STUDIES IN ANALYSIS OF PESTICIDES

A THESIS

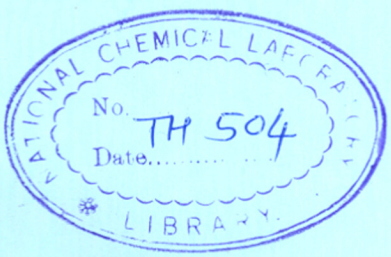
SUBMITTED TO THE

UNIVERSITY OF POONA

FOR THE DEGREE OF

MASTER OF SCIENCE

(IN CHEMISTRY)



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SEPTEMBER 1986

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C E R T I F I C A T E

Certified that the work incorporated in the thesis "Studies in analysis of Pesticides" submitted by Mrs. S.S. Kunte was carried out by the candidate under my supervision. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

(Dr.R.B. Mitra)
Supervisor

A C K N O W L E D G E M E N T

I would like to express sincerely my deep sense of gratitude to Dr.R.B. Mitra, Head, Organic Chemistry Division, NCL, under whose able guidance and encouragement, the work presented in this thesis has been carried out.

I would also like to express my gratitude towards Dr. B.B. Ghatge, Scientist, NCL, for the invaluable help throughout the course of the work.

I am thankful to Dr. L.K. Doraiswamy, Director, National Chemical Laboratory, for his kind permission to allow me to submit this work in the form of a thesis.

I acknowledge with great pleasure the help rendered by all my colleagues, especially Ms. A.L.Jadhav.

S S Kunte

(Mrs.S.S. Kunte)

National Chemical Laboratory,
Poona-411008

September 1986

GENERAL REMARKS

1. IR spectra were recorded on a Perkin-Elmer infra-cord spectrophotometer, model 137B.

2. PMR spectra were recorded on a Varian T-60 spectrometer using TMS as internal standard. The chemical shifts are given in δ values.

3. Pet.ether used refers to the fraction boiling between 60-80°C.

C O N T E N T S

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SUMMARY

Now a days several synthetic organophosphorus pesticides and fungicides are available in the market and are being used on the fields every day. The technical products are never absoutely pure and always contain numerous impurities. The activity of the pesticides may vary due to impurities and byproducts. The literature survey does not give complete analysis of the impurities in these technical products. Therefore work is undertaken for isolation and identification of the impurities present in Dimethoate, Ethion and Carboxin (technical grade products).

Chapter I deals with the general introduction of pesticides. History and classification of organophosphorus pesticides and fungicides are described in this chapter. Structure activity relationship and metabolism of organophosphorus pesticide are explained. "The methods of analysis" used for the analysis of pesticides, metabolites, formulated pesticides and residue are also mentioned in this chapter.

Chapters II, III and IV deal with the isolation and identification of impurities from Dimethoate, Ethion and Carboxin.

ISI specification for Dimethoate gives % purity of about 85 to 90%. Remaining impurities are not

investigated or identified. Impurities are isolated by doing column chromatography and preparative TLC. Identification is done by using TLC, NMR spectroscopy and Mass. Technical grade Dimethoate is 92.2% pure (NCL product), which shows volatile matter 1 to 1.5%, -S-methyl and ester impurity 3 to 5%. -S-Methyl isomer of Dimethoate (impurity) 1 to 1.5% and non-identified or more polar compound 1.5 to 2%.

Impurities from Ethion and Carboxin are also isolated by doing column-chromatography and preparative TLC. TLC, NMR and Mass spectroscopy are used for identification of impurities.

Technical grade Ethion is 95 to 96% pure, shows volatile matter 1 to 1.5%, oxo-analogue of Ethion 3 to 3.5% and unidentified impurities in traces.

Technical grade Carboxin is 97 to 98% pure, shows volatile matter 0.5 to 1%, hydroxy sulfide intermediate compound (impurity) is 0.5 to 1.0%.

CHAPTER I

General Introduction

GENERAL INTRODUCTION

The Pests and Pesticides

Massive out-break of grasshoppers cause wide spread damage to a wide range of crop species. There are a number of larvae bore in the plant stems. Sometimes the plant does not die but crop production is reduced. Number of other insect pests play their annual role and cause dramatic loss in the crop production. To bring into control this massive loss an investigation in pesticides is necessary[1].

The word pesticide is defined as any substance or mixture of substances, which is needed for preventing, destroying, repelling or mitigating any insects, pests, rodents, fungi or weeds[2]. Major applications lie in the production, storage, or transportation of food.

There are several types of pesticides which are classified as follows:

1. Insecticides
2. Miticides
3. Nematocides (to kill microscopic eelworms)
4. Rodenticides (for control of vertibrate pests)
5. Fungicides
6. Herbicides (weed killers)
7. Molluscicides (to kill slugs and snails)

Apart from the use of naturally occurring substances, such as neem leaves, tobacco leaves extract, sulfur and other inorganic chemicals are also used as insecticides. An introduction to synthetic insecticides is comparatively a recent development.

In 1930 a new era in pesticide field has been opened by introduction of synthetic organic pesticides, like orthochlorobenzene, pentachlorophenol, azobenzene, nitrophenols etc.

Upto 1950 chlorinated pesticides were very extensively used all over the world as effective pest-killers. DDT was used during II World War and post-war days. Due to persistant toxic residue effect, chlorinated pesticides were slowly outdated. However, pesticides, like other chemicals, have certain properties, that govern their effectiveness in any given system. They vapourise, oxidise, hydrolyse and metabolise.

Classification of Insecticides

Insecticdides are mainly divided into two groups:

1. Chlorinated hydrocarbon insecticides
2. Organophosphorus insecticides

1. Chlorinated Insecticides

Chlorinated insecticides are used because of their low cost and broad spectrum of activity. They have found an unique place in the control of insects and pests. But there is an important draw-back that they have high mammalian toxicity.

e.g. Aldrin, heptachlor having LD_{50} (oral) values to rats - 40 mg/Kg

Organophosphorus compound sumithion[3] having LD_{50} to rats - 500 mg/kg (low toxicity)

2. Organophosphorus Insecticides

The development of organophophorus pesticides took place in Germany during II World War. This new class of organophosphorus pesticides showed the following advantages.

1. High insecticidal and acaricidal activity
2. Wide spectrum of action on plants and pests
3. Low persistance and rapid break-down to form products which are non-toxic to mammals and animals.
4. Systemic action of a number of compounds
5. Low-dosage of a compound per unit of treated area.
6. Relative rapid metabolism and low chronic toxicity.

History

Phosphates are one of the essential constituents of the protoplasm and play an important role in the maintenance of metabolism. Some of them are nucleic acids, nucleotides, co-enzymes, metabolic intermediates and phosphides. Many phosphorous derivatives are used in day-today life as lubricants, oil additives, plasticisers, pharmaceuticals and pesticides[4].

In 1905 Harden and Young[5] had discovered the key-role played by inorganic phosphates in alcoholic fermentation in which fructose diphosphate, a metabolic intermediate was isolated. Since then many organic phosphate esters have been isolated from biological sources. Sir Todd and Cramer[6,7] have studied phosphorylation reaction in naturally occurring esters. These esters are found to inhibit the activity of cholinesterase by phosphorylation of esteric site[8]. Paraoxon, an oxo-analogue of parathion was first demonstrated as a synthetic antichòlinesterase agent[9].

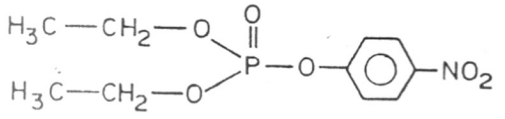
Classification of organophosphorus Insecticides

Organophosphorus insecticides are all structurally related, therefore, undergo similar reactions. Chemical classification of widely used compounds of this type is (a) Aliphatic Derivatives and

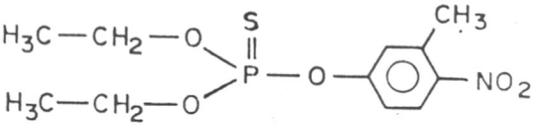
(b) Aromatic or cyclic Derivatives.

CHART — 1.

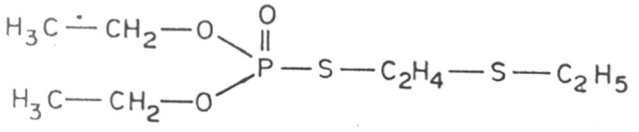
a) PARAOXON



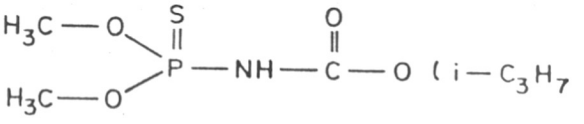
b) FENITROTHION



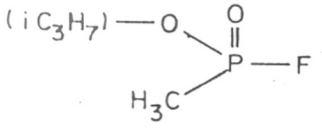
c) DEMETON — S



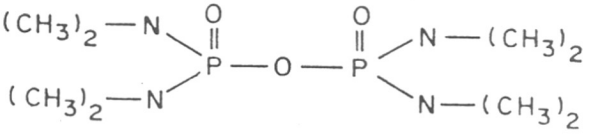
d) AVENIN



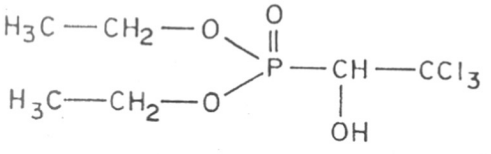
e) SARIN



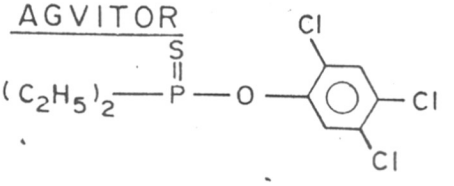
f) SCHRADAN (OMPA)



g) TRICHLORFAN



h) AGVITOR



This classification is not sufficient to explain the activity, so they are also further classified according to the potent groups in the structure.

1. Alkyl phosphates

Due to high mammalian toxicity and less stability the compounds in this group are not exploited fully e.g. Paraoxon[10] [Chart 1(a)].

2. Phosphorothionates

The compounds are thiono (P=S) derivatives having low mammalian toxicity and high stability. Many useful pesticides belong to this class e.g. Fenitrothion[11] [Chart 1 (b)].

3. Phosphorothiolates

Members of this class have (P=0) and (P-SR) linkages e.g. Demeton-S[12] [Chart 1 (c)].

4. Phosphoramide acid derivatives

They have (P-N) linkage as important feature e.g. Avenin[13] [Chart 1 (d)].

5. Phosphorofuroidic acid derivatives

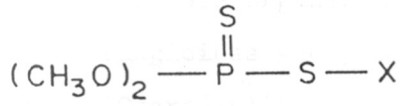
Membes of this class have (P-F) and (P=0) linkages e.g. Sarin[14] [Chart 1 (e)].

6. Pyrophosphoric acid derivatives

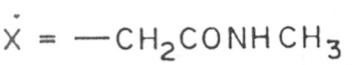
Pyrophosphoric acid derivatives constitute one of the very active pesticides e.g. Schradan[12] [Chart 1(f)].

CHART – 2.

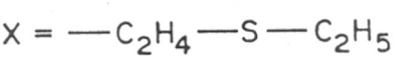
FOR PHOSPHOROTHIOLOTHIONATES



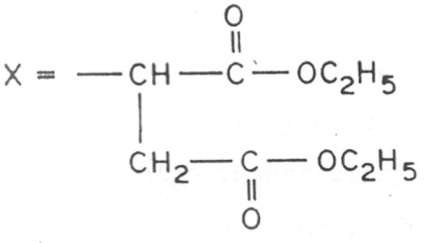
a) DIMETHOATE



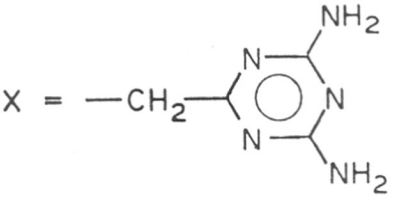
b) THIOMETON



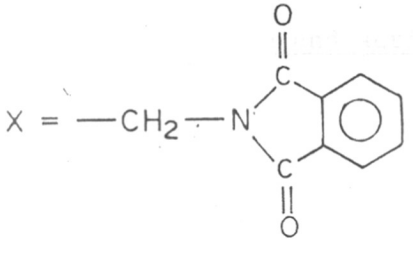
c) MALATHION



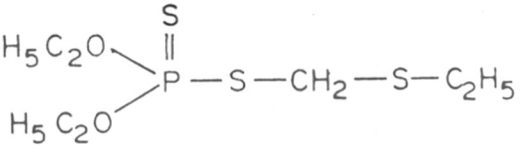
d) MENAZONE



e) IMIDAN



f) PHORATE



7. Phosphates

(P-C) linkage is the main feature of this class. Variable organic moities, used as insecticides, fungicides and plant growth regulators e.g. Trichlorfon[15] [Chart 1(g)].

8. Phosphinate Esters

In the phosphinate ester along with (P-C) linkage there is (P=S) linkage present. So these are more active than the phosphates e.g. Agvitor[16] [Chart 1 (h)].

9. Phosphoro thiolothionates

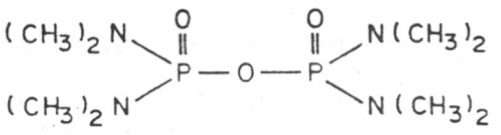
They have (P=S) thiono, (P-SR) thiolo linkage. The thiolo (P-S-R) group can be further classified as carboxy esters, amines, heterocycles, sulfides, sulfoxides and amides. Members of this class (Chart 2) are widely accepted due to low mammalian toxicity and high insecticidal activity. More than forty compounds are in market. Popular members are Thiometon[11] or Demeton-S, Dimethoate[10], Malathion[12], Menazone[17], Imidan[18] and Phorate[19].

Structure and activity

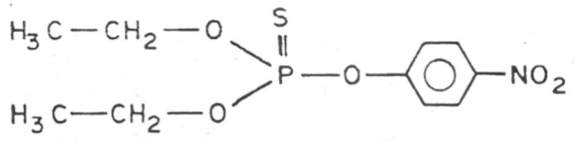
The relation between structure and activity of organophosphates was established for the first time by Holmstedt[20], Schrader[21] suggested a general formula

CHART — 3.

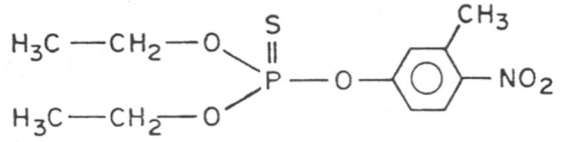
a) SCHRADAN (OMPA)



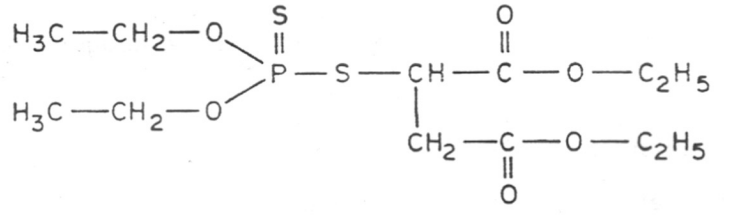
b) PARATHION



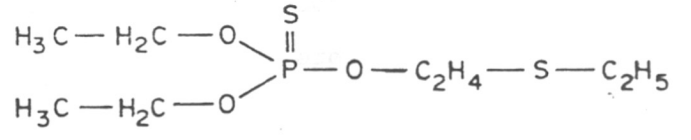
c) FENITROTHION



d) MALATHION



e) DEMETON — O



for compounds having insecticidal activity.

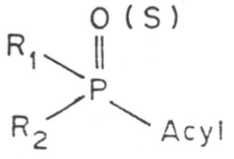
In 1941 systemic insecticide Schradan (OMPA) [Chart 3 (a)] along with other insecticides was introduced. Synthesis of parathion [Chart 3 (b)] was a great achievement in this field. Parathion being highly toxic to mammals, some lesser toxic pesticides were developed e.g. Fenitrothion[11] [Fig.3 (c)], Malathion [Chart 3 (d)] and Demeton-0 [Chart 3 (e)].

In 1950 Schrader proposed the general formula [Chart 4 (a)]. In 1964 it was modified by Clark[22] According to rule P-xyz system of pesticides, xyz may be H,C,N,0,S but z should be electron withdrawing [Chart 4 (b). The most widely accepted relationship is between inhibition costant (Ki) and rate of hydrolysis[23]. If phosphorus atom is attached with electron withdrawing groups such as -OR, -OPh, -SR, and F, the activity is enhanced as the compound hydrolysis fast.

Some compounds are potent insecticides because of the presence of certain groups. The thiono derivatives are active by virtue of S-alkylation and also conversion to an oxo-analogue. For e.g. -Paraoxon is 10,000 times active than parathion while -S-methyl analogue of malathion is more active than the parent compound by the factor of 38. Based on the electron donating capacity, amides are active in order of

CHART – 4.

(a)



(b)

$-NH_2 > -NHR > -NR_2$ but no such rule can be predicted due to their abnormal behaviour.

Eventhough several enol phosphates are very much potent-anticholinesterase agents, the activity is not accounted by hydrolysability but by protonation ability. Parasubstituted acetophenoxime phosphates are active due to the presence of electron releasing groups[24] Steric factor is also very important for inhibition action. Molecules with smaller substituents are more active than with bulkir substituent e.g. methyl phosphate has insecticidal activity, but phenyl phosphate is inactive. Some pesticides show activity due to asymmetric phosphorus atom.

Though there exist many rules to define the relationship, no perfect prediction can be made about the activity of compound. Therefore stress is given on preparation of compounds in large numbers and evaluation of their activity.

Metabolism

Enzymes and cholinesterase are inhibited by all organophosphorus compounds. The word metabolism can be defined as "the chemical change or changes that occur in foreign compounds in the body of organisms". The potency depends not only on their intrinsic enzyme

affinity but also on anticholinesterase properties acquired through vivo metabolism. The pesticides, being less polar and liophophilic in nature, readily enter into the pests through their skin. They undergo two types of transformations, activation and detoxication. In the activation transformation, conversion takes place to more active and polar form which is responsible for increase in the anticholine-esterase activity. In detoxication transformation, it is converted to lesser toxic forms so that it can be excreted out safely. Both the transformations take place simultaneously and their competency determines toxicity of a given pesticide.

