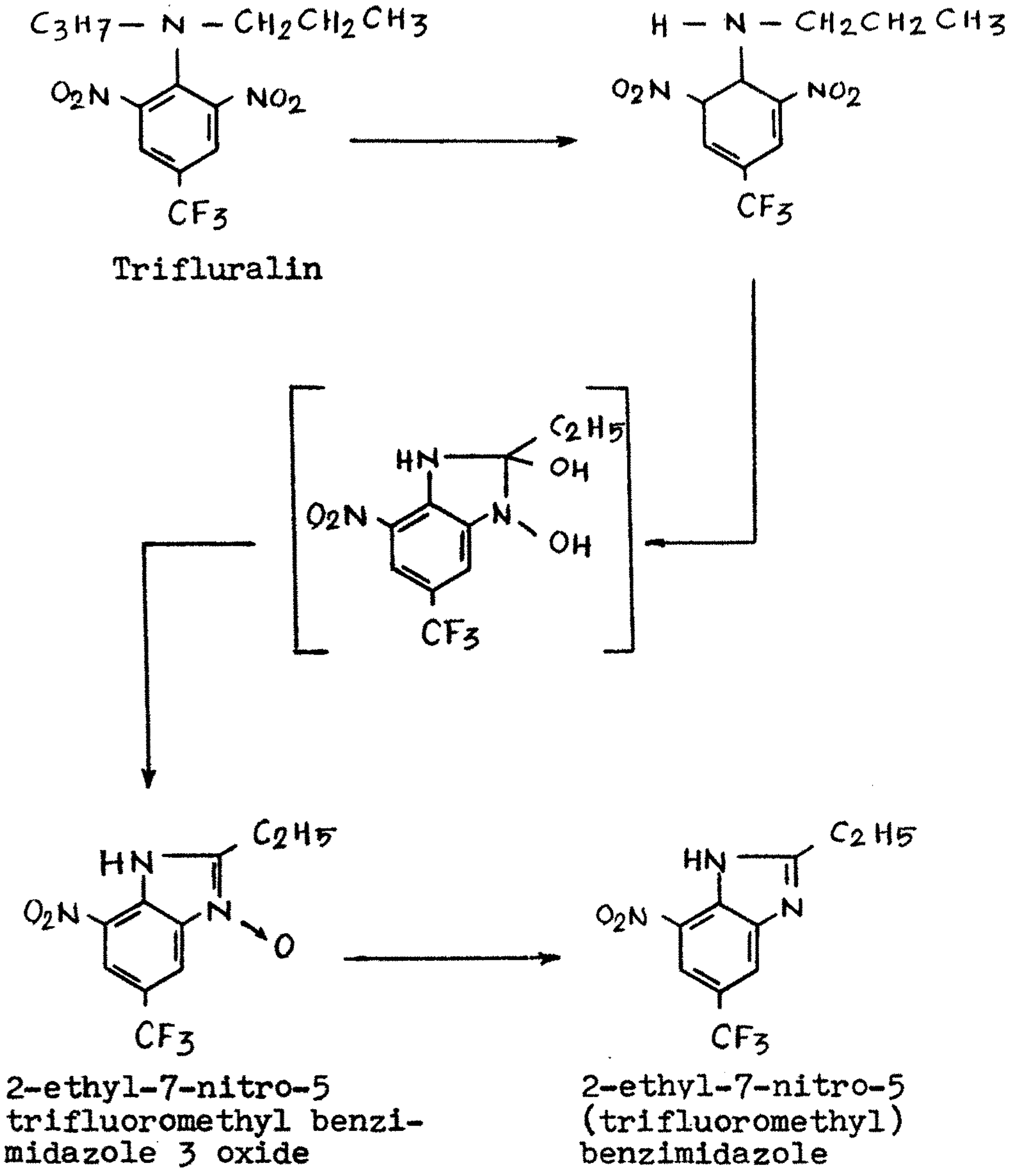


Fig 1.19 Gas chromatographic behaviour of pendimethalin in soil exposed to sunlight for 7, 15 and 30 days.

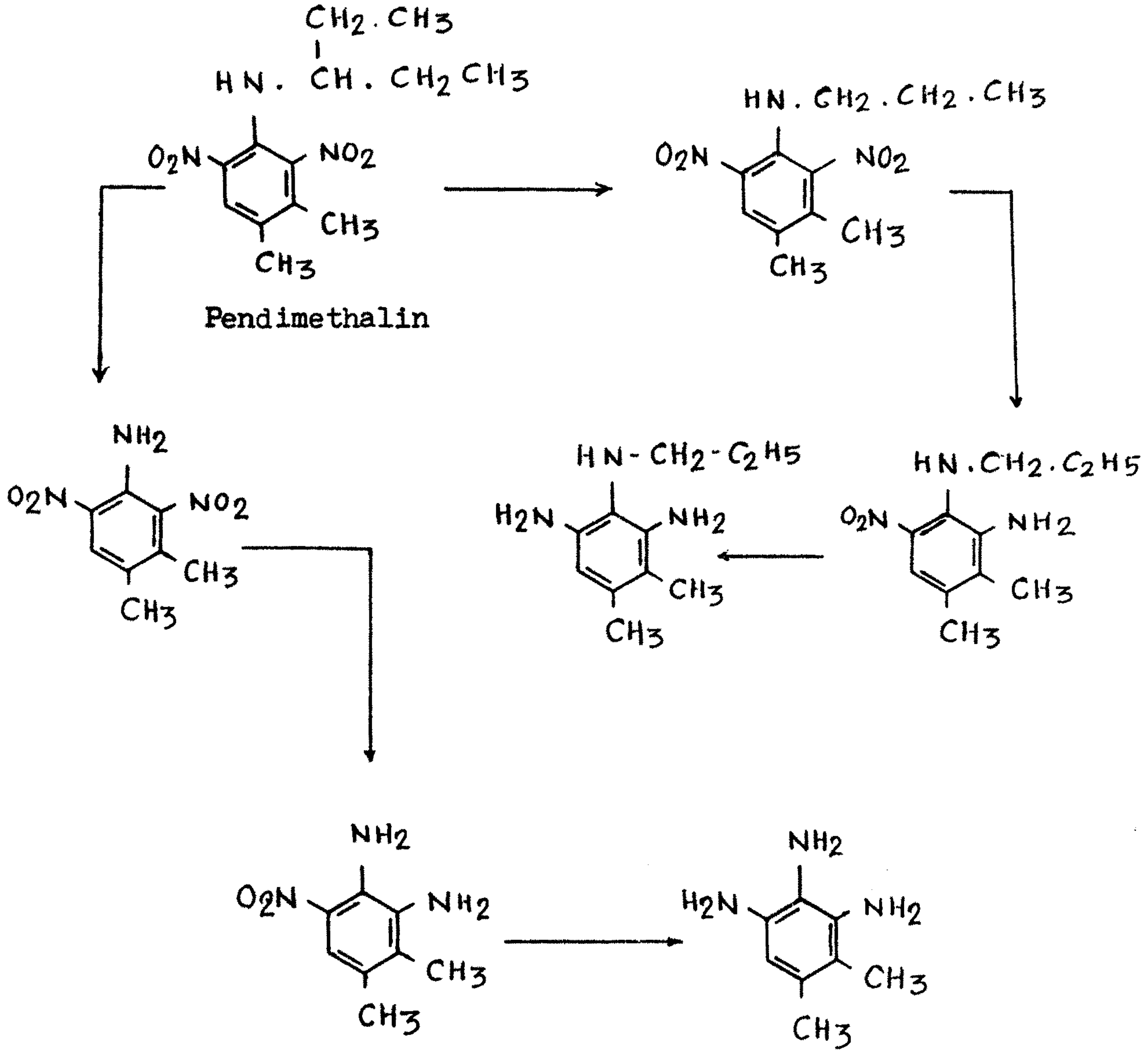
peaks corresponding to compound-A (Rt 2.2 min), compound-B (Rt 3.17) and then unknown metabolite (Rt 2.47 minute) were recorded in the chromatograms irrespective of different sampling dates. It could be further seen from the nature of the peaks that the photo degradation of pendimethalin was faster as time elapsed and compound-B was found to be the major product followed by compound-A. It may be mentioned that the discrepancies noticed between the thin layer and gas chromatographic behaviour of soil extracts could possibly be due to lower sensitivity of thin layer chromatography.

It is well known that herbicide transformation in soil can be effected by chemical, microbial and photochemical mechanisms[36]. Again, it has been reported that substituted dinitroanilines undergo photodecomposition in presence of water in sunlight after a considerable time of exposure. The degradation products of dinitroanilines in water and on dry soil after exposing to sunlight are often polar and include dealkylated, reduced and cyclic intermediates[37]. However, the possibility of formation of cyclic intermediates in the photodegradation of pendimethalin can not be considered because of the fact that initial abstraction of a hydrogen atom as postulated in the pathways of trifluralin (Scheme-I) leads to benzimidazoles (V), may be less facile from N-CHRR' compounds (pendimethalin) than those with $N-CH_2-R$ groups (trifluralin)[60].

Thus from a close scrutiny of different metabolites of substituted dinitroanilines formed as a result of photodegradation, the following degradative pathway as shown in Scheme-II can be postulated for pendimethalin.



Scheme-I : Photochemical transformation of trifluralin into cyclic metabolites.



Scheme-II. Plausible pathway of formation of different photometabolites from pendimethalin.

CHAPTER - II

Studies on the residues of some pesticides occurring in Ganga water

SECTION - A

INTRODUCTION

The perennial type Ganga river with its tidal stretch, the Hooghly and tributories running through Uttar Pradesh, Madhya Pradesh, Bihar and West Bengal form the Ganga river system, of which the Ganga basin is one of the most densely populated areas on earth. Although it serves as one of the richest sources of water, the water quality is being seriously affected day by day due to drainage of inadequately treated/untreated effluents from industrial, municipal and domestic sources. Among these various polluting sources, pesticide run-off from agricultural and municipal use through river linked waters bodies, plays a significant role in the accumulation of pesticide residues in river water and sediments.

Among the various classes of pesticides, the persistence of organochlorine pesticides like DDT, BHC, Aldrin, Dieldrin, Endosulfon, etc. in the ecosystem are well documented[115]. Many of these pesticides are being extensively used in our country for agricultural and public health programmes. As is well known these organochlorine pesticides are lipid soluble toxic organic chemicals and a low concentration of these pesticides may accumulate in the body fat of mammals that might pose problems in the long run[61]. Further, the persistence of these organochlorines in water has special significance as they are picked up by unicellular aquatic organism like plankton and in the process pesticide residues enter the food chain. It has been reported that dieldrin and DDT at a level of 10^{-5} mg/lt in water is undesirable for long time consumption by wild life[62]. DDT and Endosulfon are also toxic to fresh water fish[63].

Organic pesticides have been reported in drinking, recreational, irrigational, fish and shellfish waters of the United States wherefrom the evidence was obtained mostly from physiological responses of aquatic organisms. More recently,however, direct analyses by gas-liquid chromatography indicate that most surface waters and some ground waters contain trace amounts organic pesticides. Consequently, these raw water supplies may have to be monitored in order to avoid adverse effect from their ultimate domestic use as drinking water. Although some reports[64] concerning the pollution of water by pesticides in India are available, there appears to be no systematic investigation on the persistent types of pesticidal residues found in Ganga water. In fact, the current level of organo chlorinated hydro carbons like BHC, DDT, Endosulfon, etc. in Ganga waters has not been monitored and assessed in West Bengal.

It has been found from a survey report that organochlorines alone constitute 45-50% of total pesticide use by the farming community along both the banks of the Ganga river in West Bengal. As these persistent pesticides are expected to remain in the run-off water from the cultivator's fields which would eventually get discharged in the river, we therefore thought it worthwhile to study the levels of organochlorines like BHC, DDT, Endosulfon and its isomers/metabolites occurring in Ganga water.

SECTION - B

REVIEW OF LITERATURE

Organic pesticides like the chlorinated hydrocarbons and cyclodienes enter natural waters from direct application for control of aquatic weeds, trash fish, and aquatic insects; percolation and run-off from agricultural lands; drift from aerial and land applications, discharge of industrial waste waters from clean up of equipment used for pesticide formulation and application. Instances of pollution from all of these sources have been reviewed by Nicholson[65] (1959), Faust and Aly[66] (1964), Faust[67] (1964), Weiss[68] (1964) and West[69] (1964).

Table-1.9 summarises the suspected occurrences of some persistent organochlorinated pesticides polluting ground, surface and potable waters.

Table-1.9

Location	Pesticide	Source	Concentration	Reference
Snake,Mo. & Miss Rivers	DDT, Aldrin	Runoff	1-20 µg/1	Middleton and Lichtenberg[70] (1960)
Columbia River	Aldrin, DDT, Endrin, 2,4-D Esters	Ground and surface water	0.04-0.6 µg/1 0.02-16.0 µg/1 0.4-57.0 µg/1 Trace-18 µg/1	Hindin *et al*[71] (1964)

Table-1.9 contd.

Location	Pesticide	Source	Concentration	Reference
10 Major rivers, U.S.	DDT, dieldrin	Runoff	1-2 µg/1	Breidenbach and Lichtenberg[72] (1963)
96 Sampling stations on major rivers, U.S.	Dieldrin Endrin DDT Aldrin DDDE and DDD	Runoff Runoff Runoff Runoff Runoff	0.118 µg/1 0.094 µg/1 0.087 µg/1 0.085 µg/1 0.018 and 0.083 µg/1	Weaver[73] (1965)
	BHC and Heptachlor	Runoff	Presumptive	
Yamuna river, Delhi	DDT	Industrial waste water	0.03 mg/1 and 1.326 ppm	Pillai and Agarwal[74]
Najafgarh Drain, Delhi	DDT	DDT Factory	0.13-0.14 mg/1	Subba Rao et al[75] (1985)

The present review is restricted mostly to the persistence of organochlorinated pesticides occurring in aquatic system and the methodologies employed for separation and identification of these chlorinated pesticides from natural and potable waters.

I. Persistence in ground and surface waters

The persistence of some pesticides are controlled through the physical interaction and retention by various surfaces, by the uptake, retention, and release from various biological systems, and by hydrolytic and oxidative reactions. These chemical, physical and biological forces would determine the ultimate chemical nature of the pesticide whether it be degraded completely, *e.g.*, to carbon dioxide, water, ammonia and sulfate or incompletely to some metabolic, hydrolytic, or oxidative product. Thus, raw water supplies should be monitored for the parent compound and/or for some product of partial degradation. The effect of these breakdown products on water quality is largely unknown. Thus environmental forces that would permit entry of organic pesticides into water treatment systems are reviewed below.

(a) Chlorinated hydrocarbons

Bridges (1961)[76] reported the distribution and disappearance of endrin, 0.04 mg/l, in water, mud, vegetation and fish in a shallow pond.

Kallman *et al*[77] (1962) observed that toxaphene 0.05 mg/l, in clayton lake, New Mexico was distributed throughout the water fish, aquatic plants, and bottom sediments.

Bridges *et al*[78] (1963) determined the persistence and distribution of DDT, DDD and DDE in a farm pond. These compounds were not detected after three weeks in open waters but did persist in the mud, vegetation, and fish upto 16 months. DDT, like toxaphene, was concentrated rapidly, 30.7 ppm in 0.5 hour, by aquatic vegetation. Rainbow trout adsorbed a considerable quantity of DDT that was metabolized slowly to DDD and DDE. After 16 months, a total of 2.80 ppm was still present in the trout of which 45.0 per cent was DDT, 50.4 per cent was DDD, and 4.6 per cent was DDE. DDT was confined largely to surface waters during the first 24 hours.

Welch and Spindler[79] (1964) determined the occurrence and persistence of DDT following an aerial application for spruce budworm control in the Boulder River drainage area, Montana. DDT was detected as 0.1 µg/1 at one sampling point seven days after spraying despite precautious to prevent it from entering the stream.

Nicholson et al[80] (1964) examined levels of chlorinated hydrocarbon insecticide pollution of a stream drainage system and a potable water system. The study area was a northern Albama water-shed of approximately 400 sq.miles which was drained by 760 miles of streams tributary to the Tennesse River. Estimates were made of total tonnage of all applied insecticides and herbicides of which toxaphene, DDT, and BHC comprised over 90 per cent.

Pillai and Agarwal[74] (1979) reported the maximum level of 0.003 mg/1 and 1.326 ppm DDT in water and bottom sediment, respectively in Yamuna river in Delhi.

Joshi (1985)[81] reported the occurrence of DDT residues in fish collected from the river Hooghly.

II. Extraction from water and preliminary separation from interferences

Direct measurement and identification of organic pesticides in water may not be possible because of sensitivity and specificity requirements. Extraction, concentration, and clean up techniques must be employed prior to quantification. This portion contain a critical review of extraction and preliminary clean-up techniques that have been employed mainly for water systems.

(a) Carbon adsorption techniques

Braus *et al*[82] (1951) developed and introduced the continuous technique for isolation of synthetic organic contaminants affecting taste and odor qualities of surface waters. This field device was known as the carbon adsorption method (CAM). The CAM utilized a pre-filtering device that consisted of a borosilicate glass pipe three inches in diameter and 18 inches in length packed with 4 x 10 mesh and 30 mesh carbon. Sample volumes of 1000 and 5000 gallons were pumped through the filter at rates of 0.25 and C.56 GPM (5.1 and 10.2 GPM/ft^2) after which the carbon was removed and oven dried at 40°C for approximately two prior to solvent extraction.

Hancock and Laws[83] (1955) employed a batch-wise technique for 90 per cent recovery of BHC by passing several litres of water or sewage through a column containing one gm of carbon. Elution was accomplished with glacial acetic acid. The carbon's capacity was determined as five mg of BHC/g. Overall sensitivity of the analytical procedure was limited to five µg because of the subsequent color reaction.

Coulson and Brans[84] (1963) reported variations in adsorption efficiencies by different carbons due to inorganic contaminants of oxides of sodium, magnesium, calcium etc. Extraction with concentrated hydrochloric acid for one hour at 80°C to 90°C on a steam bath produced carbons with efficient and effective clean up properties. Pretreatment is a point often over looked in the CAM.

Breidenbach <u>et al</u>[85] (1964) provided an excellent account of procedural detail for recovery of chlorinated hydrocarbons from carbon and their subsequent separation from one another and interferences. In very general terms the dried carbon is placed into large-scale Soxhlets for continuous extraction (35 hours) with redistilled chloroform. This chloroform extract (CCE) is subsequently evaporated to dryness whereupon a residue weight is obtained.

Nicholson <u>et al</u>[86] (1964) used acetonitrile to dissolve chloroform extract residue for recovery of BHC and toxaphene. The insecticides were partitioned into petroleum ether. This ether fraction was washed with distilled water, dried with sodium sulfate and placed on florisil column where elution was effected with mixed ethers. Benzene was used to take up the dried ether residue whereupon the sample was ready for analysis.

Hindin et al[87] (1964) used either petroleum ether, or benzene extraction of chlorinated hydrocarbons and esters of 2,4-D that had been previously extracted from water by the carbon adsorption method (CAM).

Goodenkauf and Erdei[88] (1964) stated "The well known limitations of the carbon filter method make accurate quantitative results impossible".

Nicholson et al[86] (1964) determined the mean recoveries (three replications) of toxaphene and BHC from carbon as (a) from 0.5 μg/l solutions, 42 and 43 per cent respectively, and (b) from 1.0 μg/l solutions, 48 and 48 per cent respectively. These low recoveries were due apparently to poor extraction efficiencies since two insecticides were not detected in the carbon filters effluent.

Table-1.´0. Efficiency of adsorption and recovery from carbon (After Rosen and Middleton[89], 1959).

Insecticide	Per cent	
	Adsorbed	Recovered
BHC	99	80
Chlordane	99	75
DDT	98-100	76
Aldrin	97	85
DDD	96-100	82
Endrin	91	86

(b) Liquid-liquid extraction

Serial extraction

Kallman et al[77] (1962) used petroleum ether to extract toxaphene from lake water.

Lamer et al[90] (1965) tried n-hexane for recovery of six chlorinated hydrocarbons from seven rivers in the United States.

Breidenbach et al[85] (1964) suggested n-hexane for recovery of chlorinated hydrocarbons from acidified water as an alternate to the CAM.

Warnick and Gaufin[91] (1965) reported the use of Nanograde hexane for extraction of methyl parathion,parathion,dieldrin and DDT from acidified water prior to gas-liquid chromatography.

Continuous extraction

Kahn and Wayman[92] (1964) described a continuous, multichamber liquid-liquid system with an internal solvent recycle for extraction of several chlorinated hydrocarbons from natural waters. Three extraction chambers were connected in series and were charged with petroleum ether, water was pumped in to the chambers at rates of 0.5 and 1.0 l/hour. Extraction efficiencies ranged from 96 to 100 per cent for aldrin, isodrin, dieldrin, endrin and two intermidiates recovered from the waste water stream of a manufacturing facility.

Sanderson and Ceresia[93] (1965) suggest that continuous system effect higher recoveries than serial systems.

Table 1.11 shows per cent recovery data for several pesticides from water by serial and continuous systems.

Table 1.11. Liquid-liquid extraction system and per cent recoveries.

Pesticide(s)	Solvent(s)	No. of extractions	Sample volume	pH	Mean recovery (%)
DDT	3:1 Ether & *n*-hexane	4	1.5 l	4.0-5.0	88[a] (Berck[94], 1953)
Dieldrin and metabolites	*n*-hexane	4	100 ml	-	90-95 (Cueto and Hayes[95], 1962)
DDT, toxaphene, aldrin, dieldrin, BHC	1:1 Ether-pet.ether or $CHCl_3$	5	1.0 l	-	88 (Teasley and Cox[96], 1963)

Table 1.11 contd.

Pesticide(s)	Solvent(s)	No. of extractions	Sample volume	pH	Mean recovery (%)
Parathion and Diazinon	1:1 Ether-pet.ether or $CHCl_3$	5	1.01	-	90 (Teasley and Cox[96], 1963)
2,4-D, MCPA, 2,4,S-T	$CHCl_3$	3	300 ml	2.0	93 (Erne[97], 1963)
8 Cl_2-hydrocarbon	n-hexane	2	1 1	-	98 (Lamar[90] et al., 1965)

[a] Interference absent

(c) Clean up techniques

Raw waters may contain organic interference as aromatic and aliphatic compounds from industrial waste waters, pesticide formulation solvents and impurities and naturally occurring coloured compounds. These impurities must be recovered for positive identification of the organic pesticides. Thus a clean up step becomes imperative that would follow the initial extraction from grossly polluted waters. There are three consideration here.

(a) Partition chromatography

Table 1.12 summarises the apparently successful partition chromatographic clean up techniques that have been applied to natural water extracts for separation of organic interferences from organic pesticides. Columns of a silica type support appear the most useful and successful.

Table 1.12. Column chromatography clean up systems applied to the separation of organic pesticides from interference in natural waters.

Pesticide(s)	Support	Elution solvent	Identification technique	Reference
Parathion	Alumina	$CHCl_3$	Color	Nicholson et al[98] (1962)
2,4-D	Silicic acid	n-hexane	Colour and ultraviolet	Aly and Faust[99] (1963)
Toxaphene, BHC	Florisil	Pet.ether-ether	Microcoul GLC	Nicholson et al[86] (1964)
DDT	Florisil	Pet.ether-ether	Infrared and microcoul,GLC	Grazenda et al[100] (1964)

(b) Separation from natural colour compounds

Black and Christman[101] (1963) had characterised natural colour bodies in water as negative charged colloids of vegetable origin. Chemically, they are fulvic acids or aromatic polyhydroxymethoxy carboxylic acids.

Aly and Faust[99] (1963) reported successful separation of 2,4-D, 2,4 dichlorophenol, and organic colour (65 units) in a natural water by partition chromatography on a silicic acid column. n-Hexane served as the mobile solvent whereas a 1:9 mixtures of glacial acetic acid and methanol was employed as the immobile solvent.

Faust and Hunter[102] (1965) applied a rather unique technique to separate natural organic colour from Diquat and Paraquat in water. Complete removal of organic colour was effected by passage through an anion-exchange column which permitted a mean recovery of 100 per cent of Diquat and Paraquat from several natural waters.

(c) Suggested techniques

Several chromatographic and extraction clean up technique from the residue systems may have some applications to natural waters.

Crosby and Laws[103] (1964) used gas-liquid chromatography for clean up of vegetable extracts of 15 organophosphates prior to identification by infrared spectra.

Smith and Eichelberger[104] (1965) used thin layer chromatography for separation of organic interferences in the carbon adsorption method from aldrin, DDD, DDE, DDT, dieldrin, endrin, heptachlor, heptachlor-epoxide and lindane. This study was designed to clean up these chlorinated hydrocarbon pesticides prior to identification and quantification by gas-liquid chromatography. An average recovery of 91% was claimed for six of the nine pesticides.

III. Separation and identification of parent compounds, metabolites and hydrolysis products

The principles of separation and identification of organic pesticides following extraction, and clean-up from water are identical with those of plant and animal residue analyses. In proportion, however, the literature contains relatively few application of chromatography and spectrophotometry for determination of organic pesticides in water. Paper chromatography has received considerable attention in plant and animal residues but very little in water analysis. Thin layer chromatography is the current range in pesticide analysis. Gas-liquid chromatography has been explored recently for organic pesticides in water wherein most application have been concerned with seperation and identification of parent compounds. Very little attention has been given to hydrolytic and metabolic products. Spectrophotometric techniques although have been employed for organic pesticides in water, but spectroscopy can not compete, however, with the ultrasensitivity of the chromatographic techniques, which may limit it usefulness in water analysis. Thus the present review is directly focussed on TLC and GLC technique of water analysis.

(1) Thin layer chromatography

Thin layer chromatography technique exhibits considerable promise as a means of (a) clean-up prior to other technique of identification (b) separation from other pesticides, and (c) positive identification. Table 1.13 summarises pertinent conditions required for thin-layer chromatographic separation of several classes of organic pesticides.

Table 1.13. Conditions required for thin-layer chromatographic separation of organic pesticides.

Pesticides	Absorbents	Systems		Sensitivity(µg)	Reference
		Solvent	Development		
Cl_2-hydrocarbons	Alumina-G Silica gel G	n.Heptane-acetone	$AgNO_3$-2-PE	0.01,0.05 0.1	Kovacs[105] (1963)
14 Organic phosphates	Alumina-G	15 and 20% DMF-MCH	$AgNO_3$-citric acid. TBPP	0.05, 0.01	Kovacs[106] (1964)
5 Phenoxy-alkyl acids	Mix.Silica gel G and Kieselguhr G	Liq.parafin,benzene, gl, HOAC	$AgNO_3$-2-PE	-	Abbott[107] (1964)
14 Organo phosphates	Alumina-neut. Silica gel H	Benzene-acetone	BPB	0.1-0.2	Bunyan[108] (1964)

Kovacs[105] used aluminium oxide G and silica gel G for resolution of aldrin, BHC, chlordane, DDD, DDE, DDT, dieldrin, endrin, heptachlor, heptachlor epoxide, kelthane, lindane, methoxychlor, perthane, strobane, and toxaphene. This procedure was designed either as a rapid screening method or as a confirmation technique in conjunction with gas-liquid chromatography.

