STUDIES IN BIOLOGICALLY ACTIVE COMPOUNDS USING CHROMATOGRAPHY TECHNIQUES

A THESIS

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BY

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TO

GIRISH, MY HUSBAND

CERTIFICATE

Certified that the work incorporated in the thesis entitled 'Studies in biologically active compounds using chromatography technique' submitted by Mrs. Medha Girish Deo, was carried out by the candidate at the Division of Organic Chemistry I, National Chemical Laboratory, Pune-411008, under my supervision. Such material as has been obtained from other sources has been duly acknowledged in the thesis. This work was not submitted so far for any degree.

(Dr.B.B. Ghatge)
Research Guide

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

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(Mrs. M.G. Deo)

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GENERAL REMARKS

1. Numbers given to Charts, Figures and Structures in each chapter of the thesis refer to that particular chapter only.
2. References pertaining to each chapter are given at the end of that particular chapter.
3. A brief summary of each chapter is given at the beginning of that chapter.
4. Liquid Chromatography unit supplied by M/s. Waters Associates, USA was used to record retention time data.
5. Microanalysis were carried out by microanalytical section of this laboratory.

C O N T E N T S

CHAPTER - I

Introduction

SUMMARY

Use of chemicals for medicinal and pest control applications resulted in the studies of various distinctive features of these compounds which could possibly be responsible for their biological actions. A number of approaches were examined by researchers to explain the structure-activity relationships. Hansch theory of correlating organic-aqueous partition coefficients of compounds with their activity has found wide acceptance. 1-Octanol has been the solvent of choice in this method. Many successful applications of octanol-water partition coefficients to determine physicochemical parameters and possible extent of activity are well known.

Chromatography phenomenon has been very popular among experimenting chemists for separation of solutes in a mixture. With the progresses made in physical sciences during the past few decades the chromatography technique has become almost indispensible for organic chemists.

Partition chromatography involves two immiscible phases- one stationary and the other mobile. The separation phenomenon in partition chromatography is considered to be predominantly, though adbsorption may also occur to some extent. The partition chromatography may thus be considered to be a partitioning

system. With the choiee of reverse phase chromatography technique, this system may be considered to be similar to the lipid cell membrane and surrounding extra-cellular fluid. The chromatogaaphically calculated partition coefficients can also be correlated with many physiochemical parameters as well as activity. This has so far been done by relating chromatographic partition values with octanol-water partition coefficients via collander system. The present studies attempt to directly correlate the chromatographic partition coefficients with insecticidal activity.

The "chemical revolution" of the past century had led to the most remarkable advances in the chemical control of those organisms which are directly or indirectly opposed to man's well being. Vast number of synthetic agricultural chemicals had dramatically increased both the quality and quantity of the food and fibre derived from our crops and domestic animals. Similarly, our sufferings have been greatly alleviated and life expectancy enhanced by development of drugs to control diseases associated with pathogenic organisms and of chemical correctives to counterbalance natural deficiencies.

Because the one common property of all these chemicals resides in their ability to interact with living systems, they can all be said to possess some form of 'biological activity'.

1.1. Biological activity and chemical structure

All living organisms are chemically dynamic systems. They behave and function as living entities, as a direct result of extremely complex interdependent chemical reactions, which, although in continuous flux, are maintained at any given time in a delicate state of balance. The presence of a foreign chemical, within a living system, can readily upset this balance by enhancing, inhibiting, or otherwise interacting with,

one or more of the chemical reactions of components on which its integsrity depends. Such a chemical can be said to be 'biologically active.

Biological activity may take different forms and may be measured in different ways, depending on the level at which the investigation is conducted. When the critical site and mechanism of action of a chemical are known, biological activity can be measured directly, e.g. in terms of degree of inhibition or enhancement of an enzyme system. Biological activity may also be measured in an indirect manner, though in vivo observations of the end results of the chain of events, initiated by the interaction of the chemical with some unknown biochemical compounds.

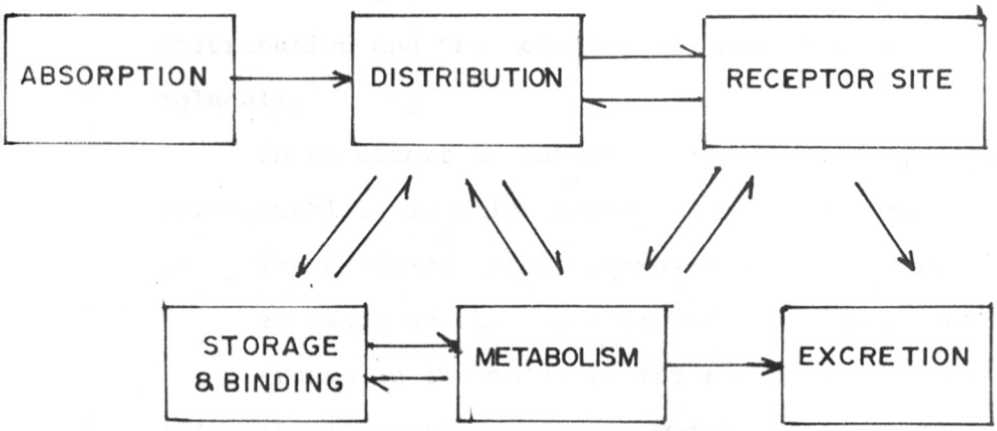
The first organic materials employed by man, for various medicinal and agricultural purposes, were generally of natural origin, and of unknown structures. As the chemical nature of the active components of these materials was recognised, and the compounds themselves were isolated, characterised, and subsequently synthesised in pure form, considerable interest was aroused in possible relationships which might exist between their chemical structures and biological effects. Interest in this area has continued to increase and today investigations involving the correlations of chemical

structure with biological activity have far reaching ramifications in many different fields.

1.1.1 Factors determining biological effect

Studies involving the correlation of biological activity with chemical structure of a compound are extremely complex,and, until recently, have been based, almost entirely, on empirical observations of a qualitative or semiquantitative nature. The biological activity of any material is governed by several major factors, which include its ability to successfully penetrate the organismsand to subsequently translocate to its site of action, its ability to avoid binding and storage in inert components and tissues, its ability to withsatand the action of degradative enzymes, and ultimately, ofcourse, its ability to interact with some essential biological receptor (Fig. 1)[1]. The extent to which a chemical satisfies any of these requirements is a function of its chemical structure and physical properties. The interpretation of data obtained in Structure - Activity investigations usually increases in complexity as the level of the investigation moves from the true in vitro system to that existing in the intact organism.

The term 'chemical structure' should not be taken in the sense of classical structure formulae, symbolising



FACTORS DETERMINING BIOLOGICAL EFFECT AND THEIR INTERACTIONS

FIG. 1.

certain organic compounds. It is the complex of spatial arrangements of, and interrelations between, the atoms in the molecule which presents informations on the possible conformations and energetic priorities in these conformations, as well as the inherent charge distribution and the mobility of these charges in the molecule.

In an effort to interpret the structure-activity relationship, two main approaches have evolved-

i) The group or moiety approach - which places emphasis on the significance of certain chemical groups in the molecule for the action[2,3] and

ii) The integral approach - which considers the molecule as a whole, and is particularly concerned with overall physico-chemical properties such as lipid/water solubility, polarity and charge distribution[4,5].

1.1.2 Quantitative Aspects of Structure Activity Relationships (QSAR)

Fundamental work on relationship between chemical structure and biological activity was carried out at the turn of the last century by Richet[6]. He established that the toxic effects of ethers, aldehydes and ketones are inversely proportional to their solubility in water. A linear relationship between surface tension and biological activity of narcotic compounds was established

by Traube[7]. Possibility of quantitative relationship between chemical structure and biological activity of a compound was noted down by Fuehner[8-10], for a series of narcotics, using number of carbon atoms as parameters. Subsequently, Crum-Brown and Fraser[11,12] put forward a quantitative relationship. According to them, physiological action ϕ, of a molecule is a function of its chemical constitution (C), thus

$$\phi = f(C)$$

Important contributions to the studies in structure activity relationships were made by Meyer[13] and Overton[14-16]. They discovered that most organic compounds, foreign to the living organism, penetrate tissue cells as though the membranes were lipid in nature. They further established that the passage across these barrier systems and the subsequent narcotic action of the chemical compounds parallel the oil-water partition coefficients of the structures investigated.

Over the years it has become increasingly apparent that biological activity can rarely be coupled to a single parameter. It has to be envisaged as the result of the interplay of various parameters. The 'partition coefficient' has since emerged as the major parameter in structure - activity relationship studies. An excellent review of the most important

events in QSAR during the last fifty years is published by Purcell, Bass and Clayton[17].

Two major approaches are presently being considered: the empirical mathematical model developed by Free and Wilson[18] and the semiempirical approach of Hansch[19-20].

i) Empirical methods

In this approach the total biological activity of a chemical compound is defined as a function of the sum of individual activity contributions of each of its substituent groups. This method is based on the assumption that in a series of analogs atleast some of the substituent groups contribute to the total activity in an essentially additive manner. The contribution of any substituent towards the total activity is expressed in terms of a denovo constant, derived mathematically from data on biological activity[18]. This method has useful potential when activity of a large series of analogs is being investigated, as it can provide guidelines to suggest the extent of activity of untested members of the series. Many successes have been reported by using this empirical mathematical method[21,22]. The objections to this approach of Free and Wilson[18], as put forward by Hansch[19], consist of the fact that each of the

empirical constants employed, combines, in one figure, the effects of such important factors as lipid solubility as well as steric and electronic effects. As a result, the constants derived from one series of analogs are not applicable to others, having different type of biological activity. Further, variations in biological activity resulting from either stereochemical configuration or from such physical properties as the degree of ionisation are not accounted for in this model.

ii) Semiempirical methods

As the limitations of purely empirical substituent constants became apparent, constants which are directly related to fundamental physico-chemical reference parameters were introduced, with the hope of universal applicability, in the quantitative expression of biological activity[19,20,23]. The relationship proposed by Hansch may be written in the form of what is known as the 'Hansch Equation", which, in turn, may be regarded as expanded Hammet[24,25] equations.

$$\log K_s = \rho \sigma + \log K_o \quad \ldots \qquad \text{eq. (1)}$$

where K_s and K_o are equilibrium constants for the reactions of substituted and unsubstituted compounds. σ is an electronic constant, that depends on the nature and the position of the substituent, and ρ is a constant associated with the type of reaction and condition under

which the reaction takes place.

$$\log (1/C) = K_1 \log P + \rho.6 + K_2 \quad \ldots \quad \text{eq (2)}$$

where C is the molor concentration of a congener necessary to give a defined biological response in any set of structures investigated. P is the partition coefficient of that congener in the octanol - water system. 6 is the Hammet substituent constant, K_1, ρ and K_2 are constants for the set under investigations and generated by regression analysis.

The usefulness of eq. (2) in QSAR has been amply demonstrated[26-28].

In order to include additional parameters a new equation was derived as follows -

$$\log (1/C) = - K_1 (\log P)^2 + K_2 \log P + \rho.6 + K_3 \quad \ldots \quad \text{eq.(3)}$$

The $(\log P)^2$ term was introduced[23,29] with the reasoning that somewhere between P = 0 and P = ∞, an ideal partition value (Po) will exist, for a given set of congeners, in a given biological system such that those members which have this value (Po) will find the sites of action, via a random walk process, in the minimum time[30]. Subsequently, validity of this assumption was supported by Penniston[31]. McFarland[32] indicated, on the basis of probability concept, that the relationship is not entirely, but sufficiently parabolic to justify fully the use of quadratic log P term in the eq. (3).

Principal objections raised against the above mentioned eq. (2) and eq. (3) may be classified into two groups:

i) It is assumed that the octanol-water system is the one of choice for portraying correct picture of what happens to a chemical structure when it is introduced into a biological system. Many doubts have been raised as to the correctness of this supposition.

ii) The eq. (3) may be modified as follows[33]

$$\log (1/C) = -K_1 (\log P)^2 + K'_2 \log P' + K''_2 \log P'' + .6 + K_3 \quad \text{eq.4}$$

In this equation original $K_2 \log P$ term is split into two terms: $K'_2 \log P'$ and $K''_2 \log P''$. Combined with the quadratic term $K_1 (\log P)^2$, the former describes the transport possibilities of the compound and the latter reflects the hydrophobic interactions on a receptor. In practise the eq. (3) is invariably preferred to eq. (4); in other words, it is assumed that the partition values of the octanol-water system provides an adequate description of the two indicated phenomena - transport and hydrophobic interactions - and that P = P''. This means that the lipophilic merits of all membranes that the compound will meet while being transported are basically not different, and are also equal to the lipophilic features of the receptor system. Again this assumption may also be questioned.

Octanol-water was proposed by Hansch and Dunn[26], as the solvent system of choice ($\rho = 1$). Its relatively poor capacity to dissolve water (water saturation concentration = 1.7 M) combined with the presence of a hydroxyl group, which can act as a hydrogen bond donor as well as acceptor, is considered to constitute reasonable model of the average macromolecular pattern in which the drug interaction takes place. Though many more organic water partitioning system have been tried[34], the octanol-water still remains as the preferential choice for estimating partition coefficients in Hansch model.

Applications of Hansch theory, in designing biologically active molecules, are wide spread. The octanol water partition coefficient is regarded as the most important physico chemical constant in the studies of Structure - Activity relationships.

Many methods are available in the literature to determine partition coefficient. These methods have been reviewed by Rekker[35]. The common method employs what is known as 'Shake-Flask' technique, which involves shaking of the solute in a suitable flask with partitioning phases and subsequent analysis of these phases to estimate the solubility.

Hansch approach to the correlation of chemical structure and activity has a great deal of flexibility

and potential. It allows the derivation of equations which not only qualitatively express the several major factors determining the observed activity, but also make possible the quantitative evaluation of each of these factors in fundamental physico-chemical terms. The constant 'log Po' is of primary importance, as this is a constant for any series of compounds and defines the optimum partition coefficient required for maximum penetration and translocation to the site of action. Further, log Po can be determined for any series of materials and in the subsequent search for new and more active compounds of that type, attempts should be made to incorporate susbtituents, which will provide a'log P' value approaching that of'log Po'.

1.2 Chromatography

'Chromatography' is a physical process of separation in which the components to be separated are distributed between two phases - a stationary phase with large surface area and a moving or mobile phase (gas or liquid) in contact with the stionary phase. The chromatographic separation results due to equilibrium distribution of components between these two different phases. Components migrate through chromatographic system only when they are in the mobile phase. Velocity of migration of a compound

depends upon its equilibrium distribution between the two phases. When equilibrium distribution favours stationary phase, compounds migrate slower. Compounds with equilibrium distribution favouring the mobile phase move faster. Thus there is difference in velocities of migration among different compounds. Separationsrresult due to these differences among velocities of migration. Conditions and parameters are manouvered to obtain optimum differential velocities of migration,thereby achieving separations of compounds from each other.

The major aims of chromatography may be summarised as follows:

i) Separation of similar and dissimilar compounds from each other in a mixture.

ii) Qualitative identification based on separation achieved.

iii) Quantitative estimation of separated compounds.

1.2.1 Historical background

Separation process has been an integral and essential part of studies in life sciences. The old Dutch word for chemistry 'Scheikunde' itself literally means'the art of separation' . Separation methods form backbone of chemical sciences and chromatography has achieved, on its merits unique position in solving

separation problems. Though references abound in ancient literature of use of chromatographic principles, knowingly or unknowingly, history of systematic study of chromatography in present day context is only about one hand half century old.