In activation, oxidative desulfuration of thio-phosphoryl sulfur atom gives corresponding oxo-analogue. The product obtained is more toxic than the parent compound. The oxidation of the thiol moiety into sulfoxide and finally into sulfones has been observed in plants, mammals and insects. Sulfones have highest anticholinesterase activity. Similarly oxidative demethylation takes place in the amino group of pesticides and increases their activity. Some rearrangement reactions and hydroxylation are also responsible for activation of the pesticides. In plants generally enzymes do not catalyse the hydrolysis of organophosphorus compounds but they do catalyse their

oxidation. Oxidation product accumulates much more in the plant than in animals. A part of the pesticide which is absorbed by the seed, is translocated throughout the growing plant and protects the plant against pest.

In detoxication, cleavage of phosphorus ester bond is involved. Some reactions are oxidative dearylation, enzymatic hydrolysis and o-dealkylation in phosphorothiolothionates, two types of cleavages take place, one is at P-S and the other at S-C bond linkage. In most of the organisms both types of cleavages occur but S-C fission is predominant. Hydrolysis of amide and reduction of nonphosphorus moiety also detoxicates the pesticides e.g. Amide in Dimethoate hydrolysis to corresponding acid[25]. Micro organisms metabolise the organophosphorus compounds fairly rapidly. Higher pH of the soil will cause increase in the rate of hydrolysis of organophosphorus pesticide, increases the oxidation and anticholinesterase activity.

Generally the organophosphorus compounds are used due to their broad spectrum of activity low cost and low toxicity to the mammals.

Fungicides

A basic principle in plant pathology is that fungicides are used for crops, that lack natural resistance to the fungus involved e.g. Phytophthora venturia inaequalis on apple.

The fungicides consists of dithiocarbamates, dicarboximides, chlorinated phenols, organomercurials and organometallic compounds.

Organometallic fungicides

A large number of metals and transition metals form fungicides, either in inorganic form or in combination with organic molecules. These compounds remain in environment for long period. Mercury is an inherently toxic element so use of mercury compounds is coming into disfavour. Many organometallic fungicides are highly water soluble.

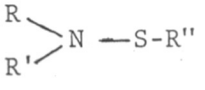
Example water soluble copper salts are extremely toxic to fungi but also interfere in the normal metabolic processes of the plant[26].

Classification of fungicides

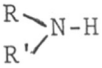
1. Non-systemic fungicides (sulfenimide)
2. Systemic fungicides

1. Sulfenimide fungicides

The sulfenimide fungicides may be structurally defined as following



R and R' may be cyclic including part of some ring or separate rings or chains. These contains atleast one carbonyl, sulfonyl, phosphonyl etc. group.

If R'' = H then 

is preferably a weak acid. R'' is a short chain polyhaloalkyl or alkenyl group.

The members of this class have limited aqueous solubility than the organometallic compounds. The short life of these fungicides in biological media suggests a low hazard associate. These are non-toxic to mammals

e.g. Captan, Difolatan [Chart 5 (a)]

2. Systemic fungicides

The introduction of systemic fungicides provided a sort of practical approach to immunity, and was regarded as the solution to problems of plant chemotherapy. Systemics are most valuable and useful agents.

These are further divided according to their structure. Due to particular presence of structure they have fungicidal activity.

a) Sufactant type

Alteration of membrane permeability. Moderate resistance and local systemic action.

e.g. Cyprex [Chart 5 (b)]

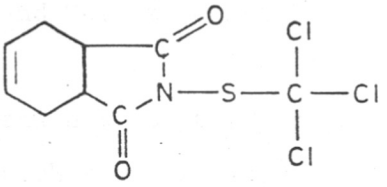
b) Benzimidazoles

In this presence of benzimidazole moiety shows activity

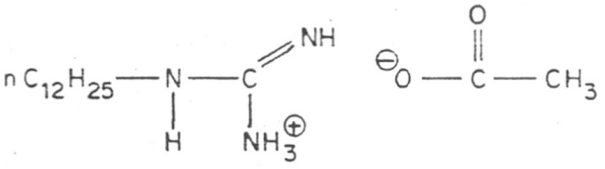
e.g. - Benomyl [Chart 5 (c)]

CHART – 5.

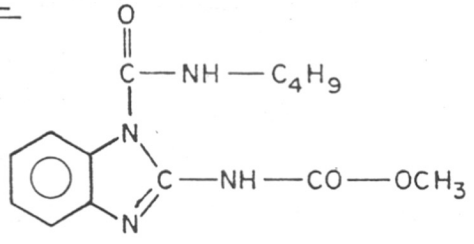
a) CAPTAN



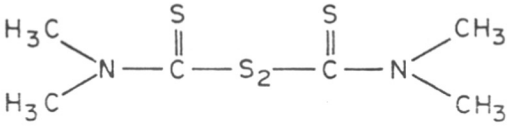
b) CYPREX



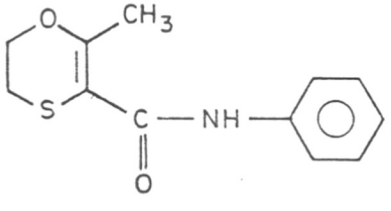
c) BENOMYL



d) THIRAM



e) VITAVAX (CARBOXIN)



c) Dithiocarbamates

Highly active as fungicides

e.g. Nabam and Thiram [Chart 5 (d)]

d) Oxathiins

In this presence of oxathiin moiety shows activity. It is an organosulphur fungicide, containing no phosphorus. Therefore it has low mammalian toxicity and relatively safe to other domestic anaimals.

e.g. Carboxin [Chart 5(e)]

Metabolism

Usually each type of fungicide has different metabolism mechanism. But finally they give products which are non-toxic. Oxathiin which is an organosulfur compound is metabolised to it's sulfoxide as a major metabolite and sulfone as the minor metabolite, in water, soil, barley and wheat. For carboxin, oxidation occurs in water and soil but hydrolysis has never been detected. It has good stability, low mammalian toxicity. Thus oxathiin fungicides are used widely.

Methods of Analysis

The following methods are applied in carrying out the analysis of pesticides:

1. Thin-layer chromatography (TLC)
2. Column chromatography

3. Gas-liquid chromatography (GLC)
4. High-performance liquid chromatography (HPLC)
5. Ultraviolet spectrophotometry (UV)
6. Infra-red spectrophotometry (IR)
7. Nuclear magnetic Resonance spectroscopy (NMR)
8. Mass spectrometry (MS)
9. Combined gas chromatography and Mass spectrometry (GCMS)

1. Thin-layer chromatography (TLC)

This is a micro type of chromatography Single drop of the solution is investigated, using different solvent systems and phases. By comparision with known standards, a number of components in the solution are identified. This technique is used for qualitative analysis of many pesticides.

2. Column chromatography

In the process, components to be separated are distributed between two phases- a stationary phase having large surface area (e.g. a porous solid) and a mobile phase (liquid) moving in contact with the stationary phase. The sample components are selectively retained by the stationary phase due to the difference in the equilibrium distribution of the sample components between the two immiscible mobile and stationary phases respectively. Actual isolation of the components is

done by column chromatography. Number of organo-phosphorus compound are isolated using this technique.

3. Gas-liquid chromatography (GLC)

Gas liquid chromatography is widely utilised technique. The process in which the components of a mixture are separated from one another by volatalizing the sample into carrier gas stream which is passing through and over a bed of solid support. The surface of support is usually coated with relatively non-volatile liquid (the stationary phase). This requires small amount of compound as compared to TLC and columnchromatography. Being versatile technique, reactions are monitored, purity of compounds is checked and number of components in the mixture are separated from one-another. Preparative Gas-chromatography is also used for isolation of components. Some organophosphorus insecticides are isolated on 5% QF-1 and 5% OV-1 coated on celite 100-150 mesh. It is used for analysis of residues in plant and soil.

4. High perferemance-liquid chromatography (HPLC)

In this technique advantage over GLC is the presence of pressure device for eluent mobility and low volume detectors. It requires very small amount of compound as compared to GLC. PPb level concentration

of compound can be detected. Since the analysis is carried out at ambient temperature, degradation and/or isomerisation due to heat is avoided.

HPLC is a novel method for analysis, but there are limitations due to nonavailability of column phases and miniature size of columns. Solvent requirement is much more. Even then by using this technique, the required analysis is possible in shortest time.
e.g. HPLC of Malathion using methanol-water solvent-system (at 25°C temperature) and using UV 254 detecter was carried out.

5. Ultraviolet spectroscopy UV

The cause of absorption in the UV, is excitation of electrons by photo energy. The possibility of utilizing UV technique for confirmation of the structure of organophosphorus compounds, having aromatic rings, their residues and metabolites, degradation products ha attracted the interest of many workers. UV spectroscopy has become useful tool in the hands of chemists, when it is utilised in combination with TLC, column chromatography and GLC. Particular compound has particular λ_{max} . So the % purity of a compound can be determined e.g. separation of impurities from Fenitrothion was carried out by following UV absorption[27] technique.

6. Infra red spectrophotometry

Absorption of infrared radiation cause a change in bond vibrations, which cause a change in molecular dipole moment. IR technique is used for the detection of functional groups in the molecule. IR spectrometry is being employed for the analysis of both technical grade pesticides and their formulations. It is also applied to the quantitative determination of many organophosphorus pesticides. Downing et al.[28] determined the DDT isomers using IR spectroscopy. Daasch[29] described a procedure for quantitative determination of each of the five known BHC isomers in the mixtures. It is also used for residue analysis[30]. Recently IR spectrophotometry procedure is included in ISI specification for Dimethoate estimation[31].

7. Nuclear magnetic resonance spectroscopy (NMR)

The magnetic properties of atomic nuclei are the spin number and the magnetic moment. Hydrogen, Fluorine, phosphorus, boron, carbon-13 and oxygen-17 have distinctive magnetic properties due to odd atomic number. The molecular or chemical environment of the nucleus produces characteristic shift. Thus NMR is used to determine the structures of organophosphorus pesticides. Structure determination for residue and metabolites is also done by NMR analysis.

8. Mass spectrometry (MASS)

Separation of ions according to their masses takes place when they are bombarded with an electron source. It is a method, which is widely used for routine analysis. The mass spectral fragmentation of major organophosphorus pesticides have been summaried[32]. It has been routinely employed in studies of the environmental degradation e.g. chemical and photochemical alteration of pesticides and other toxic substances.

9. Combined Gas chromatography and Mass spectrometry (GCMS)

In this technique, gas chromatographic technique is combined with a Mass spectrometer. As soon as the vapour of mixture is separated into it's components by GLC the individual component vapour is detected and it's mass is recorded. The coupling can be brought about using a variety of interfacial synthesis. All pesticides for which GLC separation method exists, can be analysed by GCMS.

Organophosphorus pesticides can be easily analysed by means of GCMS. The use of GCMS in pesticide analysis is described by McFadden[33], Gudzinowicz et al.[34] and Ghatge et al.[35]. Now a days GCMS is used for environmental pollutions and in residue analysis.

The work undertaken

Though synthesis and investigation from many points of view has been carried out for Dimethoate, Ethion and Carboxin, Investigation of impurities present in these organophosphorus pesticides of technical grade has not been carried out so far.

ISI specification for Dimethoate purity is 85%. Remaining 15% impurities are not investigated or identified. Impurities present in Ethion and Carboxin (technical) are not also investigated or identified. Therefore, work is undertaken for isolation of these impurities present in Dimethoate, Ethion and Carboxin. (Technical).

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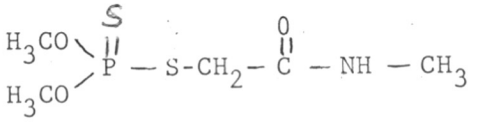
CHAPTER II

Dimethoate

DIMETHOATE

Introduction

Structural formula



Empirical formula: $C_5H_{12}NO_3PS_2$

Molecular weight: 229.3

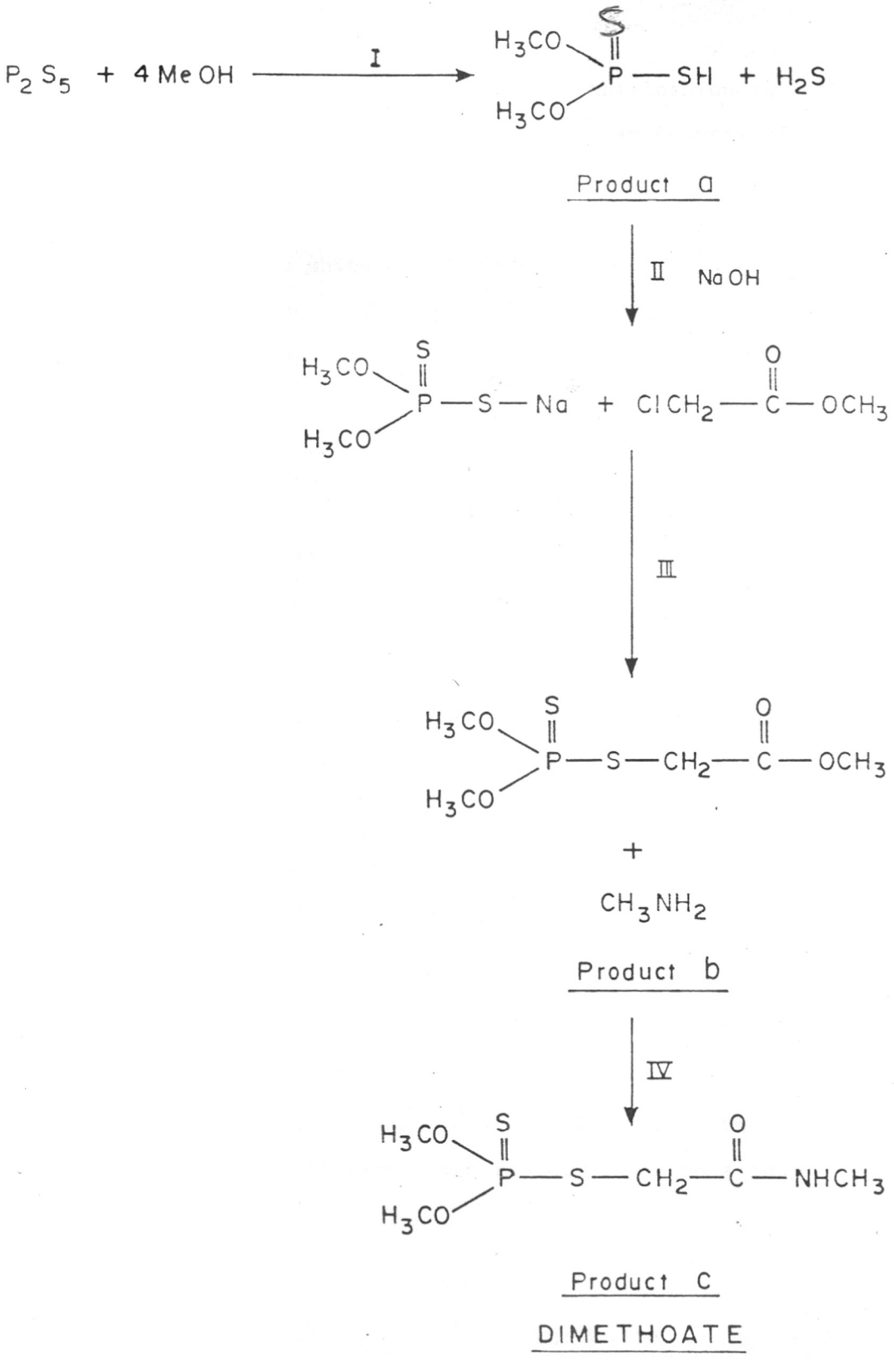
Dimethoate or Rogor, is chemically known as 0,0'-dimethyl-S-(N-methyl carbamoyl methyl) phosphorothioate. It belongs to a class of phosphorothiolothionates. It was first described by Hoegberg et al.[1] and was introduced in 1956 by American Cyanamide Company.

Alternate name: Rogor, Cygon (Trade mark of American Cyanamide Company).

Method of preparation

Its method of preparation[2] was as follows in (Scheme I). Heterogeneous reaction mixture of phosphorus pentasulfide and methanol in benzene was refluxed for 2 hrs to get a solution of 0,0-dimethyl phosphorodithioic acid [Scheme I, Prod. (a)]. Sodium salt of product (a) in aqueous solution was then allowed to react with methyl chloroacetate to get 0,0'-dimethyl-S-

SCHEME-I


P2 S5 + 4 Me OH
I
H3CO
H3CO
P — SH + H2S
Product a
II NaOH
H3CO
H3CO
P — S — Na + Cl CH2 — C — OCH3
III
H3CO
H3CO
P — S — CH2 — C — OCH3
+
CH3NH2
Product b
IV
H3CO
H3CO
P — S — CH2 — C — NHCH3
Product C
DIMETHOATE

(methoxy carbomyl methyl) phosphorothiolothionate [Scheme I, Prod. (b)] in 90% yield. Aminolysis of product (b) using methylamine gives the pesticide: Dimethoate [Scheme I, product (c)] in 87% yield.

It is a white crystalline compound (m.p.52°C). Technical product is about 85-90% pure. The nature of impurities may vary according to the mode of synthesis.

Physical properties

Dimethaote is white crystalline solid.

M.p.: 52.5°C

Solubility: 2.5% in water at room temperature. Highly soluble in lower aliphatic alcohols, ketones and chlorinated hydrocarbons. Slightly soluble in aromatic hydrocarbons and almost insoluble in aliphatic hydrocarbons[3].

Refractive index: n_D^{50} - 1.5373

Vapour pressure:

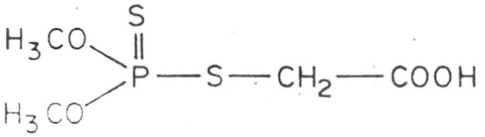
	Temperature in(°C)	Pressure
(1)	25	0.025 m.m. Hg.
(2)	40	0.165 m.m. Hg.
(3)	50	0.540 m.m. Hg.