Kovacs[106] (1964) also reported the separation and identification of 14 sulfur containing phosphate esters from crop extracts on aluminum oxide G as follows : 0.05 μg, Diazinon, demeton (thiono), Trithion, Parathion, Malathion, Ronnel, Delvav, EPN, CO-Ral, Sulfotepp, and Ethion; 0.1 μg Guthion, methyl parathion, and demeton (thiclo). Kovacs claimed that this technique was ten to fifty times more sensitive than paper chromatography.

Abbott et al[107] (1964) described a procedure for separation and identification of MCPA, MCPB, 2,4-D, 2,4-DB and 2,4,5-T from soil and water extracts. Identification was accomplished by comparison of the Rf value with a known standard.

Bunyan[108] (1964) resolved demeton-O, demeton-S, Diazinon, malathion, Mevinphos, Methyl parathion, Phorate, Phosphomidon on plants of silica gel H and alumina.

(2) Gas-liquid chromatography

GLC covered with numerous techniques for separation, identification, and estimation of organic pesticides from soil, water, food, animal and plant extract. But, GLC alone can not suffice for the positive and irrevocable identification of parent compounds, metabolites, and hydrolysis products extracted from any medium. It should be utilized in conjunction with some other form of analytical identification. It is however, an extremely attractive and glamorous analytical tool because of ultra-sensitivities : 10^{-9} g, 10^{-12} g, etc. that have been reported. These sensitivities may be necessary if the ultimate fate of organic pesticides in an aqueous environment is to be explored. A brief review follows of the more recent and major developments of gas-liquid chromatography.

(a) Water extracts

Most applications have been concerned with separation and identification of parent compound whereas very little attention has been directed toward hydrolytic and metabolic products. Table 1.14 summarises the pertinent columns and detectors used with water extracts.

Teasley and Cox[96] (1963) claimed the separation and detection of 25 to 50 mg/l of BHC, DDT, toxaphene, aldrin, and dieldrin in one litre extracts of water. Recoveries for these five chlorinated hydrocarbons averaged 88 per cent within a range of 85 to 90 per cent.

Hindin *et al*[87] (1964) evaluated several columns, temperature conditions and three detectors for separation of organic pesticides from one another and from interferences in lake sediments.

Hindin and co-workers[87] (1964) concluded that the microcoulometric detector would be more specific but less sensitive for field samples containing interferences than an electron capture detector.

Kahn and Wayman[92] (1964) separated six chlorinated hydrocarbons under the column conditions in Table 1.14 following continuous liquid-liquid extraction from water.

Breidenbach <u>et al</u>[85] (1964) have compiled the necessary procedural detail for separation of chlorinated hydrocarbons with subsequent detection by micro-coulometry and electron capture (Table 1.14).

Warnick and Gaufin[91] (1965) claimed recovery (90 per cent and greater) separation and detection of five pesticides from distilled water, tap water, and aquarium water (Table 1.14). Retention times relative to dieldrin were given for methyl parathion, parathion, OP-DDT, and P,P'-DDT.

Lamer et al[90] (1965) investigated two columns (Table 1.14) for separation of aldrin, dieldrin, O,P'-DDT, P,P'-DDT, endrin, heptachlor, heptachlor epoxide, and lindane prior to detection by electron capture. Recovery data were given from fortified distilled water (82 to 114 per cent) and natural water (78 to 123 per cent) samples. Also, data were given for occurrence of these chlorinated hydrocarbons in seven river systems in the United States.

Table 1.14. Conditions used for separation of organic pesticides by gas-liquid chromatography of water extracts.

Column			Pesticides	Sensitivity claimed	Reference
Construction and detector	Length (ft)	Temp. (°C)			
5% DC 200 oil on 60/80 chrom. P-microcoul.	6	195	Cl_2-hydrocarbons	25-50 ug/1	Teasley and Cox[96] (1963)

Table 1.14 contd.

Column			Pesticides	Sensitivity claimed	Reference
Construction and detector	Length (ft)	Temp. (°C)			
5% DOW-200 Silicone oil on 60/80 chrom. P-microcoul.	5	200-230	Cl_2-hydrocarbon 2,4-D	-	Hindin *et al*[87] (1964)
5%SE-30 silicone gum on 45/60 chrom. W-elec.cap.	4	165	Cl_2-hydrocarbons	-	Kahn and Wayman[92] (1964)
5% DOW-11-silicone grease on 60/80 chrom. W-microcoul.	3	200	Cl_2-hydrocarbons	0.001 µg/l	Bridenbach *et al*[85] (1964)
5% DOW-11-silicone grease on 60/80 chrom. W-elec.cap.	3	175	Cl_2-hydrocarbons	0.001 µg/l	Bridenbach *et al*[85] (1964)
3% SE-30 on 80/90-Ana Krom ABS-elec.cap.	4	195	Cl_2-hydrocarbons organophosphates	1.0 µg/l	Warnick and Gaufin[91] (1965)
5% DOW-11, Silicone grease on 60/80 chrom. W-elec.cap.	5	185	Cl_2-hydrocarbons	0.1 pg (lindane)	Lamer *et al*[90] (1965)
5% QF-1 on 60/80 chrom. W-elec.cap.	5	185	Cl_2hydrocarbons	-	Lamer *et al*[90] (1965)

(b) Other extracts

Since the problem of analysis for organic pesticides in aqueous environments is a relatively recent event, only a few gas liquid chromatographic techniques have appeared in the literature. This is especially true for the separation of metabolites and hydrolytic products. A brief review mainly on chlorinated hydrocarbons is given below to recent developments from plant and animal residue systems. The most suitable conditions are listed in Table 1.15.

Goulden _et al_[109] developed a halogen-sensitive detector that could be used as an alternate to electron capture.

Klein and Watts[110] (1964) claimed resolution of mixtures of the olefins of Perthane, DDD and DDT (Table 1.15). Mixtures of Perthane, O,P'-DDT, DDD, and P,P'-DDT were resolved also.

Mccully and McKinley[111] (1964) separated lindane, heptachlor, aldrin, telodrin, heptachlor epoxide, P,P'-DDE, dieldrin, O,P'-DDT, rhothane, P,P'-DDT, endrin and methoxychlor in this order (Table 1.15). These chlorinated hydrocarbons were recovered from fortified fats and oils in an average range of 73 to 112 per cent.

Burke and Giuffrida[112] (1964) determined retention times of 65 chlorinated pesticides relative to lindane (Table 1.15).

Emphasis was given to sample clean up as ensuring consistently accurate results and almost indefinite column life.

Minyard and Jackson[113] (1965) reported a unique technique, whereby chlorinated pesticides were chemically modified in the flash heater before separation of a five per cent SE-30 column (Table 1.15) and detection by electron capture. Sodium carbonate, cupric oxide, cadmium chloride, aluminium chloride, and potassium dichromate were exposed at 240°C to BHC isomers, DDD, DDE, DDT, methoxychlor, aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide, chlordane, and toxaphene. Most of the modifying agents produced degradation products that were separated more easily than the parent compounds. For example, P,P'-DDT, P,P'-DDE, and P,P'-DDD were distinguished from one another on the silicone column after exposure to sodium carbonate.

Table 1.15. Column conditions for separation of chlorinated hydrocarbons by gas-liquid chromatography[a]

Column			Sensitivity claimed	Reference
Construction[b]	Length (ft)	Temp. (°C)		
2.5% Silicone oil + 0.25% Epikote 1001 on 100/120 celite.	4	163	0.4 ng	Goulden et al[109] (1963)
2.5% of 1:1 mix. of SF-96 & 2,2 DEPD-IP[c] on 80-100 Gas Chrom. S.	3	175	-	Klein and Watts[110] (1964)
4% G.E. SE-30 + 6% D.C.Q.F.-1 on 60/80 Chrom.W.	4	175	-	Mccully and Mckinley[111] (1964)
10% DC 200 on 80/90 Anakrom ABS	6	200	0.01 ppm	Burke and Giuffrida[112] (1964)
5% SE-30 on 60/80 Chrom.W	4	195	-	Minyard and Jackson[113] (1965)

[a] All systems used the electron-capture detector.

[b] According to original author.

[c] 2,2-Diethyl-1, 3-propanediol-isophathalate polyester.

SECTION - C

EXPERIMENTAL

With a view to determining the level of organochlorinated pesticides occurring in Ganga waters, a short account of the materials and methods employed for the present investigation is described in this section.

1.1 Sampling

For monitoring the level of organochlorine pesticides in Ganga water, samples were collected from both the eastern and western banks of Ganga river. Water samples were collected from the eastern bank starting from Kalyani (Dist. Nadia) to Jagaddal (Dist. 24-Parganas) at a stretch of about 25 km. Again water samples from the western bank were collected from Kunti Ghat (Mogra-Chinsurah block) to Chandannagar (Dist. Hooghly) at a stretch of about 25 km. In addition to these, water samples were also collected from canals linking the Ganga river from both the eastern and western banks.

The water samples thus collected from different pockets of the Ganga basin were analysed for BHC, endosulfan and DDT. The experimental techniques employed for the analysis of organochlorine residues in the Ganga water involved the following steps.

1.2 Extraction and clean up

Solvent-solvent partition[114] method was used for extraction of BHC, DDT and Endosulfan residues from water samples and n-hexane (distilled) and chosen as the solvent. At the laboratory, water samples were usually allowed to stand and the supernatant liquid was decanted and extracted. One litre of water containing BHC/DDT/Endosulfan residues was taken in a separatory funnel and 100 ml of saturated NaCl solution was added and extracted vigorously three times with 200 ml, 200 ml and 100 ml of n-hexane (distilled) and then the phases were allowed to separate. All the n-hexane layers were collected and dried over anhydrous sodium sulfate. The total n-hexane layer was then evaporated to dryness by a Kuderna-Danish evaporator and the residue thus obtained was taken up in a 5 ml volumetric flask in n-hexane which was then subjected to Gas-Chromatographic analysis. The results thus obtained are shown in Section-D.

1.3 Estimation by Gas Chromatography

Gas chromatograph used was Hewlett Packard Model 5890A with Electron-Capture Detector (Scurce Ni^{63}).

(a) Operating parameters for BHC isomers

Column : 6 ft x 2 mm ID glass packed with OV-101 on chromosorb W.H.P. (80-100 mesh).

Column temperature : 160 °C

Injection temperature : 200 °C

Detector temperature : 300 °C

Carrier gas : Nitrogen (58 ml/minute).

Analytical grade 98.7% BHC (α -isomer), 99% BHC (β -isomer), 99% BHC (γ-isomer) and 99.2% BHC (δ -isomer) were used as external standards. Retention time was observed : α -3.79, β -5.95, γ-4.99, and δ -7.04 (Fig.1.20). 5-7 μl of cleaned up extract was then injected to the Gas Chromatograph.

(b) Operating parameters for the detection of DDT isomer/ metabolites

Column : 6' x 2 mm I.D. packed with OV-101 on chromosorb W.H.P. (80-100 mesh)

Column temperature : 200 °C

Injection temperature : 200 °C

Detector temperature : 300 °C

Carrier gas : Nitrogen (Flow 65 ml/min.)

Analytical grade PP'-DDT (99%), OP'-DDT (99%), PP'-DDE (98.7%), OP'-DDE (98.7%) were used as external standards. Retention time was observed : DDE (OP'-3.85, PP'-4.85), DDT (OP'-6.2, PP'-7.8). 3-7 μl of the cleaned up extract was then injected to the Gas Chromatograph (Fig.1.21).

(c) Operating parameters for the detection of Endosulfan isomers and metabolites

Column : 6' x 2 mm I.D. packed with OV-101 on chromosorb W.H.P. (80-100 mesh)

Column temperature : 200 °C

Injection temperature : 200 °C

Detector temperature : 300 °C

Carrier gas : Nitrogen (Flow 65 ml/min.)

α-Endosulfan (99%), β-Endosulfan (99%) and Endosulfan cyclic sulphate (99%) were used as external standards. Retention time observed : Endosulfan (α -4.2, β -5.5 and cyclic sulphate 7.2). 2-7 μl of the cleaned up extract was then injected to the Gas Chromatograph (Fig.1.22).

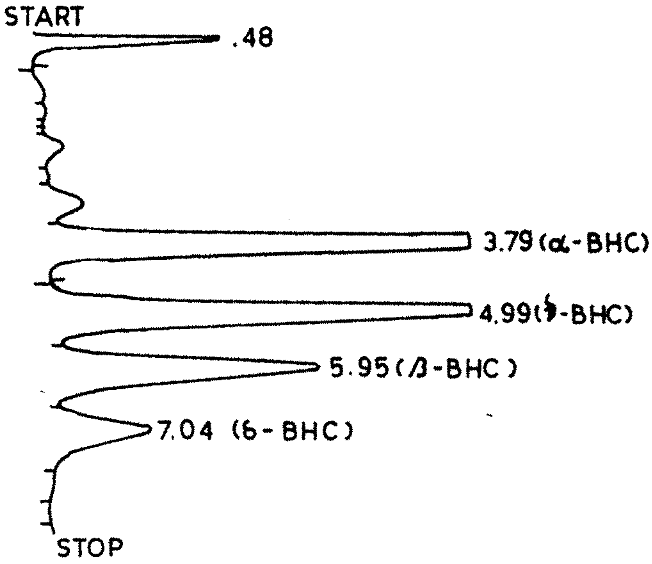


Fig. 1.20. Gas chromatogram of BHC isomers

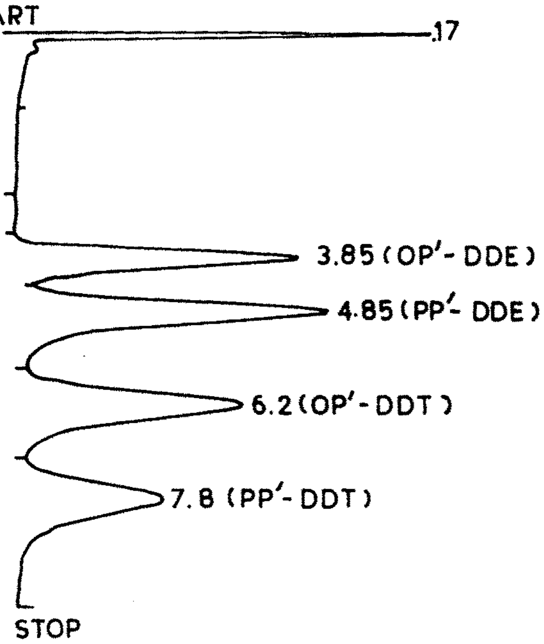


Fig. 1.21. Gas chromatogram of DDT isomer/metabolites

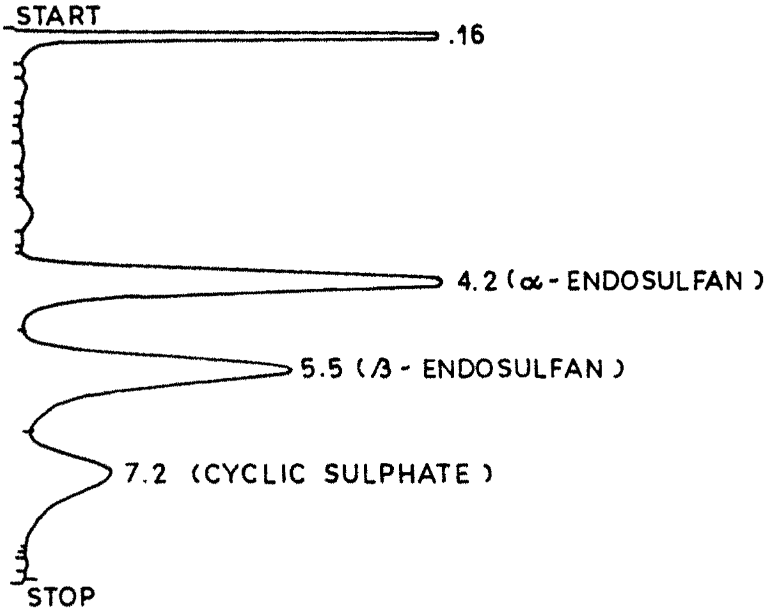


Fig. 1.22. Gas chromatogram of Endosulfan isomers/metabolites.

SECTION - D

RESULTS AND DISCUSSION

2. Recovery of BHC, DDT and Endosulfan

Recovery studies were carried out in order to establish the reliability of the analytical methods and to know the efficiency of extraction and clean-up steps employed for the present study. It was done by fortifying tap water with 0.001 and 0.0005 ppm of BHC, DDT and Endosulfan respectively. The analytical recovery data are presented in Table 1.16.

Table 1.16. Recovery of BHC, DDT and Endosulfan.

Applied concentration	Replication	Days of sampling	% of recovery		
			BHC	DDT	Endosulfan
0.001	R_1	0	85	90.5	91
	R_2	0			
	R_3	0			
0.0005	R_1	0	91	85.3	85.4
	R_2	0			
	R_3	0			

It is evident from Table 1.16 that the recovery of BHC, DDT and Endosulfan in water varied from about 85-91% respectively. This was fairly a good recovery indicating that the the method is satisfactory and could be adopted for the determination of BHC, DDT and Endosulfan in water.

Residues of BHC occurring in water of linked canals

The concentration of BHC-isomers (α- , β-, γ- and δ-) occurring in linked canals as determined by Gas Chromatography is shown in Table 1.17.

Table 1.17. Residues of BHC (α- , β-, γ- and δ-) occurring in canals linking Ganga river.

Sl. No.	Location	Amount (µg/ml)				Total
		α-BHC	β-BHC	γ-BHC	δ-BHC	
1.	Ghoshpara canal Middle point	0.0081	-	-	-	0.008
2.	Ghoshpara canal (100 mt away from the confluence)	0.007	-	-	-	0.007
3.	Benipur (Mogra-Chinsurah block)	0.001	-	0.004	-	0.005
4.	Chandrahati (Mogra-Chinsurah block)	0.001	-	0.004	-	0.005
5.	Charsarhati (Chakdah block)	0.0001	-	0.0001	-	0.0002
6.	Saraswati stream	0.0003	-	-	-	0.0003
7.	Kunti Ghat Canal (Middle point)	0.00002	-	-	-	0.00002
8.	Paint Factory Canal (Naihati)	0.00005	0.00004	-	0.00001	0.0001
9.	Paint Factory Canal (Middle Point)	0.0012	0.00005	-	0.00003	0.00128

Table 1.17 contd.

Sl. No.	Location	Amount (µg/ml)				Total
		α-BHC	β-BHC	γ-BHC	δ-BHC	
10.	Mukatapur Canal (Eastern Bank)	0.0013	-	-	0.00001	0.00131
11.	Paint Factory (Garifa)	0.0012	0.0014	0.0006	0.0018	0.005
12.	Gosai Canal (Chandannagar)	0.0097	-	-	-	0.0097

It has found from Table 1.17 that the total BHC concentration ranged from 0.00002-0.0097 ppm in 12 samples collected from linked canals. Out of these 12 samples, the γ-isomer was detected in four samples ranging from 0.0006-0.004 ppm. The highest concentration of total BHC (0.0097 ppm) was found in the Gosai canal in Chandannagar block. The highest concentration of γ-isomer (0.004 ppm) was found in Benipur and Chandrahati canal in Mogra-Chinsurah block.

Residues of BHC (α-, β-, γ- and δ-) occurring in Ganga water

The concentration of BHC-isomers (α-, β-, γ-, δ-) occurring in Ganga water as analysed by Gas Chromatography is summarised in Table 1.18.

Table 1.18. Residues of BHC (α-, β-, γ- and δ-) occurring in different spots of the Ganga river.

Sl. No.	Location	Amount (µg/ml)				Total
		α-BHC	β-BHC	γ-BHC	δ-BHC	
1.	Mogra Block	0.013	-	-	-	0.013
2.	In between Kunti Ghat and Tribeni Ghat	0.0006	-	-	-	0.0006
3.	Kunti Ghat Canal and River confluence	0.015	-	-	-	0.015
4.	Saraswati River and Hooghly River confluence	0.0096	-	-	-	0.0096
5.	Near Tribeni Ghat	0.0125	-	-	-	0.0125
6.	In between Chinsurah and Tribeni	0.0021	-	0.0004	-	0.0025
7.	Bandel Brick Field	0.00037	0.00006	0.00029	-	0.00072
8.	Bandel Church	0.0021	-	0.0001	-	0.0022
9.	Hooghly Imman Bara	0.00012	0.00002	0.00014	-	0.00028
10.	Hooghly Bridge	0.00007	0.00004	0.0001	-	0.00021
11.	Chinsurah	0.0009	-	0.001	-	0.0019

Table 1.18 contd.

Sl. No.	Location	Amount (µg/ml)				Total
		α-BHC	β-BHC	γ-BHC	δ-BHC	
12.	Opposite to Kunti Ghat 200 mt away on the eastern side	0.0026	-	0.0154	-	0.018
13.	Majher Char, Kalyani	0.0027	-	-	-	0.0027
14.	Naihati P.S. Ghat	0.00007	-	-	-	0.00007
15.	Gowala Para Kankinara (Eastern Block)	0.00007	-	-	-	0.00007
16.	Rangkal Canal confluence of the River and Canal	0.000035	0.000055	0.00006	0.00015	0.0003
17.	Khasbati (Eastern bank, near a Jute Mill)	0.00188	0.00215	0.00009	0.00028	0.0044
18.	Indian Paper Pulp, Hazinagar	0.00187	-	0.00001	0.00002	0.0019
19.	Ramprasad Ghat Halisahar	0.00114	0.00123	0.00073	0.00260	0.0057
20.	Nadia Jute Mill, Naihati	0.000014	-	-	0.000016	0.00003
21.	Bhatpara Bazar	0.00622	0.00001	-	0.00007	0.0063

Table 1.18 contd.

Sl. No.	Location	Amount (µg/ml)				Total
		α-BHC	β-BHC	γ-BHC	δ-BHC	
22.	Chalkal Ghat, Shyamnagar	-	-	-	0.000001	0.000001
23.	Reliance Jute Mill, Shyamnagar	0.00839	0.00011	-	-	0.0085
24.	Kankinara Jute Mill	0.01831	0.00019	-	-	0.0185
25.	Kankinara Ghat	0.0131	-	-	-	0.0131
26.	Anglo-Indian Jute Mill	0.0152	0.0001	-	-	0.0153
27.	Anglo-Indian Canal at confluence	0.01578	-	-	0.00002	0.0158
28.	Gowala Para, Kankinara (Mid.stream)	0.000059	0.000029	-	0.000102	0.00019
29.	Khasbati (Middle stream)	0.0000565	-	0.0000385	0.000185	0.00028
30.	Indian Paper Pulp, Hazinagar (Middle stream)	0.00015	0.00008	0.00009	0.00018	0.0005
31.	Halisahar (Middle stream)	0.00206	-	0.00067	0.00227	0.005
32.	Bandel-Sahaganj (Middle stream)	0.00026	0.00005	0.00028	-	0.00059

Table 1.18 contd.

Sl. No.	Location	Amount (μg/ml)				Total
		α-BHC	β-BHC	γ-BHC	δ-BHC	
33.	Naihati (Middle stream)	0.000015	0.000028	-	0.000017	0.00006
34.	Jagaddal Ghat (Middle stream)	0.00063	-	0.00007	-	0.0007
35.	Chandannagar (Middle stream)	0.00095	-	-	0.00005	0.001
36.	Chandannagar shoal (Middle stream)	-	-	-	0.0018	0.0018

The results indicated that out of the 36 samples collected and analysed, the total BHC concentration varied from 0.000001-0.0185 ppm of which the α-isomer could be detected in 34 samples ranging from 0.000014-0.01831 ppm. The highest amount of α-isomer as well as the total BHC could be found in water samples collected from Kankinara Jute Mill pocket.

The β-isomer was found in 14 samples ranging from 0.00001-0.00215 ppm of which the highest being found in water samples collected from Khasbati, the eastern bank of the Ganga as shown in Fig. 1.23.

The γ-isomer could be detected in 16 samples ranging from 0.00001-0.015 ppm and the highest concentration of this

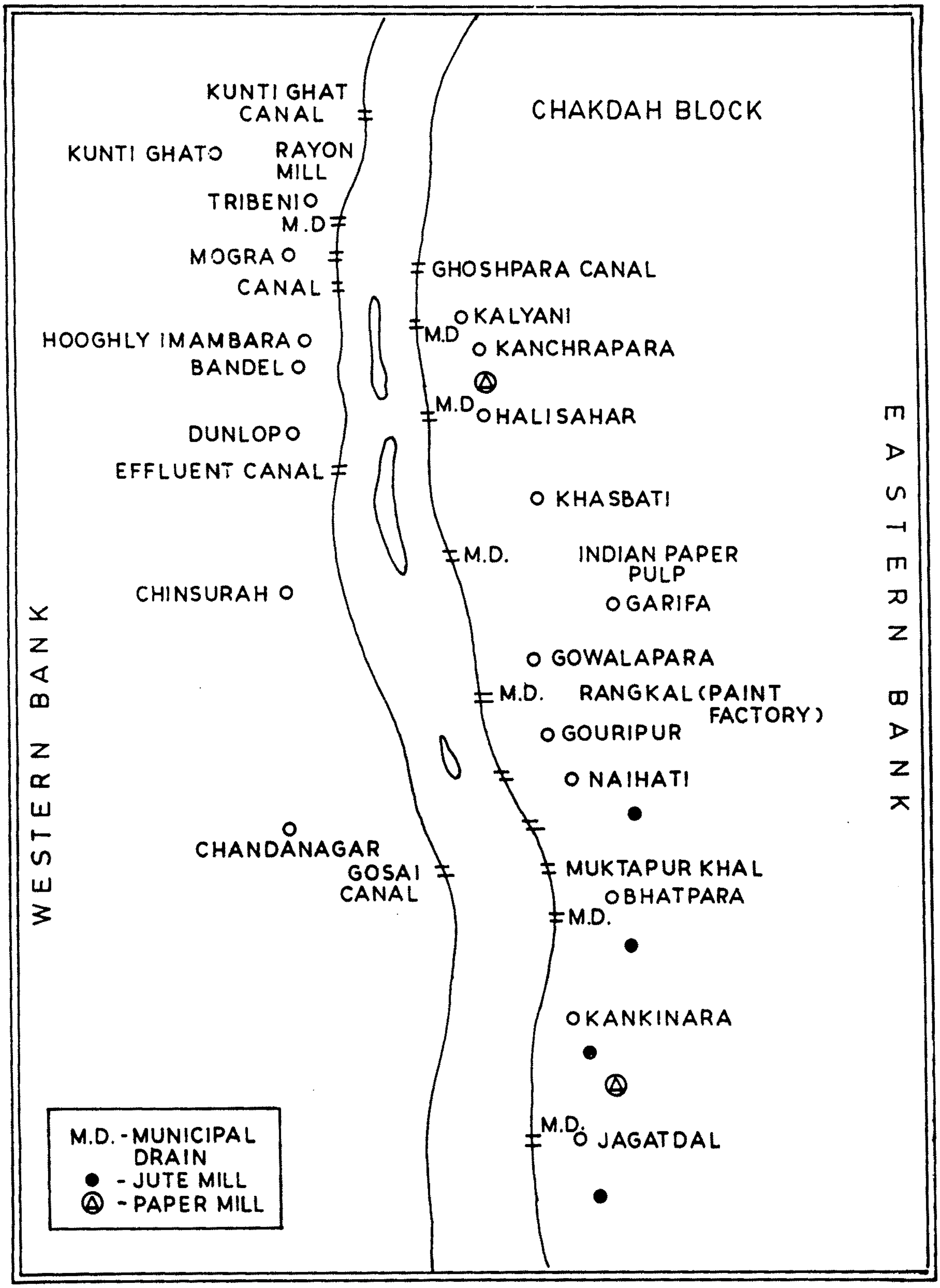


Fig. 1·23. Sampling sites under pesticides monitoring of the Ganga River.

isomer was found in water samples collected from the eastern bank which is about 200 meters away and opposite to Kunti Ghat.