Tsvet[36] is regarded as the founder of modern chromatographic technique. In 1903, he separated chlorophyll from plant pigments and hypothised the chromatography phenomenon. He also coined some of the terms in chromatography nomenclature. Many investigators had earlier employed adsorbents in tubes for examination of mixtures and for decolourisation of solutions[37-41]. Runge[41] and Goppelsroeder[42,43] had used strips and sheets of paper for partial resolution of mixtures. Although the chromatographic methods had been used sporadically in the period between 1910 and 1930[44], it was not until Kuhn's work was published[45] that chromatography came into general use. First experiments in planar chromatography were carried out by Izmailov and Shraiber[46] and Brown[47]. Fundamental work on the theory of partition chromatography was carried out by Martin and Synge[48]. During 1940's extrusion of column materials, as exemplied by Zechmeister and Cholnoky[44] and Reichstein and Shoppee[49] who worked on caretenoids, made way for elution chromatography

which became popular as 'Liquid chromatography'. By nineteen sixties thin layer chromatography (TLC) was widely practised by chemists all over the world. The highly efficient modern column chromatography started with the invention of synthetic packing materials[50]. With the appearance of high performance liquid chromatography[51] in 1967, the very face of chromatography changed as never before. The use of small particle size packings and electronic gadgetry have become standard practice. Earlier, James and Martin[52] had devised first Gas Chroamtograph in 1952, which still remains as one of the most widely practised techniques of chromatography.

1.2.2 Chromatography systems

As already mentioned, chromatography is a method of analysis in which the flow of solvent or gas promotes the separation of substances by differential migration from a narrow initial zone in a porous sorptive medium. Gas chromatography and solution-chromatography are major sub-divisions of chromatography. Gas chromatography may further be categorised as gas-adsorption and gas-solution (gas-partition, gas-liquid) chromatography. Liquid-column chromatography, thin-layer chromatography and paper chrcomatography are sub-divisions of solution

chromatography based upon the form of the sorption system. These subdivisions may be divided further with respect to the sorption phenomena upon which the separations are based e.g. solution-solid adsorption, solution-liquid adsorption, solution-ion exchange sorption and solution - solution distribution or partition. When incorporated in thin layer or in fibrous media as in paper, these modifications of the sorbent may be employed with linear, radical,, segmental and two dimensional flow of the developer.

The sorption capacity and separatory power or selectivity of a chromatographic system are inherent properties of the system itself. These qualities are usually determined empirically and extended by analogy. The overall process of chromatography is a differential migration phenomenon. The separation of the components of a mixture depends upon their special selective retention by the porous sorbent. Their migration is produced by a non-selective driving force, the flow of the developer. The differential migration results from a selective resistive action viz. the selective sorption of the components of the mixture[53]. The components are suitably detected, either within the system itself or by fraction collection for further scrutiny. In chromatography, substances may be described by

their migration relative to that of the developer. This ratio has often been called as R_f and is a characteristic of arrangement for the migration with sorbent and the developer. Each substance has a different R_F value for each sorbent and solvent combination.

1.2.3 Liquid Chromatography (LC)

A chromatography system typically comprises of (i) a stationary phase, (ii) a mobile phase and (iii) mixture of compounds to be separated. The stationary phase is generally termed as 'chroamtographic bed'. The mobile phase has been variantly referred to as 'eluent' or 'moving phase'. The solute mixture is called simply as 'sample'. The separating forces interact on the chromatographic bed to produce differential equilibrium differences for solutes resulting in 'chromatography bands' or 'zones of separations'. Travelling process of sample components from the point of introduction to the point of outlet from the chromatographic system is known as 'elution'. The retardation of compounds due to chromatographic interactions is measured by retention values expressed either in terms of time or volume. As already indicated, retention characteristics are unique for every compounds for a given set of chromatographic

parameters.

The chromatography system may be classified according to the nature of the mobile phase into gas and liquid chromatography. The Gas Chromatography (GC) distinguishes itself by the use of a gas as moving phase, in contrast with the liquid chromatography (LC) where liquids (organic solvents, water, buffer solution, etc.) are used as mobile phases. In GC compounds travel through the system in gaseous state. Compounds should be soluble in moving liquid phase, if liquid chromatography is to be attempted, as they migrate through the system in solution. Due to its very nature,,LC has become most widely practised form of chromatography of today (Fig. 2).

In LC, solubility of components in the mobile phase is the basis of separations. The choice of eluting mobile phase thus becomes a primary importance. The eluting strength of the mobile phase with respect to the stationary phase changes the chemistry of chromatography systems and dictates the extent of separations of solutes from each other e.g. with silica as stationary phase, use of acetonitrile in the place of n-hexane may eliminate any separations obtainable with n-hexane-silica system.

As the mobile phases are liquids, which are relatively non-compressible as compared with gases,

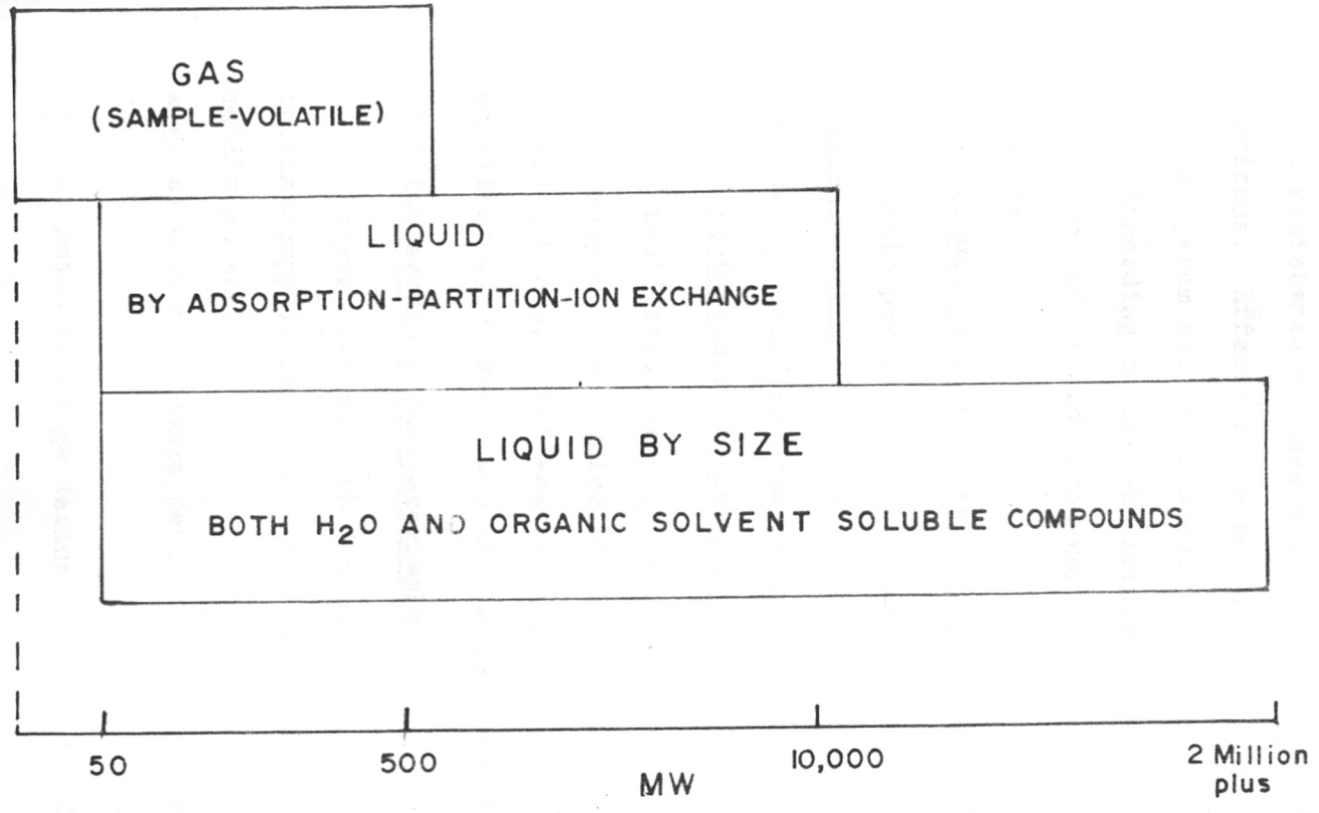


FIG. 2. CHROMATOGRAPHY METHODS - COMPARISON BY MOLECULAR WEIGHTS

diffusion problems assume less severity in LC. But in LC resistance to mass transfer is of higher magnitude. Effects of temperature on the selectivity of the system are of secondary importance.

According to the stationary phase characteristics, five types of liquid chromatography sub-types are in practice.

i) Liquid-Solid Chromatography (LSC)

Solid particles with active sites are used as stationary phases e.g. silica, alumina, cellulose, chemically bonded reverse phase packings (Fig. 3).

ii) Liquid-liquid Chromatography (LLC)

Liquid stationary phase is coated on particulate solid support. An immiscible liquid is then used as mobile phase.e.g. B-B'-oxydipropionitrile, coating on silica with iso-octane as mobile phase (Fig. 4).

iii) Ion-exchange chromatography

Polymer resins with ion-exchange sites provide chromatographic bed and buffer solutions are used as mobile phases.

e.g. a) Cation Exchange resins : strong - $SO_3^- H^+$

weak - $COO^- Na^+$

b) Anion Exchange Resins : strong - $CH_2-N^+-CH_3Cl^-$

CH_3

weak - $-N^+-H-R_2Cl^-$

iv) Gel chromatography

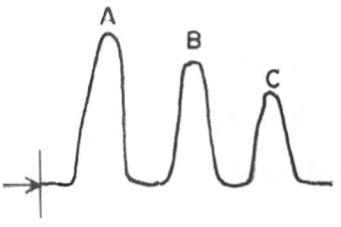
Deactivated silica or synthetic porous polymer beads (e.g. styrene-divinyl benzenes copolymers) of various molecular weight ranges are used as stationary phases. The separation takes place by molecular sizes of solutes. When polar gels in polar solvents are employed, the chromatography is termed as 'gel-filration' chromatography. When less polar resins with non-polar organic solvents are used the process is called as 'gel-permeation' chromatography (Fig. 6).

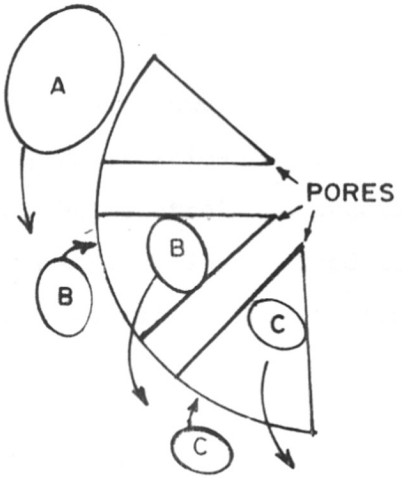
v) Reverse phase chroatography (RPC)

This recently introduced technique uses polar mobile phase and non-polar stationary phase. The chemically bonded stationary phases (e.g. Si-0-R and Si-R) are catagorised under this heading. Polard water misible organic solvents in combination with water are used as mobile phases Fig. (7).

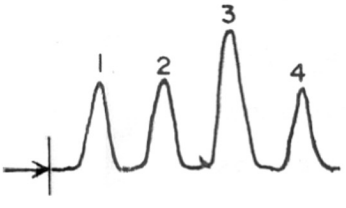
The stationary phases in LC are held in position in a suitable manner to form a chromatographic bed. The mobile phase movement across the so formed bed may be caused by either of the following ways:

i) Gravity - Stationary phase is packed in a tubular strucsture known as a 'column' which is held vertically. Reservoir containing the mobile phase is kept at a level which is higher than that of the column,





μ Styragel – 500, 500, 500, 100, 100°A
THF = 3 ML/min
Phthalates –
1 – Di-octyl –
2 – Dibutyl –
3 – Diethyl –
4 – Dimethyl –



10 μm PL Gel – 10^3, 10^5 A
THF – 2 ML/min

Polystyrene –

1 – 180,000
2 – 24,000
3 – 9,000
4 – 2,100
5 – 580
6 – O-Dichlorobenzene

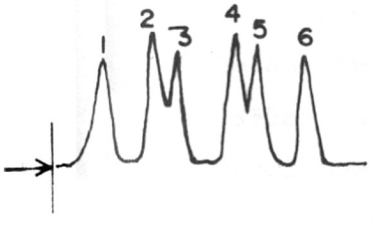


FIG. 3. GPC SEPARATIONS

generally at the top of the column itself. The liquid trickles down by gravitational force. The resulting flow has no other control and precision flow rates cannot be obtained. This method is rather tedious, slow and time consuming. This is classical, liquid column chromatography.

ii) Capillary action: The mobile phase moves against gravity by the capillary action phenomenon as in paper chromatography and thin-layer chromatography.

iii) Use of force: This method is associated with instrumental chromatography, wherein pumping devices are employed to force the liquid mobile phase across the chromatographic column at steady and reproducible rates with precision flow rate control.

The classical 'liquid-column' chromatography became very popular among experimenting chemists from 1950s. Generally a glass tube packed with suitable porous adsorbent is employed to make a column. Mobile phase trickles down at the rate of 2 to 5 ml/10 min by gravity. A number of fractions are collected and monitored by TLC.

With the advances made in physical sciences and electronics, the liquid-column chromatography was revolutionised during 1970s into what was to become modern HPLC (High Performance Liquid Chromatography).

HPLC is still basically a 'liquid column chroamtography'. Narrow mesh range particulate packings of 5 to 10 μm sizes are packed in tubular columns and the mobile phase is pumped by electronically controlled pumping devices to produce reproducible and steady flows of 3 to 7 ml/min. for analytical purposes and upto 500 ml/min. for preparative applications. Quantitative analysis has been a standard practice with the introduction of on-line detection devices in instrumental liquid-chromatography systems.

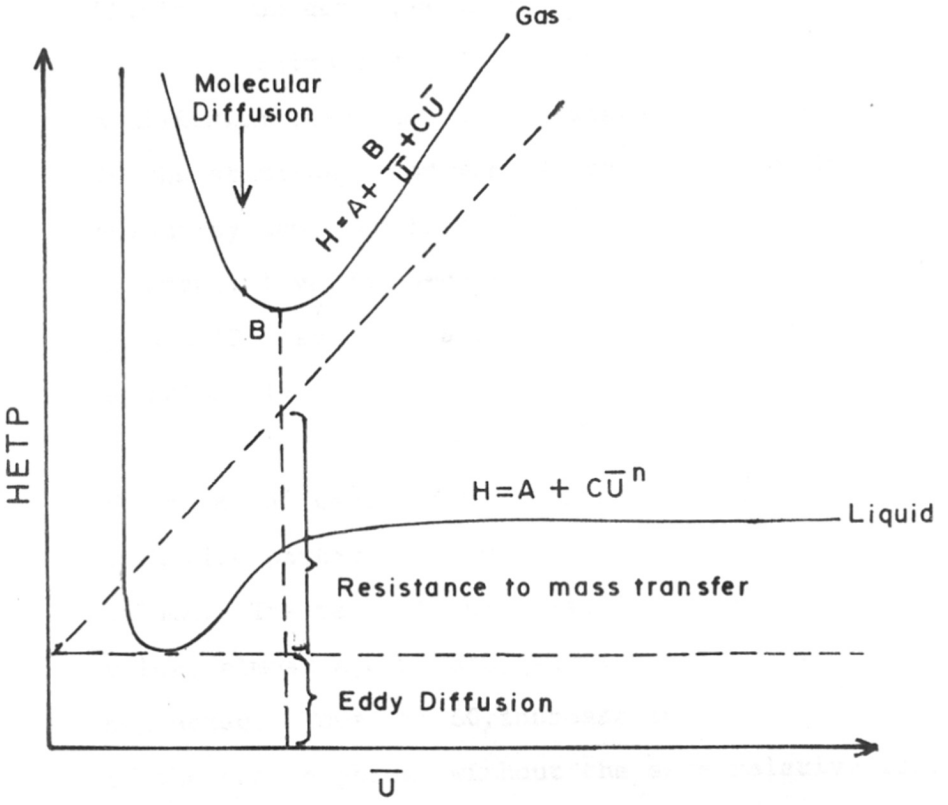
1.2.4 Van Deemter Equation

The efficiency of a chromatography system is expressed in terms of 'number of theoretical plates' a term borrowed from distillation terminology. The 'plate theory' was introduced by Martin and Synge[48] and improved by Meyer and Tompkins[54] and Glueckouf[55]. The Van Deemter Equation expression 'number of height equivalents to theoretical plates' in a chromatographic system may be written as follows:

$$\text{HETP, } H = A + \frac{B}{u} + Cu$$

where u = linear velocity

In the above equation the B term is 'diffusion term' In GC, it is of major contribution to the detriment of H. Longitudinal diffusion in a liquid is generally negligible. Therefore, in liquid chromatography the



GRAPHICAL PRESENTATION OF VAN DEEMTER EQUATION

FIG 4

middle term of the above equation, i.e. $\frac{B}{\bar{U}}$, is of little or no consequence at all.