Chemical behaviour

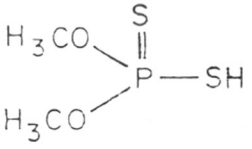
The compound decomposes slowly at elevated temperature. It hydrolyses rapidly in aqueous and

CHART – I

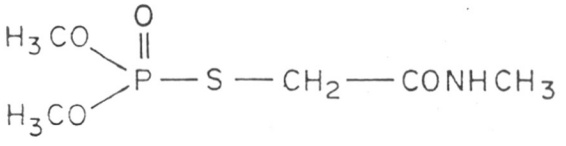
a) DIMETHOATE ACID



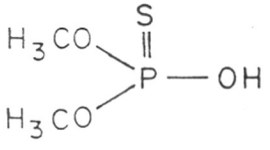
b) O,O – DIMETHYL DITHIOPHOSPHORODITHIOIC ACID



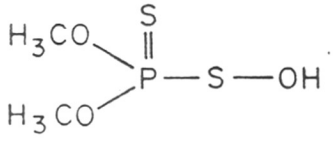
c) OXYGEN ANALOGUE OF DIMETHOATE



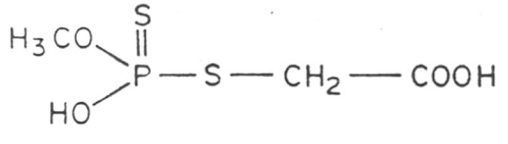
d) O,O – DIMETHYL THIOPHOSPHATE



e) O,O – DIMETHYL DITHIOPHOSPHATE



f) DESMETHYL DIMETHOATE ACID



alkaline solutions but very slowly under neutral or acidic conditions.

Biological activity

It has a broad spectrum of systemic pesticidal activity, which is particularly effective against sucking insects, such as Diptera and susceptible spider mites. It also shows contact activity. The acute oral LD_{50} of Dimethoate (young male albino rats) is 250 mg/kg.

Metabolism

Dimethoate is one of the most widely studied pesticide. Its metabolism has different routes depending upon the species[4]. The degradation of dimethoate in rat and mouse liver yields dimethoate acid [Chart I (a)] and 0,0-dimethyl dithiophosphorodithioic acid [Chart I (b)]. From sheep liver only dimethoate acid has been isolated. In many insects, phosphatase reaction is predominant over amidase reaction[5]. Under this circumstance hydrolytic cleavage is at C-N and C-S bonds, which gives dimethoate acid [Chart I (a)] or oxygen analogue of Dimethoate [Chart I (c)]. Oxygen analogue of Dimethoate rapidly hydrolyses to other non-toxic forms such as dimethoate acid, 0,0-dimethyl thiophosphate [Chart I (d)], 0,0-dimethyl dithiophosphate [Chart I (e)] and desmethyl dimethoate acid [Chart I (f)].

In olive fruit metabolism, end products are inorganic phosphates[6]. The degradation of dimethoate in rat mainly depends upon the concentration of pesticide[4]. At higher concentration (10^{-3} mole) there is C-N bond fission. But at lower concentration, C-S bond fission takes place. The effect of amidase action depends upon the species and sex. Comparative studies of mode of metabolism in plants, insects and rats has been reported by Morikawa and Saito[7]. In short O-dealkylation and carboxylation occurs in plants, while in rats C-S bond fission is predominant. General metabolic pathway can be summarised as shown in [Scheme II (a)] and [Scheme II (b)].

Formulation

Dimethoate is sold as an emulsifiable concentrate, a wettable powder or dust formulation.

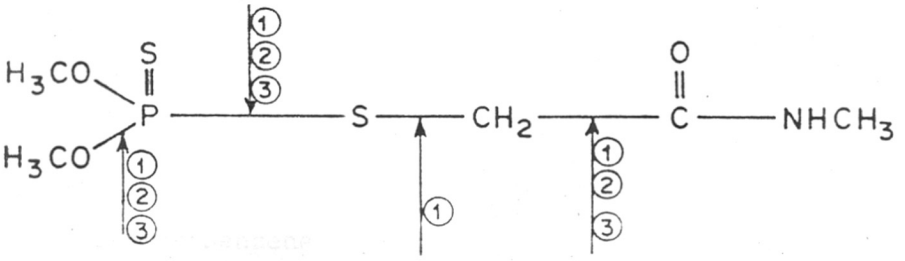
Methods used for analysis of Dimethoate

1. Volumetric method

Dimethoate forms precipitate with arseneous acid which can be measured gravimetrically. Dimethoate is hydrolyised by alkali and can be determined titrimetrically with the help of liberated methyl amine by Kjeldahl equipment.

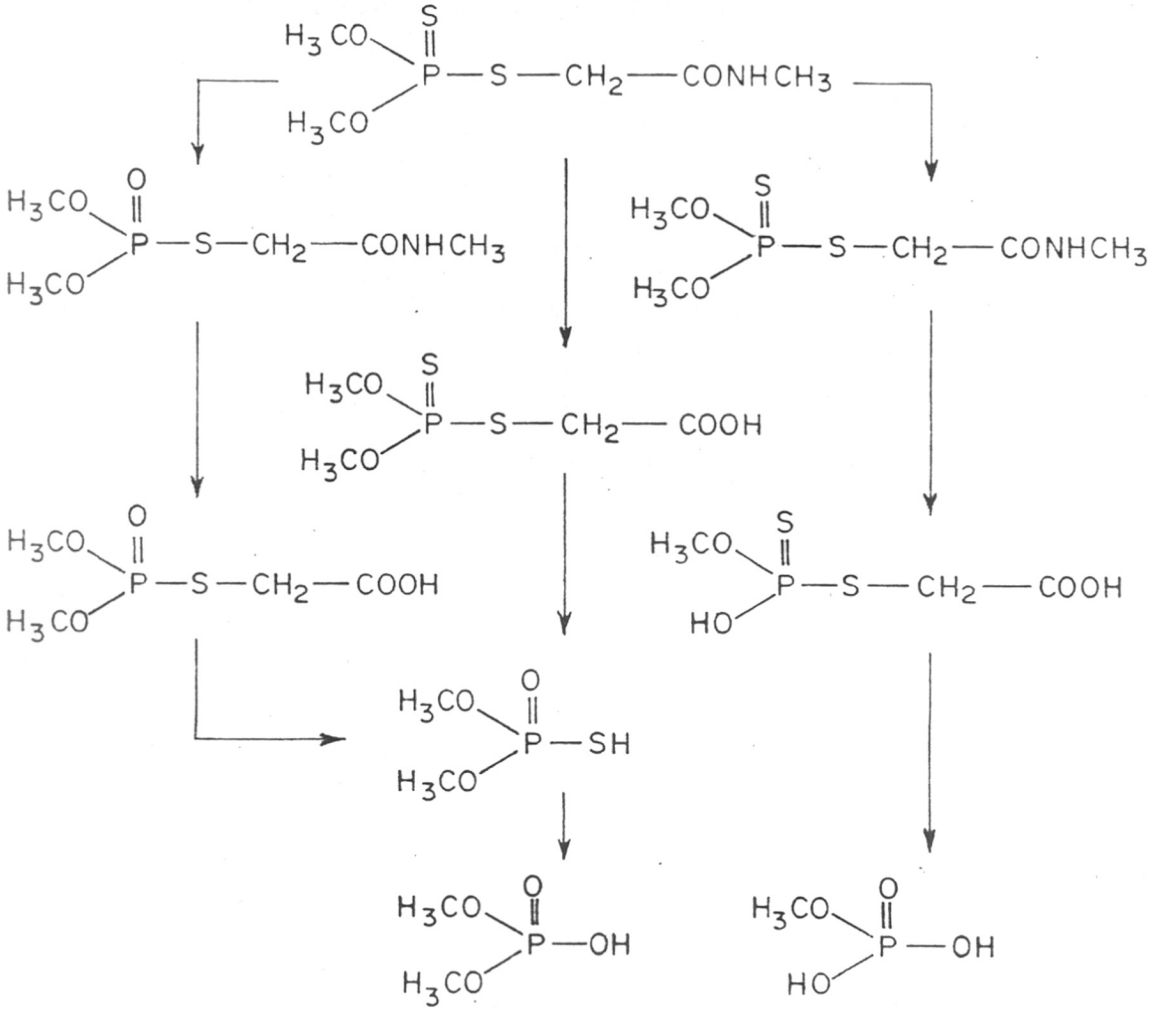
SCHEME – II

ⓐ



1) RAT METABOLISM
2) INSECT METABOLISM
3) PLANT METABOLISM

ⓑ



2. Colorimetric method

a) Dimethoate sample is estimated using 1-chloro-2, 4-dinitrobenzene as colour developing reagent[8].

b) Colorimetric method from ISI specification

Dimethoate is subjected to oxidation using nitric acid and perchloric acid to give phosphoric acid. That acid on treatment with ammonium vanadate and ammonium molybdate gives yellow colour complex. Development of colour depends upon the concentration of complex. Optical density is measured at 450 nm. The test sample is compared with the standard sample of Dimethoate.

3. Estimation of Dimethoate by IR method[9]

The sample is dissolved in carbon disulphide. The absorbance of sample is compared at the set conditions to the absorbance of standard sample of Dimethoate of known purity. The region of interest is from 9.1 to 11.2 μ. For Dimethoate, absorbance is measured at 9.8μ(1002 cm^{-1}) attributed to P-O-C bond.

EXPERIMENTAL

ISI specification for Dimethoate states 85% purity. Technical product of Dimethoate is about 85-90% pure. The impurities are to be investigated. The nature of impurities depends upon the mode of synthesis.

Thin-layer chromatography (TLC)

Several solvent systems were tried to develop the TLC pattern for good separation of components from the mixture. TLC plates were prepared in chloroform slurry by dipping the glass-plates in the big jar. Satisfactory results for separation were gained by using solvent system 60:40 acetone:pet.ether. It showed separation of components [Fig. I].

Separation of volatiles

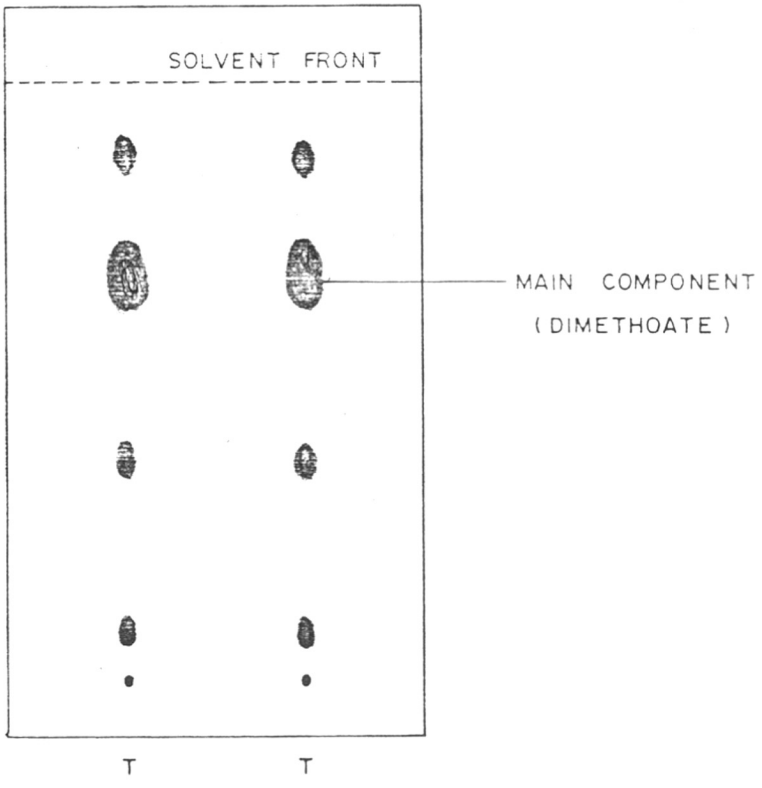
Sample was initially weighed and then dried under good vacuum for 4-5 hrs. The loss in weight was calculated as volatile matter (solvent). The average value comes to about 1 to 1.5%.

Standardisation of column chromatography ratio of silica

For isolation of impurities column chromatography was necessary. Column chromatography was first tried with (1:60) 1 gm of sample to 60 gms of silica gel

FIG.-I

SOLVENT SYSTEM — 60 : 40 ACETONE : PET. ETHER



T - TECHNICAL SAMPLE OF DIMETHOATE

(column grade) ratio. Before using in the column, the available silica gel was initially activated in the oven at about 120°C for 2 hrs, cooled to room temperature in dessicater. For 30 gms of silica gel 0.5 gm compound was taken. Column chromatography was monitored by TLC check. As we got good results for separation by using the above ratio same was utilised for large scale experiments.

Column chromatography

The Dimethoate sample was weighed accurately 1.5 gms. Then dried under good vacuum for 4-5 hrs. The loss in weight equivalent to volatile matter was about 1.2%. The available silica-gel was initially activated in the oven at about 120°C for 2 hrs, cooled to room temperature in dessicater and then used.

(a) Silica gel (column grade - 80-120 mesh) 90 gms.

(b) Technical grade sample of Dimethoate (after drying under vacuum pump)

1.49 gms.

Elution of the column

The column was packed in usual manner using pet.ether as solvent. Dimethoate was charged on the column. The eluents were collected in 50 ml fractions and were monitored by TLC.

First Eluent: [Benzene]

Benzene (200 ml) was eluted collecting four fractions of 50 ml each. These fractions were checked by TLC and it was found that compounds were stopped eluting out in the fourth fraction. Changed the solvent-system.

Second Eluent: [70:30 Benzene:Ethylacetate]

1000 ml eluents were collected and it was found that only pure dimethoate was coming out upto last fraction.

Third Eluent: [Acetone]

When nothing was coming out, acetone was used as eluent. 1500 ml acetone eluants were collected. We found some unidentified impurity in this portion.

Fourth Eluent

After acetone, methanol was used as eluent, and 1500 ml eluents were collected giving some unknown impurity.

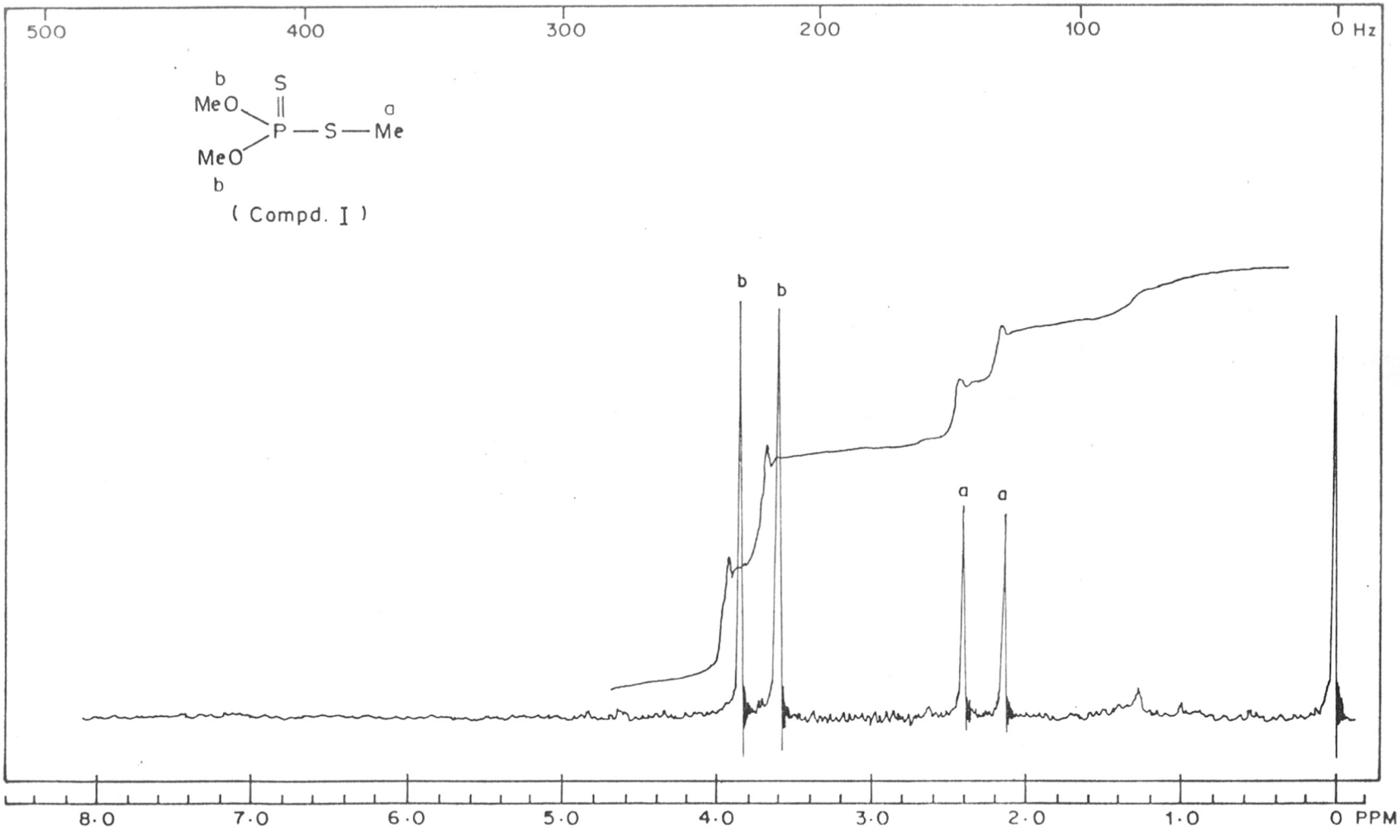

500
400
300
200
100
0 Hz
b
S
MeO
a
P — S — Me
MeO
b
(Compd. I)
b
b
a
a
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0 PPM(δ)

FIG. — II

Column Chromatography of Dimethoate

Silica gel - 90 gms (column grade, activated silica gel used)

Material loaded: 1.49 gms.