The δ-isomer could be detected in 15 samples ranging from 0.000001-0.0026 ppm, the highest being found in the water samples collected from the Ramprasad Ghat, Halisahar.

The results further indicated that 22 samples were found to contain significant amount of BHC residues ranging from 0.001-0.0185 ppm.

Thus, from the above study it was felt by the present candidate that a systematic minitoring of the BHC residues throughout the year is indispensible in order to assess the level of BHC residues occurring in Ganga water. This study is necessary for enabling us to determine the quality of potable waters.

Residues of DDT occurring in water linked canals

The concentration of DDT isomer/metabolites occurring in linked canals as determined by Gas Chromatography is shown in Table 1.19.

It was found from Table 1.19 that out of 12 samples analysed, only two samples were found to contain total DDT in the range 0.000249-0.0037 ppm.

Table 1.19. Residues of DDT isomers/metabolites (OP'-DDT, PP'-DDT, OP'-DDE and PP'-DDE) occurring in canals linking Ganga river.

Sl. No.	Location	Amount (µg/ml)				Total
		OP'-DDT	PP'-DDT	OP'-DDE	PP'-DDE	
1.	Paint Factory (Naihati) Canal (Middle point)	-	0.0001642	-	0.000085	0.000249
2.	Paint Factory Canal(Garifa)	0.0004935	0.0031978	-	-	0.0037

<u>Residues of DDT and its isomers/metabolites (OP', PP'-DDT and OP', PP'-DDE) occurring in Ganga water</u>.

The concentrations of DDT and its isomers/metabolites (OP', PP'-DDT and OP', PP'-DDE) occurring in Ganga water as analysed by Gas Chromatography is summarised in Table 1.20.

Table 1.20. Residues of DDT and its isomers/metabolites occurring in different spots of the Ganga river.

Sl. No.	Location	Amount (µg/ml)				Total
		OP'-DDT	PP'-DDT	OP'-DDE	PP'-DDE	
1.	Bandel brick field	0.000582	0.002792	0.000078	0.000117	0.0036
2.	Hooghly Imam Bara	-	0.000040	0.002922	-	0.002962
3.	Hooghly Bridge	0.000163	0.000473	0.000149	0.000081	0.00087

Table 1.20 contd.

Sl. No.	Location	Amount (µg/ml)				Total
		OP'-DDT	PP'-DDT	OP'-DDE	PP'-DDE	
4.	Halisahar	0.000133	0.000252	-	0.000069	0.00045
5.	Ramprasad Ghat, Halisahar	-	0.000582	-	-	0.000582
6.	Khasbati, Near Jute Mills (Eastern bank)	-	0.0003285	-	-	0.00033
7.	Indian Paper Pulp (Near effluent source)	0.0003957	0.002477	-	0.0000128	0.0029
8.	Indian Paper Pulp (About 20 meters away from the bank)	0.000477	0.002434	-	-	0.0029
9.	Chalkal Ghat (Eastern bank)	-	0.000066	-	-	0.000066
10.	Bhatpara Bazar (Eastern bank)	-	-	-	0.000050	0.000050
11.	Jagatddal Ghat	-	0.000246	0.000815	0.000116	0.0012
12.	Chandannagar Shoal	-	0.000006	-	-	0.000006

The results indicated that residues could be detected in 12 samples out of 36 samples collected from different pockets of both the banks of the Ganga river. The total amount of DDT varied from 0.000006-0.004 ppm of which PP'-DDT could be detected in 11 samples ranging from 0.000006-0.0028 ppm, while 5 samples were found to contain OP'-DDT ranging from 0.000133-0.000582 ppm. The highest amount of OP', PP' as well as total DDT could be detected in water samples near Bandel brick fields.

This study further indicated that OP'-DDE and PP'-DDE could be detected in 4 samples ranging from 0.000078-0.002922 ppm and in 6 samples ranging from 0.0000128-0.000117 ppm respectively, the highest amount of OP'-DDE being found in water samples near Hooghly Imam Bara.

<u>Residues of endosulfan occurring in Ganga water</u>

Table 1.21. Residues of endosulfan isomers/metabolites (α-& β-endosulfan, endosulfan cyclic sulphate) occurring in Ganga water.

Description of the locations	α-Endosulfan(in ppm) μg/ml	β-Endosulfan(in ppm) μg/ml	Endosulfan cyclic sulphate (in ppm) μg/ml	Total
1. Halisahar (Eastern bank)	0.000049	-	-	0.000049
2. Bandel Brick Field (Western bank)	0.000039	-	-	0.000039
3. Hooghly Imam Bara (Western bank)	0.000077	-	-	0.000077

Table 1.21 contd.

Description of the locations	-Endosulfan (in ppm) µg/ml	-Endosulfan (in ppm) µg/ml	Endosulfan cyclic sulphate (in ppm) µg/ml	Total
4. Hooghly Bridge (Western bank)	0.000097	-	-	0.000097
5. Jagatddal Ferry Ghat (Eastern bank)	0.000079	0.000081	-	0.000160

No endosulfan residues could be detected in water samples collected from the selected pockets of canals linked with the Ganga.

The endosulfan residues were detected in five samples out of the 36 samples collected from selected pockets of Ganga. It was observed that only α-endosulfan could be found in all the five samples in the range of 0.000039-0.000097 ppm. β-endosulfan (0.000081 ppm) could only be found in one sample. Endosulfan cyclic sulphate was not found in any one of the samples analysed.

Thus the study revealed that pesticide residues (DDT, Endosulfan) could be found in some selected pockets along both the banks of the river Ganga. PP'-DDT could be detected in 11 samples out of 35 samples. Although the concentration of DDT and its metabolites was not very high but the possibility of their accumulation in the food chain can not be ignored. The occurrence of endosulfan residues on the other hand was very much insignificant in Ganga water.

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Part - II

Studies on the chemistry of some natural pesticides

CHAPTER - I

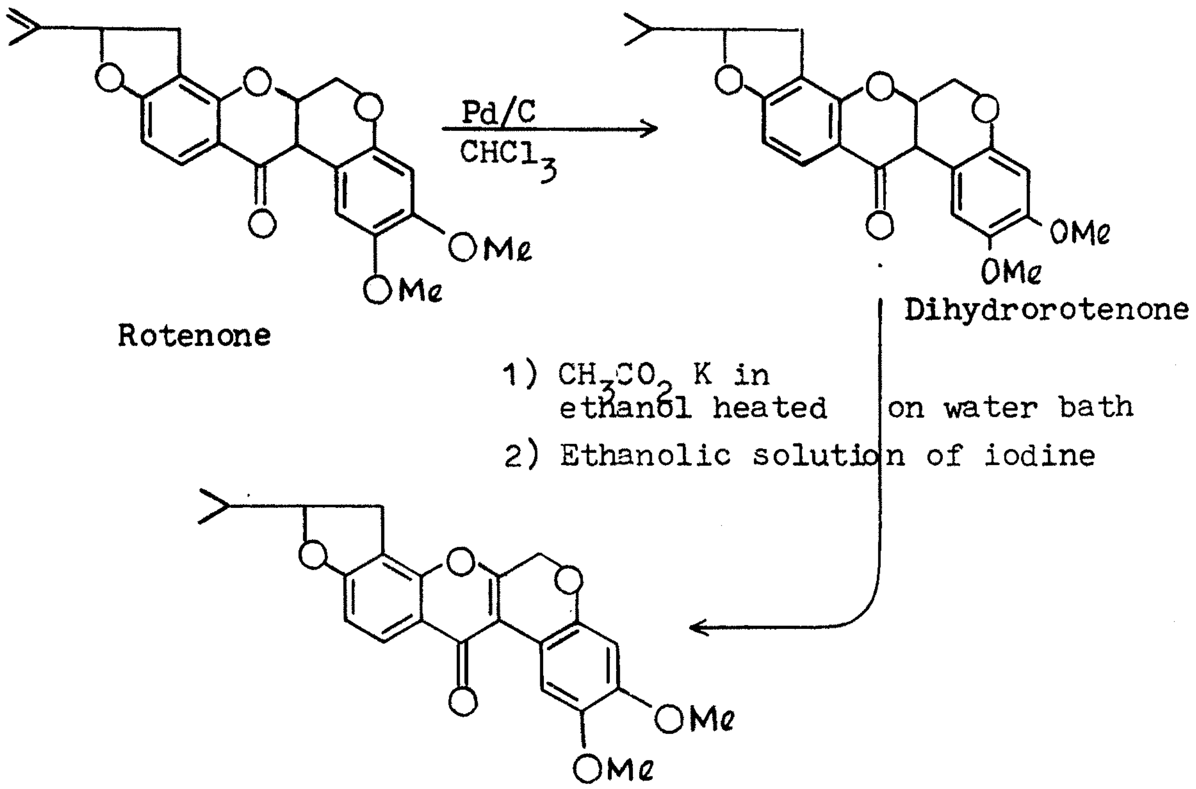
STUDIES ON THE CATALYTIC HYDROGENATION OF ROTENONE IN DIFFERENT SOLVENT SYSTEMS

SECTION - A

Rotenone is one of the most reputed naturally occurring insecticides of plant origin. The structure and synthesis of rotenone were established through the laborius work of several groups of chemists[1,2,3,4]. During the structure elucidation of rotenone a variety of chemical reactions, transformations, and systhesis was carried out and a vast amount of literature has accumulated over the years. Recently a compound possessing the modified isoflavanoid system could be isolated from the plant Tephrosia candida (Fam: Leguminosae) and the structure of this compound was settled as dehydro-dihydrorotenone (IX) from spectral and chemical evidence. The structure of (IX) was confirmed by its partial synthesis from rotenone. The synthetic pathway is depicted below.

During the synthesis of dehydrodihydrorotenone (IX) one of the major steps was hydrogenation of rotenone. The reaction conditions for hydrogenation were as follows :

(1) Solvent : Chloroform
(2) Catalyst : Pd/C
(3) Reaction time : 4 hrs.

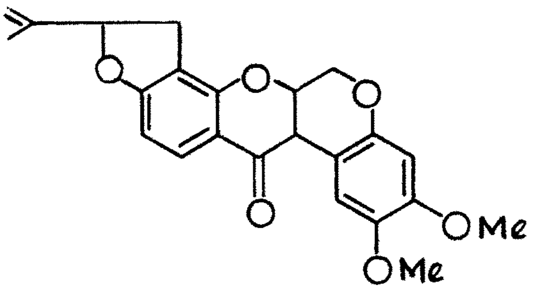


Dehydro-dihydrorotenone (IX)

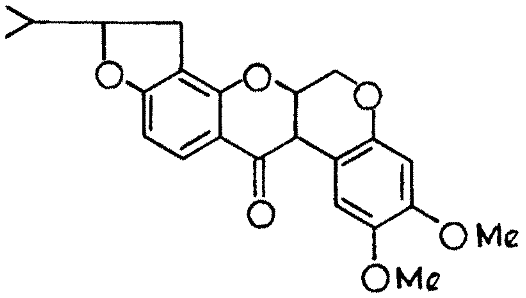
Only one product, dihydrorotenone, could be obtained from the catalytic hydrogenation of rotenone. While this work was going on, the present investigator found from a survey of literature that the earlier researchers had encountered many interesting results by using different catalysts and solvents during the catalytic hydrogenation of rotenone and obtained some major hydrogenated products along with some minor compounds. The latter compounds could not be characterised at that time. These results prompted us to undertake a study on the catalytic hydrogenation of rotenone in different solvent systems with different catalysts. For achieving this objective the investigator thought it worthwhile to present a brief review on the catalytic hydrogenation of rotenone which is presented in this section.

A brief review on the catalytic hydrogenation of rotenone

The simplest reduction product of rotenone (III), dihydrorotenone (I) was prepared by Kariyone[5] by catalytic hydrogenation with palladium-barium sulfate catalyst and described it as prisms, m.p. 216°, $[\alpha]_D^{20}$ - 220°, yielding an oxime m.p.258°.

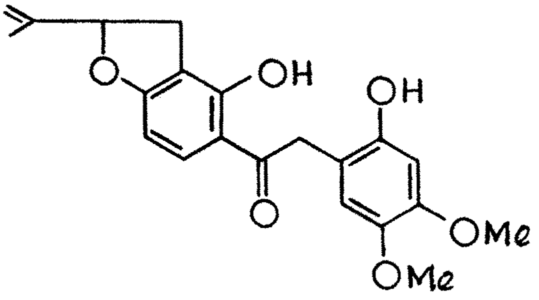


(III)



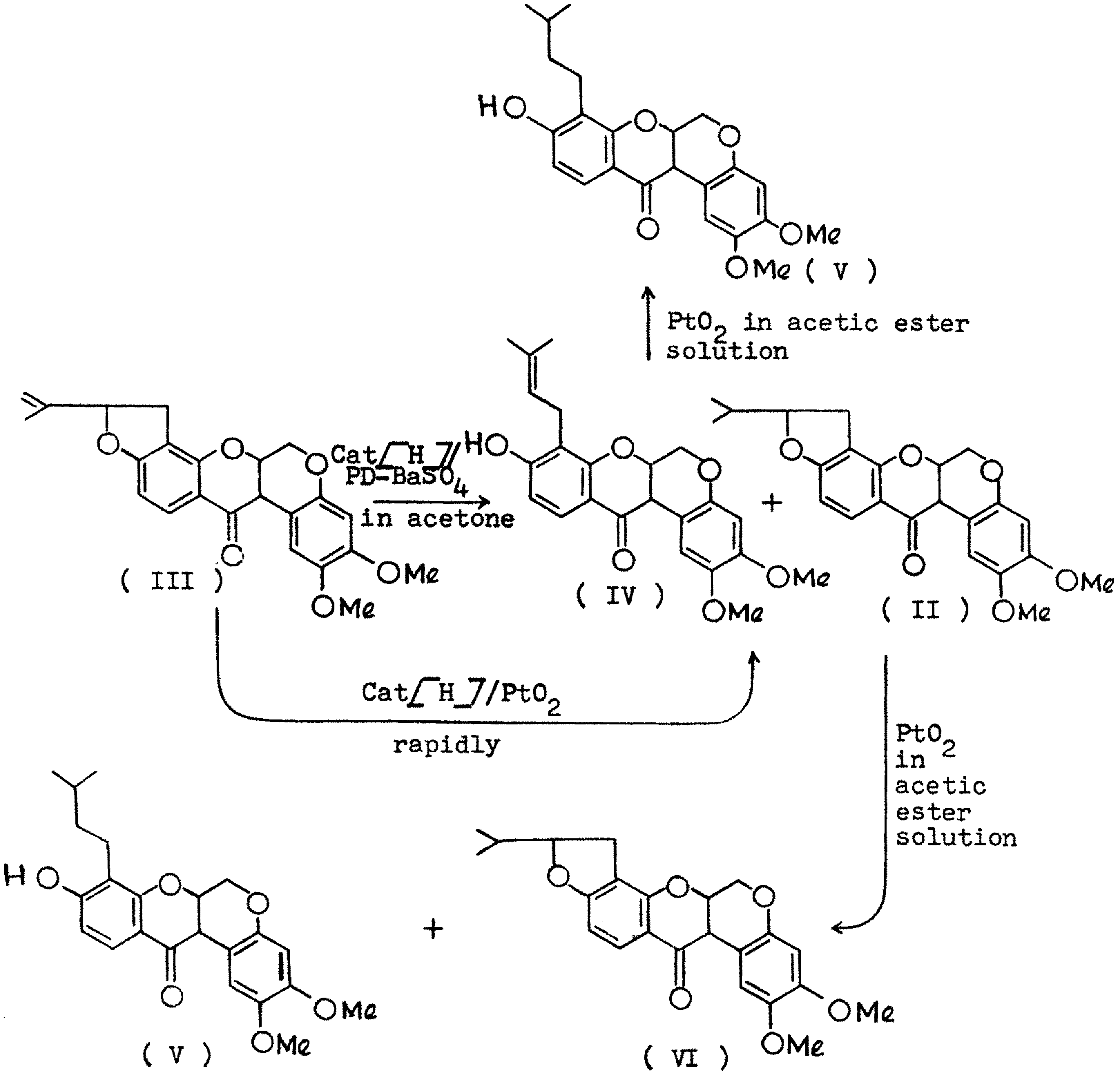
(I)

Butenandt repeated the preparation of dihydrorotenone (I) according to the directions of Kariyone, conformed the results of the Japanese investigator with respect to its physical properties and revised the formula to $C_{23}H_{24}O_6$. He employed dihydrorotenone for subsequent reactions to test the theory regarding the formation of his 'derritol' (II).



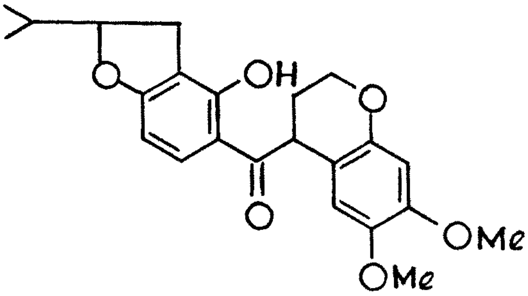
(II)

Laforge and Smith[5] made a large number of experiments on the hydrogenation of rotenone with both palladium-barium sulfate and platinum oxide catalysts in acetone and acetic ester solutions, but their results differed widely from those reported by Kariyone and Butenandt. The experimental scheme followed by them is represented below.



Haller and Schaffer[6] carried out catalytic reduction of rotenone (III) with Raney-Ni catalyst at 35-40° and reported the yield of dihydrorotenone (I) as 90-93% in benzene, in ethyl acetate or butyl acetate as 85-90% and in acetone as 60-70%.

Haller and Schaffer[7] described that the reduction of rotenone (III) in butyl acetate at 90-105° and 35-60 lb sq/in pressure with an active Ni catalyst gave quantitative dihydrorotenol (VIII); Raney catalyst at room temperature and atmospheric pressure in neutral solution solution gave 90% of dihydrorotenone (I).



(VIII)

Haller and Schaffer[7] gave a note on the hydrogenation of rotenone. Hydrogenation of rotenone (III) with platinum oxide or palladium-barium sulfate catalysts resulted in the saturation of the double bond present in rotenone (formation of dihydrorotenone) and also in the cleavage of furan oxide ring (formation of rotenonic acid) (Fig. I). They also described that with nickel catalysts, besides saturation of the double bond, cleavage of the dihydrobenzo pyrone ring took place as indicated as X-Y with the formation dihydrorotenol (VIII).

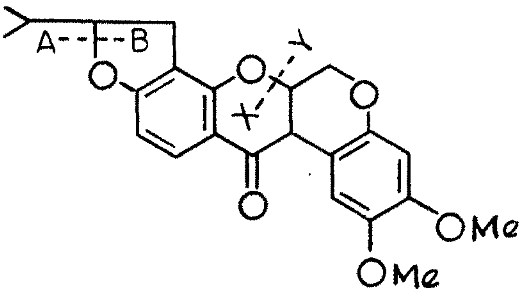
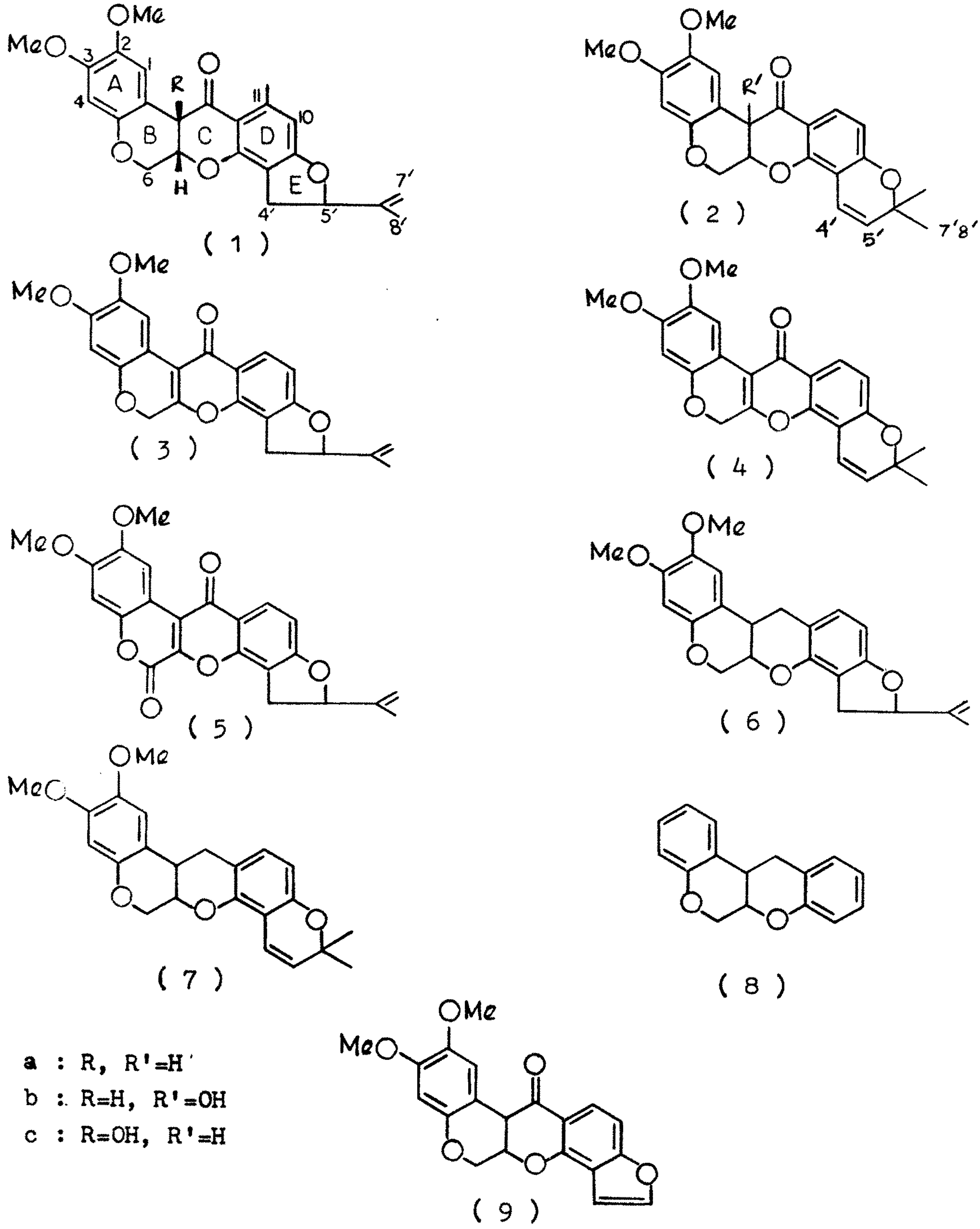


Fig. I

NMR investigation of some rotenoids

The proton magnetic resonance spectra of natural rotenoids and their relatives have been explored by Crombie *et al* and Carlson *et al*[8,9]. Much structural information of the reduction-dehydration product of rotenone was quickly accessible by these spectral data. Incidentally, these studies also allowed the assignment of NMR signals for essentially all protons of the major rotenoids. So, the present author thought it worthwhile to present a review of those NMR data obtained for various rotenoids in order to understand the structures of various minor hydrogenated products formed during the hydrogenation of rotenone in presence of various catalysts and solvent medium. The NMR data of rotenoids are presented in Table 2.1 and the corresponding structures are given in Fig.2.1.



a : R, R'=H

b : R=H, R'=OH

c : R=OH, R'=H

Fig.2.1. Structures of some rotenoids.