The third term, $C\bar{U}$, relates to the non-equilibrium resulting from resistance to mass transfer in the stationary and mobile phases within the column system. Further, $C\bar{U}$ has been found to have exponential value. With the elimination of the middle 'B' term, we have for LC, only two term equation viz.

$$H = A + C\bar{U}^n$$

The term 'A' deals with the column and is connected with flow pattern of the mobile phase inside the column. The term 'A' has been found to be small in value, almost a constant, and therefore, is normally neglected. Thus, in LC, increase in linear velocity of the mobile phase, without the same relative loss of plate efficiency, that would occur in GC, is possible. Thus, high speed liquid chromatography is in accordance with theoretical possibility. Decrease in resolution with the increase in linear velocity of the flow of the mobile phase results to a lesser extent. The high speed LC envisaged by this theory has to be carried out only with the help of instruments. The resolutions are maintained, or even incrased, by choosing smaller diameters of packing materials. The back pressure developed by smaller

packing materials is overcome with the use of high pressure pumping devices in instrumental LC Fig. (8).

1.2.5 The present status of LC

With the introduction of HPLC instruments in 1970, interest in LC was revived. The last decade has witnessed explosive growth of applications of instrumental LC in various forms of HPLC. The development of small particle size (less than 5 μm) packing materials and extra sensitive online detection devices have made HPLC, the method of choice for chemical and biochemical studies. Further, development of chemically bonded reverse phase column packings, which combine the advantages of both partition and adsorption chromatography, have opened up new areas, hitherto unexplored. The reverse phase packings are also used in TLC and the technique is known as 'High Performance TLC' (HPTLC). The analysis time of instrumental LC is typically about 10 min. Many versions of instrumental LC are now available for variety of chromatography applications. They include purely analytical work, semipreparative scale of operation, upto few grams, and gigantic preparative instruments handling upto a kilogram of sample at a time. Today the application of LC is more wide spread than anyother ferms of chromatography.

It is expected that, during next decade or so, HPLC will surpass all other chromatography techniques in terms of utility. Though the classical liquid chromatography is still being widely practised in our country, the latest developments in this field, like 'flash chromatography' for bench scale preparative work and precoated normal and reverse phase TLC plates, are being made available indigenously. The knowledge of chromatography has already become an essential prerequisite for a successful chemist in every branch of chemical sciences.

1.3 Chroamtography and Biological Activity

Quantitative Structure - Activity Relationships, (QSAR), as expounded in section 1.1 envisage, determination of hydrophobic or lipophilic nature of a compound, which in turn is correlated with its biological activity. The hydrophobicity of a compound is usually characterised by partition coefficient 'P' obtained from distribution studies of the compound between a polar and non-polar and practically immiscible solvent pair. Pioneering work of Martin and Synge[48] and Consden[56] in establishing relationship between retention, R_F, values obtained from partition chromatography and partition coefficient has led to the use of hydrophobic parameters obtained from

chromatographic measurements in QSAR models[57-59]. BateSmith and Westall[60] introduced the term,

$$Rm = \log \left(\frac{1}{R_F} - 1 \right)$$

and showed experimentally that the relationship predicted by Martin[61] was followed for a number of flavones, anthocyanins, etc. E. Tomlinson[62] had reviewed earlier work involving application of chromatographic parameters in QSAR. Recently R. Kaliszan[63] has also published a review concerning chromatographic applications in determination of many contributory parameters in QSAR.

1.3.1 Conditions for chromatographic determination of hydrophobicity

i) Control of partitioning mechanism

It is important to make chromatographic determinations in systems where paritions either is the sole process taking place or predominant over others. Partition chromatography may be carried out by means of TLC, paper chromatography and HPLC. Reverse phase techniques are prevailing in chromatography studies aimed at establishing relation between partition coefficient in QSAR and retention patterns in LC. A few successful biological correlations have been obtained using direct technique in paper chromatography[64], TLC[65] and GLC[66].

Silicone oil, 1-octanol, oleyl alcohol, liquid paraffin and chemically bonded phases are used as hydrophobic stationary phases.

When the partition chromatographic Rm value is related to the log Ps, determined in a system identical with the chromatography systems, following relationhip is obtained[63].

$$Rm = \log Ps + \log \frac{Vs}{Vm}$$

where Vm and Vs are volumes of mobile and stationary phases respectively. A slope of unity is expected only if two chromatographic phases are precisely identical with the two phases in the classical 'shake-flask' experiment[67]. The deviation from the unity may be attributed to the difference in ionic strengths in the shake-flask and chromatographic processes[68].

ii) Choice of partitioning system

Chromatography with pure water as the mobile phase is not always possible, owing to too low or too high solute polarity. Accuracy in TLC requires[69] $0.2 < R_F < 0.8$. This gives range of Rm as less than 1.5 decades. On the other hand, HPLC is capable of producing Rm values of three to four decades[70]. It is often necessary to change composition of a mobile phase in order to obtain reliable Rm values

for a series of compounds of varying polarity. Rm values of all compounds in a series for any fixed organic solvent concentration in the mobile phase can be used for QSAR purposes. Sometimes Rm values are obtained by extrapollation to a 100% water concentration in the mobile phase by using Soczewinski and Wachtmeister methods[71]. Proper care should be taken while dealing with ionisable compounds. Many methods have been reported in the literature to deal with such situations[63].

1.3.2 Present work

With the advant of HPLC and developments in chemically bonded RPHPLC packings, the chromatographic determinations of hydrophobicity have acquired new dimensions. Non-polar stationary phase columns, like octadecylsilane bonded to silica surface, provide excellent lipophilic systems for determiantion of log P values. In spite of the existence of well established[72] HPLC methods for the determination of hydrophobicity, a few practical applications in QSAR have been reported. Recently, Henry[73] and Vitali[74] reported some good correlations.

The present dissertention attempts, for the first time, to establish a relationship between insecticidal activity of series of compounds and their retention behavior expressed as

the differance in selectivities between a non-polar and an intermediate polarity column in a similar solvent system. An octadecyl silane based non-polar column and propionitrile based moderately polar column have been used to record retention times in two types of mobile phases viz. acetonitrile-water and methanol-water. The compounds selected for this study were synthesised as described in Chapter II with hexachlorocyclopentadiene as a common moiety. They were tested against 4th instar larvae of Aedes aegypti, and in some cases Musca domestica L. as well, for their insecticidal activity. Of the total fifty four compounds synthesised, forty five compounds are new. Thirty compounds exhibit insecticidal activity. The chromatography system used for these studies has been described in detail in Chapter III. Two constants have been newly derived from retention time data and have been successfully correlated with insecticidal activity, patterns of compounds in a series. Their contribution in predicting insecticidal activity of a compound in a series is discussed in Chapter IV. Further, they are also good pointers directing the right path in designing syntheses of compounds of a series which will have desired insecticidal activity.

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CHAPTER - II

Synthesis of bicyclo (2.2.1)hept-2-ene, 1,2,3,4,7,7,-hexachloro-5- and 5,6 derivatives and their insecticidal activity

SUMMARY

Compounds discussed in this chapter were synthesised with 1,3-cyclopentadiene 1,2,3,4,5,5-hexachloro (HCCP) and various dienophiles. This chapter describes their spectral data, physical properties and insecticidal activity against 4th instar larvae of Aedes aegypti and Musca domestica L. (Housefly). First part of the chapter deals with reactions of HCCP and explains types of these reactions and compounds synthesised. Their applications have also been cited. Many of them are used as pesticides while some are used in fire resistant formulations.

The present syntheses has been divided into three major schemes using Diels-Alder reactions with allyl alcohol (Scheme 1), acrylic acid and alkyl acrylates (Scheme 2) and maleic anhydride (Scheme 3). The resulting compounds viz. 5-hydroxymethyl (i) 1,2,3,4, 7,7-hexachloro bicyclo (2.2.1) hept-2-ene (ii) 1,2,3, 4,7,7-hexachlorobicyclo (2.2.1) hept-2-ene, 5-carboxylic acid and (iii) chorendic anhydride were further reacted with different compounds to synthesise nine series of compounds. Some reported compounds were also prepared for the sake of continuity of series.

Thus this chapter presents synthesis and insecticidal data of the compound.

Pesticides are chemicals designed to combat the attack of various pests on agricultural and horticultural crops. Pesticides may be divided into three major classes as follows: Insecticides, Fungicides and Herbicides (weed killers). Rodenticides for the control of vertebrate pests, nematicides used to kill microscopic eel-worms, molluscides for slugs and acaricides for killing mites are also, by convention, covered by the term pesticides. Pesticides may also be classified into two types, based on their action; viz. contact or non-systemic pesticides. Most of the earlier period pesticides were non-systemic in character whereas many of the more recent pesticides are systemic. The advantages of systemic pesticides are obvious in the sense that only the crop eating pests are affected.

Though the use of pesticides has fairly long past history, it was not until the middle of the nineteenth century that systematic scientific methods began to be applied to the problem of controlling agricultural pests. The successful introduction of dichlorodiphenyl trichloroethane (DDT) in 1940's led to the development of many different types of organochlorine compounds as potent contact insecticides[1]. Though several insecticidal chlorinated hydrocarbon cyclodiene compounds were introduced, they did not come into wide spread use until

the middle 1950's. Some of the more wellknown commercial products of this type are Aldrin, Dieldrin, Endrin, Heptachlor etc.

Development of specific resistance to a class of compounds by different pests led to a continuous search of new organic compounds to control the resisting species of pests. When it was illustrated that in the case of insects becoming resistant to DDT they also generally resist to the related compounds like DDD and methoxychlor, but not the cyclodiene insecticides, e.g. Aldrin, which falls into another cross resistance group, the development of cyclodiene group of insecticides took a new turn. Hexachloro-cyclopentadiene (I) is the key intermediate for many commercially available cyclodiene pesticidal compounds[2].

2.1 Hexachlorocyclopentadiene (HCCP) (I)

Hexachlorocyclopentadiene (I) appears in the chemical literature[3] in 1930, though possibility of knowledge of the compound before this time[4] does exist. Commercial methods for the preparation of (I) and its derivatives, for preparation of fungicides, insecticides, plant growth regulators and flame retardant resins are only about quarter century old. The extraordinary interest in this compound is reflected in the large number of derivatives reported in a relatively short period of time.

2.1.1 Physical properties of HCCP(I)

HCCP (I) is a pale yellow[5] or greenish yellow[6] liquid, with a boiling point[7] of 236-238°C at 760 mm. It has a harsh pungent odour and its viscosity[8] is n^{20} = 37.5 dynes/cm. A crystalline form of HCCP melting at -0.8°C may be converted to a more stable form, melting at 10 to 10.8°C by strong cooling or seeding[9,10].

2.1.2 Biological properties of HCCP (I)

HCCP (I) is toxic to mice and was thought to be responsible for pesticidal properties of chlordane[11]. The liquid may be absorbed through the skin and its vapour is toxic. The physiological effects of the vapour are believed to be similar to those of the carbon tetrachloride vapour, and the material should be handled with caution[12,13]. The LD_{50} value in rats is reported to be 300 mg/kg.of body weight[12]. HCCP(I) has also been claimed to exhibit fungicidal and bactericidal[13] properties.

2.1.3 Methods of preparation of (HCCP) (I)

Though many methods are reported[14], HCCP is best prepared by a two step process, from pentane and isopentane or the mixtures of the two. The hydrocarbons are first chlorinated photochemically to polychloropentanes which are passed over a porous surface-active solid, in vapour state. The octachlorocyclopentane (II) formed as an intermediate, is isolated and dechlorinated at 500°C to obtain HCCP(I) in 90% yield.

2.1.4 Reactions of HCCP (I)

HCCP (I) shows a tendency to undergo Diels Alder reaction with many dienophiles at temperatures between 20 to 200°C. It condenses even with simple olefins which normally do not react with dienes and with polynuclear aromatic hydrocarbons like naphthalene and anthracene. The condensation is the most extensively investigated reaction of the diene and has furnished a variety of new compounds which are not accessible by other methods[14]. Open chain and cyclic olefins of the type RCH = CH_2 and R-CH=CH'R containing 4 to 18 carbon Atoms have been successfully condensed[15-18] with HCCP(I). The products are 1:1 adduct which are assigned a (2.2.1) bicycloheptene (III) nomenclature, largely by analogy with the structures of non-chlorinated compounds. Acetylene and substituted acetylene react with (I) to give (2.2.1) bicycloheptadienes (IV)[19]. Chlordene (V) as an adduct of (I) is used as a starting material for commercial insecticides like chlordan (VI) and Heptachlor (VII).

Equimolar proportions of (I) and (IV) react to give hexachlorotetracyclodecadiene (Aldrin)(VIII), which can be converted to the epoxy compound Dieldrin (IX). An isomer of Aldrin known as Isodrin is produced when cyclopentadiene is condensed with

hexachloro (2.2.1) bicycloheptadiene. Isodrin when epoxidised gives Endrin which is an isomer of Dieldrin. Both Isodrin and Endrin are commercially available insecticides.

When the dienophile has multiple double bond belonging to an aromatic system such as naphthalene, it becomes difficult to isolate 1:1 adduct with (I), because the reamining unsaturation in the reacting nucleus is activated and reacts with greater ease with a second molecule of the diene to give a bis-product (X). Anthracene also yields a bis product (XI)[20-22]. The aromatic portion of the addition compound is capable of undergoing substitution reactions. (I) can be condensed with good yields with allylic compounds[23] giving substituted (2.2.1) bicycloheptenes. Compounds with activated double bonds undergo Diels-Alder reaction with (I) quite easily. The adducts with styrene and substituted styrenes are fungicides[24]. Butadiene monoxide gives an adduct with (I) which is a flame proofing agent and stabiliser for vinyl chloride[16]. A number of such addition compounds are reported in literature[14].

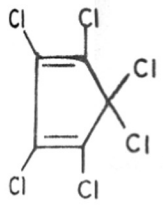
Unsaturated aldehydes, ketones, acids and esters add to (I) to form adducts, many of which are plant growth regulators causing defoliation in deciduous plants, regulating flowering and shoot development, and

in general acting as antagonist to Indole-acetic acid[25-27].