S.No. of fractions	Volume	Solvent system
1st	200 ml	benzene
2nd	1000 ml	70:30 benzene: ethyl acetate
3rd	1500 ml	acetone
4th	1500 ml	methanol

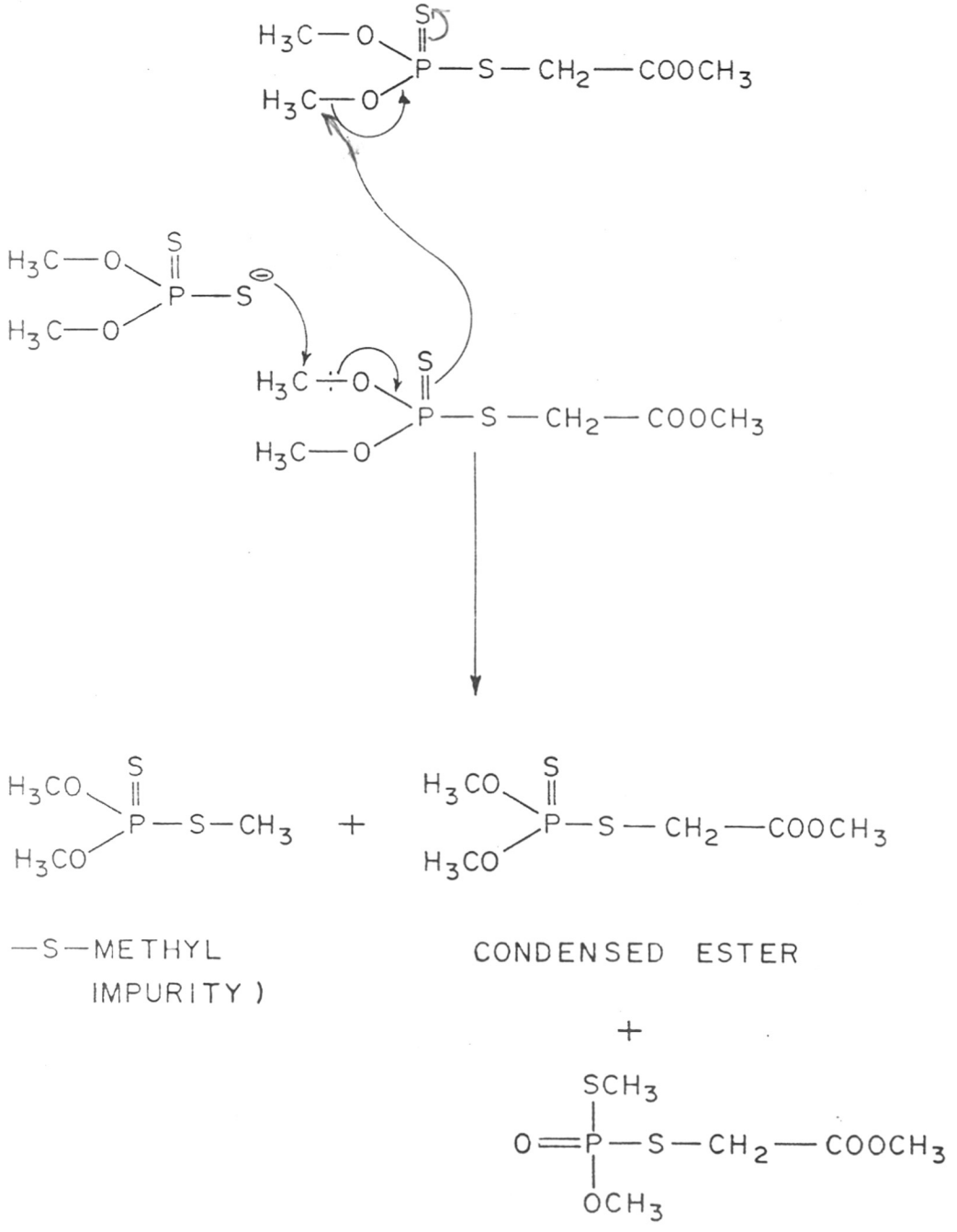
First fraction obtained from elution of benzene showed the impurity of 0,0'-dimethyl-S(methyl) phosphorothioate. The structure of this impurity was confirmed by NMR spectrum.

NMR data (a) ($-S-CH_3$) group gives signals between 2 to 2.5 δ splitting due to phosphorus (b). Two methoxy ($O-CH_3$) groups doublet at 3.9 and 3.7 δ attached to phosphorus [Fig. II].

This impurity was likely to be formed during the condensation of sodium salt of dimethoxy-dithio-phosphoric acid with monochloro acetate. The dimethoxy dithio phosphonium anion might have attacked the methoxy

CHART – II

FORMATION OF –S– METHYL IMPURITY



group of condensed ester molecule and formed -S-CH_3 derivative. The remaining condensed ester anion might have got alkylated by another molecule of ester forming product [Chart II]. The percentage ratio of the impurity mostly depends upon the time factor and temperature.

Along with -S-(methyl) impurity, sometimes there was another impurity, which was not differentiated in the same solvent system (60:40 acetone:pet.ether). In this solvent system both were having same Rf values. However, by decreasing polarity of solvent system, both -S-methyl and the other impurity showed difference in Rf values. -S- methyl was having Rf value more than that of second impurity. For clear separation 1% ethyl acetate in benzene was used as solvent system for TLC. The impurity compared and found to be intermediate ester [Fig. III] which was confirmed by NMR.

NMR data

(a) (-CH_2) group, methylene group showing doublet one at 3.56 δ and other merged into methoxy proton.

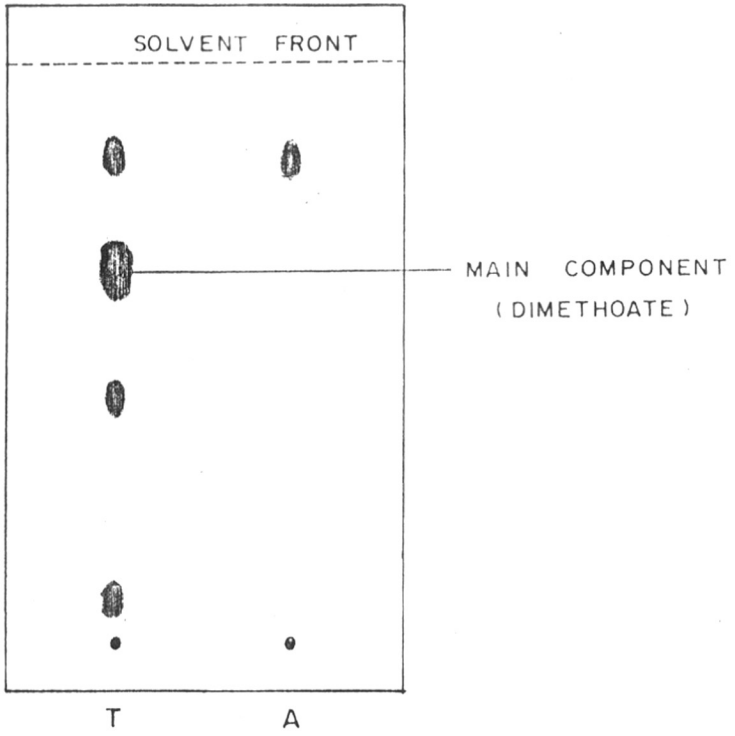
(b) (-C(=O)-OMe) group, one methyl group appears in between the 3.9 and 3.7 δ

(c) Two methoxy (-O-CH_3) groups doublet at 3.9 and 3.7 δ Splitting due to phosphorus.

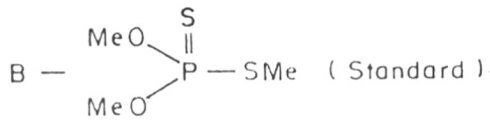
[Fig. IV]

FIG.—III.

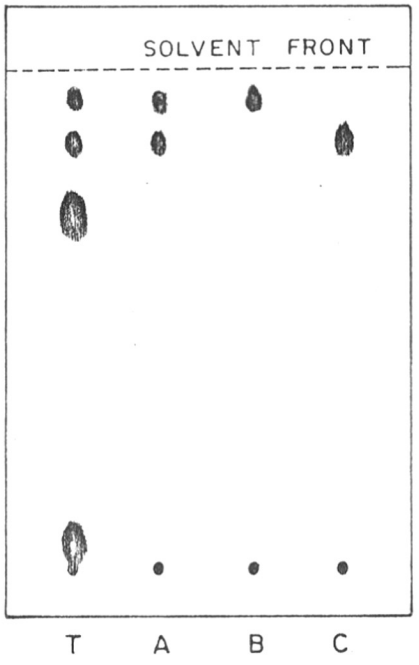
SOLVENT SYSTEM — 60 : 40 ACETONE : PET. ETHER



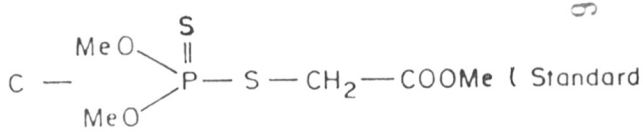
T — TECHNICAL GRADE DIMETHOATE

B —  (Standard)

SOLVENT SYSTEM — 1% ETHYLACETATE IN BENZENE



A — BENZENE FRACTION

C —  (Standard

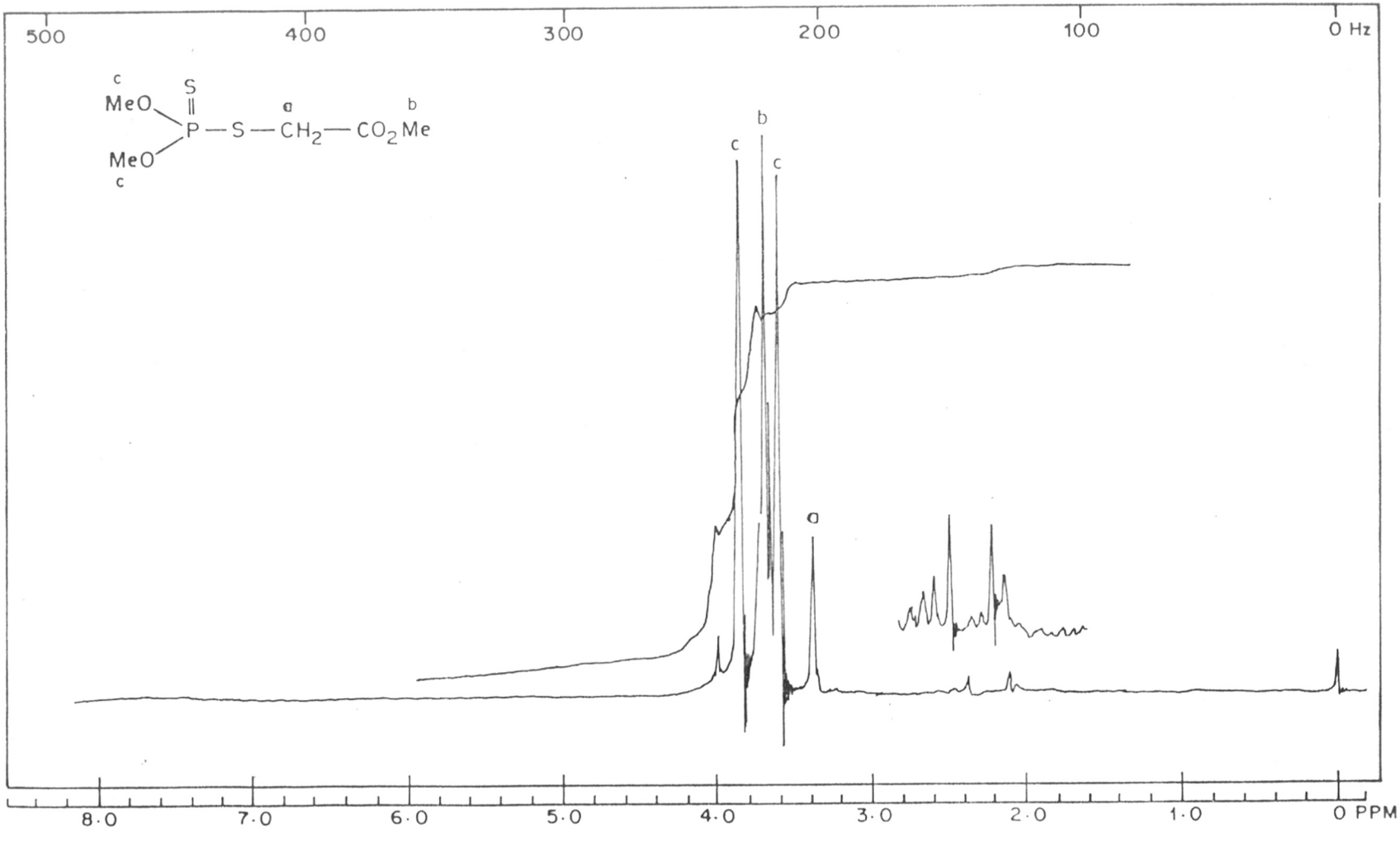

500
400
300
200
100
0 Hz
c
MeO
S
a
b
P — S — CH2 — CO2 Me
MeO
c
c
b
c
c
a
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0 PPM(δ)

FIG. IV

During the aminolysis if reaction was not 100% complete, small amount of ester was present in the technical product. Percentage of ester depends upon the rate of reaction. The total percentage of impurities of -S-methyl and ester was about 3-5% (0.075 gms).

[Chart III (a) and Chart III (b)]

Second fraction obtained in 70:30 benzene:ethyl acetate showed presence of only dimethoate. which was about 92.18% (1.36 gms) [Chart III, (c)].

NMR data

(a) Methyl ($-CH_3$) attached to nitrogen shows doublet at 2.85 . δ

(b) Methylene ($-CH_2-$) doublet at 3.56 and 3.7 δ splitting due to phosphorus atom.

(c) Two methoxy ($-O-CH_3$) attached to phosphorus gives doublet at 3.9 and 3.7 δ

(d) N-H proton at 7.9 δ [Fig. V]

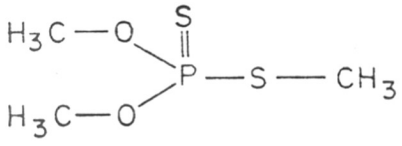
IR data

1. -N-H at 3390 cm^{-1}
2. R-C-N- carbonyl group at 1672 cm^{-1}
3. P-O-CH_3 at 1151 and 1215 cm^{-1}.
4. P-O-C at 1000 cm^{-1} (ether linkage)

[Fig. VI]

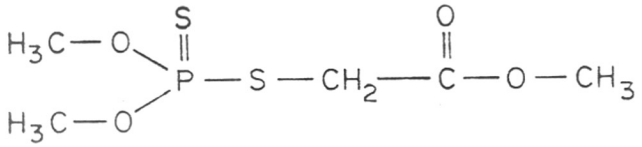
CHART – III

a) O, O'–DIMETHYL–S–(METHYL) PHOSPOROTHIOATE



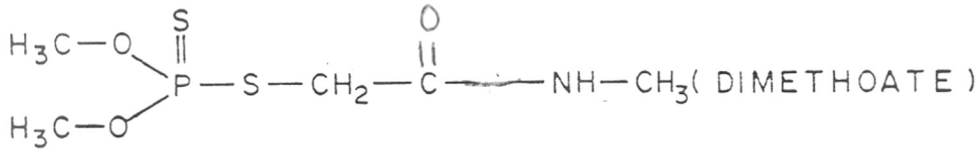
(–S–METHYL IMPURITY)

b) O, O'–DIMETHYL–S–(METHOXY–CARBONYLMETHYL)–
–PHOSPHOROTHIOLOTHIONATE



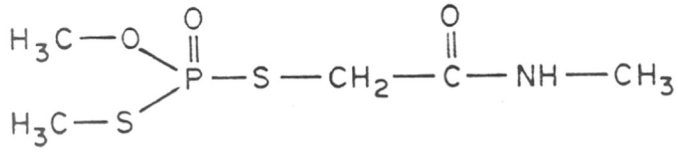
(ESTER IMPURITY)

c) O, O'–DIMETHYL–S–(N–METHYL CARBAMOYL–METHYL)
–PHOSPHOROTHIOATE



(DIMETHOATE)