Table 2.1. Chemical shifts (τ) of rotenoid protons

Compound	Solvent	H-1s	H-4s	H-6	H-6a	H-12a or H-12$^+$	Me-2, Me-3	H-10d	H-11d	H-4'	H-5'	H-7'b	Me-8'b
Rotenone (1a)	$CDCl_3$	3.23	3.55	5.41q 5.85bd	5.1	6.2	6.21 6.25	3.50	2.16	6.68q 7.07q	4.78t	4.94c 5.1	8.23b
Sumatrol (1c)	$CDCl_3$	3.14b	3.56	5.44q 5.87bd	5.2m	6.2	6.20 6.23	4.00	-	6.77q 7.17q	4.83t	4.96c 5.10c	8.26b
12-Deoxy-$\Delta^{12(12a)}$ dehydrorotenone (6)major isomer	$CDCl_3$	3.01	3.59	5.45q 5.91t	4.73m	3.41$^+$d	6.13 6.18	3.61	3.15	6.75q 7.06q	4.84t	4.92 5.10	8.23b
12-Deoxy-$\Delta^{12(12a)}$ dehydro-6'-7'-dihydrorotenone -major isomer	$CDCl_3$	3.02	3.61	5.45q 5.90t	4.73m	3.42$^+$d	6.12 6.17	3.67	3.19	6.9 7.2	5.5	-	8.96d 9.03d
6a-12a-Dehydro-rotenone (3)	$CDCl_3$	1.56	3.48	5.03s 5.03s	-	-	6.06 6.16	3.11	1.89	6.48 6.85	4.61t	4.88 5.03	8.20b
Rotenonone(5)	$CDCl_3$	1.05	3.15	-	-	-	5.99 6.06	3.04	1.86	6.31q 6.67q	4.53t	4.85 5.00	8.18b
Deguelin (2a)	$CDCl_3$	3.21b	3.57	5.38q 5.84bd	5.10m	6.2	6.21 6.25	3.57b	2.27	3.39bd	4.46d	-	8.56 8.63
α-Toxicarol(2c)	$CDCl_3$	3.14b	3.56	5.40q 5.86bd	5.16m	6.2	6.20 6.23	4.06b	-	3.46bd	4.56d	-	8.57 8.64
Tephrosin (2b)	$CDCl_3$	3.45	3.54	5.5 5.5	5.5	-	6.20 6.28	3.56b	2.30	3.42bd	4.47d	-	8.56 8.62
12-Deoxy-$\Delta^{12(12a)}$ dehydrode-guelin (7)	$CDCl_3$	3.02	3.6C	5.42q 5.87t	4.73m	3.46d	6.12 6.17	3.64b	3.19	3.40bd	4.42d	-	8.57 8.60
6a,12a-dehy-drodeguelin(4)	$CDCl_3$	1.55	3.46	5.00s 5.00s	-	-	6.06 6.15	3.15b	1.97	3.25bd	4.29d	-	8.52
Elliptone (9)	$CDCl_3$	3.23b	3.55c	5.29q 5.77bd	4.93m	6.06bd	6.22 6.26	2.87b	2.11	3.09q	2.46d	-	-

Mass spectra of some rotenoids

The cracking patterns of some rotenoids were explained by Reed and Wilson[10]. The cracking pattern showed good correlation with the known structures. This reveals that fragmentations of the dimethoxy-chroman group is common to all of them. The most abundant ion of the series excluding pachyrrhizone, is at m/z 192, and must arise as shown in Scheme-I. The formation of the other ions is shown in Scheme-II. The present investigator thought it necessary to collect the mass spectral data of some rotenoids with a view to understanding the mass fragmentation patterns of the minor hydrogenated products obtained during the hydrogenation of rotenone in presence of various catalysts and solvent media. The mass spectral data of some rotenoids are presented in Table-2.2 and the structure of rotenoids are shown in Fig.2.2.

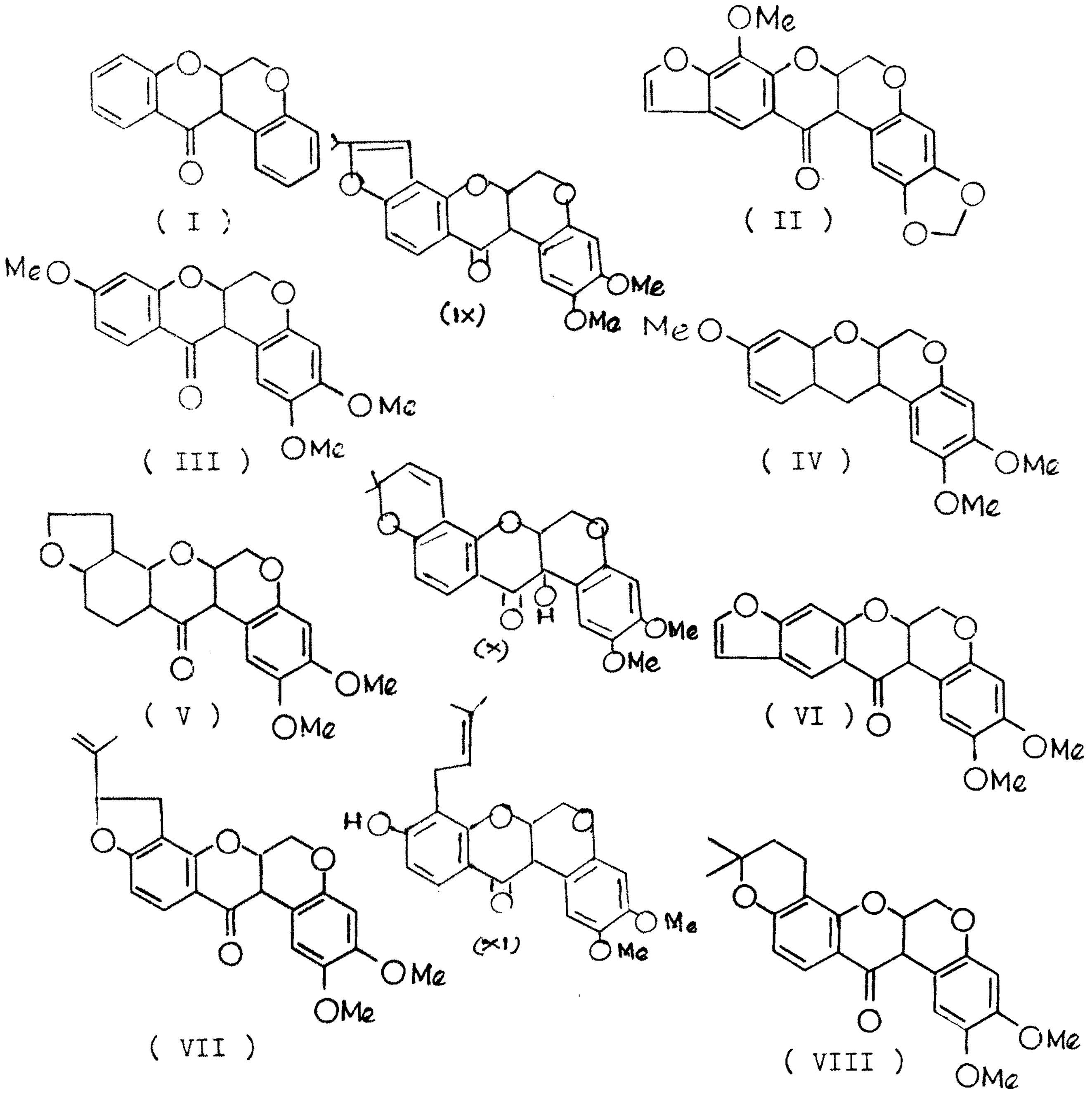
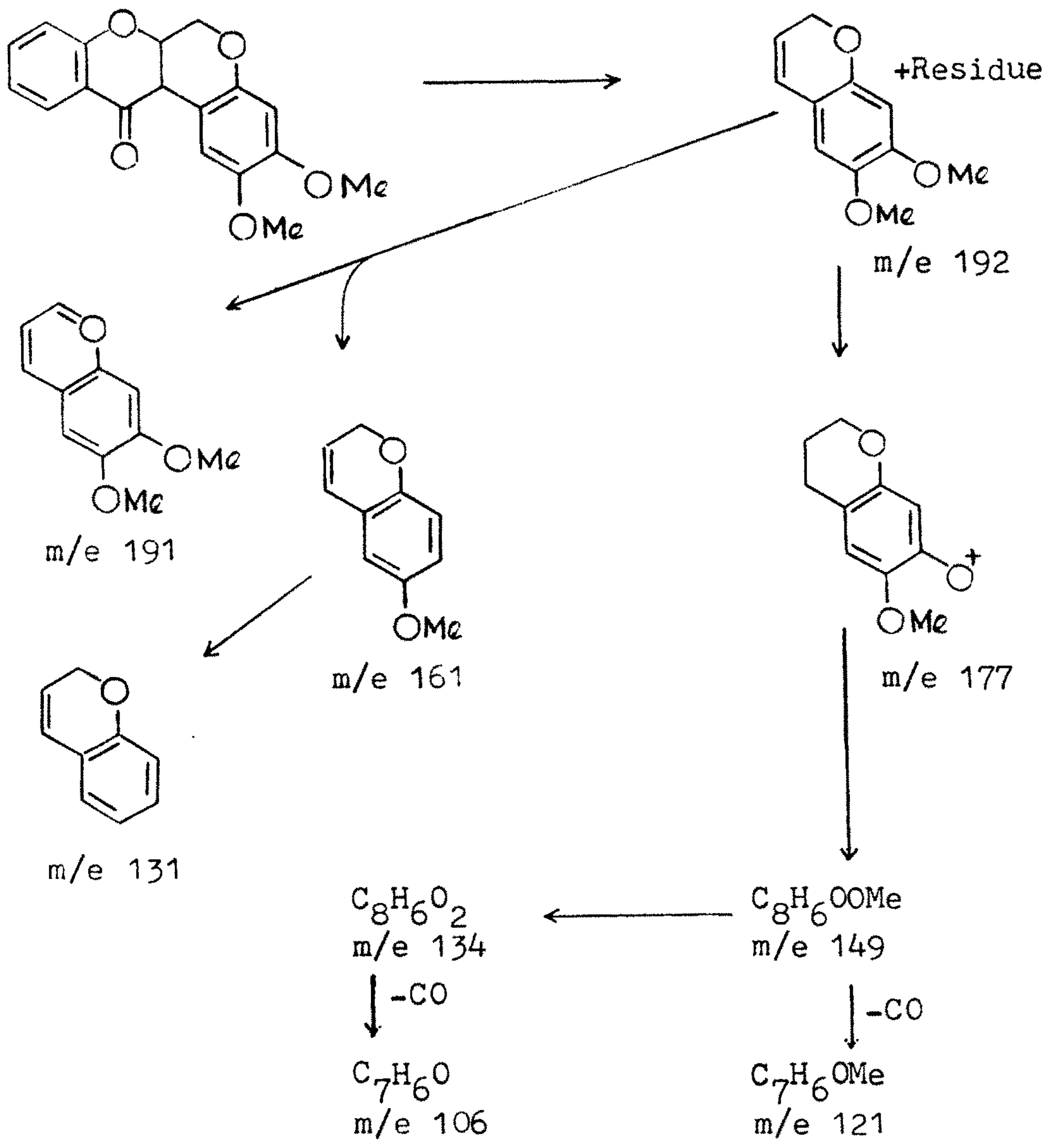
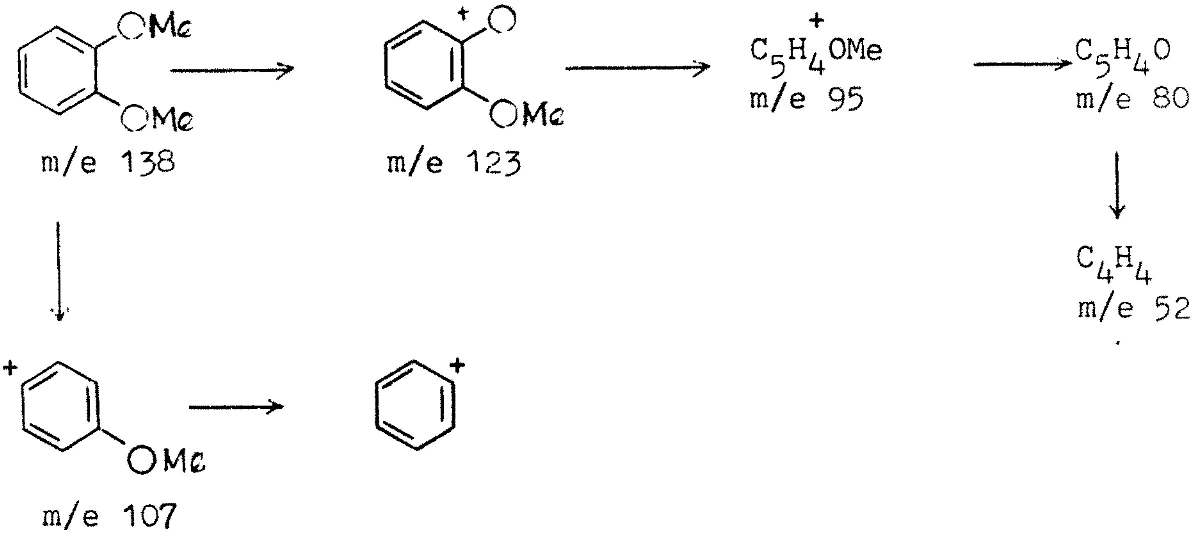


Fig.2.2. Pachyrrhizone (II), Munduserone (III), Sermundone (IV), Elliptone (V), Isoelliptone (VI), Rotenone (VII), β-dihydrorotenone (VIII), Isorotenone(ix), Toxicarol (x), Rotenonic Acid (xi).



Scheme-I. Mass fragmentation pattern of rotenoid



Scheme-II. Mass fragmentation pattern of rotenoid

Table 2.2

Mass spectra (relative intensities) of some rotenoids

m/e	(III)	(IV)	(V)	(VI)	m/e	(III)	(IV)	(V)	(VI)
359		18.4			215		0.6		
358		58.9(P)			214		0.4		
357		3.7			195	0.5	0.7	0.06	1.5
355		5.6			194	1.9	4.0	2.2	4.4
353		0.7	22.9	11.9	193	15.8	30.6	18.1	26.2
352			77.6(P)	24.8(P)	192	100.0	100.0	100.0	100.0
351			1.2	0.7	191	36.2	37.2	38.8	42.7
350			1.2	0.9	190	3.2	5.3	4.5	6.7
344		0.6			189		1.4	0.5	1.6
343	9.3	1.3			183				0.6
342	34.3(P)	0.8			180		0.6		1.6
341	0.5	0.9			179	0.8	2.5	1.5	4.8
340	0.8				178	4.8	5.1	5.2	6.2
338			0.5		177	26.4	22.9	29.6	27.7
337			1.0	1.0	176	1.9	2.2	2.7	2.9
329			0.6		175	1.8	1.5	1.8	2.8
328	0.6	0.6			168		0.7		
327	0.8	0.9			167		2.5		1.0
323			0.5		166		1.6	0.8	2.2
313	0.7	0.8			165	0.6	1.3		
312	0.5				164		2.4	2.2	3.2
311	0.5				163	1.6	1.8	2.1	3.1
307			0.5		161	1.8	1.8	4.3	7.5

Table 2.2 contd.

m/e	(III)	(IV)	(V)	(VI)	m/e	(III)	(IV)	(V)	(VI)
160		0.4	11.4	16.3	122	3.8	1.0	0.6	2.2
159		0.5		1.1	121	5.9	3.7	4.4	8.3
153		0.5		1.3	120	0.5			1.2
152	0.5	0.6	0.5	1.7	119	1.1	1.2	0.9	2.8
151	3.1	1.3	0.8	3.4	118	0.8	0.8	1.1	1.1
150	6.2	1.0	0.8	2.3	115	0.5			1.0
149	6.0	3.7	5.2	8.3	109			0.6	
148	1.2	1.1	1.0	2.5	108	1.2		1.0	
147	3.8	2.8	3.4	5.6	106	6.2		3.8	
146	1.4	1.0	1.3	2.3	105	2.0		2.9	
145		0.6	0.5	1.8	104	0.5		1.2	
139		0.8		1.3	103	3.0		1.9	
138		1.4		1.0	102	0.8		0.5	
137		1.0		1.9	97			0.6	
136		0.7		1.2	95	1.1		0.9	
135	0.8	1.1	0.8	2.2	94	1.2		0.6	
134	3.2	1.7	1.9	4.5	93	5.9		9.7	
133	0.6	0.8	1.1	3.4	92	1.2		0.6	
132		0.4	2.6	12.2	90	1.4		1.1	
130	0.9	0.5	0.7	1.6	89	1.4		1.2	
125		1.2		2.8					
124		0.7		1.7					
123	0.5	1.4		3.5					

Table 2.2 contd.

m/e	(VII)	(VIII)	(IX)	(XI)
397		5.9		4.6
396		23.7(P)		18.7(P)
395	10.9	1.0	9.5	0.7
394	38.0(P)	1.6	29.7(P)	0.8
393	2.0		1.1	
392	2.9		1.8	
382		0.6		0.5
380	0.6			
379	1.6	0.5	0.8	
378			0.5	
366	0.5			
365	1.0	0.5	0.5	
364	0.7		0.6	
363	0.9		1.0	
351	0.5			
349	0.6		0.8	0.5
341		0.6		
219			0.7	
217			0.7	
208	0.5		1.3	
207			1.3	
206			0.8	
205		1.4	0.7	0.8
204	0.6	0.5	0.5	1.0
203	2.7	0.5	2.0	1.2
202	0.4		0.5	
201	0.4		0.4	
196				0.5
195			0.5	2.5
194	1.7	1.6	1.0	7.7
193	14.2	15.7	16.3	38.6
192	100.0	100.0	100.0	100.0
191	23.5	23.3	24.3	30.3
189	0.7	0.8	1.0	3.3
188			0.5	0.8
182	1.2		2.0	0.6
181			0.9	
180			0.6	0.8
179	0.7	0.8	2.2	3.1
178	2.0	2.4	1.8	7.3
177	12.5	14.6	16.1	15.7
176	1.0	1.3	1.3	3.1
175	1.2	0.8	1.0	1.9
174	0.9		0.6	
173	0.6		0.8	
167			0.5	
166			0.5	0.5

Table 2.2 contd.

m/e	(VII)	(VIII)	(IX)	(XI)	m/e	(VII)	(VIII)	(IX)	(XI)
165	0.8	0.5	1.4	1.5	133	0.7	0.8	1.1	1.0
164	1.9	0.7	2.4	3.3	132		0.4	0.7	0.8
163	2.7	1.2	3.9	4.4	131	1.4	1.3	2.5	2.0
162	1.3	0.7	1.9	2.8	130		0.5	0.5	0.7
161	1.6	1.9	2.5	3.7	129		0.6	0.6	
160	0.7		1.1	0.7	128	0.5		0.5	
159	0.6		0.9	0.4	127			0.5	
153			0.5		126			0.5	
152			0.5	0.5	125			1.4	
151		0.7	1.7	1.3	124			0.8	
150		0.7	0.1	3.4	123	0.5	0.8	2.5	0.7
149	2.4	5.2	4.2	9.6	122		0.6	1.2	1.7
148	0.6	1.0	1.1	1.9	121	1.8	3.3	3.6	4.7
147	2.0	2.1	2.7	3.3	120			1.0	0.7
146	1.1	0.8	1.0	1.3	114	1.0	0.9	2.2	1.3
145	0.6	0.5	0.8	0.6	118	0.7	0.7	1.5	1.4
143			0.7		117	0.6		1.1	0.6
141			0.6		116			0.6	0.5
139			0.5		115	0.7	0.5	1.2	0.8
137		0.5	1.2		113				0.5
136			0.7	0.5	111			2.4	
135	0.6	0.8	1.6	1.2	109			2.9	0.7
134	1.4	1.5	2.1	2.6	108			0.8	

Table 2.2 contd.

m/e	(VII)	(VIII)	(IX)	(XI)	m/e	(VII)	(VIII)	(IX)	(XI)
107			2.3	1.5	104			0.9	0.7
106			3.9	3.6	103			3.6	2.1
105			2.2	1.9	102			0.5	0.6

m/e	(X)	m/e	(X)	m/e	(X)
411	6.4	208	1.5	191	35.6
410	28.1(P)	207	0.7	190	8.8
409	1.0	206	0.5	189	2.5
408	1.2	205	1.4	188	1.3
396	9.7	204	5.8	187	2.0
395	19.7	203	20.7	181	1.1
394	1.0	202	1.4	180	4.5
381	1.5	201	1.2	179	22.5
380	1.3	199	1.0	178	4.3
379	1.3	198	6.2	177	24.6
229	0.9	197	0.5	176	4.3
220	1.0	195	1.0	175	3.6
219	3.4	194	2.9	174	0.7
218	3.5	193	25.2	165	1.5
217	7.9	192	100.0	164	2.9

Table 2.2 contd.

m/e	(X)	m/e	(X)	m/e	(X)
163.2(m)	4.1	135	3.9	115	1.2
162	3.5	134	3.9	111	0.6
161	3.7	133	4.3	110	0.5
160	1.2	132	1.0	109	1.1
159	1.6	130	1.1	108	0.9
152	1.0	123	0.7	107	3.5
151	4.3	122	1.3	106	6.5
150	1.7	121	7.7	105	3.2
148	3.6	120	1.1	104	0.9
147	6.4	119	3.2	103	3.7
146	2.3	118	2.6	102	1.2
145	1.0	117	0.8	101	0.3
136	1.1	116	0.5		

m/e	(II)	m/e	(II)	m/e	(II)
367	10.5	351	0.2	336	0.9
366	36.5(P)	350	0.4	335	0.9
365	1.9	349	0.6	323	0.5
364	3.8	338	0.6	322	0.4
363	0.6	337	0.7	321	0.8

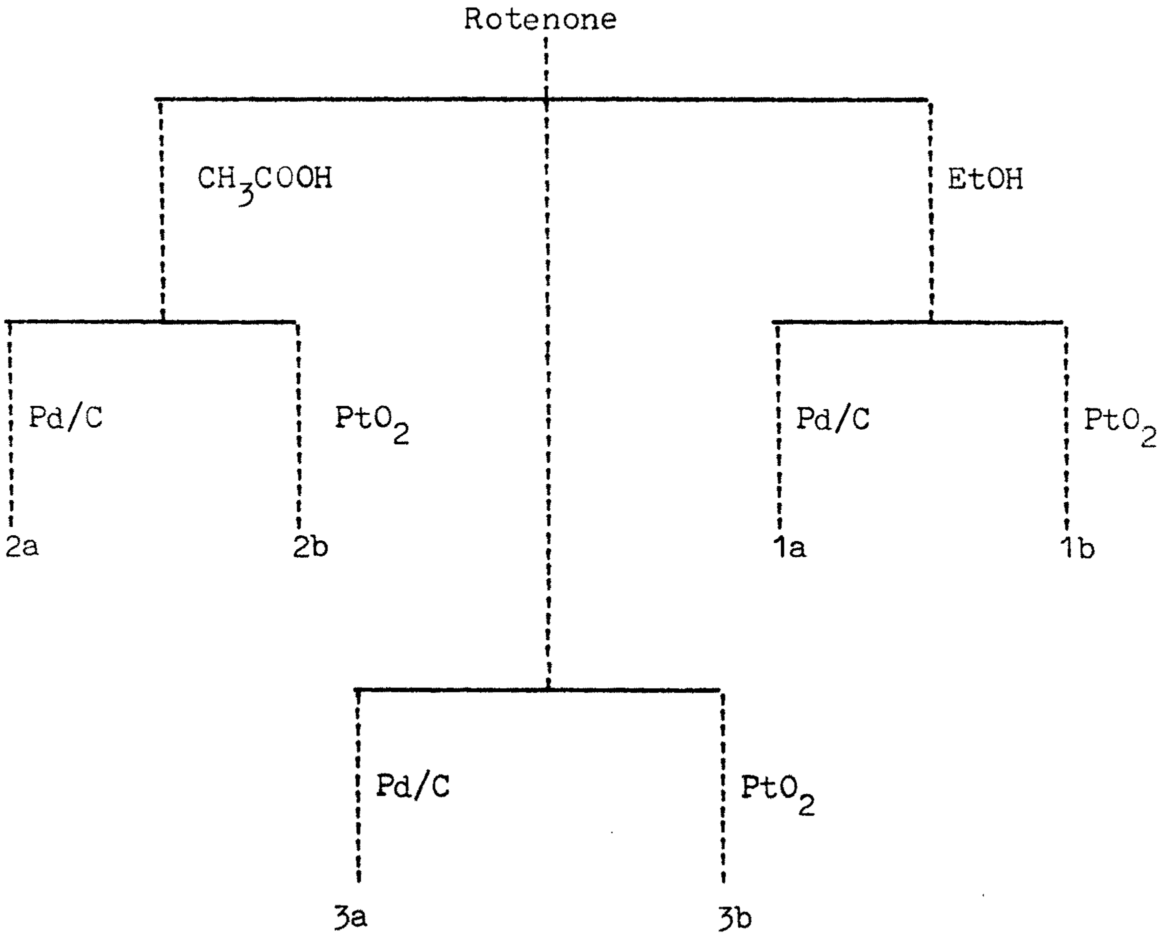
Table 2.2 contd.

m/e	(II)	m/e	(II)	m/e	(II)
320	0.5	168	0.4	142	0.2
309	0.7	167	1.0	141	0.4
308	0.8	166	0.6	140	0.5
307	1.8	164	1.6	139	1.6
306	1.0	163	9.0	138	3.1
305	0.7	162	6.2	137	1.2
192	2.9	161	4.4	136	1.2
191	6.5	160	1.7	135	1.3
190	9.0	159	0.5	134	1.0
189	1.8	158	0.3	133	3.7
183	0.9	157	0.5	132	1.3
182	0.5	156	0.2	131	0.8
181	0.5	155	0.5	130	0.3
180	0.5	154	0.4	129	0.6
179	1.2	153	0.7	128	0.5
178	2.7	152	0.8	127	0.5
177	19.3	151	1.5	126	0.6
176	100.0	150	1.7	125	1.2
175	59.8	149	1.7	124	0.9
174	3.7	148	2.7	123	1.5
173	1.1	147	7.9	122	1.1
172	0.7	146	2.1	121	1.4
171	0.6	145	1.3	120	1.0
170	0.2	144	0.7	119	2.8
169	0.4	143	0.4	118	1.6
				117	1.4
				116	0.5
				115	0.6
				114	0.2

SECTION - B

STUDIES ON THE CATALYTIC HYDROGENATION OF ROTENONE IN DIFFERENT SOLVENT SYSTEMS

As a part of our research programme we became interested to undertake a study on the catalytic hydrogenation of rotenone using different solvent systems and catalysts in order to isolate and characterise the major and minor hydrogenated products. The scheme adopted for the catalytic hydrogenation of rotenone is shown below :



Catalytic hydrogenation of rotenone in ethyl alcohol medium in presence of Pd/C catalyst (1a) furnished two minor products along with dihydrorotenone as the major one. After usual work up the reaction product was subjected to column chromatography over silica gel. Elution of the chromatogram with petrol ether-benzene (1:1) afforded a colourless solid which crystallised from ethyl alcohol as colourless needles, m.p. 157°. This compound is being designated as compound-A in the subsequent sections. Further elution of the chromatogram with benzene afforded a brown solid which crystallised from a mixture of chloroform-petrol ether as light brown crystals, m.p. 143°. The latter is being designated as compound-B in the subsequent sections.

Catalytic hydrogenation of rotenone in presence of PtO_2(1b) with the same solvent system afforded a colourless compound m.p. 165°, after crystallisation from ethyl alcohol. The physical properties of the compound were found to be in well aggrement with those reported for dihydrorotenone (m.p., m.m.p., co-TLC).

When the catalytic hydrogenation of rotenone was carried out in acetic acid medium in presence of Pd/C catalyst (2a) for 4 hours, a yellowish compound was obtained which crystallised from ethyl alcohol, m.p. 132-133°. The latter could not be detected due to poor yield.

A brown coloured compound, m.p. 154° was obtained from the hydrogenation of rotenone after hydrogen had been absorbed for 3 hours in presence of PtO_2 catalyst (2b) in the same solvent system. Characterisation and structure elucidation of the said compound was also not possible by the present investigator due to poor yield of the material.