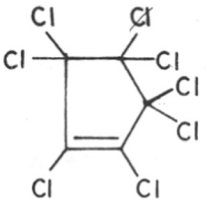
Chlorendic acid (XII) and chlorendic anhydride (XIII) which are commercially available[28,29] are prepared with maleic anhydride and (I) or as an oxidation product of chlordene[30]. The acid is remarkably stable to alkali. The chlorine atoms are not affected even by prolonged refluxing with aqueous or alcoholic bases[28-31].

(XII) and (XIII) may be converted to corresponding diesters by heating with simple alcohols and acid catalysts[31] or by Diels-Alder reaction with alkyl maleates[28]. Monoesters are prepared from anhydride and monohydric alcohols at room temperature without catalyst. Many of these esters exhibit lubricant characteristics[32]. Polyhydric alcohols react with (XIII) to produce unusually flame resistant alkyd type resin[33]. Reaction of dihydric alcohols and (XIII) result in soluble thermoplastic linear polyester resins while polyhydric alcohols give thermosettings resins[31]. (XIII) can be used to acylate benzene and toluene with aluminium chloride[29].

HCCP (I) can be chlorinated photochemically[6] or in the presence of aluminium chloride[34] to yield (II). It also undergoes dimerisation reaction to a chlorocarbon $C_{10}Cl_{12}$ when heated with aluminium



(I)



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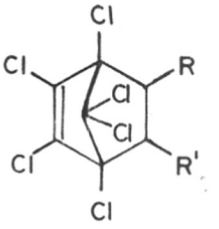

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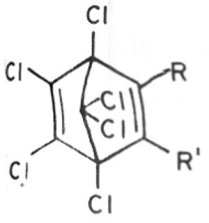

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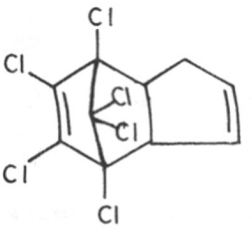

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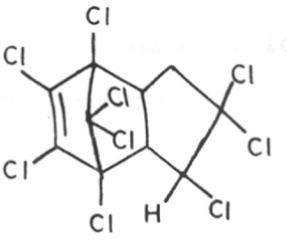

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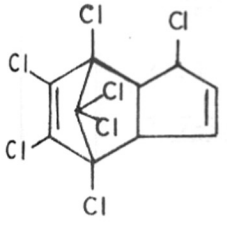

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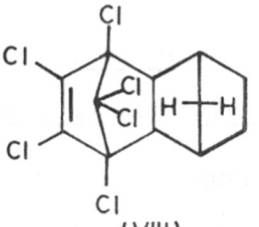

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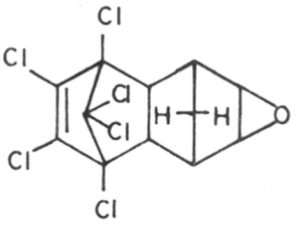

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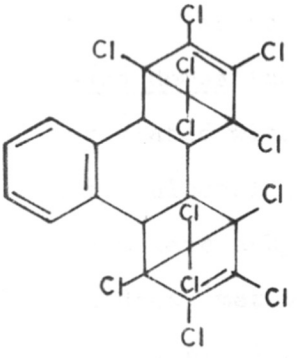

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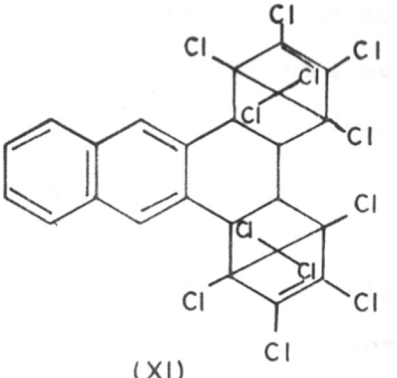

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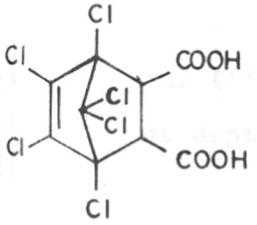

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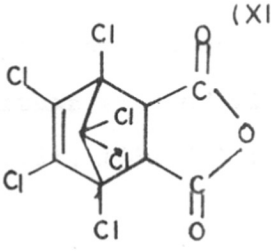


(XIII)

FIG. I.

chloride without a solvent[6].

Catalytic hydrogenation of (I) proceeds stepwise 1,2,3,4-tetrachlorocyclopentadiene obtained initially gives cyclopentane on further hydrogenation[35]. The hydrogenation catalysed by platinum, stannous chloride and zinc-hydrochloric acid result in the same intermediate. HCCP (I) may be decomposed by alkali in a controlled manner to form dialkoxy ketals[5]. The analogous thioketals are used as extreme pressure additives for lubricating oil and rust inhibiting compound in mineral oil[36].

2.1 Synthesis of bicyclo (2.2.1) hept-2-ene 1,2,3,4, 7,7-hexachloro, 5- and 5,6 derivatives

Forty five new compounds were derived from (I) and their insecticidal activity tested against 4th instart larvae of Aedes aegypti (mosquito larvae) and Musca domestica L. (housefly). Three schemes were formulated with (I), using Diels Alder reactions with allyl alcohol (Scheme 1), acrylic acid (Scheme 2) and maleic anhydride (Scheme 3). The resulting compounds 5-hydroxy methyl 1,2,3,4,7,7-hexachloro, bicyclo (2.2.1) hep-2-ene (XIV)[23], 1,2,3,4,7,7-hexachloro bicyclo (2.2.1) hept-2-ene, 5-carboxylic acid (XVI)[27,37] and (XIII) were further reacted with different compounds as depicted in Fig.(2), (3), (4) respectively to

SCHEME–1

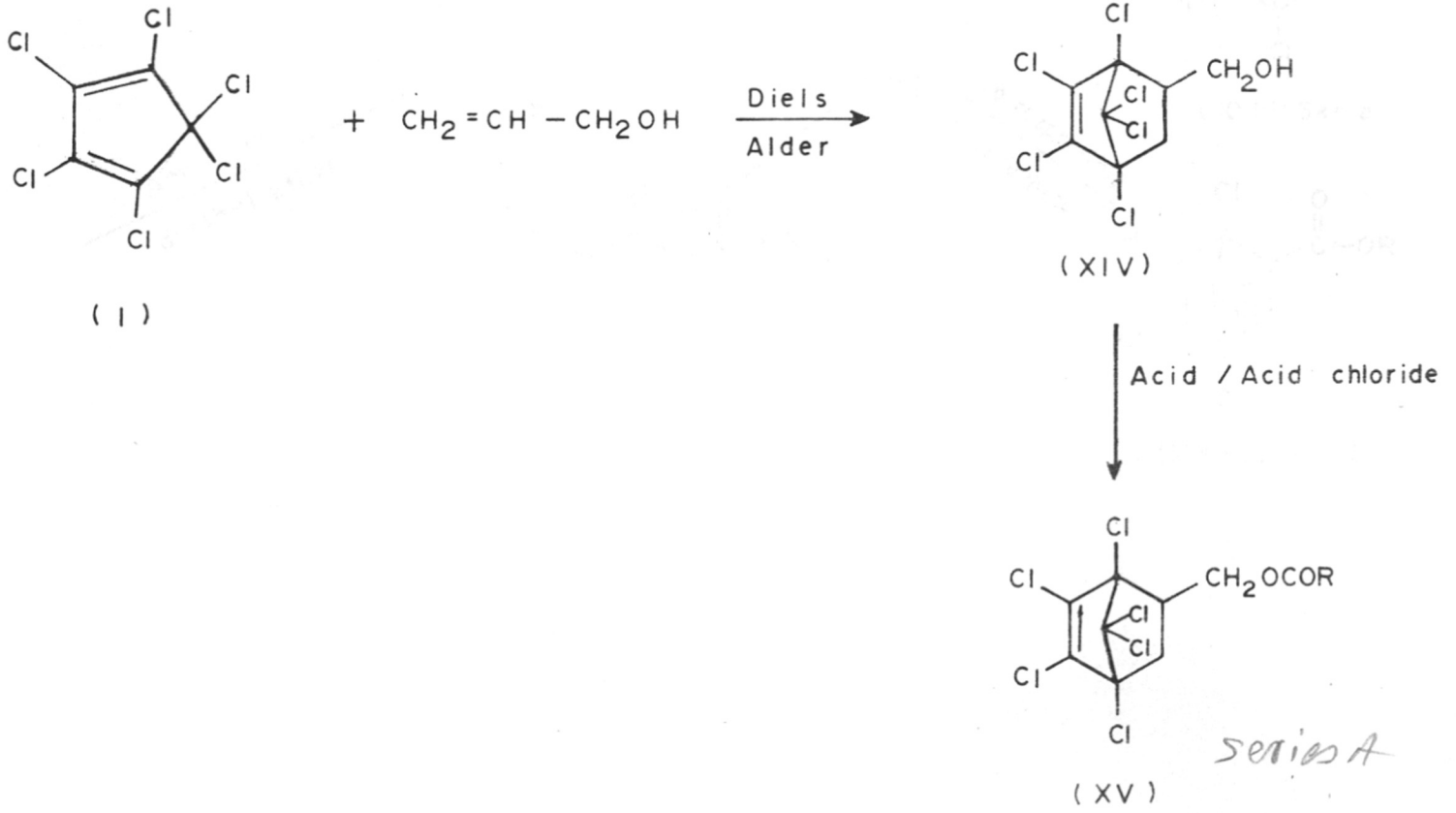


FIG. 2.

50

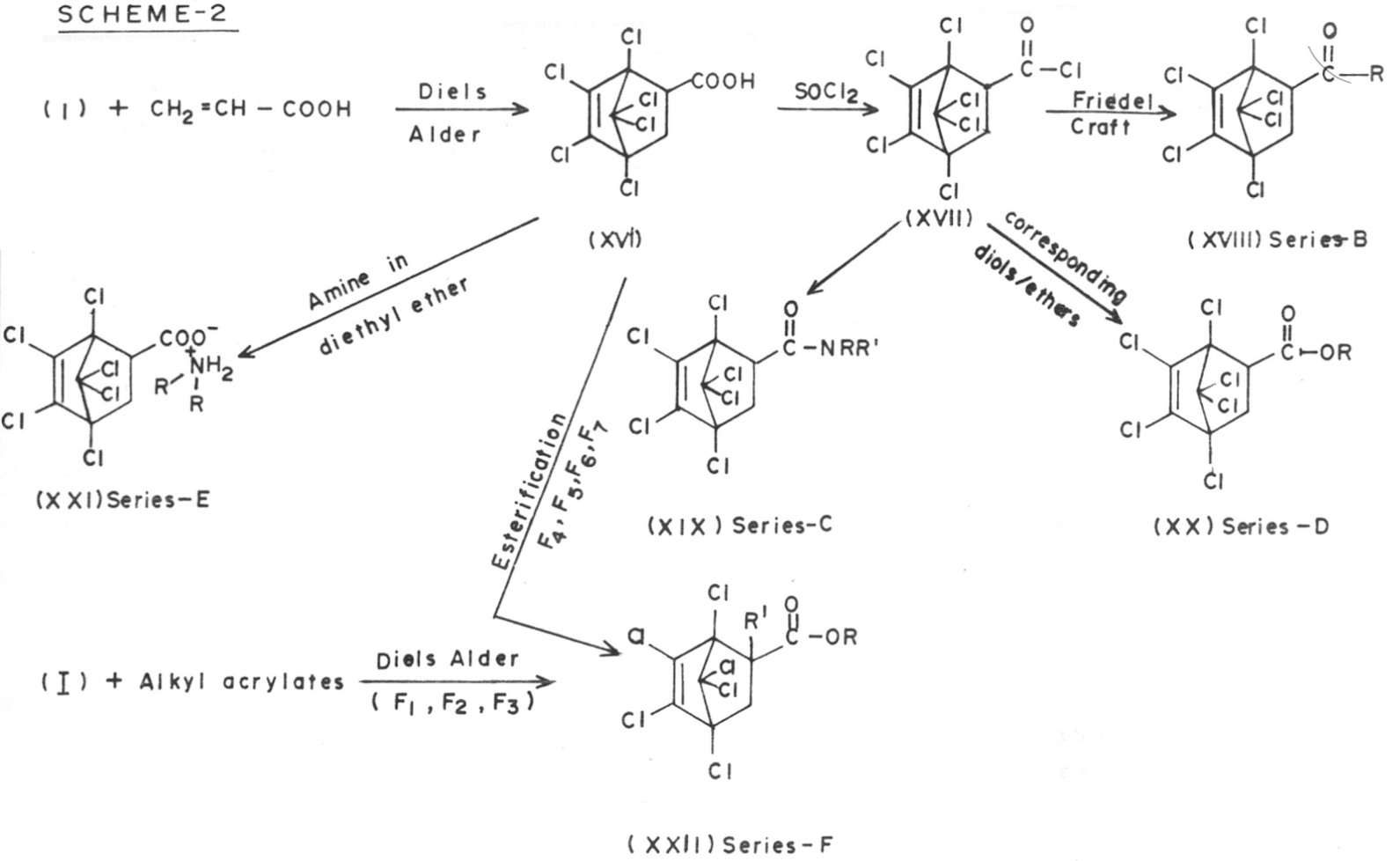

SCHEME-2
(I) + CH2=CH-COOH
Diels Alder
COOH
SOCl2
C-Cl
Friedel Craft
C-R
(XVI)
(XVII)
(XVIII) Series-B
Amine in diethyl ether
COO⁻
NH2
R
R
(XXI) Series-E
Esterification F4, F5, F6, F7
C-NRR'
(XIX) Series-C
corresponding diols/ethers
C-OR
(XX) Series-D
(I) + Alkyl acrylates
Diels Alder
(F1, F2, F3)
R'
C-OR
(XXII) Series-F

FIG. 3

SCHEME – 3

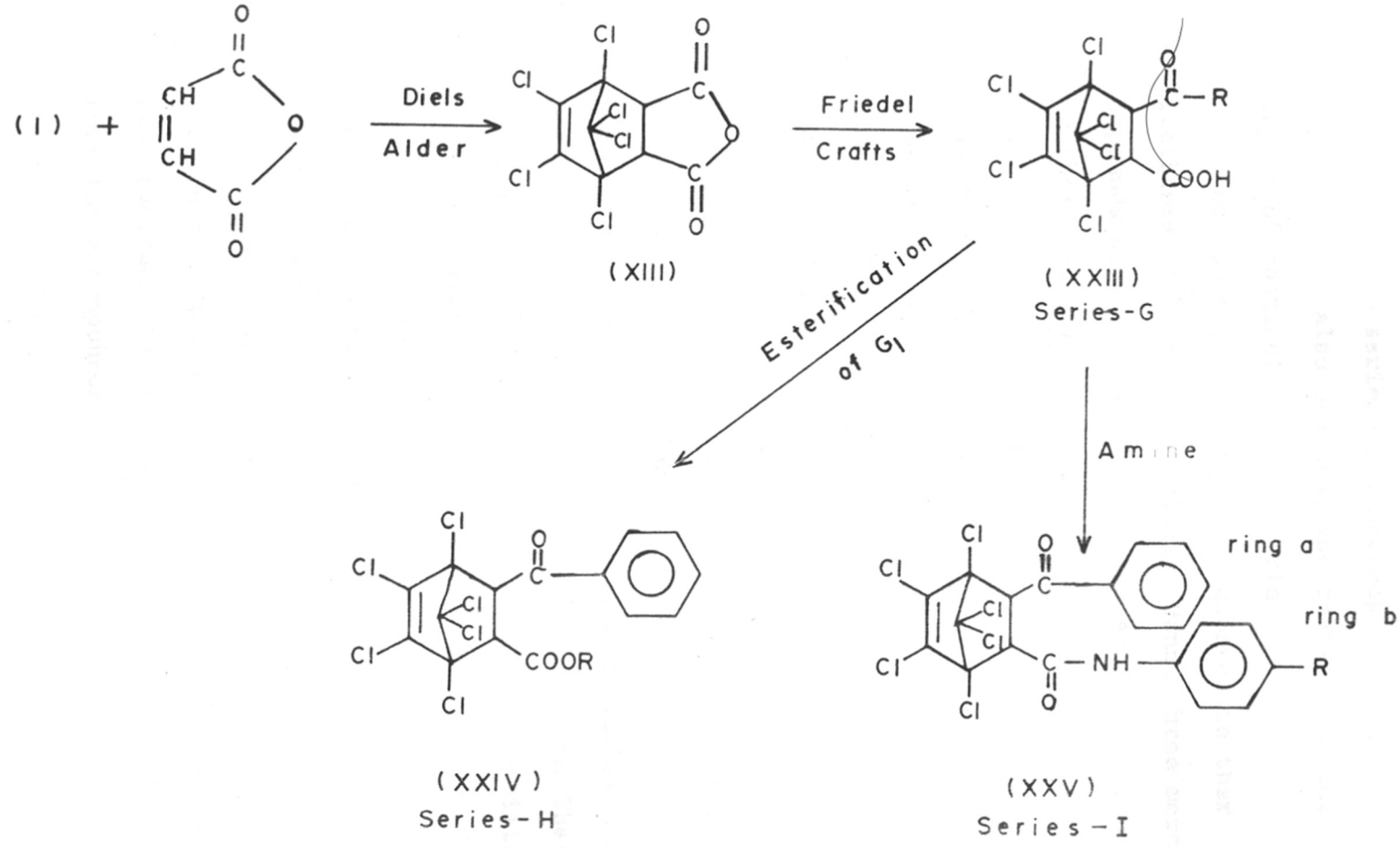


FIG. 4.