d) –S– METHYL ISOMER OF DIMETHOATE



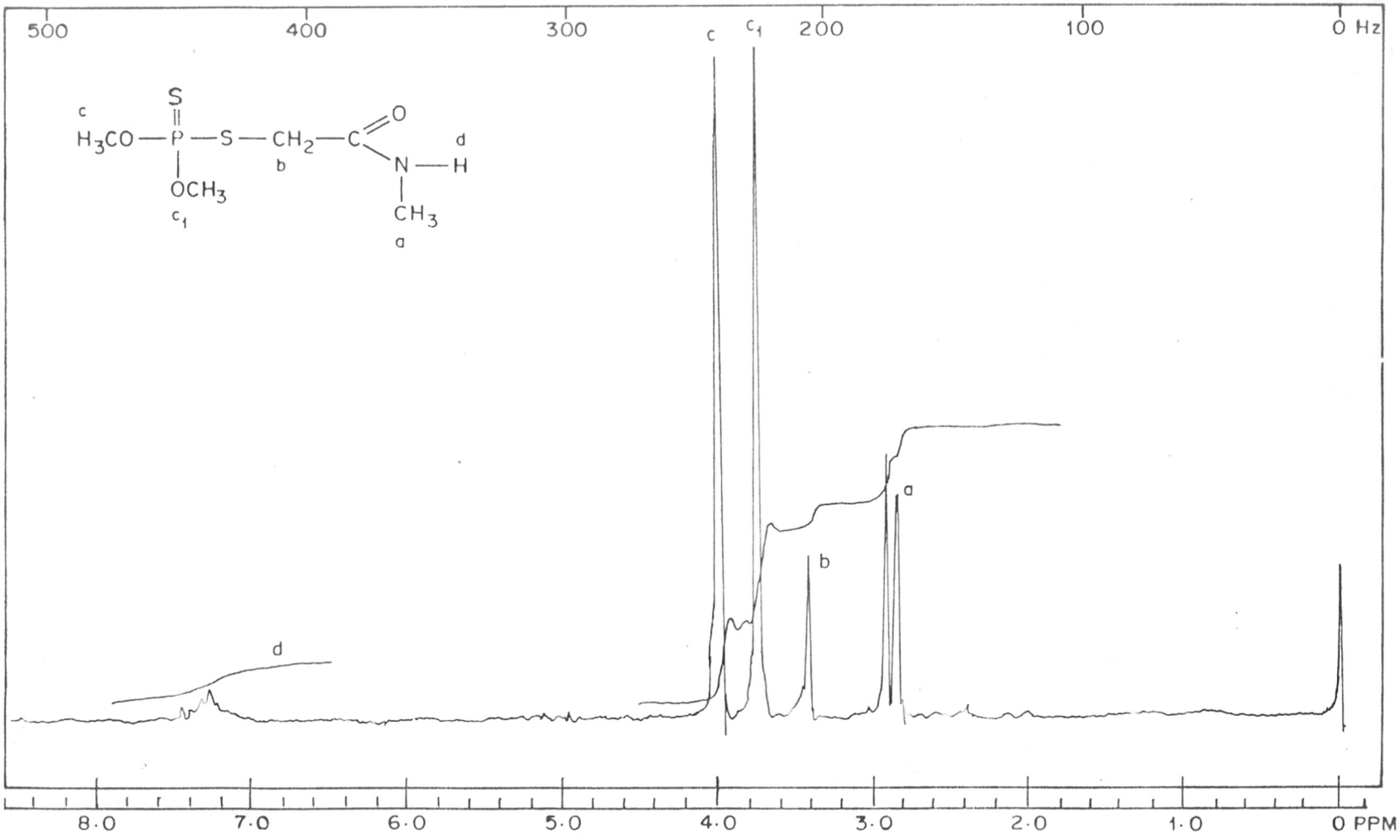
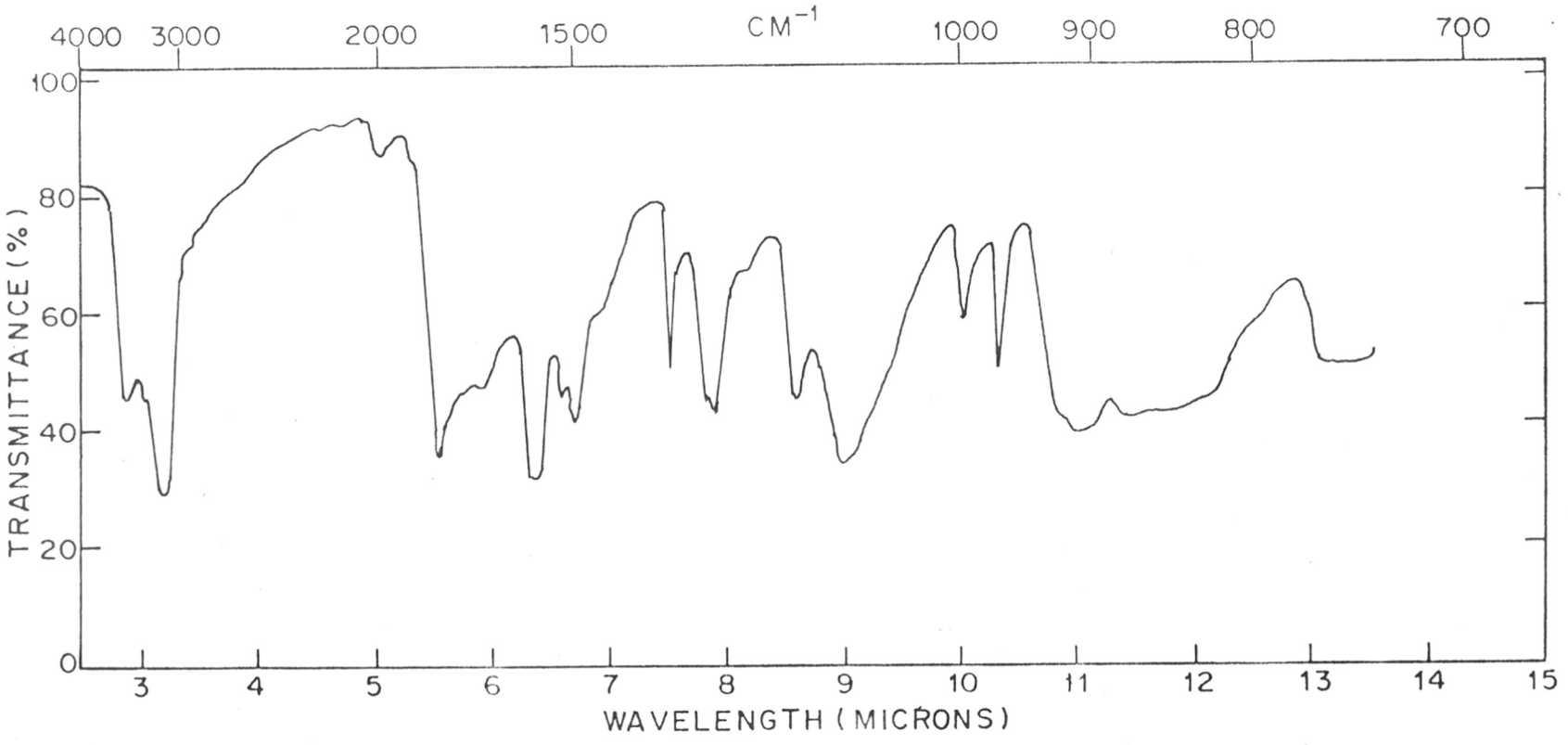


FIG. — V



DIMETHOATE

FIG.– VI.

Now the third fraction eluted with acetone did not show single spot in TLC (0.022 gms). This fraction was purified by preprative TLC. The main impurity fraction was separated by running the plate thrice with the solvent-system 70:30 benzene:ethylacetate. The recovered pure compound was found -S-methyl isomer of Dimethoate [Chart III (d)] which was confirmed by NMR and mass. This impurity was about 1 to 1.5%. Molecular weight was determined by the mass spectrophotometer was 229.

NMR data

a) (-S-Me) group shows doublet at 2 to 2.5 δ due to phosphorus.

b) Methyl attached to nitrogen shows doublet due to N-H proton coupling at 2.85 δ

c) Methylene ($-CH_2$) doublet at 3.56 and 3.7 δ

d) One methoxy attached to phosphorus gives doublet at 3.95 and 3.75 δ

[Fig. VII]

Fourth fraction eluted with methanol was found to be about 1.8 to 2% (0.022 gms). It seems that this product was very polar non-organic material. Esterification of this compound was tried by using diazomethane but it was not successful because of its colloidal form. This might be some fine silica gel in colloidal form or non-organic material.

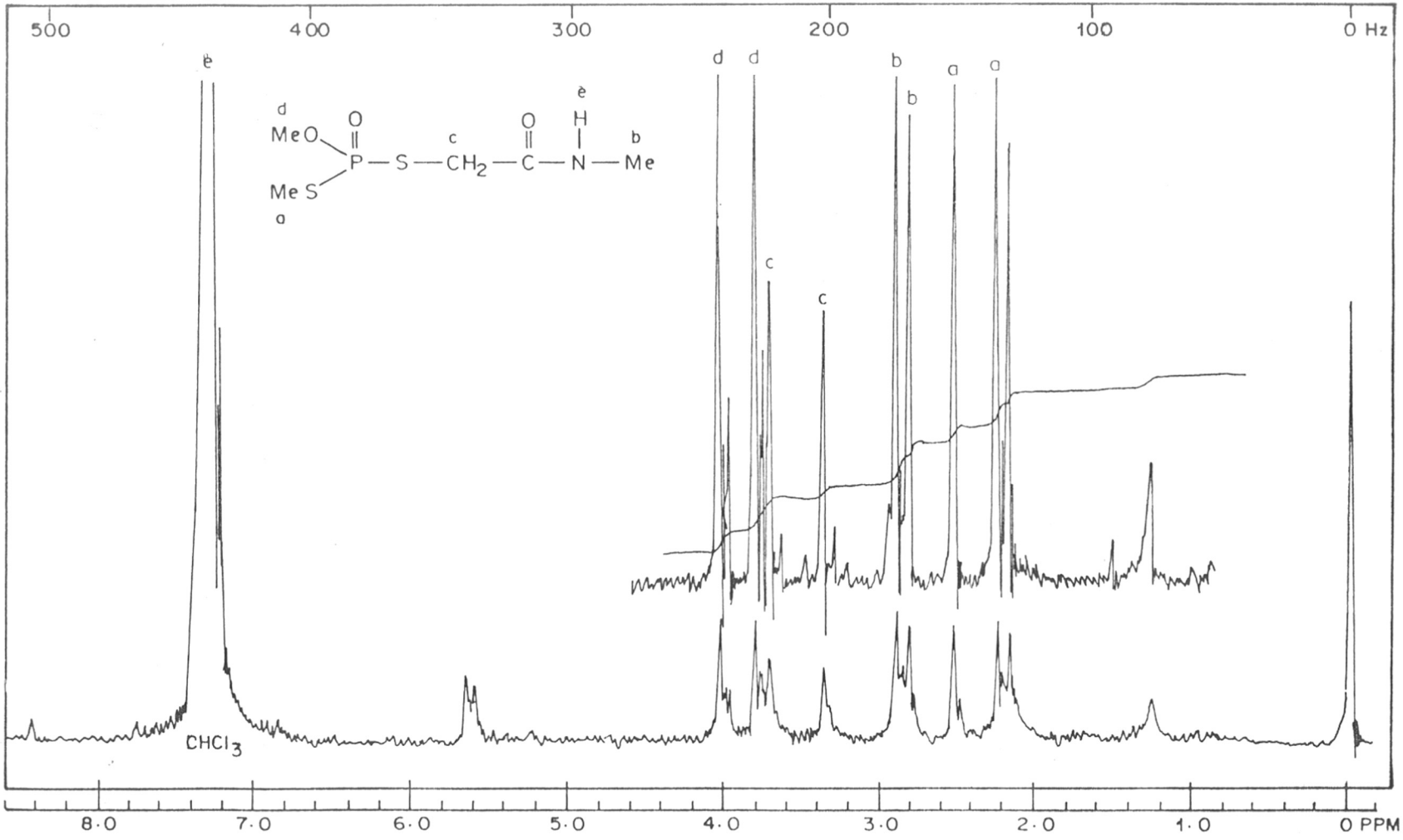

500
400
300
200
100
0 Hz
e
d
MeO
O
P
S
c
CH2
O
C
H
N
b
Me
Me S
a
d d
c
c
b
b
a
a
CHCl3
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0 PPM (δ)

FIG. — VII.

Assay for Technical grade Dimethoate

1. Volatile matter (solvent - 1 to 1.5%
2. -S methyl and ester impurity - 3 to 5%
3. Dimethoate - 92.2%
4. -S methyl isomer of Dimethoate (impurity)- 1 to 1.5%
5. Non-identified more polar compound - 1.5 to 2%.

REFERENCES

1. Hoegberg E.I. and Cassaday J.T., J.Am.Chem.Soc., 73, 557 (1951).
2. Hoegber E.I., Cassaday J.T. and Gleissener B.D., US Patent 2,494,283 (1948).
3. Analytical methods for Pesticides, Plant Growth Regulators and Food Additives by Gunter Zweig Vol.No.II, page No.171 to 172.
4. Uchida T., Dautermann W.C. and O'Brien R.D., J.Agri.Food Chem., 12, 49 (1964).
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6. Sauti R. and Giacomalli R., J.Agric.Food Chem., 10, 257 (1962).
7. Morikowa O. and Saito T., Batyu-Kagku, 31, 130 (1966).
8. Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives by Gunter Zweig Vol. No. II page No.176 (George et al., 1966).
9. ISI Specification for Dimethoate Ammendment to ISI 3902 (1975) in Feb. 1980.

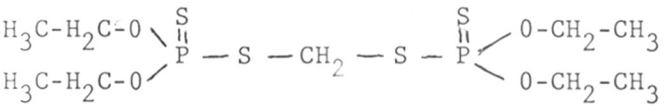
CHAPTER III

Ethion

E T H I O N

Introduction

Structural formula



Empirical formula: $C_9H_{22}O_4P_2S_4$

Molecular Weight: 384.5

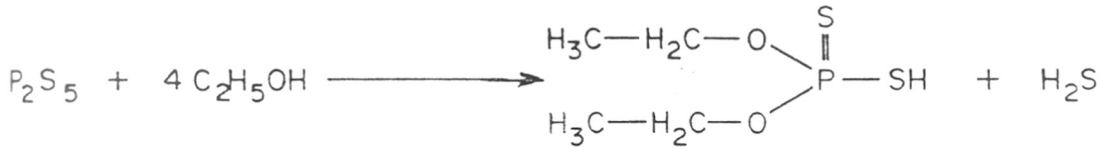
Ethion is chemically known as 0,0,0'0'-tetraethyl S-S'-methylene bis phosphorodithioate. Ethion[1-3] belongs to a class of phosphorothiolothionates group of organophosphorus pesticides. It is a non-systemic insecticide, acaricide and scale control agent.

Ethion[4] was discovered and developed by the Niagara Chemical Division Food Machinery and Chemical Corporation and was covered by US Patent 2,873,328(1959).

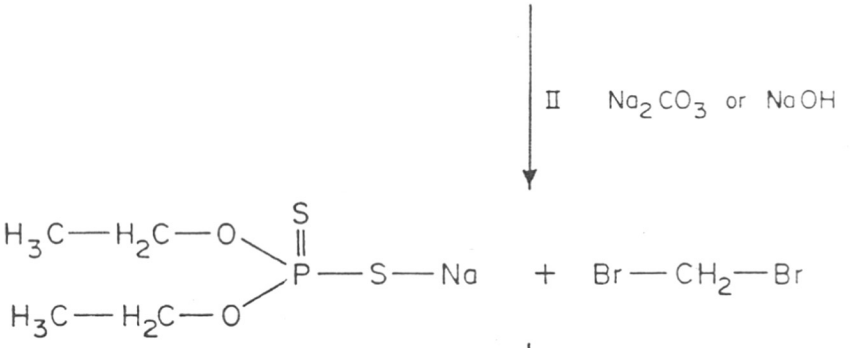
Method of preparation

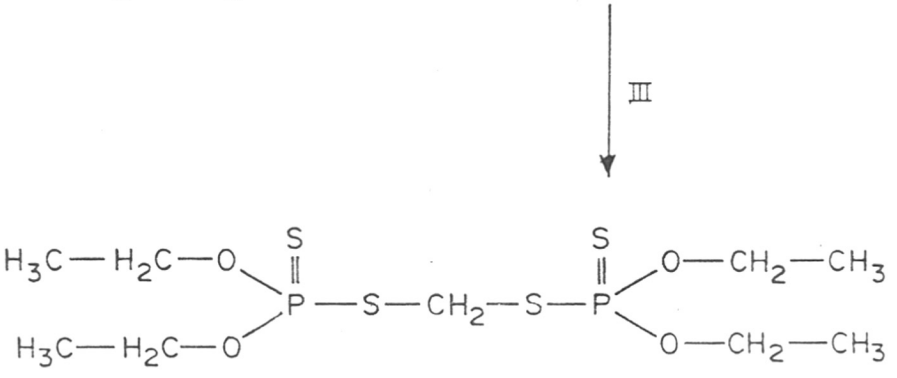
In the preparation of Ethion [Scheme I] a heterogenous reaction mixture of phosphorus pentasulfide and ethanol in benzene was refluxed for 2 hrs. to get a solution of 0,0-diethylphosphorodithioic acid [Product (a), Scheme I]. The sodium salt of the acid was prepared by addition of aqueous sodium

SCHEME — I



Product a





Product b

(ETHION)

hydroxide to the benzene solution of the acid. This aqueous solution of sodium salt was allowed to react with methylene bromide in presence of ethanol to give Ethion [Product (b), Scheme I]. The technical grade Ethion was 94 to 95% pure.

Physical properties

Ethion is water white to amber coloured, non-volatile liquid which solidifies in the range of -12°C to -15°C.

Vapour pressure: At 25°C 1.5×10^{-6} mm Hg.

Density: At 20°C 1.215 to 1.230.

The solubility of Ethion[5] is given below:

Miscible in	5% miscible in	Slightly soluble in
1. Benzene	1. Kerosene	1. water
2. Ethyl alcohol		
3. Hexane		
4. Xylene		
5. Methylated naphthalene		

Chemical behaviour

Ethion undergoes to acidic hydrolysis, basic hydrolysis and slow air oxidation.

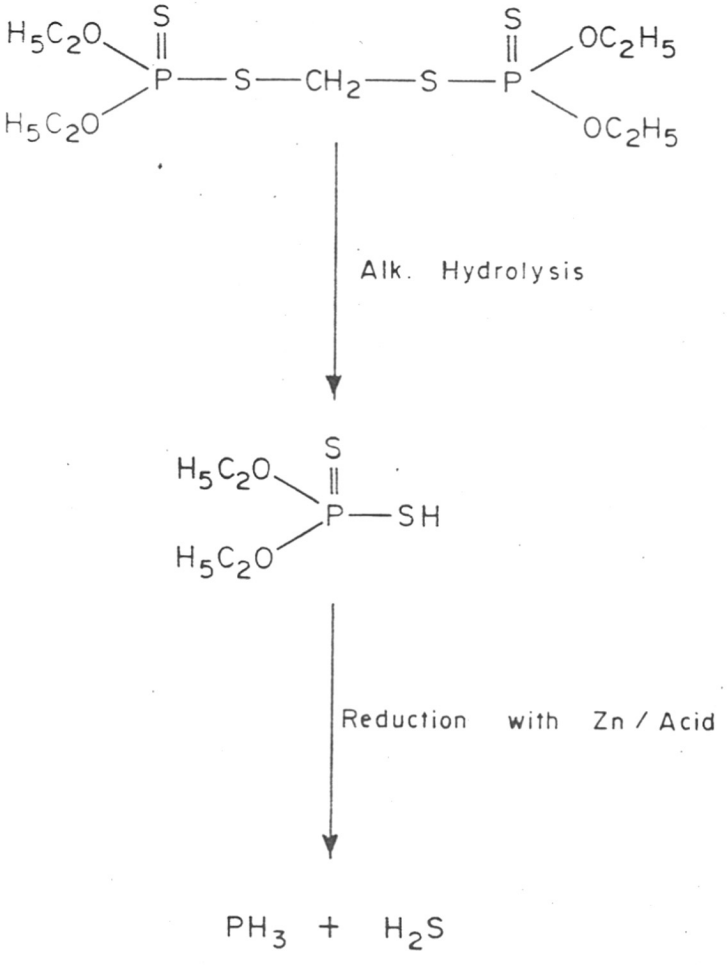
Eichel-berger et al.[6] have studied the persistance of pesticides in river water and found that maong 28 organophosphorus pesticides, including ethion and azodrin was stable for a period of 8 weeks. Cowart and co-workers[7] have studied the hydrolysis rate of ethion along with other phosphorus pesticides, they concluded that hydrolysis in water increased with decreasing sulfur content of organophosphorus pesticides.

Ethion was susceptible to alkaline hydrolysis to yield 0,0-diethyl dithiophosphoric acid.[8] Ruzika[9] has identified the oxidation product of ethion. He oxidised ethion with per acetic acid solution, aqueous solution of bromine and $KMnO_4$ in acetone.

Cook et al.[10] has studied the effect of UV light on ethion. According to him this effect was similar to sunlight and prolonged exposure to UV light had converted ethion to highly polar compounds.

Reduction mechanism of pesticide ethion has been studied by Lee-Myung Yun[11]. He found that after alkaline hydrolysis ethion was reduced with zinc and acid to H_2S and PH_3 which was identified using $Ph(oAc)_3$, $AgNO_3$ and $Hg\ Br_2$ papers [Scheme II].