The catalytic hydrogenation of rotenone was carried out in dry $CHCl_3$ with Pd/C (3a) for 4 hours. After usual work up a single compound, m.p. 148° could be obtained. It could not be characterised. However, the catalytic hydrogenation of rotenone in the same solvent system for 4 hours in presence of PtO_2 catalyst afforded a minor compound, yellowish crystalline in nature, m.p. 162-163°. It was proved to be unreacted rotenone.

The structure elucidation of compound-A and -B has been incorporated in the next section.

SECTION-C

C.1 Physical and spectral properties of compound-A

C.1.1 Homogeneity

On thin layer chromatogram using the following solvent systems (Table 2.3), compound-A produced a single iodine staining spot indicating its homogeneity.

Table . 2.3

No.	Solvent system	Rf.
1.	Benzene-ethyl acetate(9.5:0.5)	0.71
2.	Benzene-ethyl acetate (9:1)	0.81

C.1.2 Physical properties of compound-**A**

Compound-A crystallised from ethanol as white needles, m.p. 157°. It was highly soluble in chloroform, benzene, ethyl acetate and insoluble in water.

C.1.3 Molecular formula of compound-A

The elemental analysis of compound-A gave the emperical formula $(C_{23}H_{26}O_5)_n$ and since its molecular weight was found to be 382 by mass spectrometry, the molecular formula of compound-A should be $C_{23}H_{26}O_5$.

C.1.4 Spectral properties of compound-A

C.1.4.1 Ultraviolet spectrum

The ultraviolet spectrum of compound-A (Fig. 2.3) was studied and showed UV absorption maxima at 227 and 288 nm (in ethanol).

C.1.4.2 Infrared absorption spectrum

The IR spectrum (Fig.2.4) of compound-A in KBr was studied and the probable assignments of the important peaks are listed in Table-2.4.

Table - 2.4

Position of absorption peak cm^{-1}	Intensity	Probable assignment
2980	Strong	Aromatic side chain
1610	Medium	
1510	Medium	
1470	Strong	gem-dimethyl
1400	Weak	
1350	Weak	

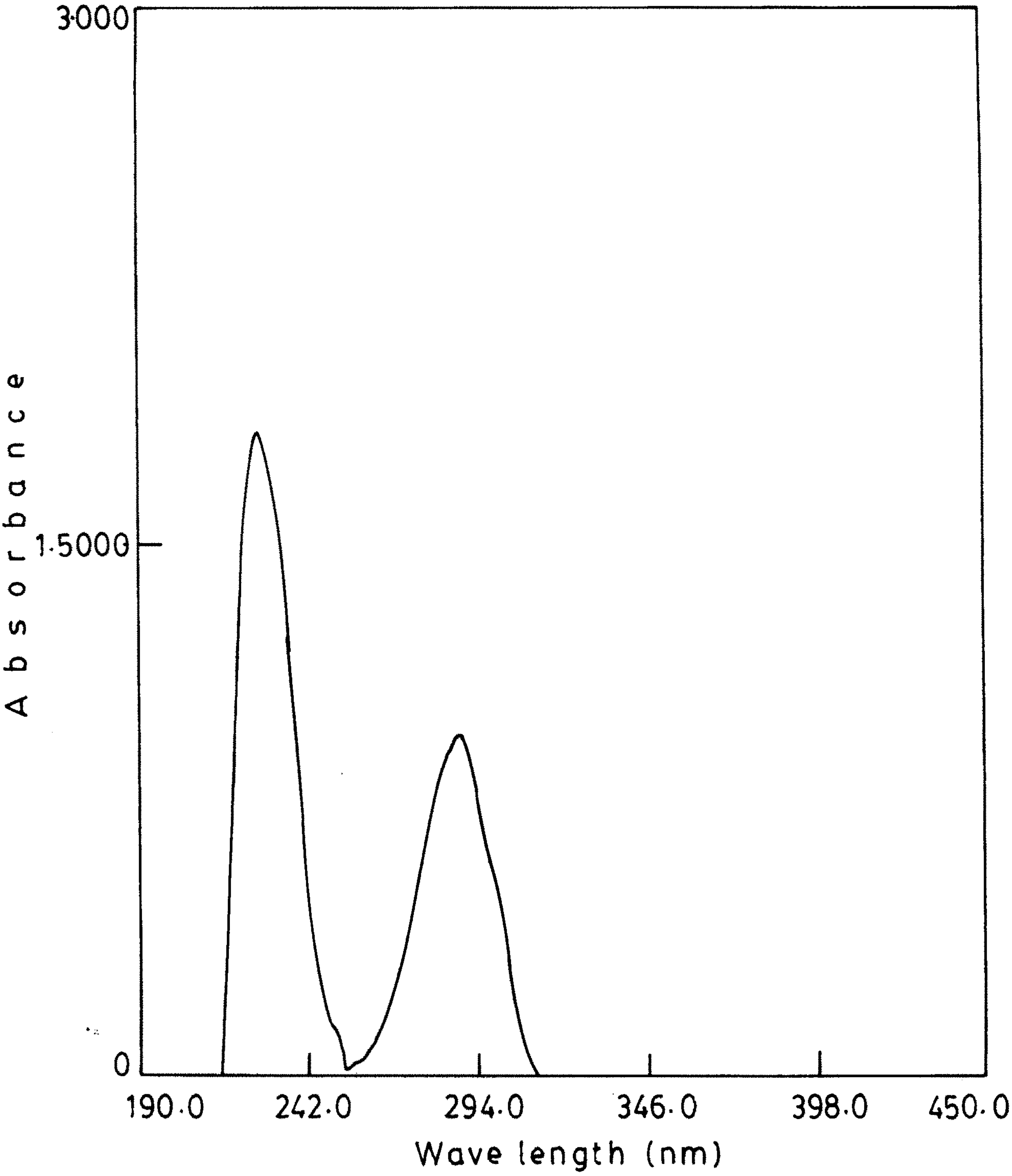


Fig. 2·3. UV spectrum of Compound - A

Table 2.4 contd.

Position of absorption peak cm^{-1}	Intensity	Probable assignment
1315	Medium	Aromatic
1230	Medium	
1190	Medium	
1140	Medium	
1120	Weak	
1090	Medium	
1060	Medium	
1040	Weak	
990	Weak	
960	Weak	
880	Weak	

C.1.4.3 Nuclear Magnetic Resonance Spectrum

The NMR spectra (Fig.2.5) of compound-A was determined in a 100 MHz instrument using deuterochloroform as the solvent and TMS as the interval standard.

Table - 2.5

Chemical δ	Shift τ	No. of protons	Multiplicity	Probable assignment
6.64	3.36	1	Singlet	C_1-1$\underline{H}$
3.76	6.24	6	Singlet	2-OCH_3

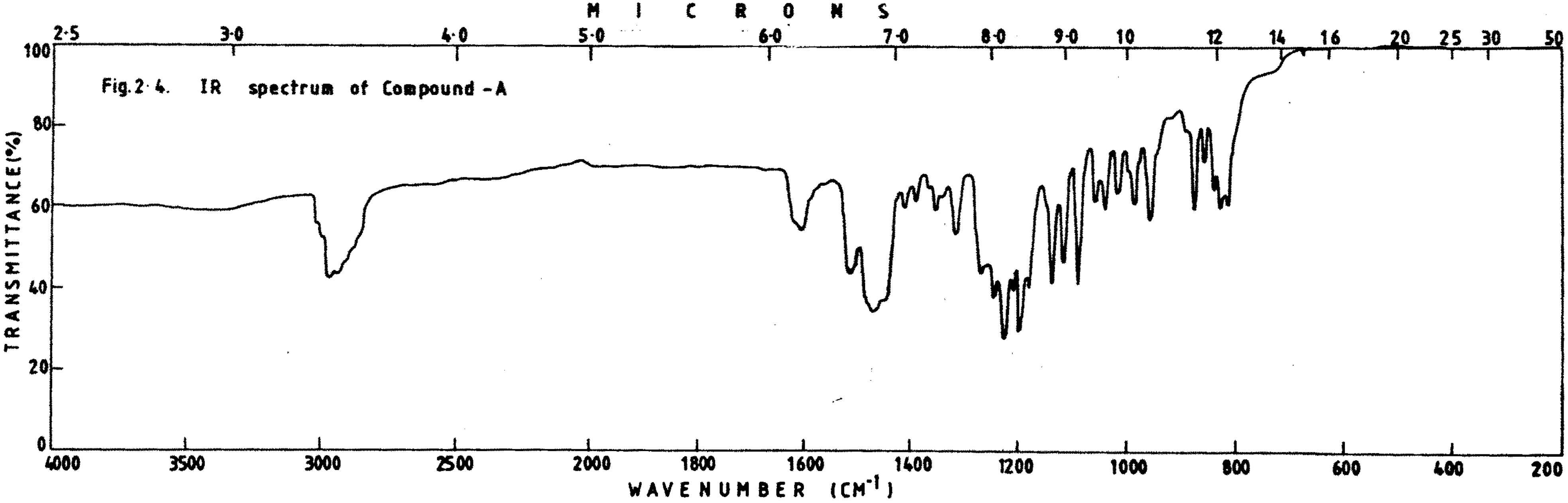


Fig.2·4. IR spectrum of Compound -A

Table-2.5 contd.

Chemical	Shift	No. of protons	Multiplicity	Probable assignment
6.38	3.62	1	Singlet	C_4-1H
4.62	5.38	2	Multiplet	C_6-2H
6.36	3.64	1	Doublet	C_{10}-1H
6.76	3.24	1	Doublet	C_{11}-1H
4.26	5.74	2	Doublet	C_{12}-2H
3.14	6.86	2	Multiplet	$C_{4'}$-2H
2.62	7.38	2	Triplet	$C_{5'}$-2H
0.92	9.08	3	Singlet	1-CH_3
0.98	9.02	3	Singlet	1-CH_3

C.1.4.4 Mass spectrum

The mass spectrum of compound-A (Fig. 2.6) exhibited, besides the molecular ion at m/z 382 (M^+ 95%), a number of diagnostic peaks at m/z 192 (100%), m/z 191 (81%), m/z 177 (54%), m/z 176, m/z 149, m/z 134 and m/z 106.

C.2 Physical and spectral properties of compound-B

C.2.1 Homogeneity

On thin layer chromatogram using the following solvent systems (Table-2.6), compound-B produced a single iodine staining spot indicating its homogeneity.

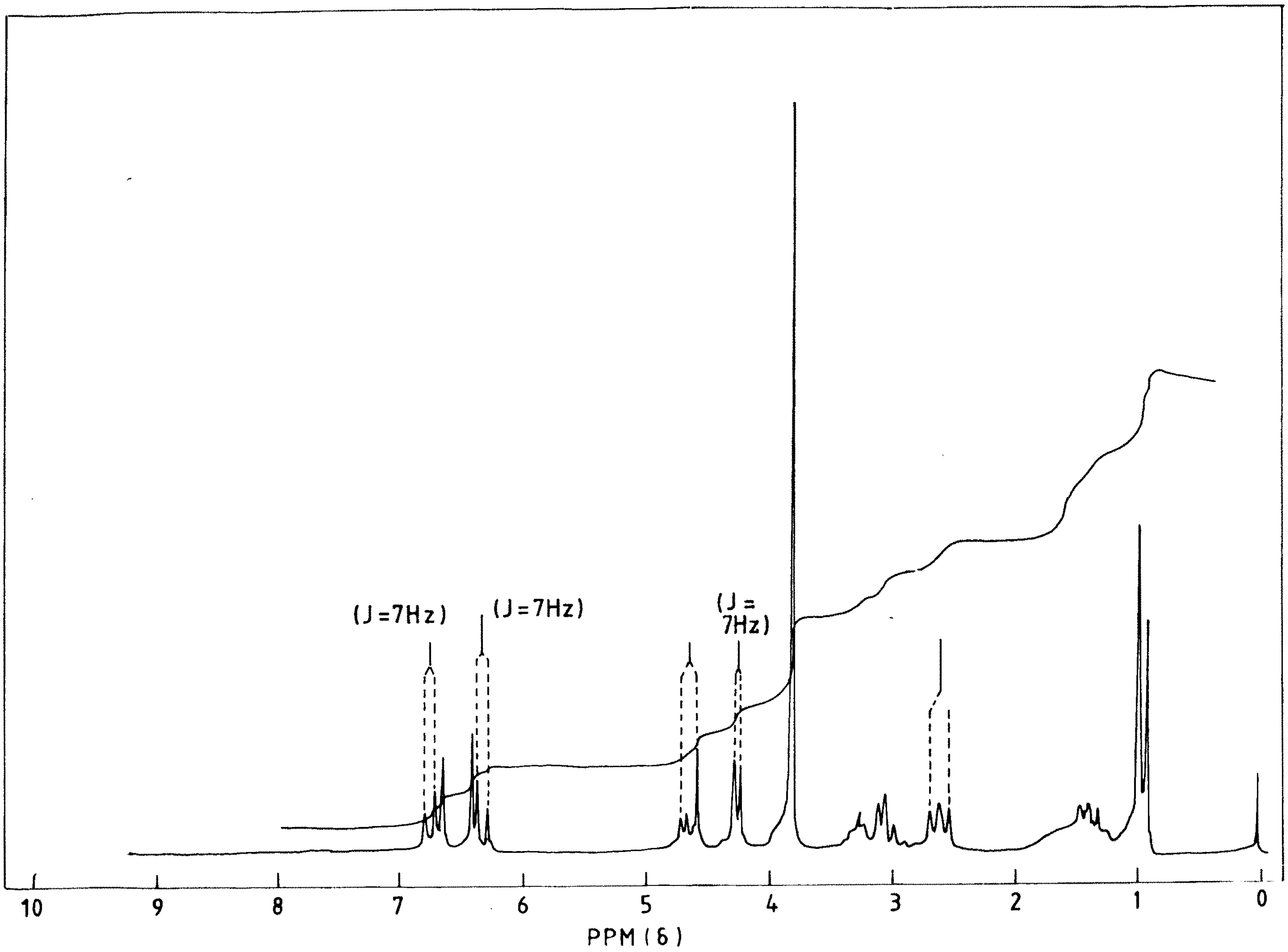


Fig. 2·5. NMR spectrum of Compound-A

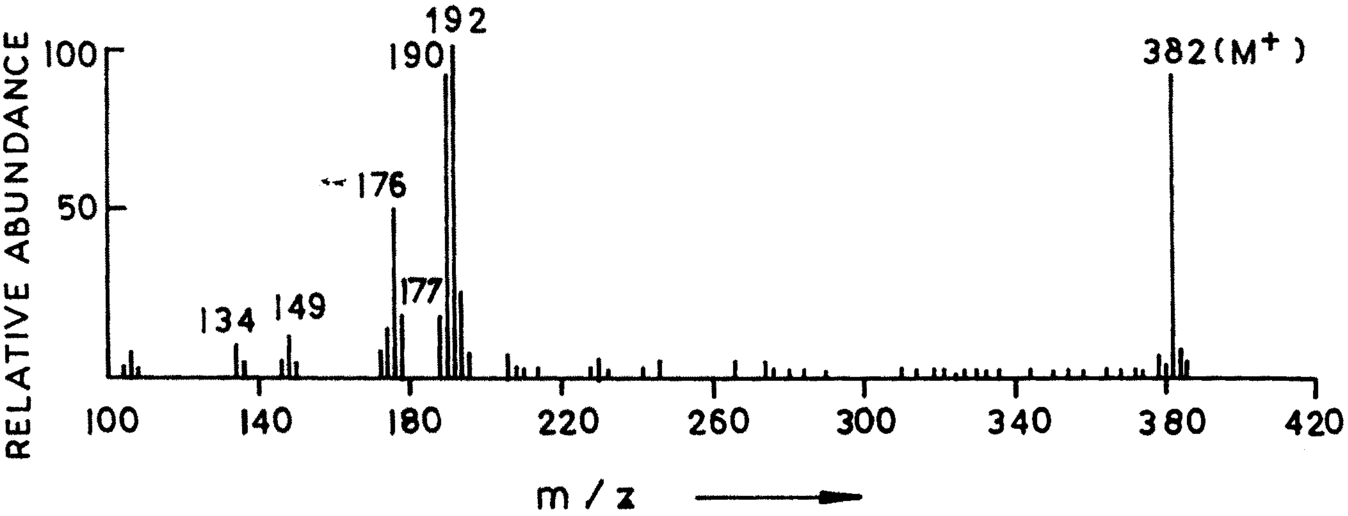


Fig. 2·6. Mass spectrum of Compound - A

Table - 2.6

No.	Solvent system	Rf.
1.	Benzene-ethyl acetate (9.5 : 0.5)	0.62
2.	Benzene-ethyl acetate (9 : 1)	0.70

C.2.2 Physical properties of compound-B

Compound-B crystallised from petrol ether-chloroform (9:1) as light brown crystals, m.p. 143°. It was highly soluble in chloroform, benzene, ethyl acetate and insoluble in petrol ether and water.

C.2.3 Molecular formula of compound-B

The elemental analysis of compound-B gave the emperical formula $(C_{23}H_{28}O_5)_n$ and since its molecular weight was found to be 384 by mass spectrometry the molecular formula of compound-B should be $C_{23}H_{28}O_5$.

C.2.4 Spectral properties of compound-B

C.2.4.1 Ultraviolet spectrum

With a view to determining the nature of chromophoric groups in compound-B, its ultraviolet spectrum (Fig.2.7) was studied. It showed UV absorption maxima at 237, 287 and 300 nm (in ethanol).

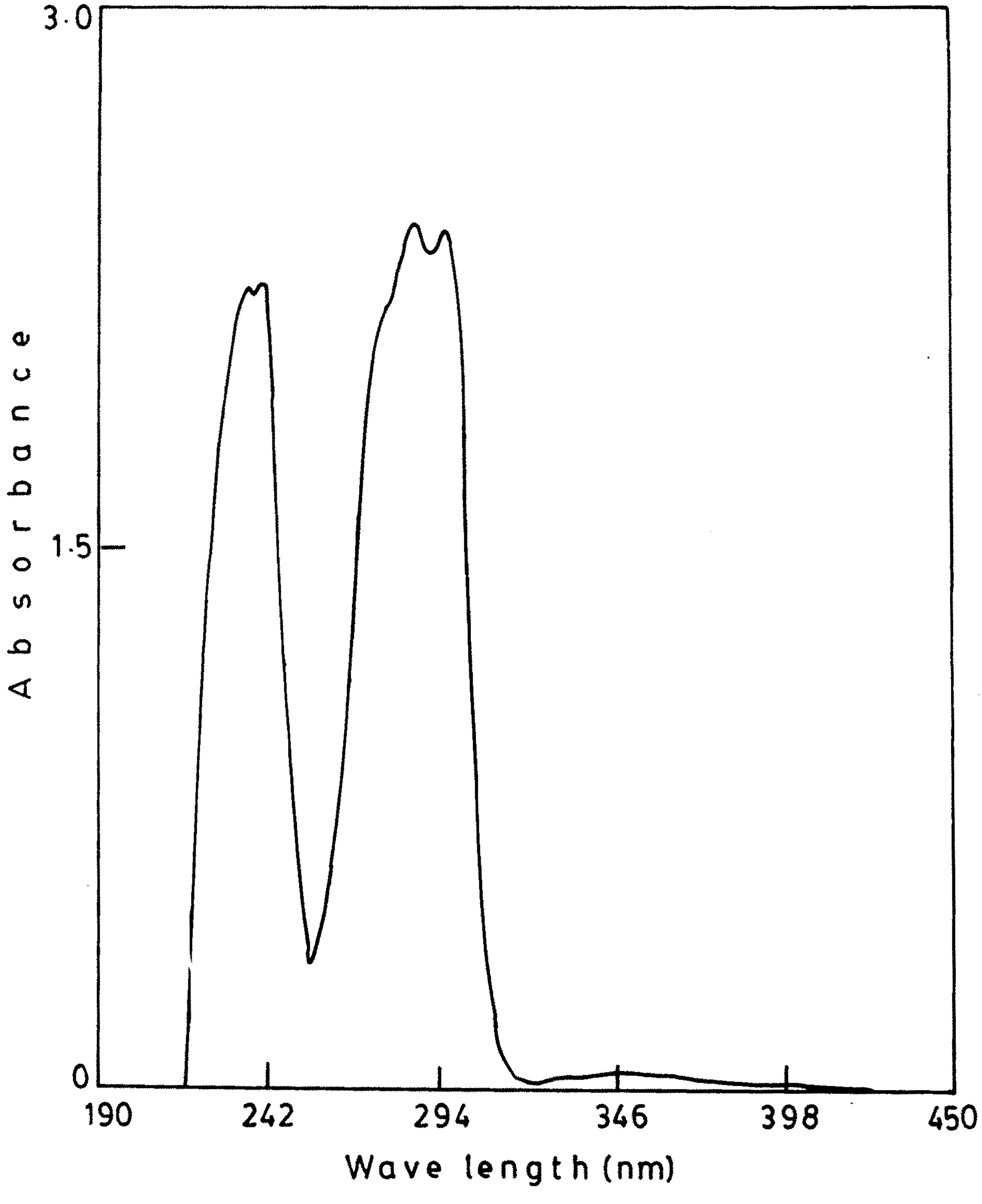


Fig. 2·7. UV spectrum of Compound - B

C.2.4.2 Infrared absorption spectrum

The IR absorption spectrum (Fig.2.8) of compound-B in KBr was studied and the probable assignments of the important peaks are listed in Table-2.7.

Table-2.7

Position of absorption peak cm^{-1}	Intensity	Probable assignments
3400	Strong	Phenolic-OH
2960	Strong	Aromatic side chain
1610	Medium	
1520	Medium	
1410	Weak	
1360	Weak	Aromatic
1200	Wesk	
1120	Weak	
1090	Weak	
1050	Medium	
870	Medium	Isopropyl group
840	Weak	
810	Weak	
800	Weak	
630	Weak	
550	Weak	

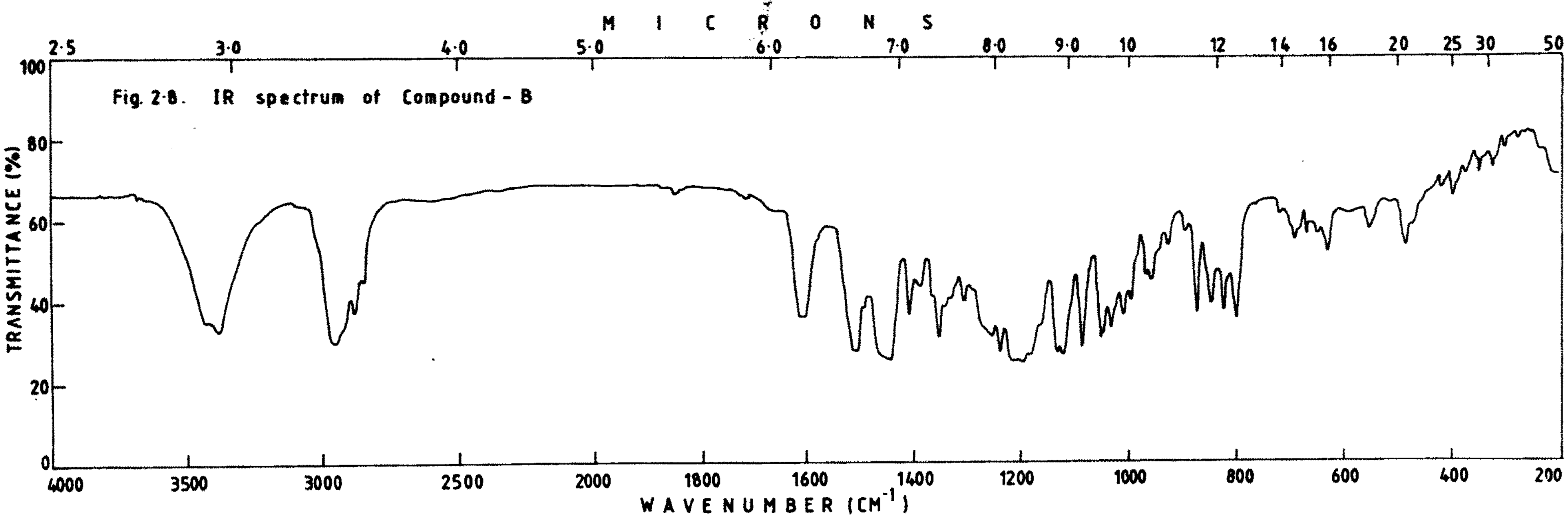


Fig. 2·8. IR spectrum of Compound - B

C.2.4.3 Nuclear Magnetic Resonance

The NMR spectra (Fig. 2.9) of compound-B was determined in 100 MHz instrument using deuterochloroform as the solvent and TMS as the internal standard.

Table-2.8

Chemical δ	Shift τ	No. of protons	Multiplicity	Probable assignment
6.6	3.4	1	Singlet	$C_1$1H
3.8	6.2	6	Singlet	2-OCH_3
6.4	3.6	1	Singlet	C_1-1H
4.68	5.32	2	Double doublet	C_6-2H
6.36	3.64	1	Doublet	C_{10}-1H
6.8	3.2	1	Doublet	C_{11}-1H
4.2	5.8	2	Doublet	C_{12}-2H
3.24	6.76	2	Multiplet	$C_{4'}$-2H
1.92	8.08	2	Sextet	$C_{5'}$-2H
2.76	7.24	1	Triplet	$C_{6'}$-1H
0.92	9.08	3	Singlet	$-CH_3$
1.06	8.94	3	Singlet	$-CH_3$

C.2.4.4 Mass spectrum

The mass spectrum of compound-B (Fig.2.10) exhibited, besides the molecular ion at m/z 384, (M^+ 96%) some diagnostic peaks at m/z 192 (100%), m/z 193 (20%), m/z 191 (30%), m/z 177, m/z 149 and m/z 136.