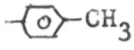
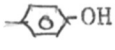
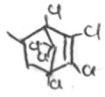
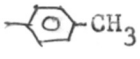
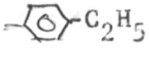
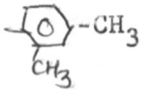
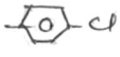
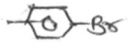
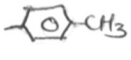
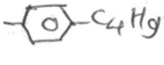
prepare various series of compounds. Some reported compounds were also prepared and tested, for the purpose of continuity of the series.

Compounds (A_1 and A_2) were reported in the literature[23,38] as insecticides. Hence three more compounds were synthesised (Series A) from compound (XIV) and their insecticidal activity was tested. The newly synthesised compounds showed marginal insecticidal activity as compared to A_1 and A_2. The CH_2OCO linkage of series A was replaced by keto group (C=O) in the side chain for series B. These compounds were found to be slightly more active. As the series B compounds are lipophilic in nature, with a view to increase their activity, a nitrogen linkage was introduced to synthesise series C. However, the insecticidal activity pattern did not change substantially; the compounds were inactive or marginally active. Therefore, in an effort to make the molecule more hydrophilic, a free hydroxyl group or ether linkage was introduced (Series D). This modification did change the insecticidal activity substantially and all but one compound were found to be inactive. Further, one more hydrophilic series was synthesised comprising of quaternary ammonium salts (Series E). The compound E_7 was reported as a defoliating agent[27]. In this series, only three compounds had marginal to moderate activity

against mosquito larvae. Series F was a modification of series D, synthesised to remove free hydroxyl group and to introduce alkyl chain. Though four compounds (F_1 and F_3)[39], (F_2)[49] and (F_6)[27,41] were known, only (F_6) was reported for detoliating activity. Majority of the compounds of this series showed moderate to very good results. The compound F_5 showed 100% mortality against both test species.

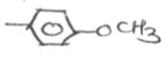
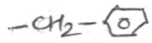
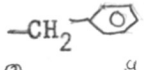
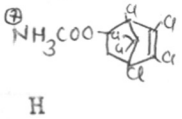
The Scheme-3 resulted in the disubstituted products. Series G comprises of 7 compounds with a free carboxylic group. Synthesis of compound G_1 is reported in the literature[29] but not its insecticidal activity. All the compounds in this series were either inactive or exhibit marginal activity. Assuming that the inactivity was attributable to the free hydroxyl group, compound G_1 was esterified to prepare series H. However, the resulting compounds were also found to be inactive. Only compound H_3 showed moderate activity against houseflies. The compound G_1 was then further derivatised with p-substituted anilines and the resulting anilides were subjected to tests (Series I). As was evident from the data, these compounds showed marginal to moderate activity against mosquito larvae yielding an improvement over earlier two series G and H.

Table II (1)

S.No.	Compd.	R	R'	% Mortality at 5 ppm[a] Mosquito	Housefly[b]
1	2	3	4	5	6
1	A_1	$-CH_3$	-	50	
2	A_2	$-CH_2CH_3$	-	27	
3	A_3		-	7	
4	A_4		-	77	
5	A_5		-	4	
6	B_1		-	14	
7	B_2		-	34	
8	B_3		-	10	
9	B_4		-	37	
10	B_5		-	14	
11	B_6		-	7	
12	C_1	H		8	
13	C_2	H		10	
14	C_3	H		14	

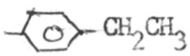
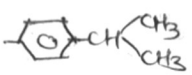
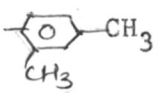
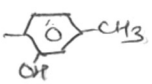
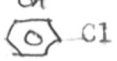
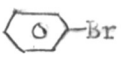
cont....

Table II (1) continued

1	2	3	4	5	6
15	C_4	H		15	
16	C_5	H		7	
17	C_6	H		0	
18	C_7	CH_3	$-CH_3$	0	
19	D_1	$-(CH_2)_2OH$	-	0	
20	D_2	$-(CH_2)_3OH$	-	0	
21	D_3	$-(CH_2)_4OH$	-	0	
22	D_4	$-CH_2CHOHCH_2OH$	-	0	
23	D_5	$-(CH_2)_2O(CH_2)_2OH$	-	0	
24	D_6	$-(CH_2)_2OCH_3$	-	17	
25	D_7	$-(CH_2)_2OC_2H_5$	-	0	
26	E_1	H	$-C_2H_5$	0	0
27	E_2	H	$-n\text{-}C_4H_9$	0	0
28	E_3	H	$-C(CH_3)_3$	00	0
29	E_4	$-CH_3$	$-CH_3$	45	0
30	E_5	H		4	0
31	E_6	H		7	0
32	E_7	H		0	45
33	F_1	$-CH_3$	H	14	5
34	F_2	$-C_2H_5$	H	16	30

cont.....

Table II (1) continued

1	2	3	4	5	6
35	F_3	CH_3	$-CH_3$	24	5
36	F_4	n-C_3H_7	H	10	0
37	F_5	iso-C_3H_7	H	97	100
38	F_6	n-C_4H_9	H	44	40
39	F_7	n-C_5H_{11}	H	0	00
40	G_1		-	0	0
41	G_2		-	0	0
42	G_3			0	10
43	G_4		-	0	0
44	G_5		-	0	0
45	G_6		-	0	100
46	G_7		-	0	0
47	H_1	$-n-C_3H_7$	-	0	0
48	H_2	$-n-C_4H_9$	-	00	0
49	H_3	$-iso-C_4H_9$	-	0	20
50	I_1	H	-	10	0
51	I_2	$-CH_3$	-	20	0
52	I_3	$-n-C_4H_9$	-	10	12
53	I_4	$-OCH_3$	-	17	0

Serie A to D could not be tested against houseflies

2.2.2 Experimental

IR spectra were recorded on a Perkin-Elmer 599B Infrared Spectrophotometer (ν_{max} in cm^{-1}). PMR spectra were recorded in CCl_4 solution on T-60 (Varian) and in $CDCl_3$ on FT-80A (Varian) and WH-90 MHz FT-NMR (Brucker) spectrometers using TMS as an internal standard. Purity of the compounds was checked by either GC or HPLC. All m.ps. were uncorrected.

Scheme 1

Series A: (XVI) 5-Substituted hydroxy methyl, 1,2,3,4,7, 7-hexachloro, bicyclo (2.2.1) hept-2-ene.

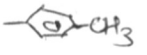
(XIV) was esterified by two methods:

Method (1): By using acid chloride

To an ice-cooled and stirred etheral solution of 3.31 gms (0.01 mole) of compound (XIV), 3.80 gms (0.01 mole) of compound (XVII) were added. Et_3N was taken in catalytic amount. It was then stirred overnight at room temperature. The reaction mixture was poured in excess water. The ether layer was separated, washed withwater (10 ml x 2), dried over Na_2SO_4 and distilled to give ester A_5 (82%). A_5 was recrystallised from aqueous methanol. A_1 was synthesised using acetyl chloride.

Method (2): By using acid

3.31 gms (0.01 mole) of (XIV) was dissolved in 50 ml of dry xylene. 1.36 gms (0.01 mole) of p-toluic acid and p-toluene sulphonic acid (catalytic amount) were added to xylene solution. This mixture was refluxed for three hours. Xylene was then removed by steam distillation. The residue was taken up in chloroform, neutralised by sodium bicarbonate and dried over Na_2SO_4. Chloroform was evaporated to obtain A_3 in 70% yield. Compound A_2 and A_4 were prepared similarly.

A_3: R = 

Recrystallised from pet.ether (60-80°C).

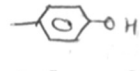
M.p. 87°C.

Elemental analysis Found: C, 42.41; H, 2.93; Cl, 46.90

calculated for $C_{16}H_{12}Cl_6O_2$ C,42.76; H, 2.67; Cl, 47.44.

IR bonds: 1730 (- C(=O)-O-), 1610 (C=C), 1190 (C-O-C-).

PMR: δ 2.0 (2H, m, C_6-H), 2.28 (3H, s, Ar-CH_3), 2.73 (1H, dd, J = 8 and 12 Hz, C_5-H), 4.30 (2H, d, J=6 Hz, -CH_2-COO-), 7.20 and 7.95 (2H each, d each, J = 8 Hz each, Ar-H).

A_4 R = 

Recrystallised from CCl_4.

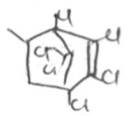
M.p. 162°C.

Elemental analysis Found: C, 39.54; H, 2.38; Cl, 46.78

calculated for $C_{15}H_{10}Cl_6O_3$ C, 39.91; H, 22.17; Cl, 47.21.

IR: 1690 (C=O), 1610 (C=C).

PMR: δ 1.90 (2H, m, C_6-H), 2.76 (1H, dd, J = 9.6 and 12.8 C_5-H), 4.2 (2H, d, J = 6.4 Hz - CH_2COO-), 6.60 (1H, s, -OH), 6.76 and 7.84 (2H each, d each, J = 8 Hz each, Ar-H). Fig. (5).

A_5: R = 

Recrystallised from aqueous methanol.

M.p. 130°C.

Elemental analysis Found: C, 29.08; H, 1.21; Cl, 63.52

Calculated for $C_{16}H_8Cl_{12}O_2$ C, 29.78; H, 1.22; Cl, 64.74.

IR: 1745 (-C=O), 1605 (C=C), 1190 (C-O-C).

PMR: δ 2.56 (4H, m, C_6 - protons from two bicyclo systems), 3.60 (2H, dd, J=6 and 10 Hz, C_5-H from two bicyclo systems), 4.10 (2H, q, J=6 Hz, $-CH_2COO$-).

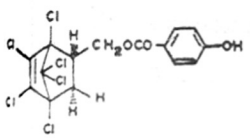
Scheme - 2

Series B: (XVIII)

Ketone 1,2,3,4,7,7-hexachloro-5-yl, bicyclo (2.2.1) hept-2-ene

Friedel-Crafts reaction

To an ice-cooled and stirred solution of 3.60 gms (0.01 mole) of compound (XVII)[42] in benzene (50 ml), 1.33 gms (0.01 mole) of anhydrous $AlCl_3$ were added. After two hours, 10 ml water was added slowly which was



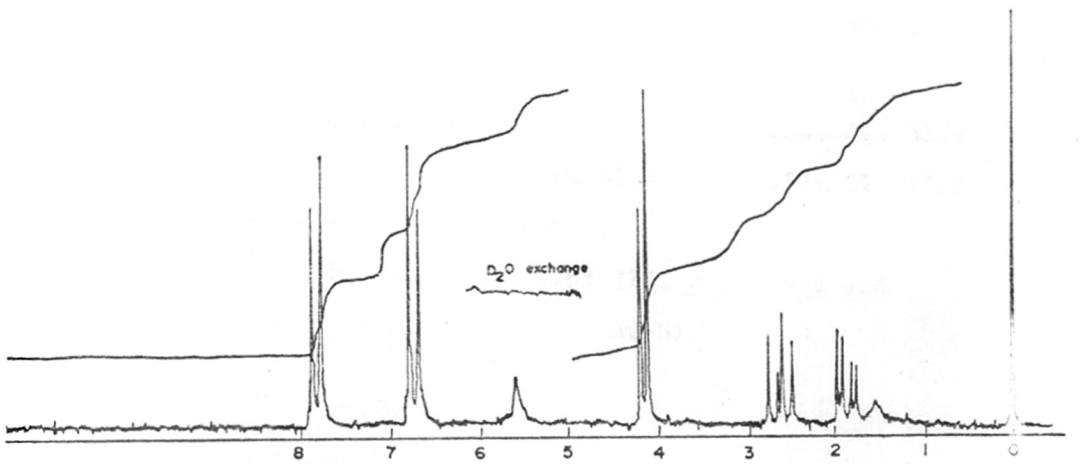


FIG (5) 5-[(4-HYDROXYL) BENZOYL]-HYDROXYMETHYL 1,2,3,4,7,7-HEXACHLORO BICYCLO (2·2·1) HEPT-2-ENE

followed by 10 ml of 10% HCl. This mixture was kept overnight. Excess of benzene was removed by steam distillation. Residue was extracted by chloroform (50 ml x 2) and dried over Na_2SO_4. chloroform was evaporated to yield 80% B_1. The compounds B_2, B_3, B_4, B_5 and B_6 were prepared in same manner.

B_1 R = 

Recrystallised from aqueous methanol

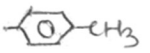
M.p. 82°C.

Elemental analysis Found: C, 41.85; H, 2.34; Cl, 51.88

Calculated for $C_{14}H_8Cl_6O$ C, 41.48; H, 1.97; Cl, 52.59.

IR : 1680 (>C=O), 1600 (>C=C<).

PMR: δ 2.67 (2H, m, C_6-H), 4.63 (1H, dd, J = 6 and 8 Hz C_5-H), 7.67 (5H, m, Ar-H).

B_2 R = 

Recrystallised from aqueous methanol.

M.p. 63°C.

Elemental analysis Found: C, 42.55; H, 2.03; Cl, 49.50

Calculated for $C_{15}H_{10}Cl_6O$ C, 42.96; H, 2.37; Cl, 50.84.

IR: 1680 (>C=O), 1600 (>C=C<).