SCHEME – II


H5C2O
S
P
S
CH2
S
P
OC2H5
H5C2O
OC2H5
Alk. Hydrolysis
H5C2O
S
P
SH
H5C2O
Reduction with Zn / Acid
PH3 + H2S

Metabolism

Metabolism of ethion was studied by Rao and others[12] by liver homogenates from different species. They found that ethion disappeared by hepatic oxidative desulfuration, system of four different species in the presence of NAD and NADA. They have reported that compound was detoxicated via oxidation of thionosulfur, in the activation stage [Scheme III].

Heinicke[13] have studied the effect of ethion on photosynthesis of red delicious apple leaves and found that ethion effects the reduction rate in photosynthesis.

Formulation:

Ethion is available in following formulations:

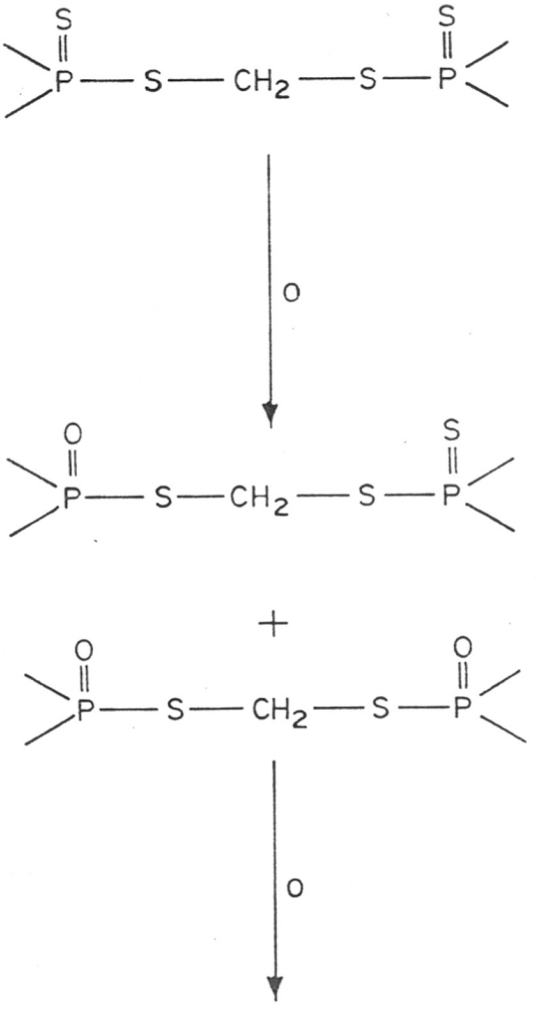
1. 25% W.P. (wettable powder)
2. 30% E.C. (Emulsifiable concentrate)
3. 4% dust.
4. 5%, 8% and 10% grannules

Methods used for analysis

1. Copper complex method

The method involves the alkaline hydrolysis of ethion and the 0,0' diethyl dithiophosphoric acid was converted to yellow complex copper salt by copper sulfate[14,15]. The copper complex salt was measured at absorbtion 418 mm by colorimetry.

SCHEME — III.



2. Colorimetric method using nitric acid and perchloric acid as oxidation agents

Ethion was subjected to oxidation using nitric acid and perchloric acid to give phosphoric acid. That acid on treatment with ammonium vanadate and ammonium molybdate gave yellow coloured complex. Development of colour depends upon the concentration of complex. Optical density was measured at 450 nm. The test sample was compared with the standard sample of ethion.

3. IR method

Absorbance of 959 cm^{-1} and 1017 cm^{-1} attributed to P-O-C bonds were measured[16].

The very intense P-O-C absorption peak at 1017 cm^{-1} imparted sensitivity to IR measurement, while secondary strong P-O-C peak at 959 cm^{-1} enhanced specificity.

4. Gas liquid chromatography

GLC technique was used successfully for the analysis of ethion. Egan[17] developed the method for GLC separation in sub-microgram amounts. In this analysis electron capture detector (E.C.D.) has been used.

In our laboratory a process for the synthesis of ethion and analysis of ethion has been developed.

Analysis of ethion (technical grade) has been carried out successfully using SE-30, or QF-1 (5%) at 200°C with thermal conductivity detector (TCD) and/or flame ionisation detector (FID).

EXPERIMENTAL

Tehcnical grade ethion product was about 94 to 96% pure. This percentage of purity varies from 94 to 98%. The remaining 2 to 6% portion has been investigated.

Thin-layer chromatography (TLC)

Several solvent systems were tried, to develop the TLC pattern for good separation of components from the mixture. TLC plates were prepared in chloroform slurry by dipping the glass plates in a big slurry jar. Satisfactory results for separation were gained by using solvent system 90% benzene to 10% ethylacetate. Components of ethion (technical) show clear separation [Fig. I].

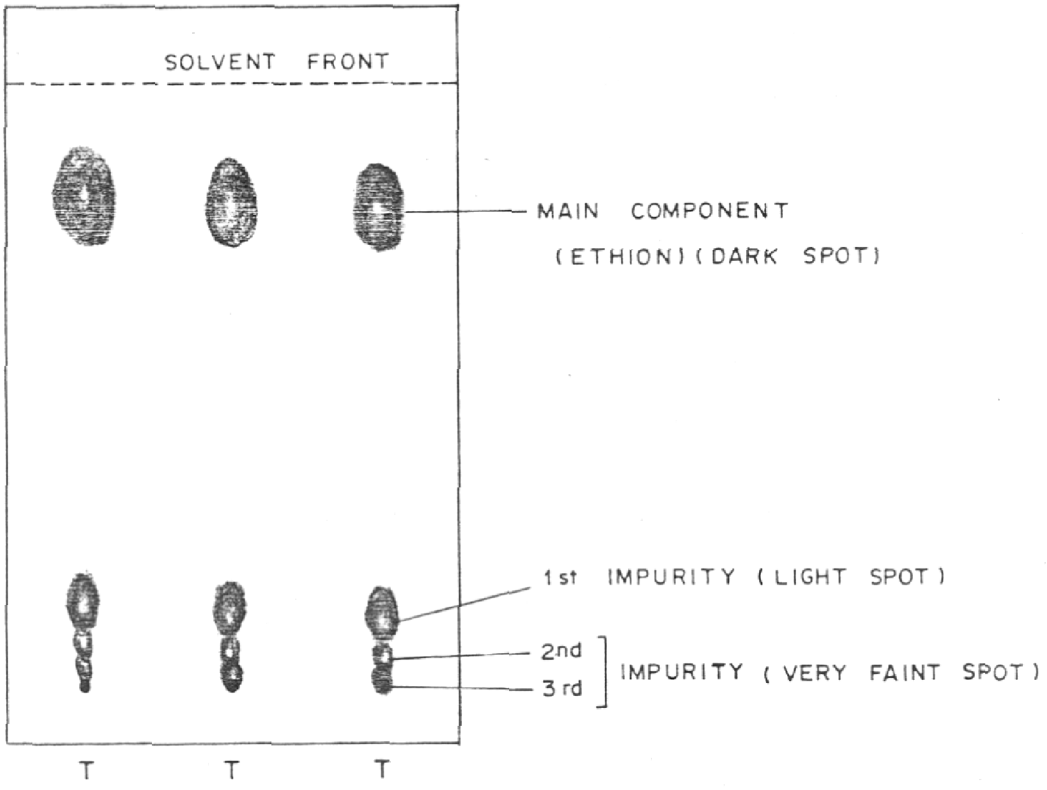
Separation of volatiles

GLC analysis of ethion always shows low boiling compounds, probably solvents. Sample was initially weighed and then dried under good vacuum for 4-5 hrs. The loss in weight was calculated as volatile matter (solvent). The average % of volatile matter was 1 to 1.5%.

FIG.—I.

SOLVENT SYSTEM —

90 % BENZENE : 10 % ETHYLACETATE



T — TECHNICAL GRADE SAMPLE OF ETHION

Standardisation of column chromatography ratio of silica

For isolation of individual impurity column chromatography was used. Column chromatography was first tried with (1:30) 1 gm of sample to 30 gms of silica gel (column grade) ratio. Before using in the column available silica gel was initially activated in the oven at about 120°C for 2 hrs, cooled to room temperature in dessicator. For 30 gms of silica gel 1.0 gms compound was taken. Column chromatography was monitored by TLC. We could achieve the required separation and isolation using (1:30) ratio so the same ratio was continued for large scale.

Column chromatography

The ethion sample was weighed accurately (5 gms), then dried under good vacuum for 4-5 hrs. The loss in weight was calculated as volatile matter which was about 1%. The available silica gel was initially activated in the oven at about 120°C for 2 hrs, cooled to room temperature in desiccator and then used.

a) Silica gel (column grade 80-120 mesh) - 150 gms.
b) Technical sample of Ethion (after drying under vacuum pump) - 4.95 gms.

Elution of the column

The column was packed in usual manner using pet.ether as solvent. Ethion was charged on the column. The eluents were collected in 50 ml fractions and were monitored by TLC.

First Eluent [Benzene:pet.ether]

60 : 40

Total 500 ml eluents were collected. When ethion (pure) stopped coming down, the solvent polarity was changed. The eluted material was found to be pure ethion (4.75 gms).

Second Eluent): [Benzene]

When mixed solvents were found ineffective the column was eluted with only benzene (about 200 ml). The eluted portion showed all the impurities (0.175 gms).

Third Eluent: [Ethyl acetate]

The column was further eluted by ethyl acetate (100 ml). The total eluents were containing hardly any (0.02 gms) material.

Column chromatography of Ethion

Silica gel - 150 gms (column grade, activated silica gel used)

Material loaded - 4.95 gms.

S.No. of fractions	Volume	Solvent system
1st	500 ml	Benzene:pet.ether 60 : 40
2nd	200 ml	Benzene
3rd	1.00 l	Ethylacetate

The benzene and ethylacetate elution showed more or less same TLC pattern and therefore they were mixed together. It was not showing separation by column chromatography (Fig. II). So further separation of impurities was tried on preparative TLC.

Preparative TLC

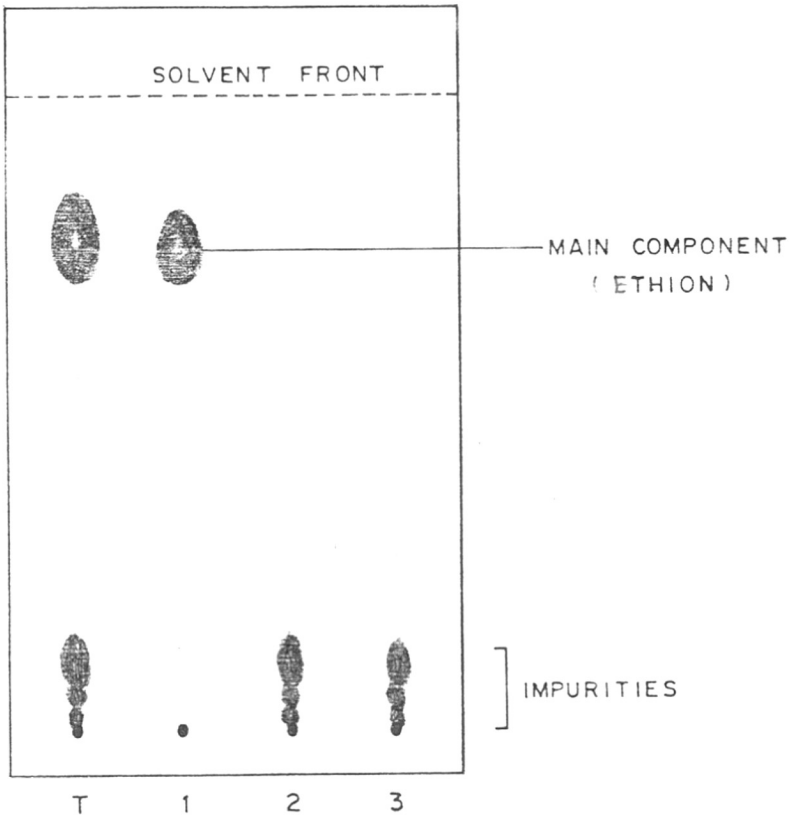
The preparative TLC of mixed impurities (0.195 gms) was carried out in the usual way and the major impurity was separated out (0.180 gms). The quantity of other two spots are very less in that even after extraction it was difficult to collect properly and thus was left without further processing.

NMR of the Ethion (pure) taken in CCl_4 shows the following signals.

FIG.—II.

SOLVENT SYSTEM —

90% BENZENE ; 10% ETHYLACETATE



T — TECHNICAL GRADE SAMPLE OF ETHION

1 — PURE ETHION (1st FRACTION)

2 — ALL IMPURITIES (BENZENE FRACTION)

3 — ALL IMPURITIES (ETHYL ACETATE FRACTION)

NMR data;

(a) Methyl ($-CH_3$), four methyl groups occurs at the 1 to 2 δ as clear triplet.

(b) Methylene ($-CH_2$) Five groups occur between 3.9 to 4.5 δ as multiplet.

[Fig. III]

IR data;

(a) $P-O-CH_2-CH_3$ at 1151 and 1215 cm^{-1}

(b) P-O-C at 1017 cm^{-1} (ether linkage)

[Fig. IV]

The major first impurity was characterised carefully using modern instrumental technique such as elemental analysis, NMR, IR and Mass.

I Elemental analysis - (Found)

Sulphur	-	26 to 27%
Phosphorus	-	16 to 17%

Calculated for - $C_9H_{22}O_5P_2S_3$

Sulphur	-	26.09%
Phosphorus	-	16.85%

While Ethion having molecular formula - $C_9H_{22}O_4P_2S_4$ requires

Sulphur	-	33.34%
Phosphorus	-	16.14%

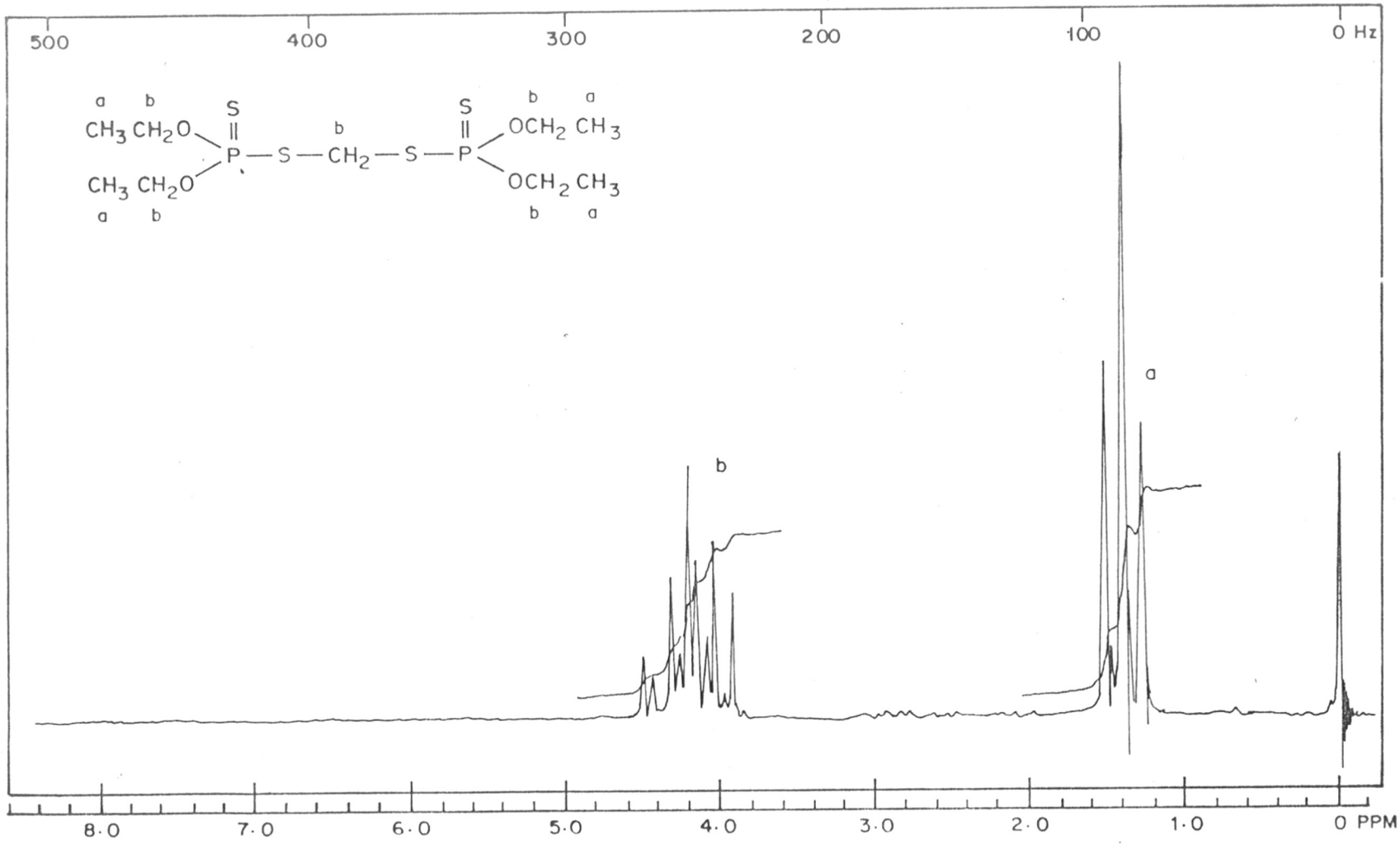
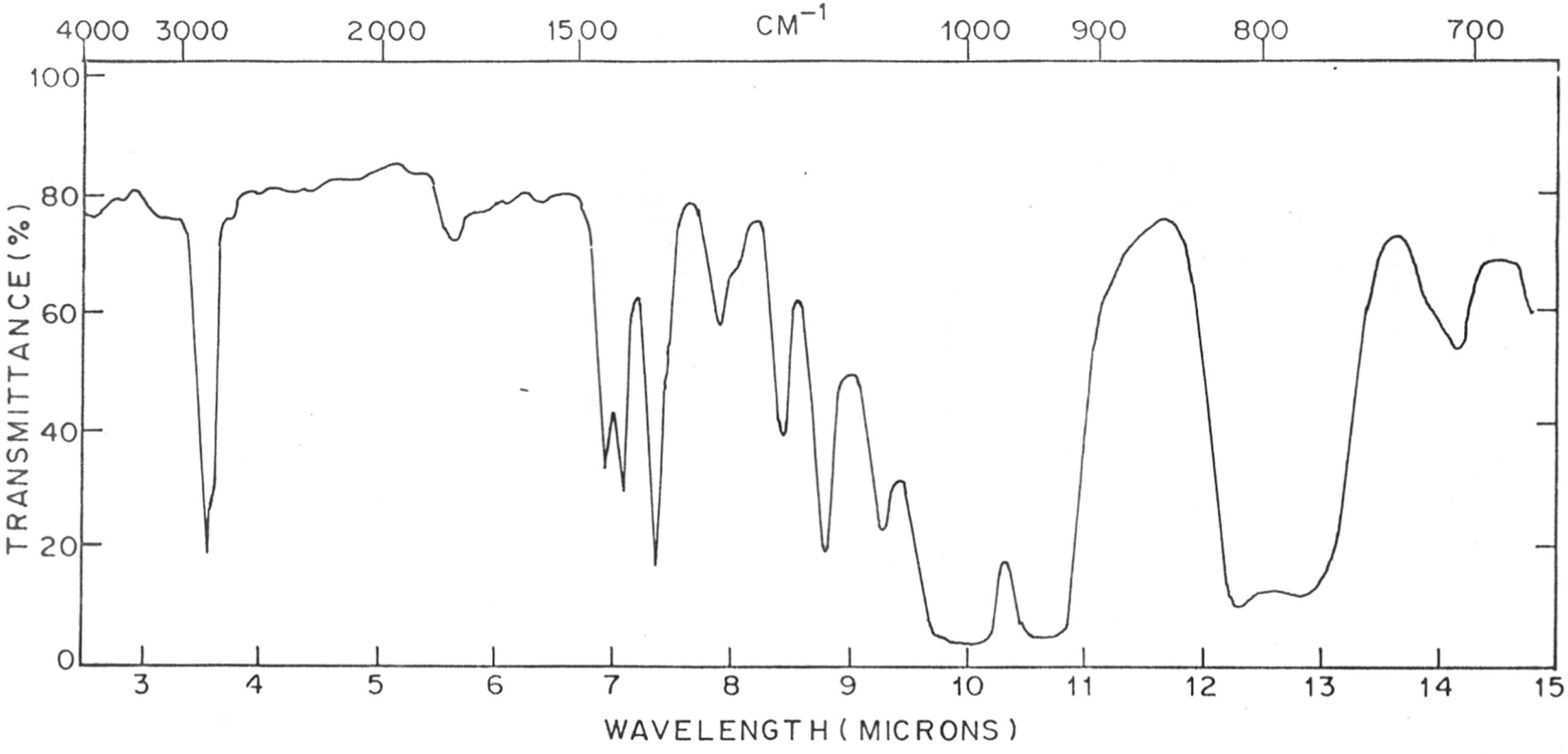