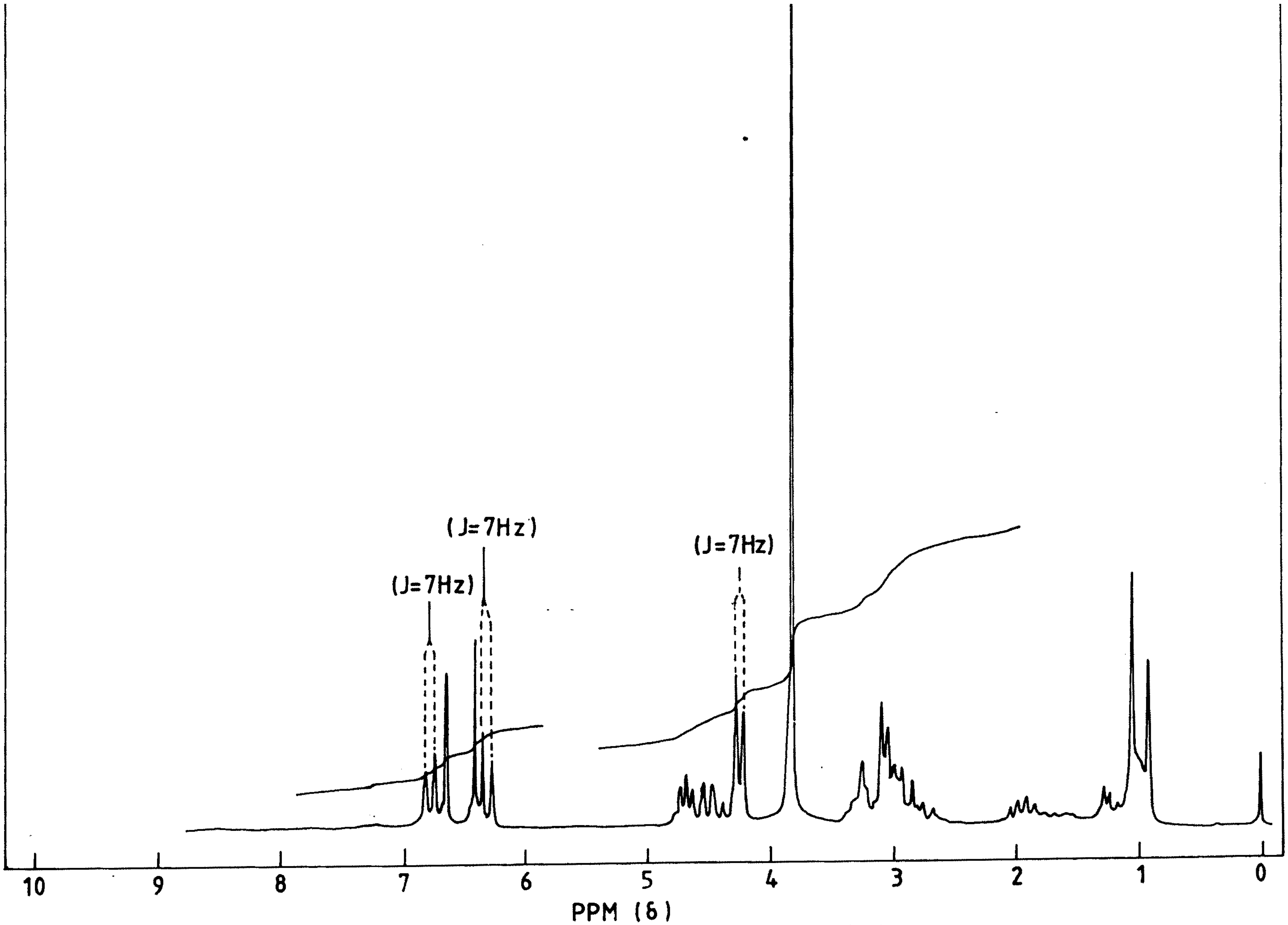


Fig. 2·9. NMR spectrum of Compound -B

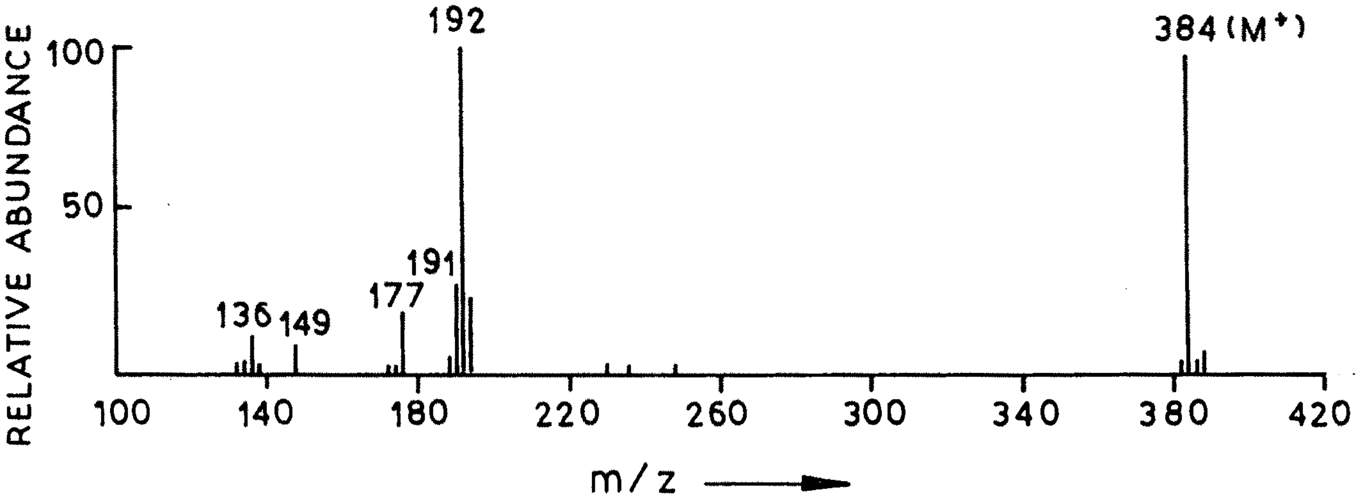


Fig. 2·10: Mass spectrum of Compound -B

SECTION - D

D.1 Conclusion drawn from spectral data and structure elucidation of compound-A

Compound-A was crystallised from ethanol as colorless needles, m.p. 157°. Elemental analysis and mass spectrometrically derived molecular weight (M^+ 382) established its molecular formula as $C_{23}H_{26}O_5$. The UV absorption spectrum of the compound $[\lambda_{max}^{EtOH}$ 227, 288$]$ spoke in favour of a rotenoid system.

The IR spectrum of the compound-A was quite informative as it revealed the absence of a keto group being present in a rotenoid system. A comparison of IR spectra of some rotenoids[9] having $>C = O$ group with compound-A is shown below.

Table-2.9

Sl. No.	Compound	ν_{max}^{KBr} cm^{-1}
1.	Rotenone	2950, 1680, 1610, 1510, 1460, 1410, 1350, 1310
2.	Deguelin	1670, 1605, 1580, 1520, 1350
3.	Compound-A	2980, 1610, 1510, 1470, 1315, 1230, 1190, 990.

It could be understood from the Table-2.9 that compound-A did not contain a $>C = O$ group.

From a careful analysis of the NMR spectrum of compound-A it was evident that compound-A contained two methoxyl groups (3.76δ, *S*, 6H). The appearance of two doublets at 6.36δ (J = 7 Hz) and 6.76δ (J = 7 Hz) provided ample evidence for the presence of two *ortho*-protons in compound-A. From these two NMR signals coupled with the IR spectrum it was assumed that compound-A was devoid of a keto group. The absence of keto functionality was further evident from its NMR spectrum. The signals at 4.26δ (2*H*, J = 7 Hz) could be readily assigned to the C-12 methylene protons of a rotenoid system. Further the NMR spectrum disclosed the presence of two more aromatic protons at 6.3δ (*S*, 1H) and 6.64δ (*S*, 1H) which could be assigned to C_4-H and C_1-H of the A-ring of rotenoid skeleton. A multiplet centred at 4.62δ could be attributed to the C_6-methylene protons of B-ring.

The NMR spectrum also showed characteristic signals for the presence of a γ, γ-dimethyl pyran system (two siglets at 0.92δ and 0.98δ for two *gem*-$C\langle{}^{CH_3}_{CH_3}$; a triplet centred around at 2.62δ for two methylene protons and two other methylene protons around 3.14δ as a multiplet).

The mass spectrum of compound-A showed in addition to the molecular ion peak at m/z 382 (M^+), some diagnostic peaks. It is well known that rotenoid type of molecules show two prominent peaks of which one is a base peak formed by RDA fragmentation of the molecular ion peak. Incidentally, compound-A gave two prominent peaks at m/z 192 (base peak) and at m/z 191 which could be accounted for a RDA fragments (Chart 2.1). From these

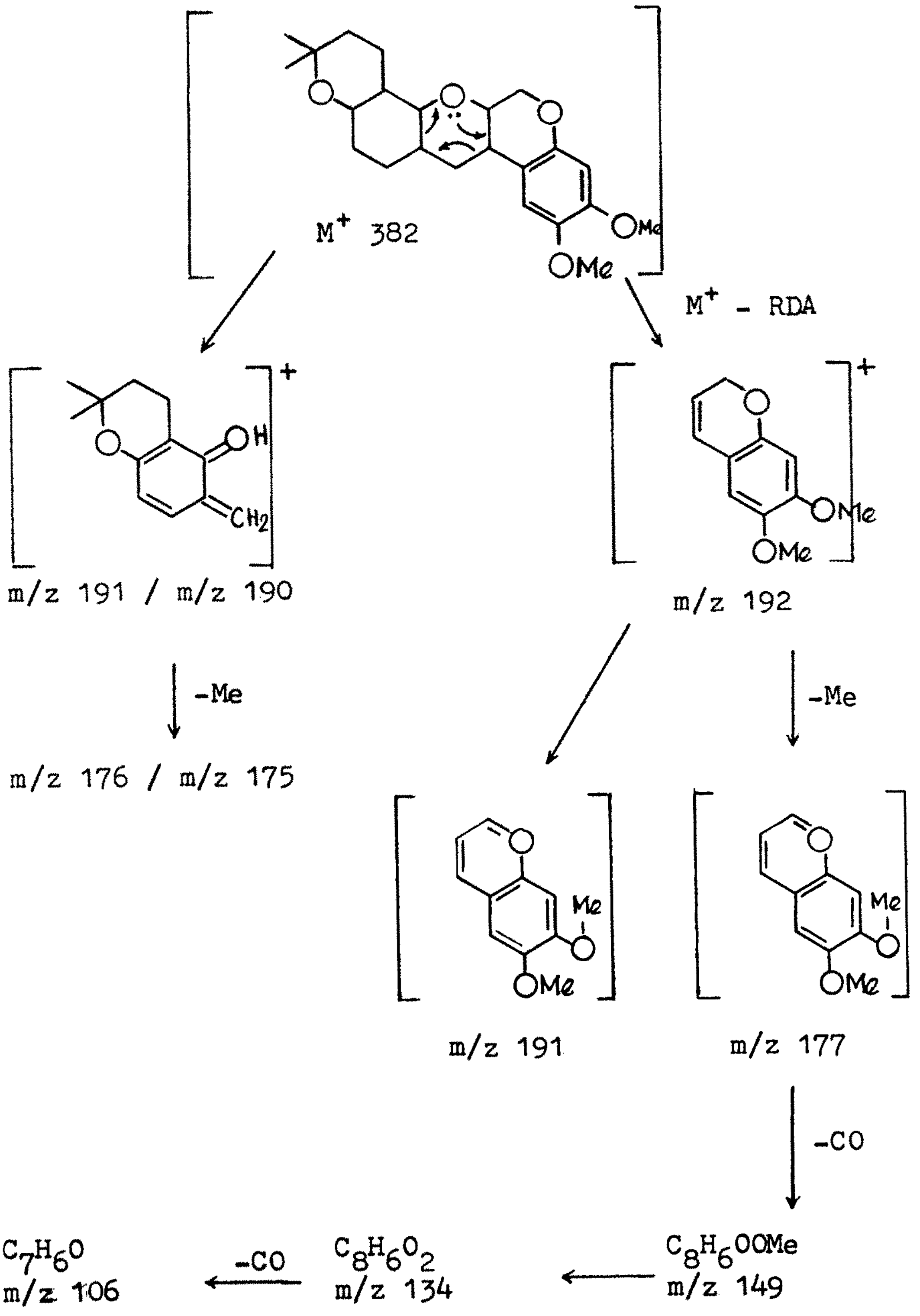
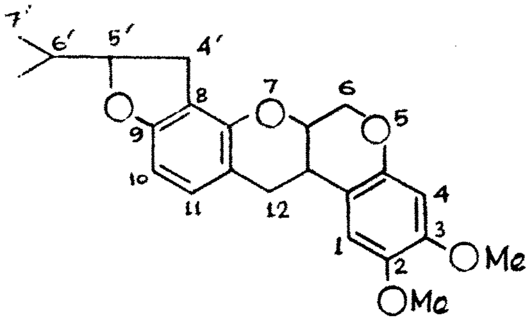
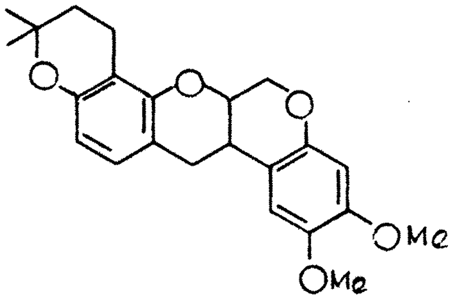


Chart-2,1. Mass fragmentation pattern of compound-A facts it was resonable to conclude that compound-A did not possess any ketonic functionality. The peak at m/z 177 could arise due to loss of a methyl group from the peak at m/z 192. The loss of CO from the ion fragment at m/z 177 could give rise to a peak at m/z 149. The peak at m/z 134 could arise by loss of another methyl group. Again, the loss of CO from the ion fragment at m/z 134 might give rise to a peak at m/z 106. The peak at m/z 176 could result by the expulsion of a methyl group from the peak at m/z 191.

On the basis of the above facts it is quite logical to represent the structure of compound-A as either I (12-deoxy-6'-7'-dihydrorotenone) or II (12-deoxy-dihydro-deguelin).



(I)



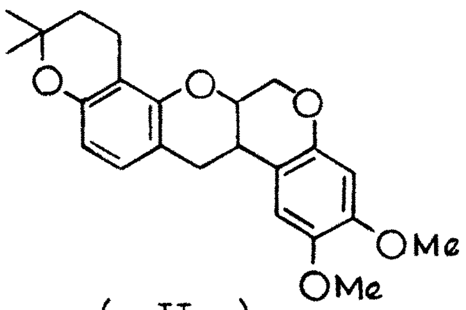
(II)

A comparison of the NMR spectra of some rotenoid derivatives[9] with compound-A would provide further circumstantial evidence (Table-2.10) about the absence of $C_{5'}$-methine proton (Ring E) in the NMR spectrum of compound-A.

Table-2.10

Sl. No.	Compound	δ -value of $C_{5'}$-H
1.	Rotenone	5.22
2.	12-Deoxy-dehydro-rotenone	5.16
3.	6a, 12a,-Dehydrorotenone	5.39
4.	Compound-A	-

The absence of a methine proton ($-\overset{|}{\underset{|}{C}}-H$) can only be explained if it is assumed that the E-ring is a six membered pyran system. The structure of compound-A could be retionalised on the basis of structure (II). The NMR data as discussed above were found to be in excellent agreement thereby confirming once again that compound-A possessed the proposed structure (II).



(II)

D.2 Structure elucidation of compound-B

Compound-B was crystallised from a mixture of petrol ether-chloroform as light brown crystals, m.p. 143°. Elemental analysis and mass spectrometrically derived molecular weight (M^+ 384) established its molecular formula as $C_{23}H_{28}O_5$. The UV absorption spectrum of the compound [λ_{max}^{EtOH} 237, 287, 300] provided evidence in favour of a rotenoid system.

The IR spectrum of compound-B was quite significant as it revealed the presence of a phenolic-OH group [ν_{max}^{KBr} 3400 cm^{-1}] and the absence of a keto group from the rotenoid system. A comparison of the IR spectra[9] of some rotenoids having both keto and hydroxyl groups with compound-B are presented below.

Table-2.11

Sl. No.	Compound	ν_{max}^{KBr} cm^{-1}
1.	Rotenone	2950, 1680, 1610, 1510, 1460, 1410, 1350, 1310
2.	Tephrosin	3500, 1670, 1580, 1520, 1450, 1340
3.	Compound-B	3400, 2960, 1610, 1520, 1410, 1360

From this table it was evident that compound-B contained a hydroxyl group and it was devoid of a keto functionality.

From a careful analysis of the NMR spectrum of compound-B, it was evident that compound-B contained two methoxyl groups (3.8 δ , S, 6H). The appearance of two doublets at 6.36 δ (J=7Hz) and 6.8 δ (J = 7 Hz) provided evidence for the presence of two ortho-protons in compound-A. The signals at 4.2 δ (J = 7 Hz) suggested the presence of two methylene protons attributable to the C-12 positions of the rotenoid system. Further the NMR spectrum also disclosed the presence of two more aromatic protons at 6.6 δ (S, 1H) and 6.4 δ (S, 1H) which could be assigned respectively to C_1-H and C_4-H of the A-ring of a rotenoid system. The appearance of a double-doublet at 4.68 δ could be attributed to two methylene protons present at C-6 position of the B-ring.

The NMR spectrum also revealed the characteristic signals for an isopentyl side chain (two singlets at 0.92 and 1.06 δ for two methyl groups; a multiplet centred around at 3.24 δ for two methylene protons, a sextet at 1.92 δ for two central methylene protons and methine proton signals around 2.76 δ as triplet).

The mass spectrum of compound-B showed, in addition to the molecular ion peak at m/z 384 (M^+), some more diagnostic peaks. Compound-B like the earlier hydrogenated product compound-A gave two fragments characteristic of rotenoid system. Compound-B gave two prominent peaks at m/z 192 (base peak) and at m/z 191 which could be accounted for RDA fragments (Chart-2.2). The peak at m/z 177 arose due to the loss of a methyl group from the ion peak at m/z 192. The peak at m/z 149 might arise due to loss of CO from the ion fragment at m/z 177. The peak at m/z 136 resulted by the expulsion of an isobutyl group from the peak at m/z 193. These fragmentations were found to be consistent with the presence

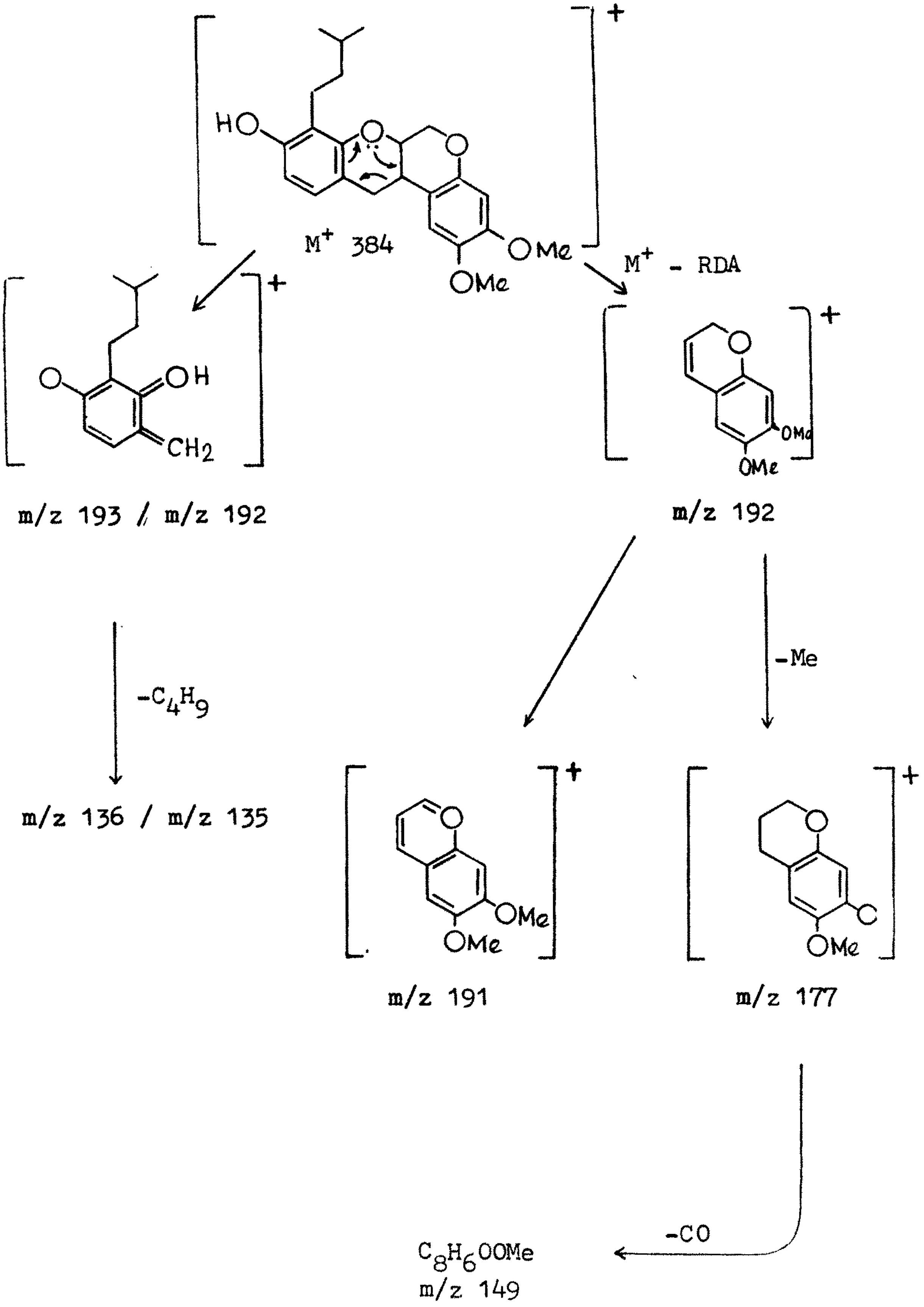
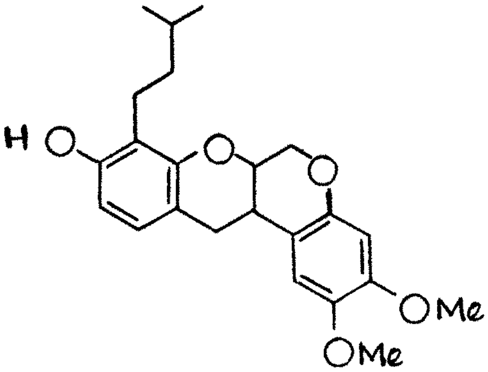


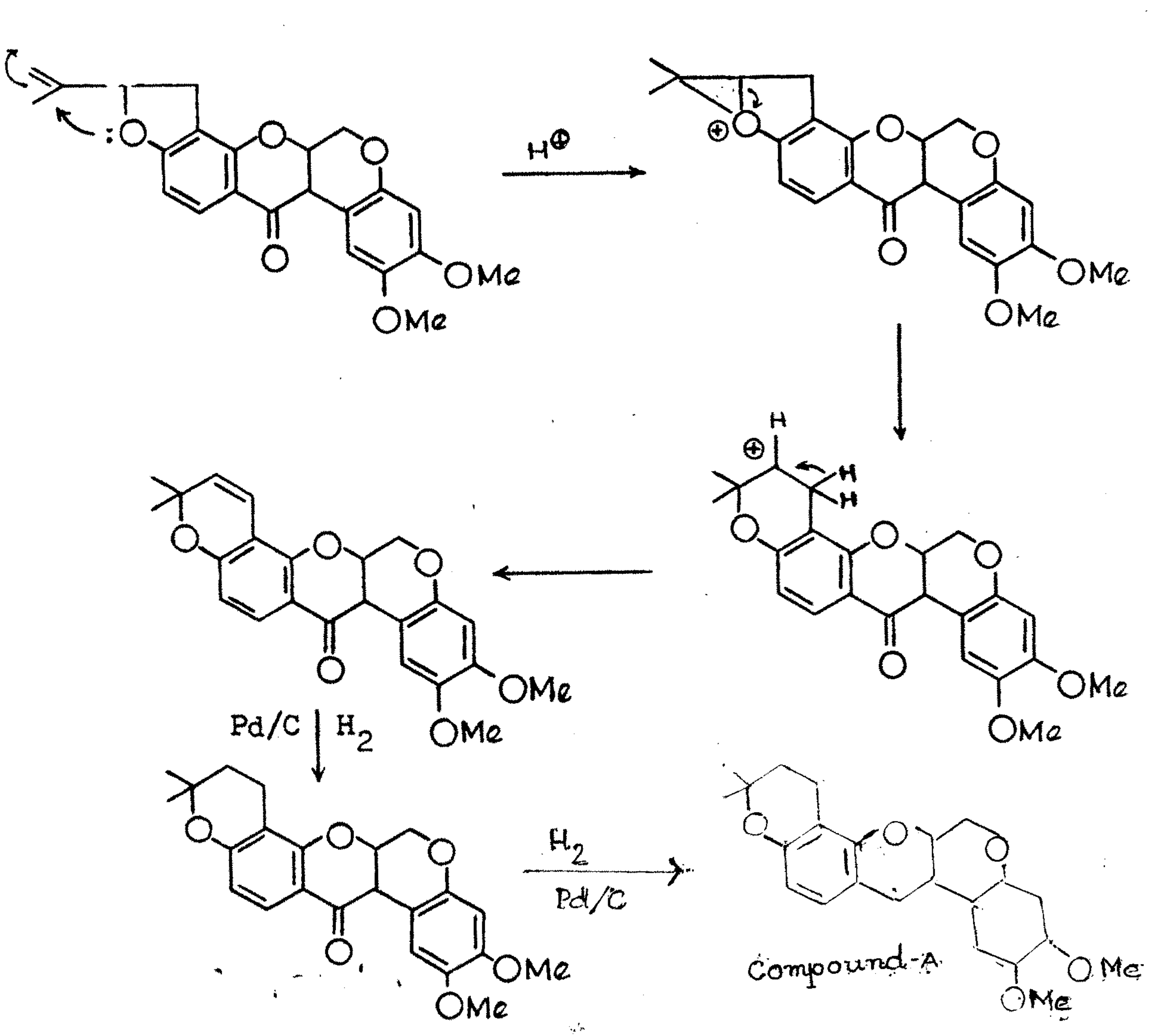
Chart-2.2. Mass fragmentation pattern of compound-B of an isopentyl side chain instead of an isopropyl side chain in the E-ring of rotenoid system.

On the basis of above facts it was evident that compound-B possessed an isopentyl side chain at C-8 and that it was devoid of an E-ring. The structure of compound-B was thus settled as (III).