PMR: δ 2.50 (3H, s, Ar-CH_3), 2.70 (2H, m, C_6-H), 4.60 (1H, dd, J=6 and 8 Hz, C_5-H), 7.25 and 7.87 (2H each, d each, J= 8 Hz each, Ar-H).

B_3 R = 

Recrystallised from aqueous methanol.

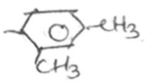
M.p. 91°C.

Elemental analysis Found: C, 44.29; H, 2.52; Cl, 48.80

Calculated for $C_{16}H_{12}Cl_6O$: C, 44.34; H, 2.77; Cl, 49.19

IR: 1675 (>C=O), 1600 (>C=C<).

PMR: δ 1.36 (3H, t, J=8 Hz, primary methyl), 2.70 (4H, m, benzylic -CH_2 and 2H from C_6 are together), 4.67 (1H, dd, J = 6 and 8 Hz, C_5-H), 7.30 and 7.90 (2H each d each, J = 8 Hz each, Ar-H). Fig. (6).

B_4: R = 

Recrystallised from aqueous methanol.

M.p. 120°C.

Elemental analysis Found: C, 44.09; H, 2.59; Cl, 48.69

Calculated for $C_{16}H_{12}Cl_6O$ C, 44.34; H, 2.77; Cl, 49.19.

IR: 1675 (>C=O), 1600 (>C=C<).

PMR: δ2.30 and 2.45 (3H each, s each, two methyls on aromatic ring) 2.60 (2H, m, C_6-H), 4.56 (1H, dd, J=6 and 8 Hz C_5-H), 7.27 (3H, m, Ar-H).

B_5: R = 

Recrystallised from aqueous methanol

M.p. 132°C.

Elemental analysis Found: C, 37.89; H, 1.45; Cl, 55.97

Calculaeed for $C_{14}H_7Cl_7O$ C, 38.23; H, 1.60; Cl, 56.54.

IR: 1680 (>C=O); 1610 (>C=C<).

PMR: δ 2.70 (2H, m, C_6-H), 4.60 (1H, dd, J=6 and 8 Hz, C_5-H), 7.50 and 7.93 (2H each, d each, J=8 Hz each, Ar-H).

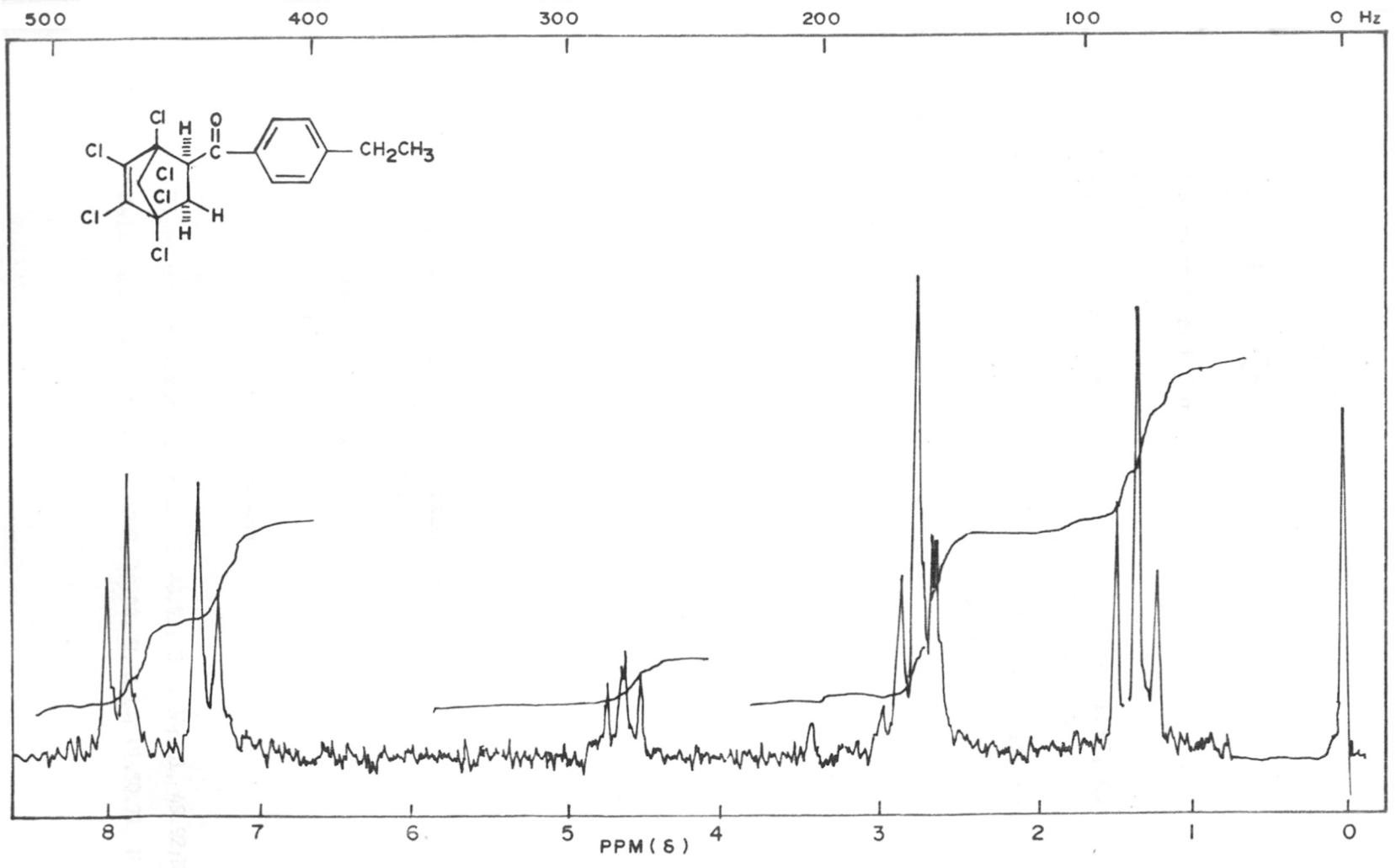
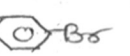


FIG (6) 5(4-ETHYL BENZOYL) 1,2,3,4,7,7-HEXACHLORO BICYCLO(2·2·1) HEPT-2-ENE

B_6 : R = 

Recrystallised from aqueous methanol.

M.p. 149°C.

Elemental analysis Found: C, 34.36; H, 1.37; Cl, 43.87; Br = 15.93

Calculated for $C_{14}H_7BrCl_6O$ C, 34.71; H, 1.47; Cl, 44.01; Br, 16.53.

IR: 1675 (>C=O), 1600 (>C=C<).

PMR: δ 2.67 (2H, m, C_6-H), 4.57 (1H, dd, J=6 and 8 Hz, C_5-H), 7.57 and 7.83 (2H each, d each, J = 8 Hz each Ar-H).

Series C: (XIX) N-Substituted-1,2,3,4,7,7-hexachloro bicyclo (2.2.1) hept-2-ene, 5-carboxamide

To a stirred solution of 3.60 gms (0.01 mole) of compound (XVII) in benzene (50 ml), 0.93 gms (0.01 mole) aniline was added[43] at room temperature. After 1 hour, the reaction mixture was poured on crushed ice and treated with dil. HCl. Benzene layer was washed by water (10 ml x 2) and dried over anhydrous Na_2SO_4. Benzene was evaporated to give C_1 in 84% yield. All other compounds of series C were synthesised similarly.

C_1 : R = H, R' = 

Recrystallised from benzene

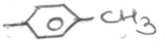
M.p.178°C

Elemental analysis Found: C, 40.03; H, 2.08; Cl, 49.92;N,2.99

Calculated for $C_{14}H_9Cl_6NO$ C, 40.00; H, 2.14; Cl,50.71; N, 3.33

IR: 3240 (-NH), 1655 (-C(=O)NH), 1600 (C=C).

PMR: δ 2.70 (2H, m, C_6-H), 3.52 (1H, dd, J=4.8 and 6.4 Hz, C_5-H), 7.36 (5H, m, Ar-H), 7.50 (1H, s, -NH). Fig. (7)

C_2 R = H, R' = 

Recrystallised from benzene

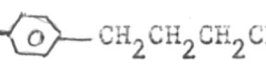
M.p. 184°C.

Elemental analysis Found: C, 40.81; H, 2.33; Cl, 48.92; N, 3.10

Calculated for $C_{15}H_{11}Cl_6NO$ C,41.47; H, 2.53; Cl, 49.08; N, 3.22.

IR: 3330 (-NH), 1660 (C(=O)NH), 1600 (C=C).

PMR: δ 2.24 (3H, s, Ar-CH_3), 2.64 (2H, m, C_6-H), 3.80 (1H, dd, J = 4.8 and 6.4 Hz, C_5-H), 7.10 and 7.40 (2H each, d each, J = 8 Hz each, Ar-H), 7.55 (1H, s -NH).

C_3 R=H, R' = 

Recrystallised from pet. ether (60-80°C).

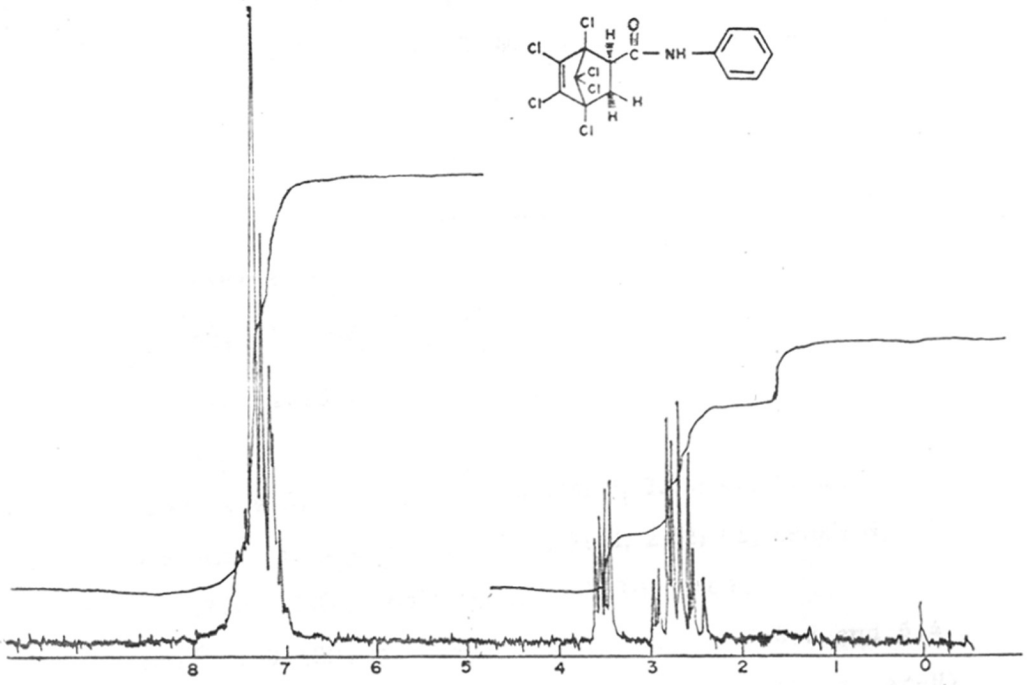
M.p.150°C.

Elemental analysis Found: C, 45.38; H, 3.57; Cl, 44.75; N, 2.94

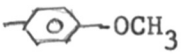
Calculated for $C_{18}H_{17}Cl_6NO$ C,45.30; H, 3.25; Cl, 43.87; N, 2.62

IR: 3290 (-NH), 1660 (-C(=O)NH), 1600 (C=C).

PMR: δ 0.91 (3H, t, J=6.4 Hz, primary -CH_3), 1.44 (4H, m, -CH_2-CH_2-), 2.60 (4H, m, benzylic -CH_2 and C_6-H together), 3.56 (1H, dd, J=4.8 and 6.4 Hz, C_5-H), 7.2 and 7.36 (2H each, d each, J=8 Hz, each, Ar-H), 7.50 (1H, s, -NH).



FIG(7) N-PHENYL 1,2,3,4,7,7-HEXACHLORO BICYCLO (2·2·1) HEPT-2-ENE 5-CARBOXAMIDE

$\underline{C_4}$: R=H, R' = 

Recrystallised from benzene.

M.p.190°C.

Elemental analysis Found: C, 39.54; H, 2.25; Cl, 46.58; N, 3.02

Calculated for $C_{15}H_{11}Cl_6NO_2$ C, 40.00;H,2.44; Cl, 47.33; N, 3.11

IR: 3280 (-NH), 1660 (-C$\overset{O}{\underset{NH}{<}}$), 1610 (C=C), 1235 (-C-O-C-).

PMR: δ 2.64 (2H, m, C_6-H), 3.76 (4H, m, C_5-H)and Ar-OCH_3), 6.84 and 7.40 (2H each, d each, J = 8 Hz each, Ar-H), 7.50 (1H, s -NH).

$\underline{C_5}$: R=H, R'= -CH_2-

Recrystallised from benzene.

M.p.161°C.

Elemental analysis Found: C, 41.23; H, 2.43; Cl, 48.50; N, 2.94

Calculated for $C_{15}H_{11}Cl_6NO$ C, 41.47; H, 2.53; Cl, 49.08; N, 3.22

IR: 3280 (-NH), 1655)(-C$\overset{O}{\underset{NH}{<}}$), 1610 (C=C).

PMR: δ 2.60 (2H, m, C_6-H), 3.60 (1H, dd, J=4.8 and 6.4 Hz, C_5-H), 4.37 (2H, m, benzylic -CH_2), 7.20 (5H, s, Ar-H), 7.45 (1H, s, -NH).

$\underline{C_6}$: R = H, R'= 

Recrystallised from pet.ether (60-80°C).

M.p.168°C.

Elemental analysis Found: C, 39.03; H, 3.41; Cl, 49.68; N, 3.01

Calculated for $C_{14}H_{15}Cl_6NO$ C, 39.44; H, 3.52; Cl, 50.00; N, 3.29.

IR:3280 (-NH), 1645 (-C$\overset{O}{\underset{NH}{<}}$), 1605 (C=C).

PMR: δ 1.50 (11H, m, ⬡), 2.50 (2H, m, C_6-H), 3.23 (1H, dd, J=4.8 and 6.4 Hz, C_5-H), 5.60 (1H, s, -NH).

C_7 : R = CH_3 , R' = CH_3

Recrystallised from pet.ether (60-80°C).

M.p.53°C.

Elemental analysis Found: C, 32.16; H, 2.27; Cl, 56.96; N, 3.58

Calculated for $C_{10}H_9Cl_6NO$ C, 32.26; H, 2.42; Cl, 57.26; N, 3.76

IR: 1655 (C(=O)N(Me)Me), 1610 (C=C).

PMR: δ 2.50 (2H, m, C_3-H), 2.83 and 3.10 (3H each, s each, $-CH_3$ each).

Series D: (XX) 5-(Substituted) 1,2,3,4,7,7-hexachloro, bicyclo (2.2.1)hept-2-ene carboxylic acid

To an ice cooled and stirred solution of glycerine, etheral solution of compound (XVII) (3.64 gms, 0.01 mole) were added. Few drops of pyridine were added and the mixture was stirred for two hours at room temperature. The reaction mixture was poured in excess water and extracted by ether. Ether layer was dried over Na_2SO_4. The ether was evaporated and residue was purified by column chromatography to obtain D_4 in 90% yield.

All other D series compounds were prepared by same manner.