500
400
300
200
100
0 Hz
a b
CH3 CH2O
S
P
S
CH2
b
S
P
b a
OCH2 CH3
CH3 CH2O
OCH2 CH3
a b
b a
b
a
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0 PPM(δ

FIG. – III.



ETHION

FIG. – IV.

NMR data (a) Methyl ($-CH_3$) groups are four, occurs at the 1 to 2 δ as triplet (not clear) (b) Methylene ($-CH_2$) five groups occur between 3.9 to 4.5 δ as multiplet [Fig. V].

IR data

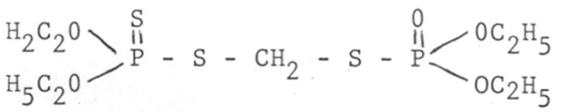
(a) P-0-CH_2-CH_3 at 1151 and 1215 cm^{-1}

(b) P-0-C at 1017 cm^{-1} (ether linkage)

[Fig. VI]

Mass - M^+ 368 - Molecular weight was determined by the mass spectrophotometer was 368.

The above mentioned characteristics indicate the structure which is likely to be



Molecular formula - $C_9H_{22}O_5P_2S_3$

Assay of Ethion [Technical grade]

1. Volatile matter (solvent) - 1 to 1.5%
2. Main component (Ethion) - 95 to 96%
3. Oxo analogue of Ethion - 3 to 3.5% (as impurity)
4. Moisture - 0.1 to 0.3%
5. Unidentified impurities - traces

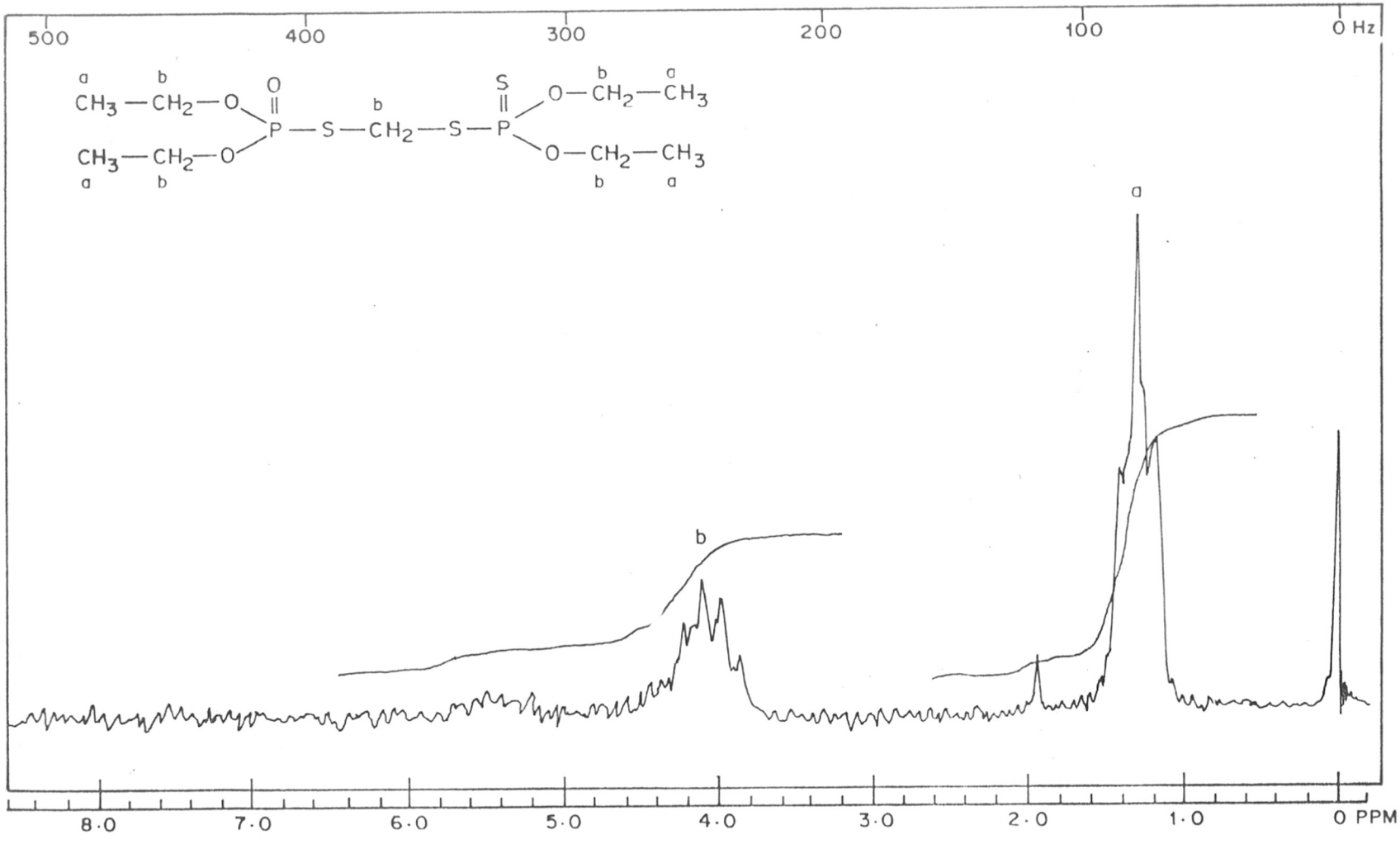
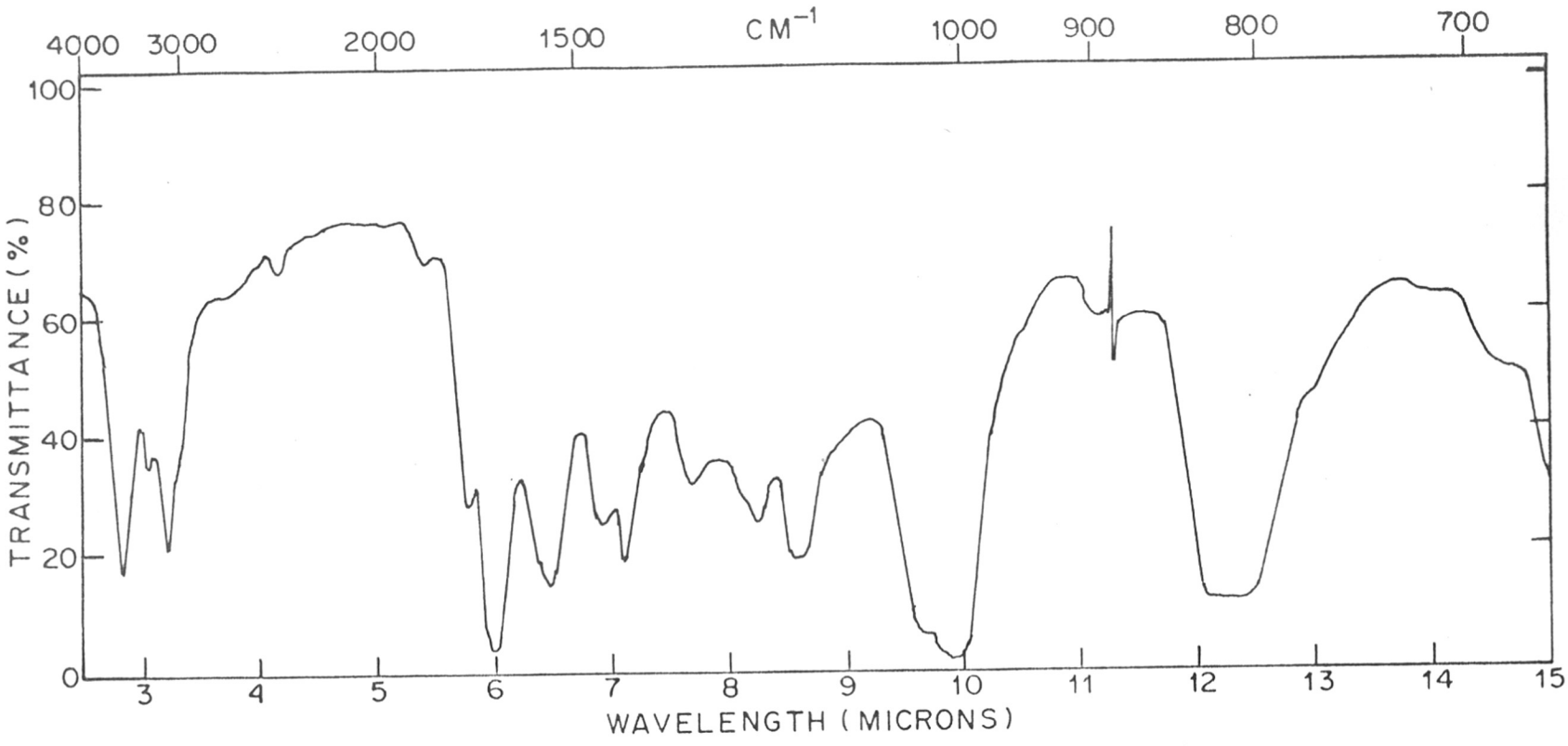

500
400
300
200
100
0 Hz
a
b
CH3 — CH2 — O
O
P — S — CH2 — S — P
S
O — CH2 — CH3
b
a
CH3 — CH2 — O
a
b
b
a
b
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0 PPM(δ)

FIG. V.



ETHION IMPURITY

FIG. – VI.

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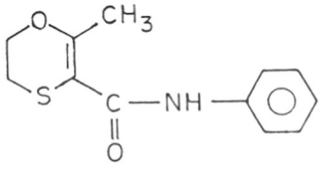
CHAPTER IV

Carboxin

C A R B O X I N

Introduction

Structural formula



Empirical formula: $C_{12}H_{13}NO_2S$

Molecular weight: 235

Carboxin or vitavax is chemically known as (5,6-dihydro-2-methyl-1,4 oxathiin, 3 carboxanilide). The nomenclature used in chemical abstract is 2,3-dihydro-6-methyl-5-carbamoyl-1:4 oxathiin. It was commonly known as carboxin. It was introduced in 1966 by Uniroyal Inc. under the code No.D 735 with trade mark as "vitavax" . The process was covered with US Patent 3249, 499, 339, 3202; 3454391. Its fungicidal properties were described by Von Schmeling B and Kulkaim[1]. It was systemic fungicide.

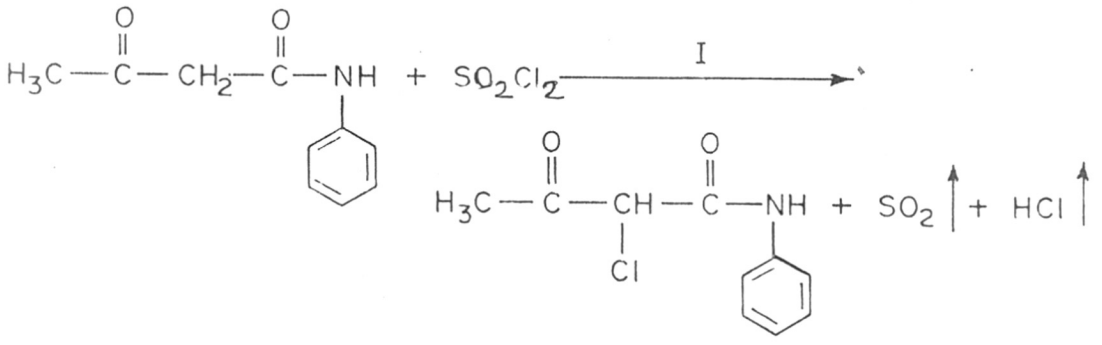
Method of preparation

Its method of preparation was as follows in [Scheme I]. Preparation of carboxin was described in three steps, (a) chlorination (b) condensation (c) cyclisation.

SCHEME — I

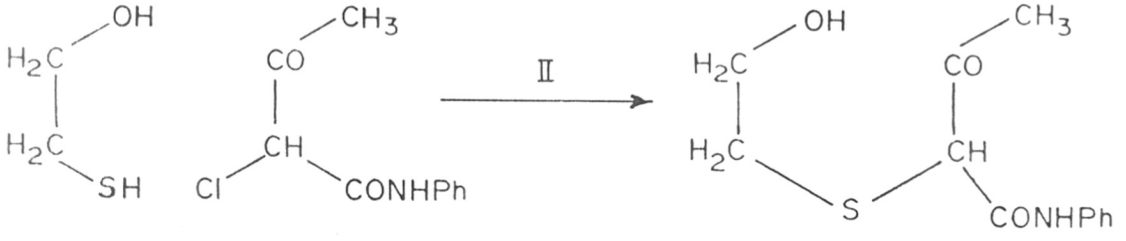
CARBOXIN SYNTHESIS

a) CHLORINATION —



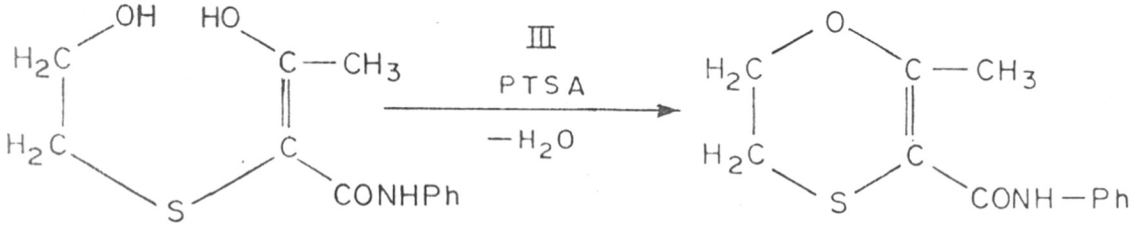
Product a

b) CONDENSATION —



Product b

c) CYCLISATION —



Product c

a) Chlorination

To the mixture of acetoacetanilide and benzene (dry), sulphuryl chloride was added slowly with constant stirring. After the reaction was complete, $NaHCO_3$ was added to it till the solution was alkaline to litmus.

b) Condensation

First reaction was continued [Scheme I, Product (a)] without isolation, 2-mercapto-ethanol was added to above reaction mixture with constant stirring. Stirring was continued for 2 hrs. Excess of benzene layer was removed, washed with water and dried to get keto intermediate [Scheme I, Product (b)].

c) Cyclisation

p-Toluene sulphonic acid and keto product [Scheme I, Product (b)] were taken in dry benzene and mixture was refluxed using Dean-Stark apparatus till water separation stopped (for 7 hrs.) Benzene layer was washed with water to remove p-toluene-sulphonic acid and benzene was removed by distillation. The product (carboxin) [Scheme I, Product (c)] formed was crystallised from methanol. Technical grade product of carboxin was about 97 to 98% pure.

Physical properties

Carboxin is a white crystalline solid with yellowish tinge. Almost odourless. It was stable in neutral medium. It has very little or no deterioration effect on storage.

M.p. 91.5 to 92°C.

Solubility: At 25°C solubility carboxin was given below.

Solvent	Solubility
1. Water	170 ppm
2. Acetone	60% (w/w)
3. Hexane	15% (w/w)
4. Dimethyl sulphoxide	15% (w/w)
5. Ethanol	11% (w/w)
6. Methanol	31% (w/w)

Advantages over other pesticides

Carboxin has the following advantages:

1. It is an organosulphur pesticide containing no phosphorus and having fungicidal action.
2. It is a highly water-insoluble fungicide with good stability.
3. It is non-corrosive.
4. It has low mammalian toxicity and it is thus relatively safe to other domestic animals.