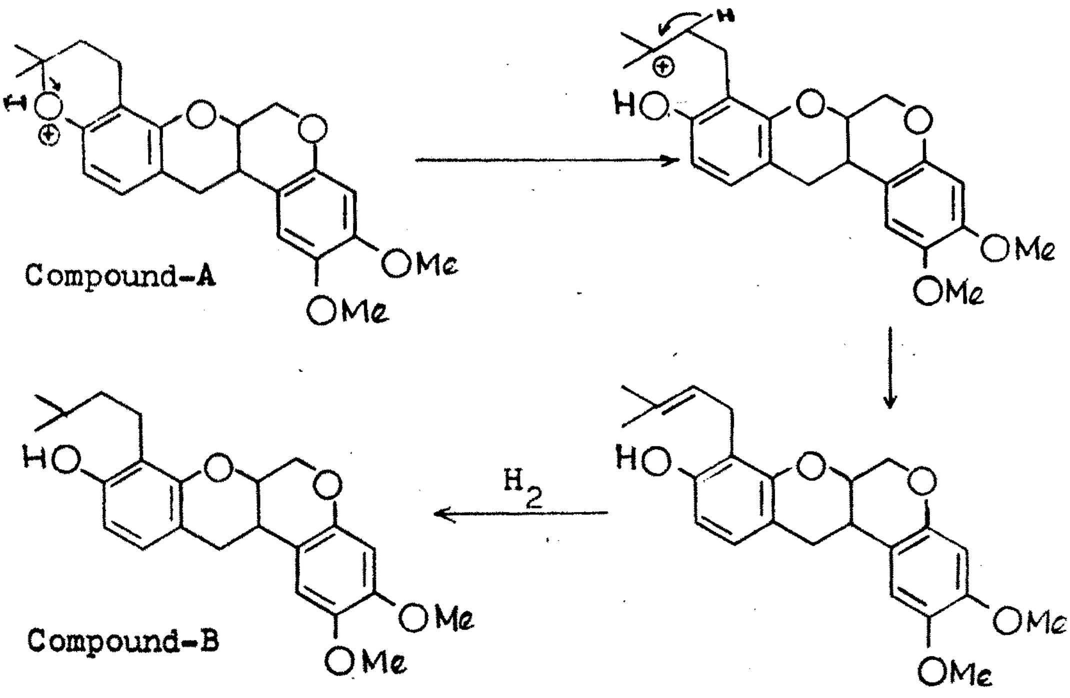


(III)

A plausible mechanism for the formation of compound-A and compound-B from rotenone has been postulated (Scheme III and IV).



Scheme-III. Plausible mechanism for compound-A



Scheme-IV. Plausible mechanism for compound-B

SECTION - E

EXPERIMENTAL

E.1 Hydrogenation of rotenone in ethyl alcohol

E.1.1 In presence of Pd/C catalyst

Rotenone (500 mg) was added to ethyl alcohol (25 cc) containing 50 mg of palladium-charcoal catalyst and the solution was shaken in hydrogen atmosphere for 4 hours. The reaction was discontinued after the absorption of about 320 c.c. of hydrogen. Then, the solution was filtered off and concentrated to a small volume. The latter was chromatographed over silica gel (10 gm, BDH, 60-120 mesh). The chromatogram was eluted with solvents of increasing polarity (Table-2.12). Petrol ether-benzene (1:1) eluates on evaporation gave a colourless solid which crystallised from ethyl-alcohol as colourless needles, m.p. 157°, designated as compound-A, yield 40 mg. Elution of the chromatogram with benzene afforded a brown solid which was further crystallised from a mixture of petrol ether-chloroform (9:1) as light brown crystals, m.p. 143°, yield 100 mg, and designated as compound-B.

Table-2.12

Fractions	Solvents	Volume	Residue on evaporation
1-10	Petrol ether	100	Red oily residue
11-20	Petrol ether-Benzene (1:1)	100	Colourless residue*

Table-2.12 contd.

Fractions	Solvents	Volume	Residue on evaporation
21-34	Benzene	100	Brown coloured residue**
35-40	Benzene-ethyl acetate	100	Light red residue

* From this fraction compound-A was isolated.

** From this fraction compound-B was isolated.

Spectral properties of compound-A

UV : λ_{max}^{EtOH} nm 227, 288

IR : ν_{max}^{KBr} cm^{-1} 2980 (Aromatic side chain), 1470, 1610, 1400 (*gem*-dimethyl), 1320, 1230, 1190, 1140, 1120, 1090, 1060, 1040, 990, 960, 880 (Aromatic).

NMR : (δ, $CDCl_3$) : 6.64 (*S*, 1*H*, C_1-*H*), 3.76 (*S*, 6*H*, 2-OCH_3), 6.38 (*S*, 1*H*, C_4-*H*), 4.62 (*m*, 2*H*, C_6-2*H*), 6.36 (*d*, 1*H*, C_{10}-*H*), 6.76 (*d*, 1*H*, C_{11}-*H*), 4.26 (*d*, 2*H*, C_{12}-2*H*), 3.14 (*m*, 2*H*, $C_{4'}$-2*H*), 2.62 (*t*, 2*H*, $C_{5'}$-2*H*), 0.92 (*S*, 3*H*, 1-CH_3), 0.98 (*S*, 3*H*, 1-CH_3).

Mass : 382 (M^+), 192 (M^+ -190), 191 (M^+-191), 177 (M^+-205), 176 (M^+-206), 149 (M^+-233), 134 (M^+-248), 106 (M^+-276).

Spectral properties of compound-B

UV : λ_{max}^{EtOH} nm 237, 287, 300

IR : ν_{max}^{KBr} cm^{-1} 3400 (Phenolic-OH), 2960 (Aromatic side chain), 1360, 1200, 1220, 1090, 1050 (Aromatic), 870, 840, 810 (Isopropyl group).

NMR : (δ, $CDCl_3$) : 6.6 (*S*, 1*H*, C_1-*H*), 3.8 (*S*, 6*H*, 2-OCH_3), 6.4 (*S*, 1*H*, C_1-*H*), 4.68 (*dd*, 2*H*, C_6-2*H*), 6.36 (*d*, 1*H*, C_{10}-*H*), 6.8 (*d*, 1*H*, C_{11}-*H*), 4.2 (*d*, 2*H*, C_{12}-2*H*), 3.24 (*m*, 2*H*, $C_{4'}$-2*H*), 1.92 (Sextet, 2*H*, $C_{5'}$-2*H*), 2.76 (*t*, 1*H*, $C_{6'}$-H), 0.92 (*S*, 3*H*, 1-CH_3), 1.06 (*S*, 3*H*, 1-CH_3).

Mass : 384 (M^+), 193 (M^+-191), 192 (M^+-192), 191 (M^+-193), 177 (M^+-207), 149 (M^+-235), 136 (M^+-248).

E.1.2 In presence of PtO_2 catalyst

Rotenone (500 mg) was subjected to hydrogenation with 40 mg of PtO_2 catalyst in the same solvent system. The uptake of hydrogen after about 3.5 hours was 290 c.c. The solution was filtered and concentrated from which a colourless solid crystallised out. It was purified by recrystallisation from ethyl alcohol as colourless needles, m.p. 165°C, yield 150 mg. This compound was identified as dihydrorotenone by direct comparison with an authentic sample (m.p., m.m.p., co-TLC).

E.2 Hydrogenation of rotenone in acetic acid

E.2.1 In presence of Pd/C catalyst

Rotenone (500 mg) was added to glacial acetic acid (300 c.c.) containing 50 mg of Pd/C catalyst. The uptake of hydrogen was 300 c.c. after about 4 hours. The solution was then diluted with benzene and extracted with dilute Na_2CO_3 solution to free it from acetic acid and washed with water. The benzene solution was then dried over anhydrous Na_2SO_4 and concentrated to a small volume from which a yellowish compound crystallised out. It was then purified by recrystallisation from ethyl alcohol, m.p. 132-133°, yield 10 mg. The structure of the compound could not be established due to its poor yield.

E.2.2 In presence of PtO_2 catalyst

Rotenone (500 mg) was subjected to hydrogenation for 3 hours in presence of PtO_2 catalyst (50 mg) in the same solvent system. A brown coloured compound, m.p. 154° was obtained following the same procedure as in the previous case. The spectral studies could not be carried out due to paucity of the material.

E.3 Hydrogenation of rotenone in anhydrous chloroform

E.3.1 In presence of Pd/C catalyst

Rotenone (500 mg) was dissolved in dry $CHCl_3$ (30 c.c.) and subjected to hydrogenation in presence of Pd/C catalyst.

The consumption of hydrogen in 4 hours was 220 c.c. The solution was then filtered off from the catalyst and concentrated to a small volume. A crystalline compound separated out which was again purified by dissolving in hot alcohol. The m.p. of the compound was observed to be 148°. It could not be characterised.

E.3.2 In presence of PtO_2 catalyst

Rotenone (500 mg) was subjected to hydrogenation with 40 mg of PtO_2 catalyst in the same solvent system. About 190 c.c. of hydrogen was taken up in 4 hours and the product was worked up as in previous case. A yellowish crystalline compound, m.p. 162-163° was obtained which could be detected as unreacted rotenone by direct comparison with an authentic sample (Co-IR, Co-TLC).

CHAPTER - II

CHEMICAL INVESTIGATION OF DIDYMOCARPUS PODOCARPA

SECTION - A

Under a research programme on naturally occurring oxygen heterocycles, we became interested to undertake phytochemical investigations on some flavanoid-bearing plants. While surveying the literature on flavanoids, it came to the notice of the present investigator that a limited number of chalcones substituted at various positions in A-rings are found in some Didymocarpus species belonging to Gesneriaceae family. Thus, the plant Didymocarpus pedicellata (Fam : Gesneriaceae), which is reputed for its medicinal properties[11], elaborates a number of chalcones[12-16], highly oxygenated at various positions in A-rings and it has been found that all of these chalcones possess unsubstituted B-rings[12,13]. From another Didymocarpus species, D.aurentiaca, growing abundantly in the hilly areas of West Bengal, two new C-methylated chalcones and two α-pyrone derivatives[17-19]were isolated. The B-rings of these two C-methylated chalcones were also found to be devoid of substituents in B-rings. Interestingly, both of these Didymocarpus species elaborate phloroglucinol derived chalcones and flavanones. Very recently, phytochemical investigation of another Didymocarpus species viz., D.podocarpa furnished 7,8-epoxy 5,6-dehydrokawain from the petrol ether extract of this plant. All these information prompted us to undertake further phytochemical investigation on D.podocarpa (Fam: Gesneriaceae) to isolate oxygen heterocycles having biological properties.

SECTION - B

Morphology of *Didymocarpus* species

Family : Gesneriaceae

Seventy species are found in India, Malaya and China. The name of some of the important species are listed below :

1. *Didymocarpus pedicellata*
2. *D.aurentiaca*
3. *D.pyamaea* Clarke
4. *D.macrophylla* Wall
5. *D.cinerea* Don Proder
6. *D.andersoni* Clarke
7. *D.oblonga* Wall
8. *D.aromatica*
9. *D.villosa* Don Proder
10. *D.podocarpa*
11. *D.leucocalyx*
12. *D.pulhere*

Didymocarpus podocarpa

Habitat

Subalpine Sikkim; Lachen, altitude 10-12,000 ft, J.D.H., Sinchul, altitude 800 ft, J.D.H.; Singalelah, altitude 8000 ft., Trentler, C^{20}.

General habit

Stem 2-8 in., ashy-pubescent, leafless except at the summit. Leaves opposite, attaining 6 by 4 in., pubescent only on the nerves beneath; petiole 0-1 in., mostly short. Peduncles 2-4, 1-2½ in., bracts 1/5 in., wide rotund, coloured subconnate; pedicels 1/3-2/3 in., glabrous. Calyx nearly 1/4 in., campanulate. Corolla nearly 1 in., purple, glabrous or sparsely pilose without capsule-stalk longer than, some times nearly twice as long as, the calyx-differs from the 3 preceding dosely allied species by the larger flowers and larger long-pedicelled capsule.

Since the genus Didymocarpus elaborates a number of flavonoids along with some terpenoids especially diterpenoids and 2-pyrone derivatives, so it is quite logical to present a brief summary of the constituents occurring in various Didymocarpus species in Section-C.

SECTION - C

Table - 2.13

List of naturally occurring compounds isolated from Didymocarpus species.

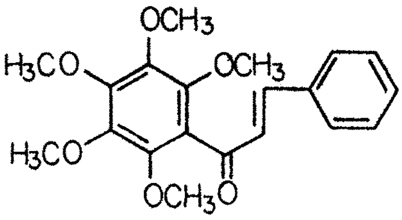
Sl. No.	Name of the compound, molecular formula, melting point	Structure	Plant source	Reference
1	2	3	4	5
1.	Pedicellin $C_{20}H_{22}O_6$ 93-94°		Didymocarpus pedicellata	14,15, 21

Table-2.13 contd.

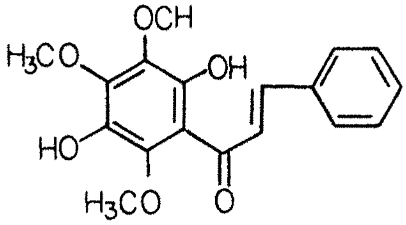
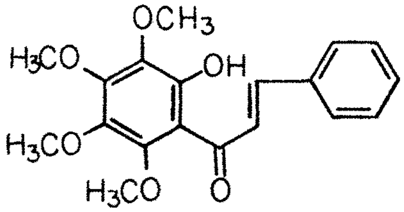
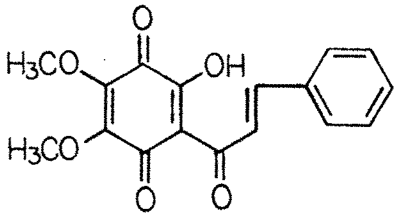
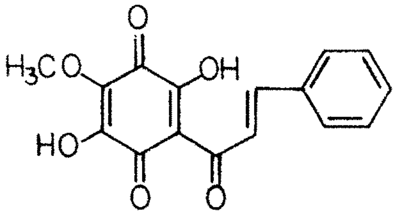
1	2	3	4	5
2.	Pedicin $C_{18}H_{18}O_6$ 139-40°		*D.pedicellata*	14,15, 21
3.	Methyl pedicin $C_{19}H_{20}O_6$		*D.pedicellata*	16
4.	Methylpedicinin $C_{17}H_{14}O_6$ 110°		*D.pedicellata*	14,21, 22
5.	Pedicinin $C_{16}H_{12}O_6$ 203-5°		*D.pedicellata*	14,21, 22

Table-2.13 contd.

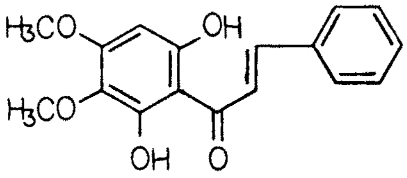
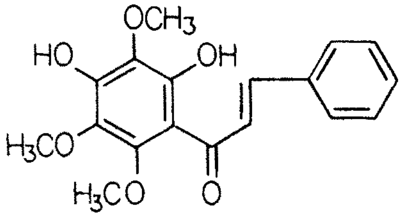
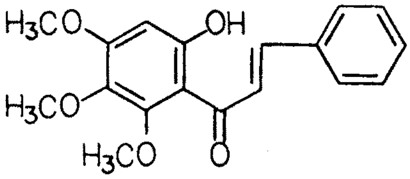
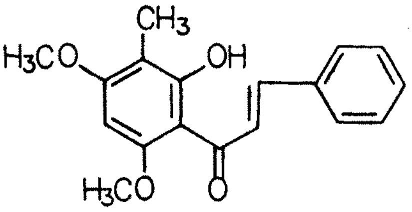
1	2	3	4	5
6.	Pashanone $C_{17}H_{16}O_5$ 147-9°		*D.pedicellata*	15
7.	Isodidymocarpin $C_{18}H_{18}O_6$ 139-140°		*D.pedicellata*	23
8.	2'-hydroxy-4' 5',6'-trimethoxy chalcone $C_{17}H_{18}O_5$ m.p.101-2°		*D.pedicellata*	24
9.	Aurentiacin $C_{18}H_{18}O_4$ m.p.140-141°		*D.aurentiaca*, *Pityrograma pallida*	25,26

Table-2.13 contd.

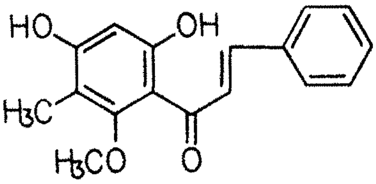
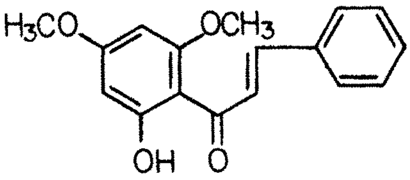
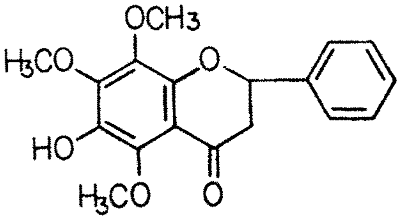
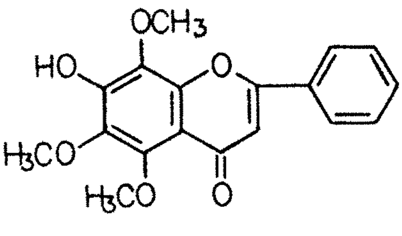
1	2	3	4	5
10.	Aurentiacin-A $C_{17}H_{16}O_4$ 205-6°		*D.aurentiaca*	27
11.	Flavokawin-B		*D.corchorifolia*	38
12.	Isopedicin $C_{18}H_{18}O_6$ 103-104°		*D.pedicellata*	11
13.	Didymocarpin $C_{18}H_{18}O_6$ 103-104°		*D.pedicellata*	28

Table-2.13 contd.

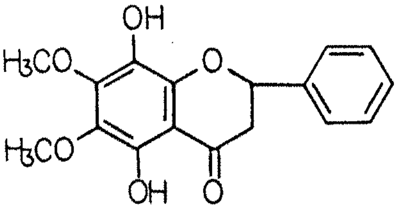
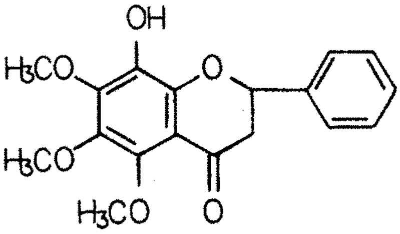
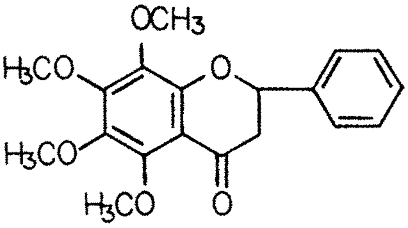
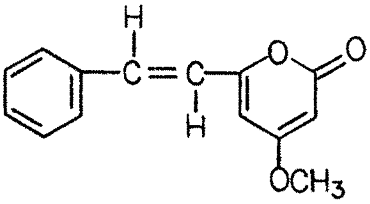
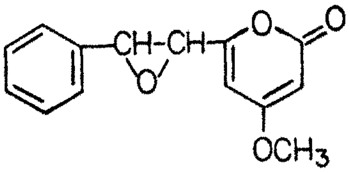
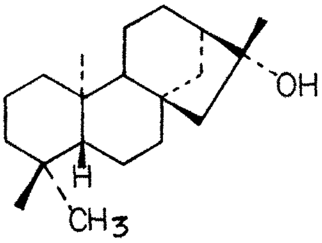
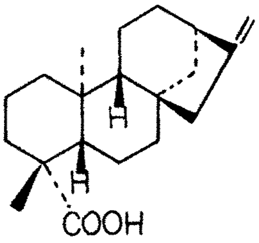
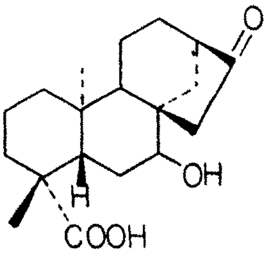
1	2	3	4	5
14.	Didymocarpin-A $C_{17}H_{16}O_6$ 213-215°		*D.pedicellata*	29
15.	Didymoflavanone $C_{18}H_{18}O_6$ m.p.87-8°		*D.pedicellata*	24
16.	5,6,7,8-tetra-methoxyflavanone $C_{19}H_{19}O_6$ 103-4°		*D.pedicellata*	24
17.	5,6-Dehydrokawain $C_{14}H_{12}O_3$ 141°		*D.aurentiaca*	30

Table-2.13 contd.

1	2	3	4	5
18.	7,8-Epoxy-5, 6-Dehydro kawain $C_{14}H_{12}O_4$ 125-26°		D.aurentiaca D.podocarpa	30, 31
19.	ent-16 - - Kauranol $C_{20}H_{34}O$ 213-16° $[\alpha]_D$ - 47°		D.oblonga	32
20.	ent-16-Kaurene- -19-oicacid $C_{20}H_{30}O_2$ 172-74° $[\alpha]_D$-114°		D.oblonga	32
21.	Didymocblongin $C_{20}H_{30}O_3$ 239-43° $[\alpha]_D$-63°		D.oblonga	33

SECTION-D

CHEMISTRY OF COMPOUND - X

Isolation

The whole plant of Didymocarpus podocarpa was collected during the month of October-November from Darjeeling area of West Bengal. The air dried powdered whole plant was exhaustively extracted with petroleum ether (b.p.60-80°). The petroleum ether extract on concentration gave a thick oily residue which was chromatographed over silica gel. Elution of the chromatogram with petroleum ether-benzene (1:1) afforded a yellowish orange solid which was rechromatographed over silica gel and petroleum ether-benzene (4:1) eluates afforded an orange solid which crystallised from a mixture of petrol ether-benzene as orange plates, m.p. 141°. This compound is designated as compound-X in the subsequent sections.

Homogeneity

On thin layer chromatograms, using the following solvent systems (Table-2.14), compound-X produced a single iodine staining spot indicating its homogeneity.

Table - 2.14

No.	Solvent system	Rf.
1.	Benzene-chloroform (9 : 1)	0.9
2.	Benzene-chloroform (9.5:0.5)	0.7

Properties and colour reactions

Compound-X crystallises from petroleum ether-benzene (9.5:0.5) as orange plates, m.p. 141°. It is highly soluble in benzene, chloroform, ether and ethanol, moderately soluble in petrol ether (b.p. 60-80°) and insoluble in water.

Compound-X gave a brown colour with freshly prepared alcoholic ferric chloride solution indicating the presence of a phenolic hydroxyl group.

Molecular formula of compound-X

The elemental analysis of compound-X gave the emperical formula $(C_{18}H_{18}O_4)_n$ and since its molecular weight was found to be 298 by mass spectrometry, the molecular formula of compound-X should be $C_{18}H_{18}O_4$.

Spectral properties of compound-X

Ultraviolet spectrum

With a view to determining the nature of the chromophoric groups of compound-X, its ultraviolet spectrum (Fig. 2.11) has been studied. It shows UV absorption maximum at 343 nm (in ethanol). Compound-X does not produce any remarkable shift of maxima on treatment with sodium acetate.

Infrared absorption spectrum

The IR absorption spectrum (Fig.2.12) of compound-X in nujol has been studied and the probable assignments of the important peaks are listed in Table-2.15.

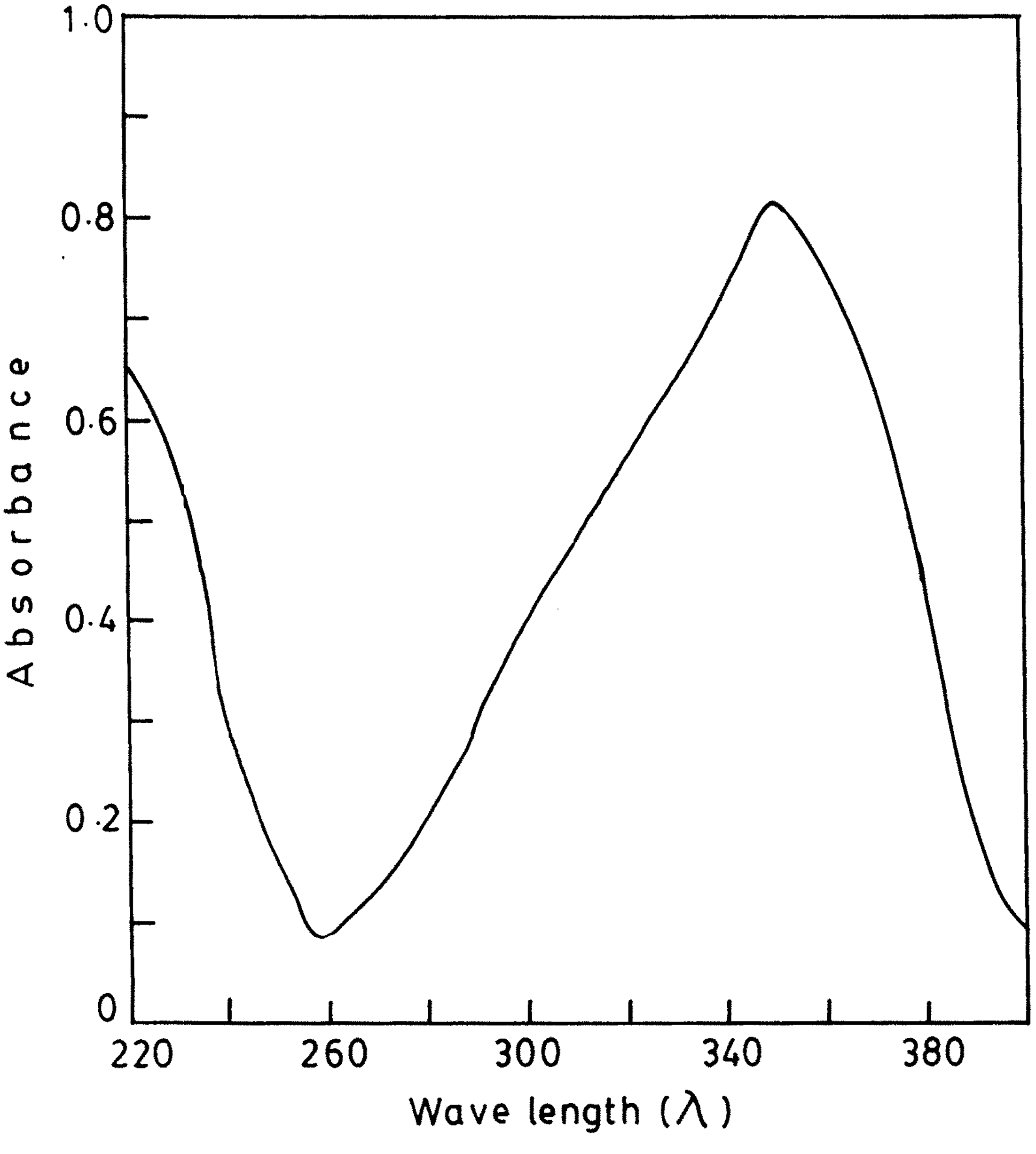


Fig. 2.11. UV spectrum of Compound - X

The presence of a monosubstituted benzene ring in the compound-X is indicated by the appearance of a peak at 70 cm^{-1}[34]. The peak at 1615 cm^{-1} may be attributed to a chelated $>C = O$ function. The strong peaks at 1600, 1550 and 1125 cm^{-1} are indicative of the presence of aromatic ring. A peak at 3200 cm^{-1} may be due to the presence of a chelated phenolic -OH group.