D_1 : R = $-CH_2CH_2OH$

Elemental analysis Found: C, 30.48; H, 1.99; Cl, 53.82

Calculated for $C_{10}H_8Cl_6O_3$: C, 30.85; H, 2.06; Cl, 54.76

IR: 3400 (-OH), 1740 (-C(=O)-O-), 1610 (C=C), 1200 (C-O-C).

PMR: δ 2.40 (1H, s, -OH), 2.67 (2H, m, C_6-H), 3.70 (3H, m, C_5-H and $-CH_2$-OH), 4.27 (2H, m, -COO-CH_2).

D_2 R = $-CH_2CH_2CH_2OH$

Elemental analysis Found: C, 32.28; H, 2.19; Cl, 52.03

Calculated for $C_{11}H_{10}Cl_6O_3$ C, 32.75; H, 2.48; Cl, 52.85

IR: 3340 (-OH), 1740 (-C(=O)-O-), 1610 (C=C), 1200 (C-O-C).

PMR: δ 1.85 (2H, m, $-CH_2CH_2OH$), 2.57 (2H, m, C_6-H), 2.94 (1H, s, -OH), 3.60 (2H, m, C_5-H and $-CH_2OH$), 4.20 (2H, t, J=6 Hz $-COOCH_2-$).

D_3 R = $-CH_2CH_2CH_2CH_2OH$

Elemental analysis Found: C, 34.30; H, 2.72; Cl, 50.83

Calculated for $C_{12}H_{12}Cl_6O_3$, C, 34.53; H, 2.88; Cl, 51.08.

IR: 3340 (-OH), 1745 (-C(=O)-O-), 1610 (C=C), 1200 (C-O-C).

PMR: δ 1.70 (4H, m, $-CH_2-CH_2CH_2-CH_2-$), 2.30 (1H, s, -OH), 2.60 (2H, m, C_6-H), 3.57 (3H, m, $-CH_2OH$ and C_5-H), 4.10 (2H, t, J=6 Hz, $-COOCH_2-$))Fig. (8).

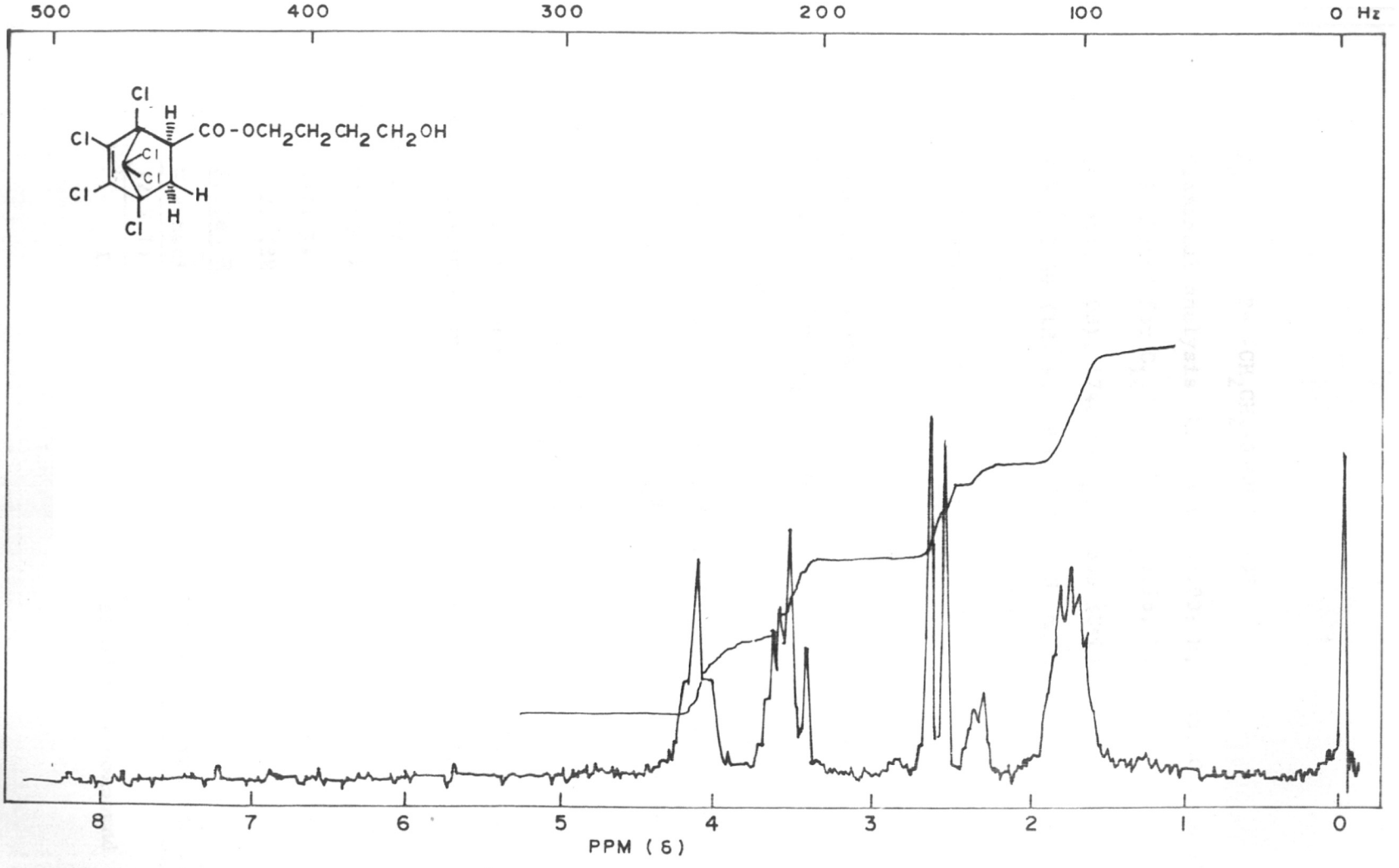
D_4 R = $-CH_2CHOHCH_2OH$

Elemental analysis Found: C, 30.98; H, 2.15; Cl, 49.92

Calculated for $C_{11}H_{10}Cl_6O_4$ C, 31.50; H, 2.39; Cl, 50.84.

IR: 3390 (-OH), 1745 (-C(=O)-O-), 1605 (C=C), 1200 (C-O-C).

PMR: δ 2.50 (2H, m, C_6-H), 3.57 (4H, m, C_5-H, -CH-OH and CH_2-OH), 4.13 (2H, m, $-COOCH_2-$), 5.70 (2H, s, two -OH groups).



FIG(8) 5-(4-HYDROXY PROPYL) 1, 2, 3,4,7, 7-HEXACHLORO BICYCLO(2 2·1) HEPT-2-ENE CARBOXYLIC ACID

D_5 R= $-CH_2CH_2-O-CH_2CH_2-OH$

Elemental analysis Found: C, 33.00; H, 2.59; Cl, 48.73.

Calculated for $C_{12}H_{12}Cl_6O_3$ C, 33.26; H, 2.77; Cl, 49.19.

IR: 3430 (-OH), 1740 ($-C\langle^{O}_{O}$), 1605 (C=C), 1200 (C-O-C).

PMR: δ 2.40 (1H, s, -OH), 2.57 (2H, m, C_6-H), 3.60 (7H, m, C_5-H and $-CH_2OCH_2CH_2-$), 4223 (2H, t, J=4 Hz, $-COOCH_2-$).

D_6 R = $-CH_2-CH_2-O-CH_3$

Elemental analysis Found: C, 32.68; H, 2.42; Cl, 51.99

Calculated for $C_{11}H_{10}Cl_6O_3$ C, 32.75; H, 2.48; Cl, 52.85.

IR: 1740 ($-C\langle^{O}_{O}$), 1610 (C=C), 1205 (-C-O-C-).

PMR: δ 2.56 (2H, m, C_6-H), 3.26 (3H, s, $-OCH_3$), 3.50 (3H, m, C_5-H and $-COO-CH_2\underline{CH_2}-$), 4.13 (2H, t, J=5 Hz, $-COOCH_2$).

D_7 R = $-CH_2CH_2-O-CH_2CH_3$

Elemental analysis Found: C, 34.46; H, 2.53; Cl, 50.69

Calculated for $C_{12}H_{12}Cl_6O_3$ C, 34.53; H, 2.88; Cl, 51.08.

IR: 1730 ($-C\langle^{O}_{O}$), 1605 (C=C), 1200 (C-O-C).

PMR: δ 1.20 (3H, t, J = 7 Hz, primary methyl), 2.60 (2H, m, C_6-H), 3.53 (5H, m, C_5-H and $-CH_2-O-CH_2-$), 4.20 (2H, t, J=5 Hz, $-COOCH_2-$) Fig. (9).

Series E - Compound (XXI)

N(substituted) ammonium 1,2,3,4,7,7-hexachloro, bicyclo (2.2.1) hept-2-ene-5-carboxylate:

These compounds were synthesised from compound (XVI)

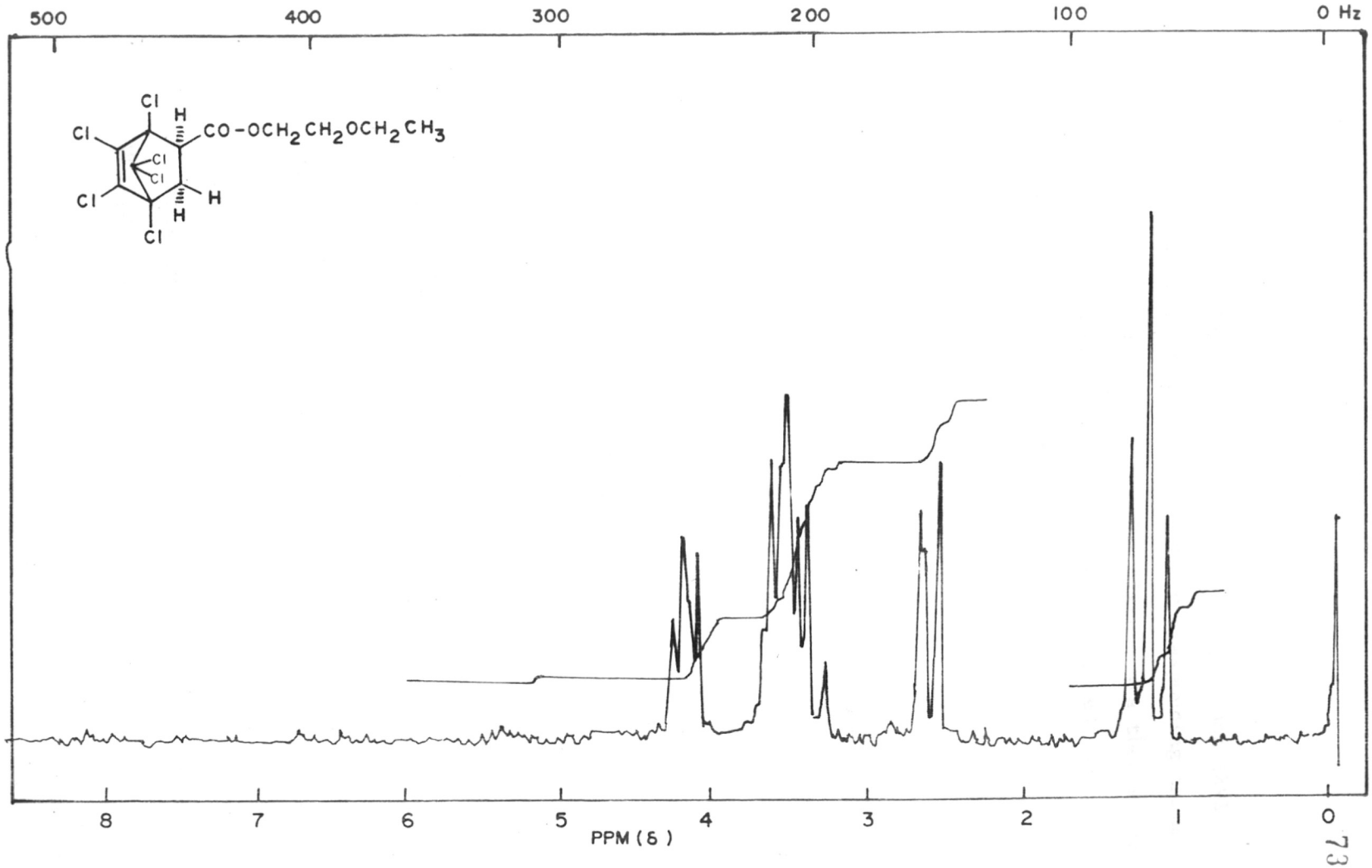


FIG (9) 5-(2-ETHOXY ETHYL) 1,2,3,4,7,7-HEXACHLORO BICYCLO(2·2·1) HEPT-2-ENE, CARBOXYLIC ACID

and corresponding amines. 0.01 mole of compound (XVI) was dissolved in excess of ether and corresponding amine (0.01 mole) was added to it. The mixture was then stirred for 1 hour at room temperature. Solid settled in the solution slowly. Yield was 90%.

E_1 $R' = H$, $R' = -CH_2CH_3$

Recrystallised from chloroform

M.p. decomposed at 260°C.

Elemental analysis Found: C, 29.58; H, 2.63; Cl, 53.50; N, 3.21

Calculated for $C_{10}H_{11}Cl_6NO_2$ C,30.77; H, 2.82; Cl, 54.62; N, 3.59.

IR: 1650 ($\overset{+}{N}H_3$), 1600 (C=C), 1550 (-C$\overset{O}{\underset{O^-}{<}}$).

E_2 R = H, $R' = -CH_2CH_2CH_2CH_3$

Recrystallised from chloroform

M.p. 173°C

Elemental analysis Found: C,34.21; H,3.04;Cl, 50.87; N, 3.42

Calculated for $C_{12}H_{15}Cl_6NO_2$ C, 34.53; H, 3.36; Cl, 51.09; N, 3.55.

IR: 1650 ($\overset{+}{N}H_3$), 1600 (C=C), 1540 (C-C$\overset{O}{\underset{O^-}{<}}$).

E_3 R = H, $R' = -C(CH_3)_3$

Recrystallised from chloroform

M.p. 209°C.

Elemental analysis Found: C, 34.11; H, 3.13; Cl, 50.70; N, 3.50.

Calculated for $C_{12}H_{15}Cl_6NO_2$ C, 34.53; H,3.36; Cl, 51.09; N, 3.55

IR: 1635 ($\overset{+}{N}H_3$), 1600 (C=C), 1565 (-C$\overset{O}{\underset{O^-}{<}}$).

E_4 R = CH_3, R' = CH_3

Recrystallised from chloroform.

M.p. 164°C.

Elemental analysis Found: C, 30.01; H, 2.26; Cl, 53.90; N, 3.25

Calculated for $C_{10}H_{11}Cl_6NO_2$ C,30.76; H, 2.82; Cl, 54.62; N, 3.59.

IR: 1640 ($\overset{+}{N}H_2$), 1605 (C=C), 1565 (-C$\overset{O}{\underset{O}{}}$).

E_5 R = H, R' = 

Recrystallised from chloroform.

M.p. 206°C.

Elemental analysis Found: C, 36.82; H, 3.71; Cl, 47.50; N, 3.10

Calculated for $C_{14}H_{17}Cl_6NO_2$ C,37.84; H, 3.83; Cl, 47.97; N, 3.15

IR: 1625 ($\overset{+}{N}H_3$), 1600 (C=C), 1560 (-C$\overset{O}{\underset{O}{}}$).

E_6 R=H, R' = $-CH_2$- 

Recrystallised from chloroform

M.p. 159°C

Elemental analysis Found: C, 39.48; H, 2.57; Cl, 46.77; N, 3.00

Calculated for $C_{15}H_{13}Cl_6NO_2$ C, 39.83; H, 2.87; Cl, 47.07;;N, 3.09.