Biological activity

Carboxin is a well established fast acting broad specstrum systemic fungicide. It was used on wheat, cotton, ground nut , cereals, peanuts and vegetables for controlling smuts, bunts and rhizoctonia. The two polymorphic forms did not differ in fungicidal activity. The acute LD_{50} for rats is 3820 mg/kg. The acute dermal LD_{50} for rabbits is more than 8000 mg/kg. Albino rats fed on diets containing 600 ppm carboxin for two years, suffered with detectable symptoms. The LD_{50} for 75% wettable powder vitavax formulation is 4.5 ppm for blue gill sunfish. The LD_{50} is greater than 5620 ppm for bobwhite quail and greater than 4640 ppm for mallard ducks.

Metabolism

Chin et al. [1970 (a) and (b)] reported that carboxin (vitavax) was metabolised to its sulfoxide as the major metabolite and sulfone as the minor metabolite in water, soil, barley and wheat. The oxidation of this sulfur atom was of some interest since it represents a case of somewhat sterically hindered sulfur; still undergoing significant oxidation metabolism[2]. Carboxin seed treatment on barley and wheat was absorbed by seedlings. In the plants, it was mainly oxidised to sulfoxide (5,6-

dihydro-2-methyl-1:4 oxathiine -3-carboxanilide 4-oxide) but small amount of sulfone (5,6-dihydro-2-methyl-1:4 oxathiine-3-carboxanilide-4,4'-oxide) was also found. As plant approaches maturity the extractable oxathiine residues were converted to insoluble anilide complex, probably with lignin No hydrolysis of carboxin was detected in plants[3].

Oxidation occurs in water and soil but hydrolysis has never been detected in either case. The oxidation of carboxin in water was retarded by high pH . . . . . At 2 and 4 pH traces of further oxidation products occur. The oxidation of carboxin in soil occurs and was complete within two weeks[4].

$$R-S-R' \xrightarrow{O} R-\overset{\overset{O}{\|}}{S}-R' \xrightarrow{O} R-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-R'$$

Formulations

Carboxin was marketed in the form of 75% wettable powder, 34% active flowable (liquid suspension) and 25% wettable powder formulation. It was also combined with thiram in dry and flowable formulations. Formulations of 75% wettable powder was suggested for use for the seed treatment of cereals and ground nuts at 3-6 oz/100 lbs.

Methods used for analysis

1. IR method[5]

Carboxin was analysed by infra red spectrum based on measurement at 5.97, 6.30 and 7.75 μ m in comparison with carboxin standard using benzene as solvent 0.2 gm active ingredient dissolved in 25 ml benzene and IR spectrum was recorded in slow scanning speed.

2. Volumetric method[6]

Carboxin was oxidised by H_2O_2 to its sulphoxide and excess of H_2O_2 was back titrated with standard sodium thiosulphate.

a) Analysis of residue was done by hydrolysis and determination of the aniline formed was done by a method, in which aniline was coupled with p-dimethyl amino benzaldehyde[7].

b) Carboxin residue was analysed by GLC using specific nitrogen detector[8].

EXPERIMENTAL

Technical carboxin product was about 97 to 98% pure. This purity percentage depends upon the method of synthesis. The remaining impurities were not characterised completely. These impurities were investigated to obtain complete analysis of the product.

Thin-layer chromatography: TLC

Several solvent systems were tried to develop the TLC pattern for good separation of components from the mixture. TLC plates were prepared in chloroform slurry by dipping the glass-plates in a big slurry jar. Satisfactory results for separation were gained by using solvent system 10% ethyl acetate in 90% benzene [Fig. I].

Separation of volatiles

Sample was initially weighed and then dried under good vacuum at 80°C for 3-4 hrs. The loss in weight was calculated as volatile matter (likely to be alcohol which was used for washing). The average value comes to about 0.4 to 0.5%.

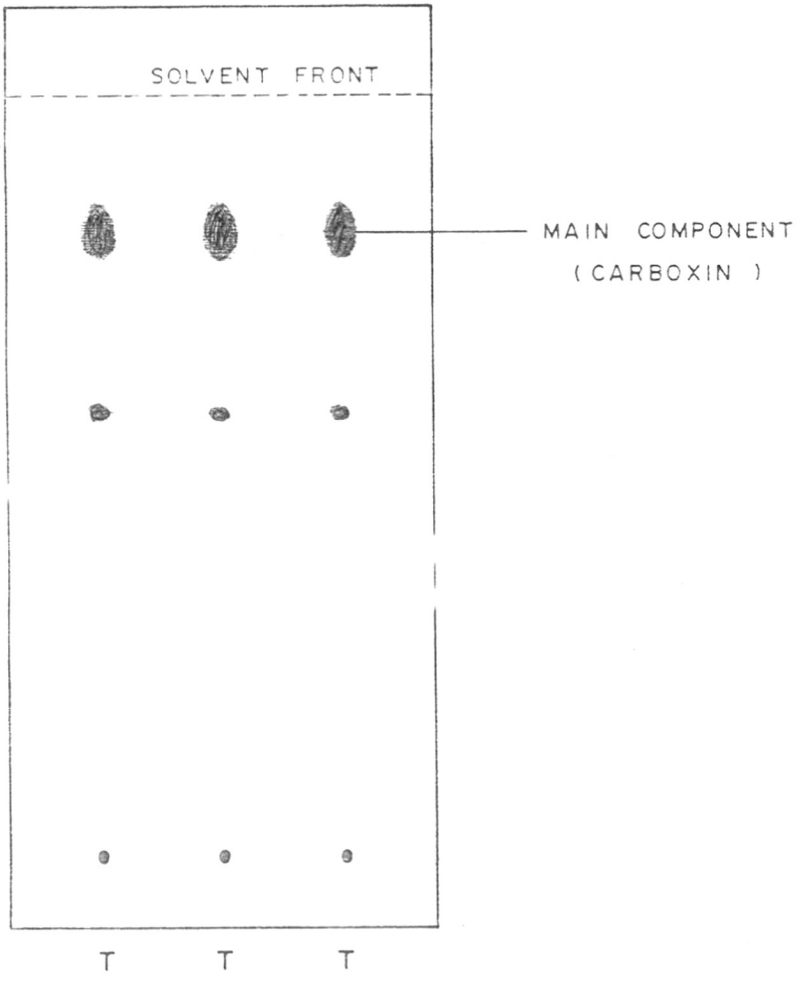
Moisture content

The moisture content of dried sample was estimated using Karl-Fischere apparatus. The % moisture of carboxin was about 0.2 to 0.3%.

FIG.—I.

SOLVENT SYSTEM —

10 % ETHYLACETATE IN 90 % BENZENE



T — TECHNICAL GRADE CARBOXIN

Standardisation of column chromatography

ratio of silica

For isolation of individual components of the mixture column chromatography was necessary. Column-chromatography was first tried on 30 gm of silica gel (column-grade). The ratio taken for compound to silica gel was (1:30) ratio. Before using in the column the available silica gel was initially activated in the oven at about 120°C for 2 hrs, cooled to room temperature in dessicator. For 30 gm of silica gel 1 gm compound was taken. Column chromatography was monitored by TLC. As clear cut separation and isolation was possible by using this ratio same was continued for large scale experiments.

Column chromatography

The carboxin sample was weighed accurately (5 gms). Then dried under good vacuum for 3-4 hours at 80°C. The loss in weight equivalent to volatile matter was about 0.5%. The available silica gel was initially activated in the oven at about 120°C for 2 hrs, cooled to room temperature in dessicator and then used.

a) Silica gel (column grade - 80 -120 mesh) - 150 gms.

b) Technical grade sample of carboxin (after drying under vacuum pump) - 4.97 gms.

Elution of the column

The column was packed in usual manner using pet.ether as solvent. Carboxin was charged on the column. The eluents were collected in 50 ml fractions and monitored by TLC.

First Eluent - [Benzene]

The column was initially eluted with benzene. The eluent was containing pure carboxin. 700 ml of fractions were collected. TLC check showed that the fractions collected after that were not containing anything. Weight obtained for pure carboxin was 4.80 gms.

The polarity of solvent system was changed.

Second Eluent: [90:10 Benzene:Ethyl acetate]

The column was further eluted with a mixture of benzene and ethylacetate [90:10]. The total eluent was 100 ml. It was containing 0.15 gm of material. The residue remaining on column was 0.025 gms.

Column chromatography of carboxin

Silica gel - 150 gms (column grade, activated silica gel).

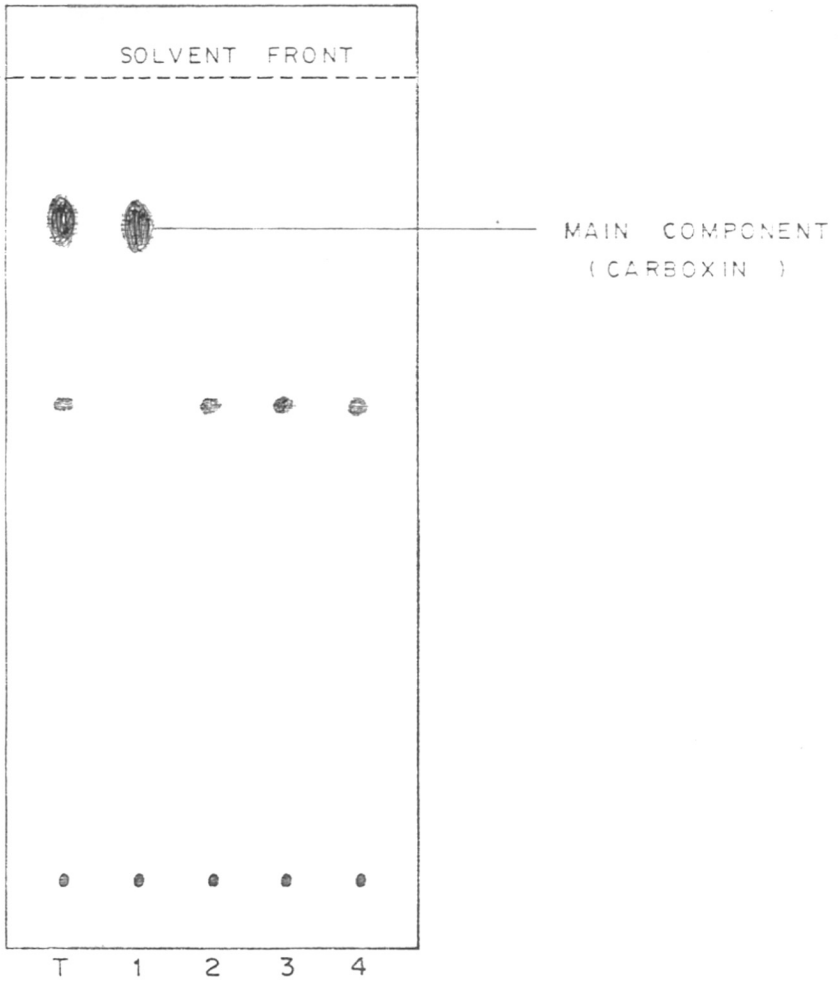
Material loaded - 5 gms.

S.No. of fractions	Volume	Solvent system
1st	700 ml	Benzene
2nd	100 ml	90:10 Benzene: Ethyl acetate

FIG. —II

SOLVENT SYSTEM —

10 % ETHYLACETATE IN 90 % BENZENE



T — TECHNICAL GRADE CARBOXIN

1 — PURE CARBOXIN

2 — IMPURITY ISOLATED FROM COLUMN

3 — STANDARD HYDROXY INTERMEDIATE

4 — IMPURITY ISOLATED BY EXTRACTION

The unknown impurity was analysed by comparative TLC, IR, NMR and Mass Spectroscopy.

It was confirmed that the major component of impurities was hydroxy sulfide intermediate.

The residue was in traces and found difficult to identify. This hydroxy sulfide intermediate might be there due to incomplete cyclisation reaction or due to reverse reaction in presence of traces of water. This isolated impurity was compared with standard intermediate by TLC [Fig. II].

NMR data

(a) Methyl (CH_3 - $\overset{O}{\overset{\|}{C}}$ -) attached to carbonyl shows singlet at 2.3 δ

(b) Methylene (-S-CH_2) attached to sulphur shows triplet at 2.55 δ

(c) -C<u>H</u>- show singlet at 3.17 δ

(d) Methylene (-S-CH_2-<u>CH_2</u>-OH) attached to hydroxy shows at 3.6

(e) Pheny protons (-CONH<u>Ph</u>) shows multiplet at 7 to 7.8 δ

(f) O<u>H</u> and <u>NH</u> protons at 450 offset shows singlet at 8 δ [Fig. III]

IR data

(1) -NH, OH at 3250 cm^{-1}

(2) -$\overset{O}{\overset{\|}{C}}$NHPh- carbonyl at 1675 cm^{-1}

(3) -$\overset{O}{\overset{\|}{C}}$-$CH_3$ carbonyl at 1625 cm^{-1}

(4) -$\overset{O}{\overset{\|}{C}}$-<u>$CH_3$</u> Aliphatic group at 1380 cm^{-1}

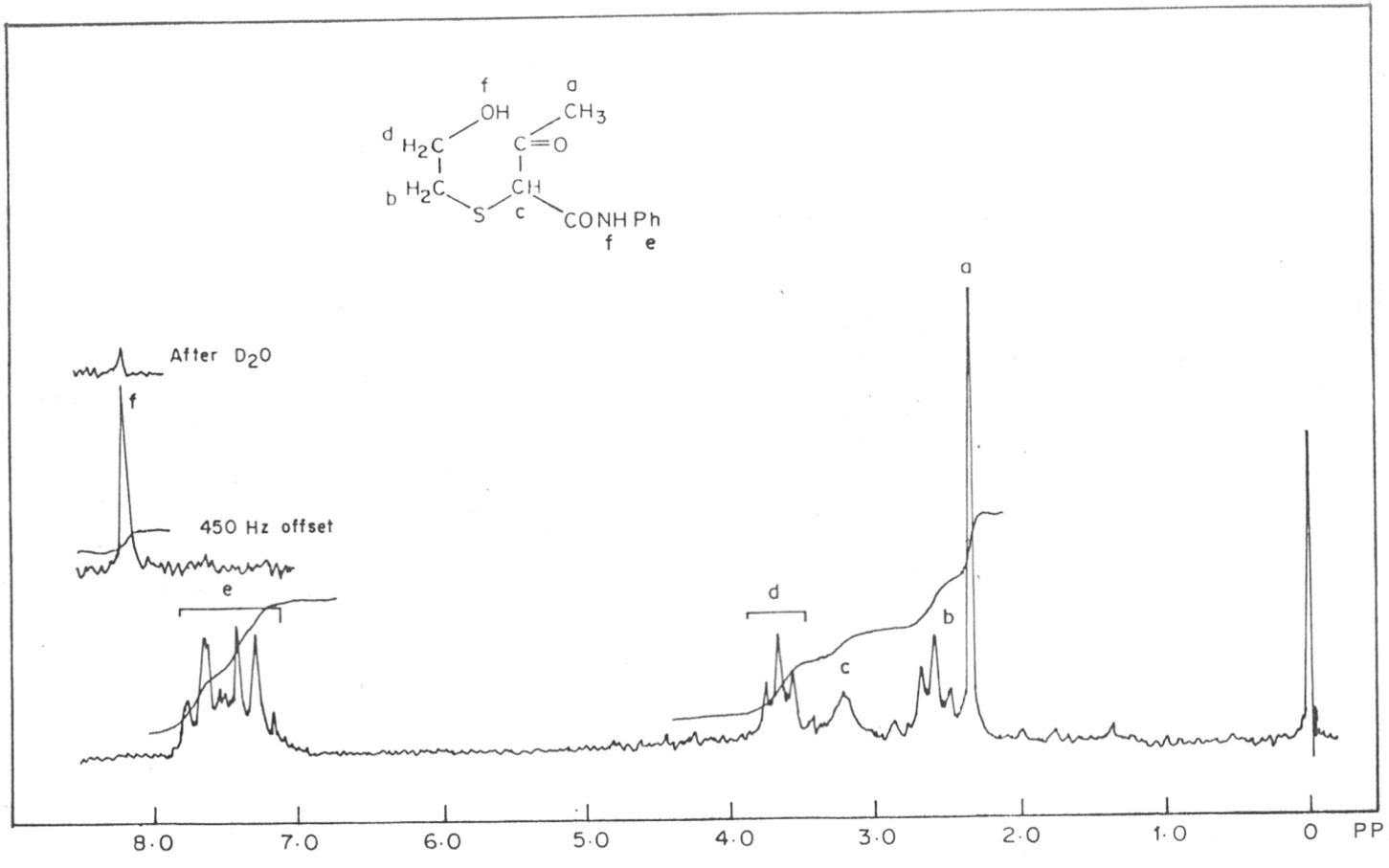
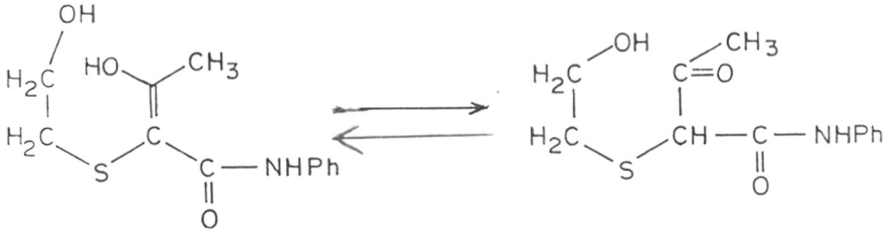

f
a
OH
CH3
d H2C
C=O
b H2C
CH
S
c
CONHPh
f e
After D2O
f
450 Hz offset
e
d
c
b
a
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM(δ)

FIG. III

Mass M^+ 253

Molecular weight was determined by the mass spectrophotometer was 253.

Hydroxy sulfide intermediate



Isolation of the impurity by another method

Technical sample of carboxin (5 gms) was taken in 300 ml benzene. The benzene solution was extracted by 1% NaOH solution three times, total (150 ml) using 50 ml solution each time. Being hydroxy intermediate the impurity forms complex with Na^+. The water layer was separated (150 ml) and was acidified by acetic acid. It was then extracted (150 ml water layer) with chloroform twice (100 ml). Chloroform was removed by distillation to obtain hydroxy intermediate. It was compared with standard product by TLC [Fig. II]. By using this technique we have isolated the impurity very easily and quickly.

Assay of technical grade carboxin

1. Volatile matter (alcohol) - 0.5 to 1.0%
2. Main component (carboxin) - 97 to 98%.
3. Hydroxy compound (impurity I) - 0.5 to 1.0%.
4. Moisture - 0.2 to 0.5%.
5. Residue - 0.1 to 0.2%.

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