Table - 2.15

Position of absorption peak cm^{-1}	Intensity	Probable assignment
1615	Strong	$>C=O$ (chelated)
700	Strong	Monosubstituted benzene ring
1600	Strong	Aromatic
1550	Strong	
1125	Medium	
790	Medium	
745	Strong	
3200	Weak	Chelated phenolic -OH

Nuclear Magnetic Resonance Spectrum of Compound-X

The NMR spectrum (Fig.2.13) of compound-X was determined in a 60 MHz instrument using deutero-chloroform as the solvent and TMS as the internal standard.

The NMR spectrum of compound-X signals for two highly deshielded protons at 7.7δ as singlet which are the α- and β-protons of a chalcone system. The appearance of a sharp

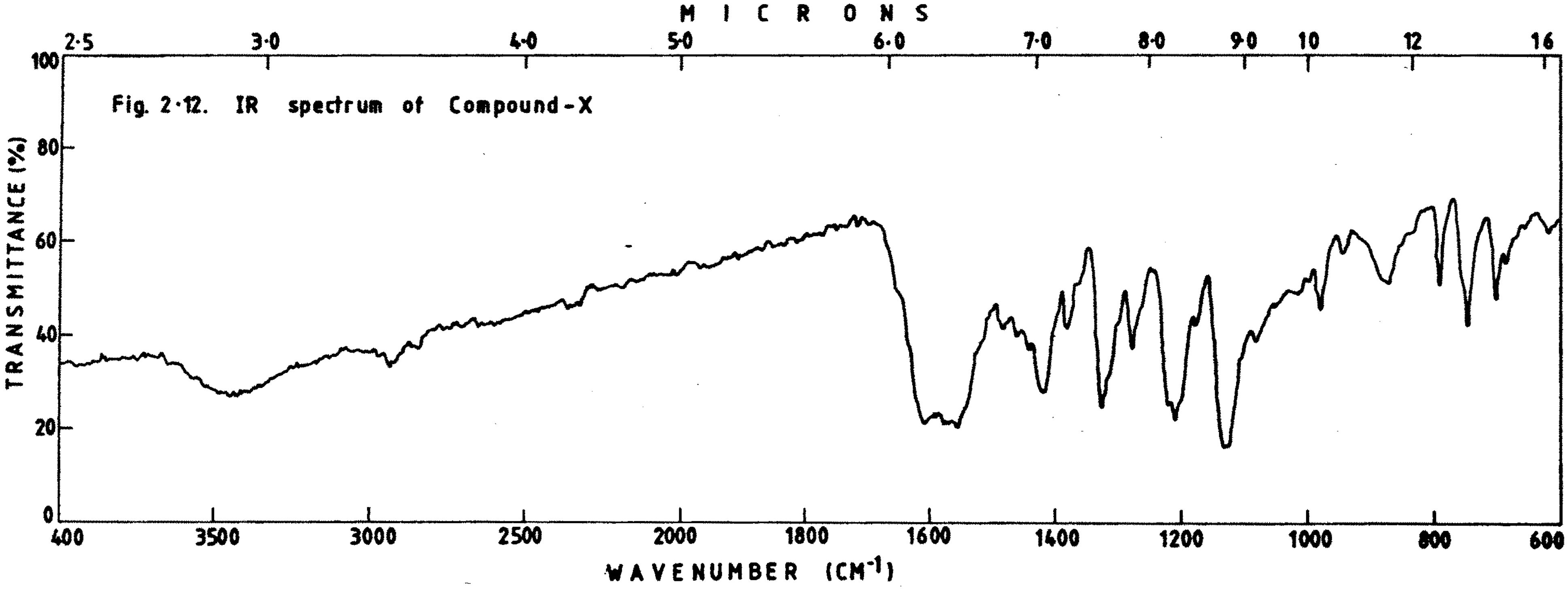


Fig. 2·12. IR spectrum of Compound-X

singlet at 14.06 δ, which disappears on D_2O exchange, may be assigned to a chelated phenolic proton. The 3H-singlet at δ 2.06 may be due to the presence of an aromatic methyl group in the compound. In addition to these protons, an aromatic singlet at 6.03 δ and five aromatic protons around 7.43 δ as multiplet along with two-OCH_3 groups appearing as two singlets at 3.95 δ and 4.0 δ are discernible in the NMR spectrum of compound-X. The proton-chemical shifts and their probable assignments are shown in Table-2.16.

Table - 2.16

Chemical δ	Shift τ	No. of protons	Multiplicity	Probable assignment
7.7	2.3	2	Singlet	*trans*-olefinic protons
14.06	-2.06	1	Singlet	Phenolic-OH
2.06	7.94	3	Singlet	Methyl protons
6.03	3.97	1	Singlet	Aromatic
7.43	2.57	5	Multiplet	Aromatic
3.95	6.05	3	Singlet	$-OCH_3$
4.00	6.00	3	Singlet	$-OCH_3$

Mass spectrum of compound-X

The mass spectrum of compound-X (Fig.2.14) exhibits, besides the molecular ion peak at m/z 298 (M^+), a number of diagnostic peaks at m/z 297, m/z 221 and m/z 195.

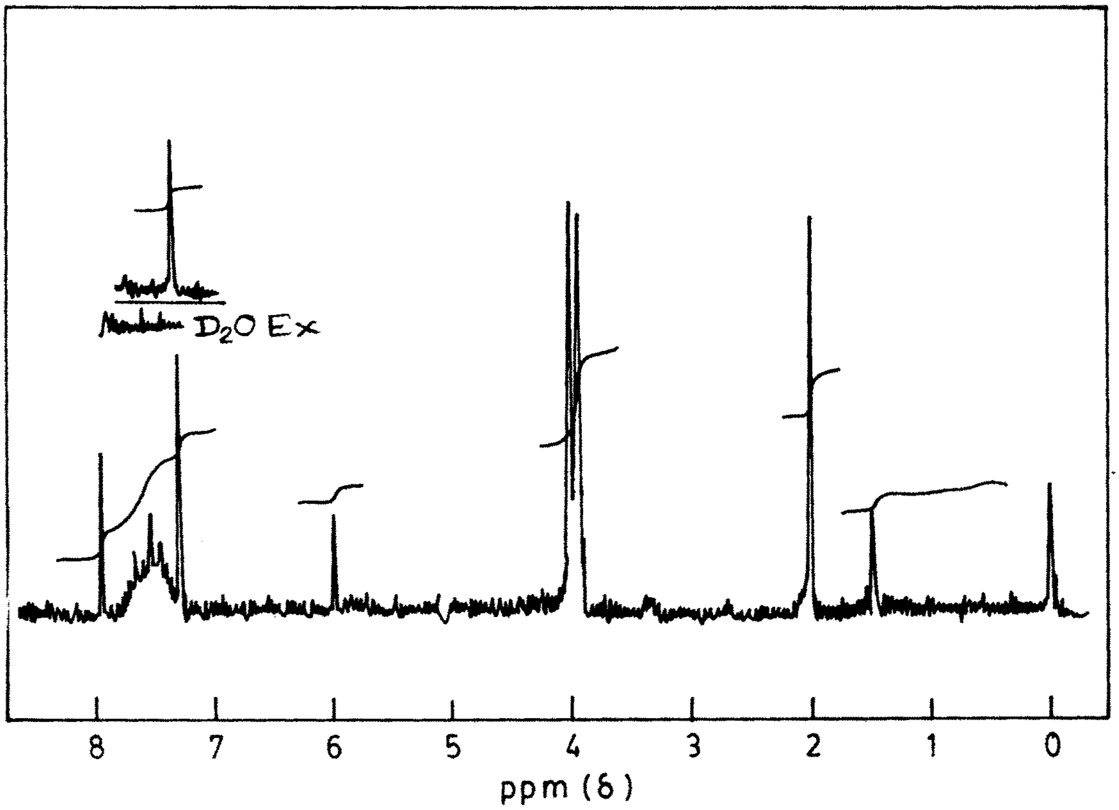


Fig. 2·13. NMR spectrum of Compound - X

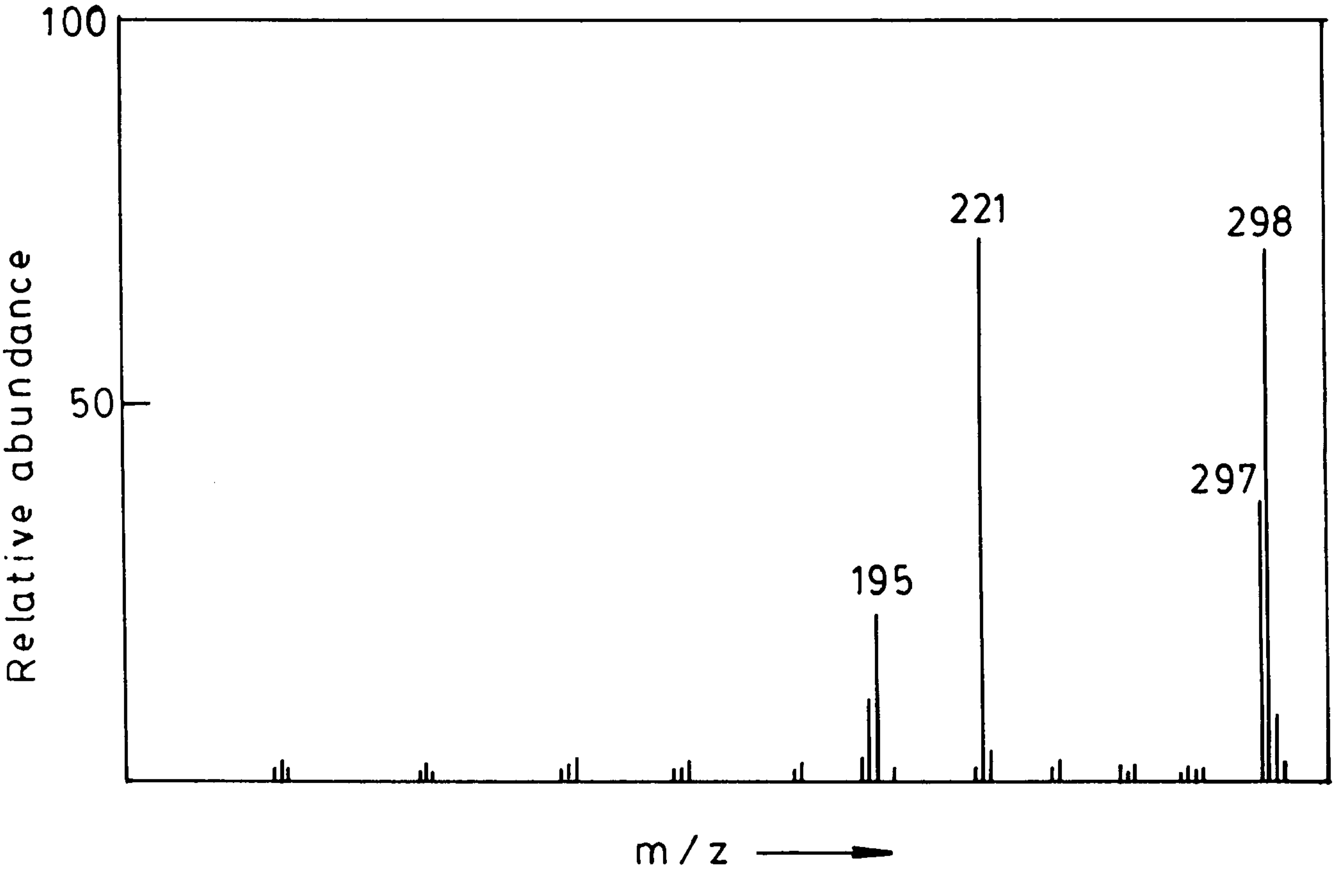


Fig. 2.14. Mass spectrum of Compound - X

Conclusion drawn from spectral data of compound-X

The UV spectrum of compound-X shows only one maximum at 343 nm which does not suffer any bathochromic shift on the addition of sodium acetate. This phenomenon indicates the absence of a -OH group at the para-position of ring-B[34]. Furthermore, the UV spectrum speaks in favour of phloroglucinol substitution pattern in ring-A of compound-X. A comparison of UV absorption spectra of some chalcones[35] having phloroglucinol substitution pattern in ring-A is shown in Table-2.17.

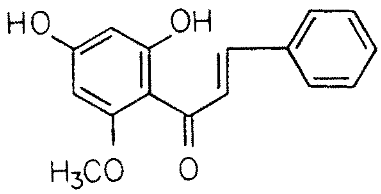
Table-2.17

Sl.No.	Compound	$^{EtOH}_{max}$ nm
1	2',4',6'-trihydroxy-4-methoxy-chalcone	358
2	2'-hydroxy-4'-6'-dimethoxy-chalcone	338
3	2',4'-dihydroxy-6'-methoxy-chalcone	345
4	Aurentiacin	343
5	Compound-X	343

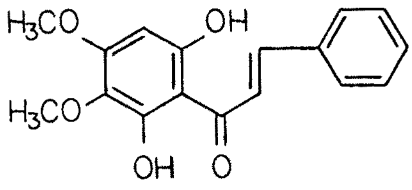
The IR spectrum of compound-X is quite informative as it reveals the presence of a chelated carbonyl function (1615 cm^{-1}), a complex aromatic substitution pattern (1600, 1550, 1125, 745 and 790 cm^{-1}) and a monosubstituted benzene ring (700 cm^{-1}).

Thus from UV and IR data, it may be inferred that the A-ring of compound-X possesses a phloroglucinol type of substitution pattern and that the B-ring of this chalcone is probably unsubstituted.

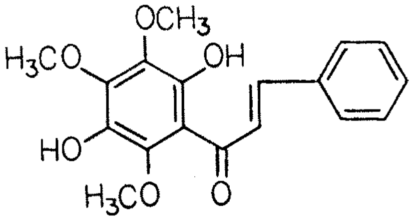
For further information regarding the structure of compound-X, its NMR spectrum was thoroughly examined. Generally, it has been observed that the trans-olefinic protons of a chalcone system appear around δ 7.3-8.0 as to pairs of doublets[36]. It has also been observed that the NMR spectra of cardamonin[37] (1) pashanone[34] (2) and pedicin (3) signal around 7.6-7.8 δ as a sharp singlet for the α- and β-protons.



(1)



(2)

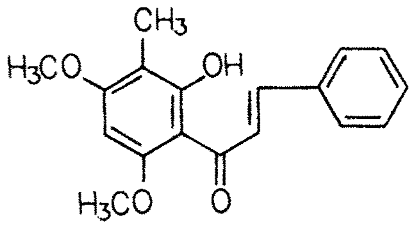


(3)

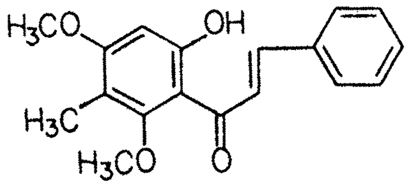
So, the appearance of a singlet (2H) at δ 7.7 speaks in favour of a chalcone system to be present in compound-X. Since compound-X appears to be a chalcone derivative from NMR and UV data, it would be worthwhile to examine its mass spectral fragmentation pattern.

In the mass spectrum of compound-X the appearence of two prominent peaks at m/z 221 and m/z 195 may arise by loss of a phenyl group and a styrene fragment respectively from the molecular ion thereby providing further evidence as to the unsubstituted nature of the B-ring of this chalcone derivative. The mass spectral data also suggest that three of the four oxygen atoms of compound-X are most probably situated in the A-ring of this chalcone. The mass fragmentation pattern of compound-X is shown in Chart-2.3.

On the basis of above discussion, the compound-X might be represented as (4) or (5).



(4)



(5)

Finally, compound-X was identified as 4',6'-dimethoxy-3'-methyl-2'-hydroxy-chalcone (6) by direct comparison with an authentic sample of aurentiacin obtained from <u>Didymocarpus aurentiaca</u> and the compound-X was indeed found to be identical with

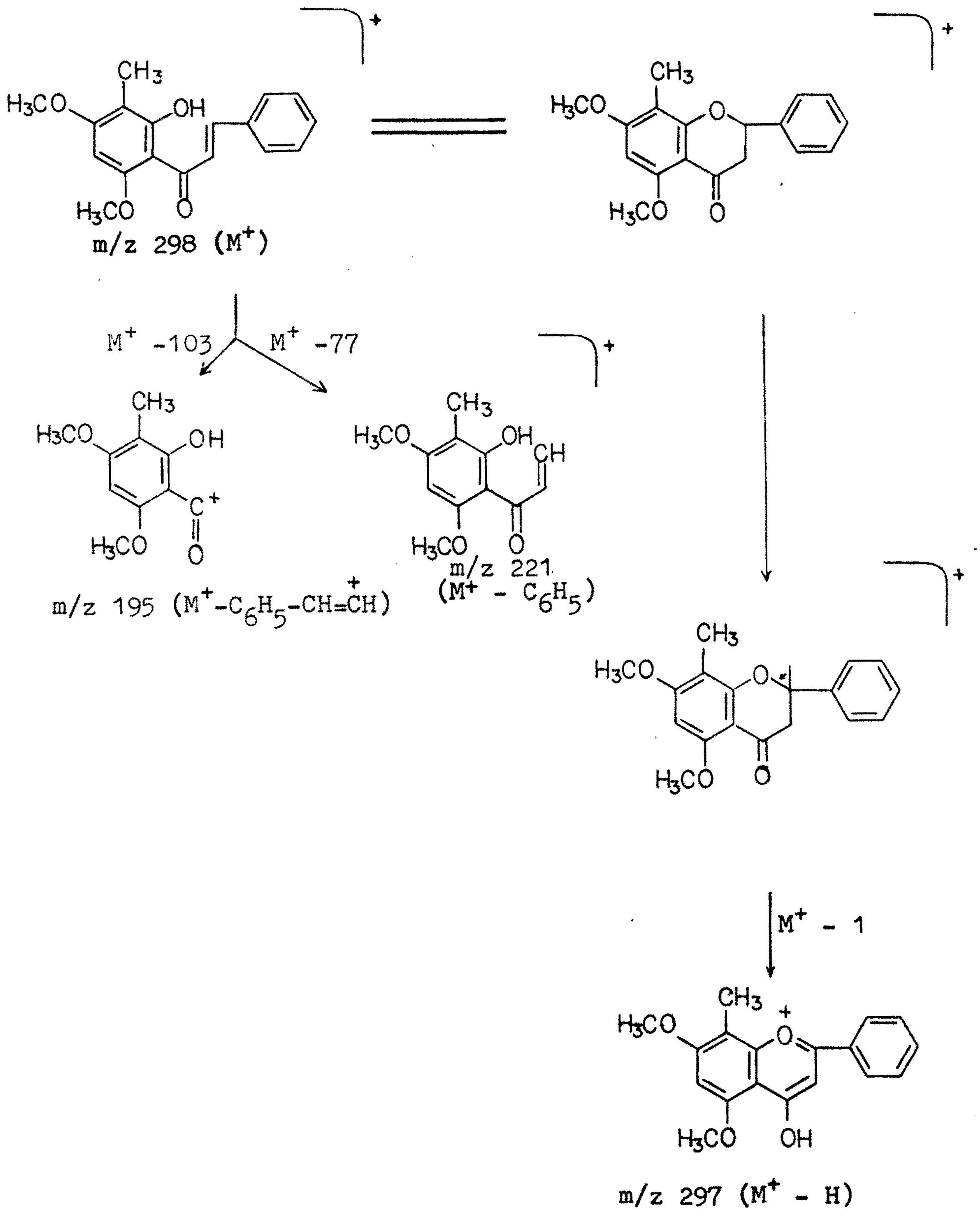
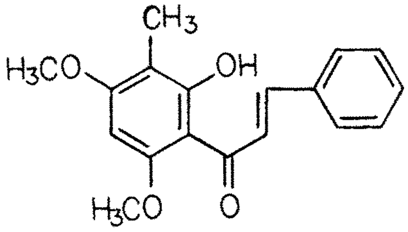


Chart-2.3. Mass fragmentation pattern of compound-X aurentiacin in all respects [m.p., m.m.p., Co.TLC and superimposable IR (Fig. 2.15)]. On the basis of comparison study, the structure of compound-X should be represented as (6).



(6)

The occurrence of 4',6'-dimethoxy-3'-methyl-2'-hydroxychalcone (6) is being reported for the first time from <u>Didymocarpus podocarpa</u>.

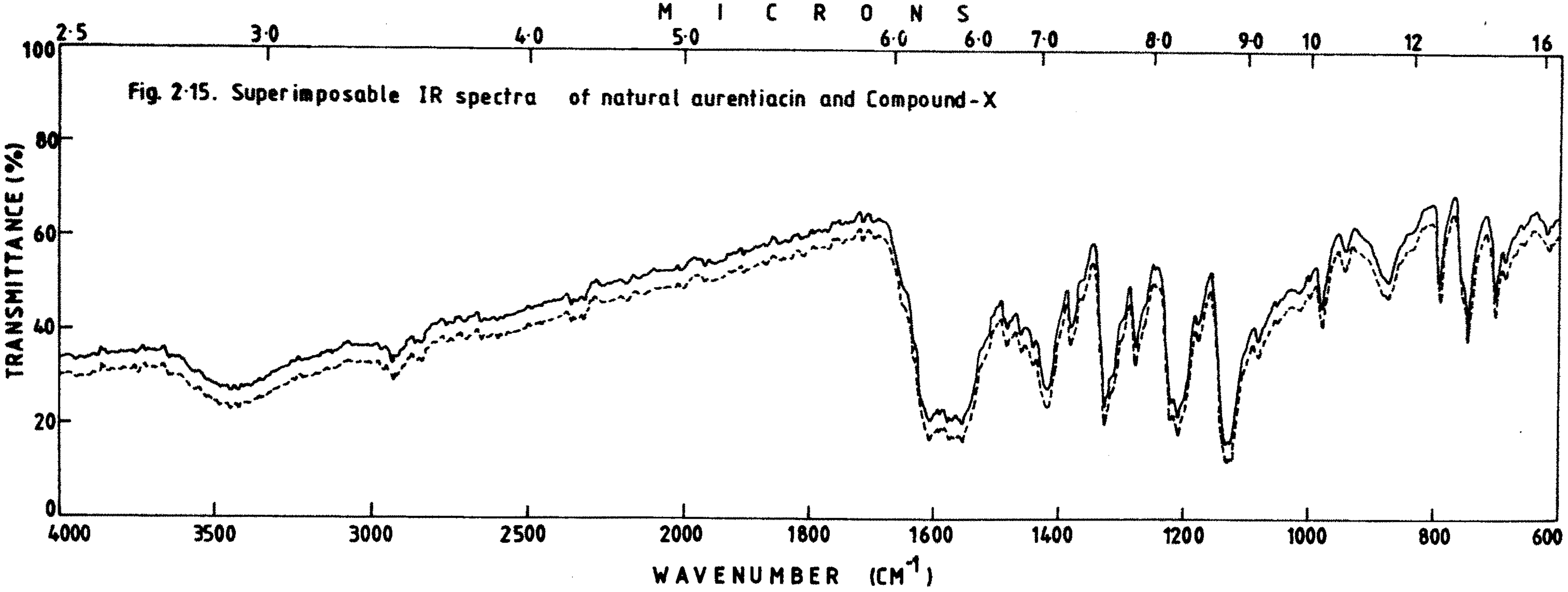


Fig. 2·15. Superimposable IR spectra of natural aurentiacin and Compound-X

SECTION - E

EXPERIMENTAL

General

Melting points were determined on a Kofler block or sulphuric acid bath and are uncorrected. Petroleum ether refers to light petroleum, b.p. 60-80°, unless otherwise mentioned. Ultraviolet spectra were measured in ethanol solution with Beckman DU-6 Spectrophotometer. NMR spectra were determined with a Varian A-60 instrument in $CDCl_3$ with TMS as internal standard (S = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). Molecular weight was determined by mass spectrometry. Mass spectra were recorded in an AEI-MS 9 mass spectrometer using a direct insertion probe and operating at 70 EV. Silica gel (Merck) was used for thin layer chromatography (0.35 mm thickness) and iodine was used for developing the plates. The analytical samples were routinely dried over P_2O_5 at 80° for 24 hours in Vacuo.

Isolation of compound-X

Air dried finely powdered whole plant of Didymocarpus podocarpa (1 kg) was exhaustively extracted (40 hours) with petroleum ether in a soxhlet apparatus. The solvent was evaporated and the concentrated extract (15 gm) was chromatographed over silica gel (BDH, 300 gms, 60-120 mesh). The chromatograph was eluted with solvents of increasing polarity (Table-2.18) Petroleum ether-benzene (1:1) eluates on solvent evaporation gave a yellowish solid-A (1.15 gm) which was further purified by rechromatography (Table-2.19) over silica gel. Elution of the chromatogram with petroleum ether-benzene (9:1) afforded an orange coloured crystalline solid designated as compound-X.

Table-2.18

Fraction	Solvents	Volume (cc)	Residue on evaporation
1-3	Petroleum ether	250	Nil
4-8	Petroleum ether-benzene (4:1)	250	Gum
9-20	Petroleum ether-benzene (2:1)	250	Red oil Trace yellow solid with impurities
21-38	Petroleum ether-benzene (1:1)	250	Yellow solid (compound-X) with trace impurities

Table-2.19

Fractions	Solvents	Volume (cc)	Residue on evaporation
1-7	Petroleum ether	25	Nil
8-12	Petroleum ether-benzene (9.5:0.5)	25	Gum
13-20	Petroleum ether-benzene (9:1)	25	Red oil
21-60	Petroleum ether-benzene (4:1)	25	Orange yellow solid (compound-X)
61-80	Petroleum ether-benzene (1:1)	25	Trace of orange solid with impurities
81-85	Benzene (100%)	25	Nil

Purification of compound-X

The fractions (21-60) obtained from the chromatogram (Table-2.19) were mixed and the orange yellow solid obtained after solvent evaporation was repeatedly crystallized from petroleum ether-benzene (9.5:0.5) as bright orange plates, m.p. 141° (yield 0.005%).

UV : λ_{max}^{EtOH} nm 343 (log 3.8)

$\lambda_{max}^{EtOH + NaOEt}$ nm 344;

IR : ν_{max}^{nujol} cm^{-1}, 1615, 700, 1600, 1550, 1125, 790, 745, 3200

NMR : 2H-(S 7.7 δ , *trans*-olefinic), chelated phenolic -OH (S, 14.06 δ disappeared on D_2O exchange); one -3H (S 2.06 δ , $-CH_3$), two-3H (S, 3.95 and 4.0 δ , two $-OCH_3$), 5 ArH (m, 7.43 δ), 1 ArH (S, 6.03 δ).

Mass : m/e 298 (M^+), m/e 297 (M^+-1), m/e 221 (M^+-77) and m/e 195 (M^+-103).

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