IR: 1620 ($\overset{+}{N}H_3$), 1605 (C=C), 1560 (-C$\overset{O}{\underset{O}{}}$).

Series F Compound (XXII) 1,2,3,4,7,7-hexachloro bicyclo (2.2.1) hept-2-ene, 5-carboxylic acid-alkyl ester

3.45 gms (0.01 mole)of compound (XVI) were dissolved in 50 ml of n-propanol. To this solution p-toluene sulphonic acid was added (in catalytic amount) and the mixture was refluxed for 14 hours. About one-half

of n-propanol was removed by distillation and residue was poured in excess water and neutralised by $NaHCO_3$. The chloroform extract of this solution was dried over anhydrous Na_2SO_4 and the chloroform was evaporated to get F_4 in 65% yield. F_5 and F_6 were synthesised using 2-propanol and n-pentanol respectively.

F_4: R = H, R' = $-CH_2CH_2CH_3$

Recrystallised from aqueous methanol.

M.p. 81°C.

Elemental analysis Found: C, 34.00; H, 2.36; Cl, 54.81.

Calculated for $C_{11}H_{10}Cl_6O_2$ C, 34.10; H, 2.58; Cl, 55.04.

IR : 1730 (>C=O), 1600 (>C=C<), 1200 (-C-O-C-).

PMR: δ 0.89 (3H, t, J = 6 Hz, primary methyl), 1.60 (2H, m, $-CH_2CH_3$), 2.53 (2H, m, C_6-H), 3.41 (1H, dd, J=6 and 10 Hz, C_5-H), 3.96, (2H, t, J=7 Hz, $-COOCH_2-$).

F_5 R = H, R' = $-CH(CH_3)_2$

Recrystallised from aqueous methanol.

M.p. 51°C.

Elemental analysis Found: C, 34.04; H, 2.77; Cl, 54.35

Calculated for $C_{11}H_{10}Cl_6O_2$ C, 34.10; H, 2.58; Cl, 55.04.

IR: 1725 (>C=O), 1600 (>C=C<), 1100 ($-C(CH_3)_2$), 1206 (>C-O-C) Fig. (10)

PMR: δ 1.26 (6H, d, J = 6 Hz, isopropyl methyls), 2.56 (2H, m, C_6-H), 3.50 (1H, dd, J = 6.4 and 10 Hz, C_5-H), 5.0 (1H, m, $-CH(CH_3)_2$) Fig. (11).

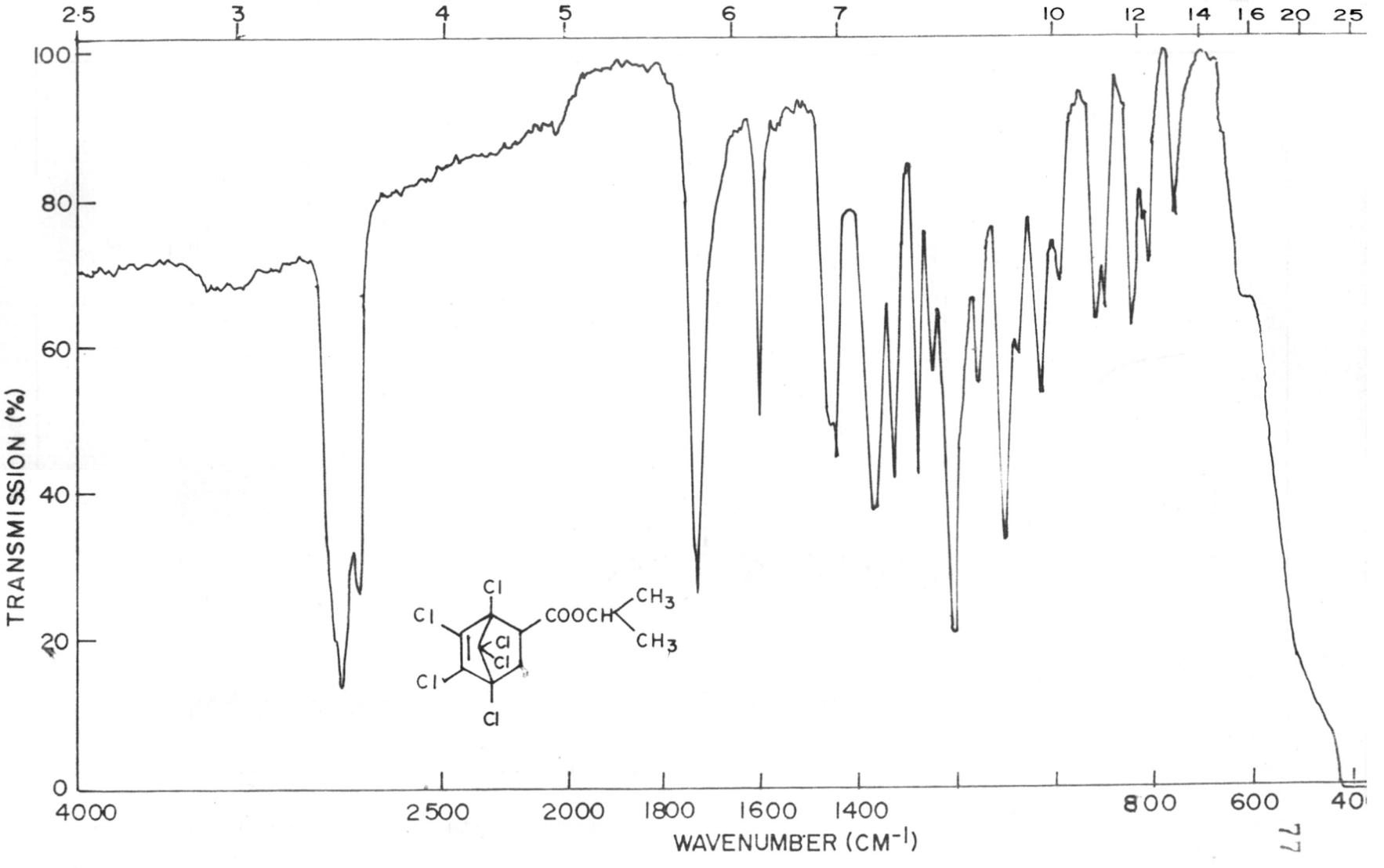


FIG (10) ISOPROPYL, 1,2,3,4,7,7 HEXACHLORO BICYCLO (2·2·1) HEPT-2-ENE, 5-CARBOXYLIC ACID

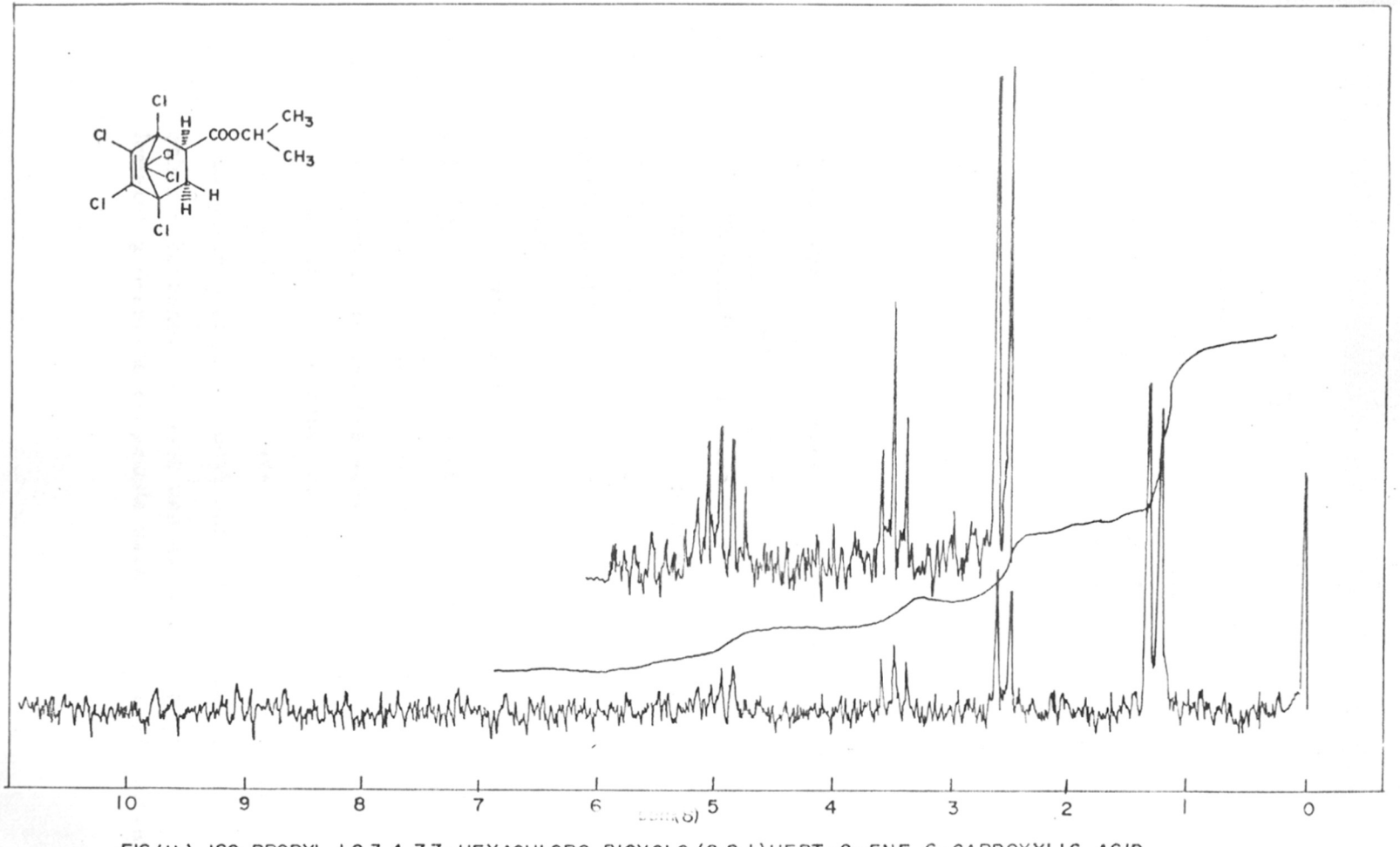


FIG (11) ISO PROPYL, 1,2,3,4,7,7-HEXACHLORO BICYCLO (2·2·1) HEPT-2-ENE 6-CARBOXYLIC ACID

F_7 R = H, R' = $-CH_2(CH_2)_3CH_3$

Viscous liquid

Elemental analysis Found: C, 37.05; H, 2.98; Cl, 50.96

Calculated for $C_{13}H_{14}Cl_6O_2$ C, 37.59; H, 3.37; Cl, 51.33.

IR: 1730 (C=O), 1600 (C=C), 1200 (C-O-C),

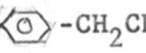
PMR: δ 0.96 (3H, t, J = 6 Hz, primary methyl), 1.46 (6H, m, $-(CH_2)_3$), 2.67 (2H, m, C_6-H), 3.57 (2H, m, $-OCH_2-$), 4.1 (1H, dd, J=6 and 10 Hz, C_5-H).

Scheme - 3

G-Series - (XXIII)

5-Keto-aryl, 1,2,3,4,7,7-hexachloro, bicyclo (2.2.1) hept-2-ene-6-carboxylic acid[29].

Chlorendic anhydride (3.71 gms, 0.01 mole) was dissolved in 10 ml of dry ethylbenzene. To this solution was added all at once 3.85 gms (0.29 mole) of anhydrous $AlCl_3$. An immediate exothermic reaction was controlled by cooling. After the reaction had subsided, the mixtue was hydrolysed by cautious addition, with stirring of 10 ml of water followed by 20 ml of 10% HCl. The mixture was allowed to stand overnight and steam distilled to remove the ethylbenzene. The solid which remained in the residue was filtered with suction, digested with boiling water. The reamining solid was decolourised with charcoal and crystallised from pet. ether + benzene. Solvent was evaporated to get G_{12}. Remaining G-series compounds were synthesised similarly.

G_2 R =  $-CH_2CH_3$

Recrystallised from pet.ether + benzene.

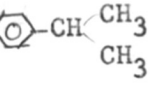
M.p. 148°C.

Elemental analysis Found: C, 42.08; H, 2.49; Cl, 44.02

Calculated for $C_{17}H_{12}Cl_6O_3$ C, 42.77; H, 2.52; Cl, 44.65.

IR: 3050 (acid -OH) 1715 (acidic -C(=O)), 1670 (conjugated >C=O), 1600 (>C=C<).

PMR: δ 1.28 (3H, t, J=7.5 Hz, primary methyl), 2.72 (2H, q, J=7.5 Hz, benzylic $-CH_2$), 4.0 (1H, d, J=10 Hz, C_5-H), 4.90 (1H, s, -COOH), 5.0 (1H, d, J=10 Hz, C_6-H), 7.28 and 7.80 (2H each, d each, J = 10 Hz each Ar-H).

G_3 R = 

Recrystallised from aqueous methanol.

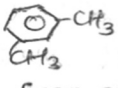
M.p.184°C.

Elemental analysis Found: C, 43.25; H, 2.69; Cl, 42.66

Calculated for $C_{18}H_{14}Cl_6O_3$ C, 43.90; H, 2.85; Cl, 43.29.

IR: 3040 (acidic -OH), 1720 (acidic -C(=O)), 1670 (conjugated >C=O), 1600 (>C=C<),

PMR: δ 1.28 (6H, d, J=7.5 Hz isopropyl methyls), 3.0 (1H, m, $-CH(CH_3)_2$), 4.04 (1H, d, J=10 Hz, C_5-H), 4.36 (1H, s, -COOH), 5.0 (1H, d, J=10 Hz, C_6-H), 7.28 and 7.92 (2H each, d each, J = 10 Hz each, Ar-H).

G_4 R = 

Recrystallised from aqueous methanol.

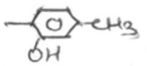
M.p.215°C.

Elemental analysis Found: C, 41.91; H, 2.31; Cl, 44.13

Calculated for $C_{17}H_{12}Cl_6O_3$ C,42.77; H, 2.52; Cl, 44.65.

IR: 3045 (acidic -OH), 1725 (acidic -C=O), 1670 (conjugated C=O), 1600 (C=C).

PMR: δ 2.35 and 2.43 (3H each, s each, two methyl groups on aromatic ring), 4.22 (1H, d, J=10.58 Hz, C_5-H), 4.90 (1H, s, -COOH), 5.02 (1H, d, J=10.13 Hz, C_6-H), 7.22 (2H, d, J=7.49 Hz, Ar-H), 7.91 (1H, m, Ar-H). Fig. (12).

G_5 R = 

Recrystallised from aqueous methanol.

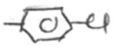
M.p.164°C.

Elemental analysis Found: C, 39.91; H, 2.00; Cl, 43.72.

Calculated for $C_{16}H_{10}Cl_6O_4$ C, 40.00; H, 2.08; Cl, 44.46.

IR: 3350 (acidic -OH) 1760 (acidic -C=O), 1700 (conjugated C=O), 1600 (C=C).

PMR: δ 2.30 (3H, s, Ar-CH_3), 4.07 (1H, d, J=10.27 Hz, C_5-H), 4.14 (1H, d, J=10.27 Hz, C_6-H), 4.94 (2H, m, -COOH and -OH), 6.67 (1H, m, Ar-H), 7.10 (2H, m, Ar-H).

G_6 R = 

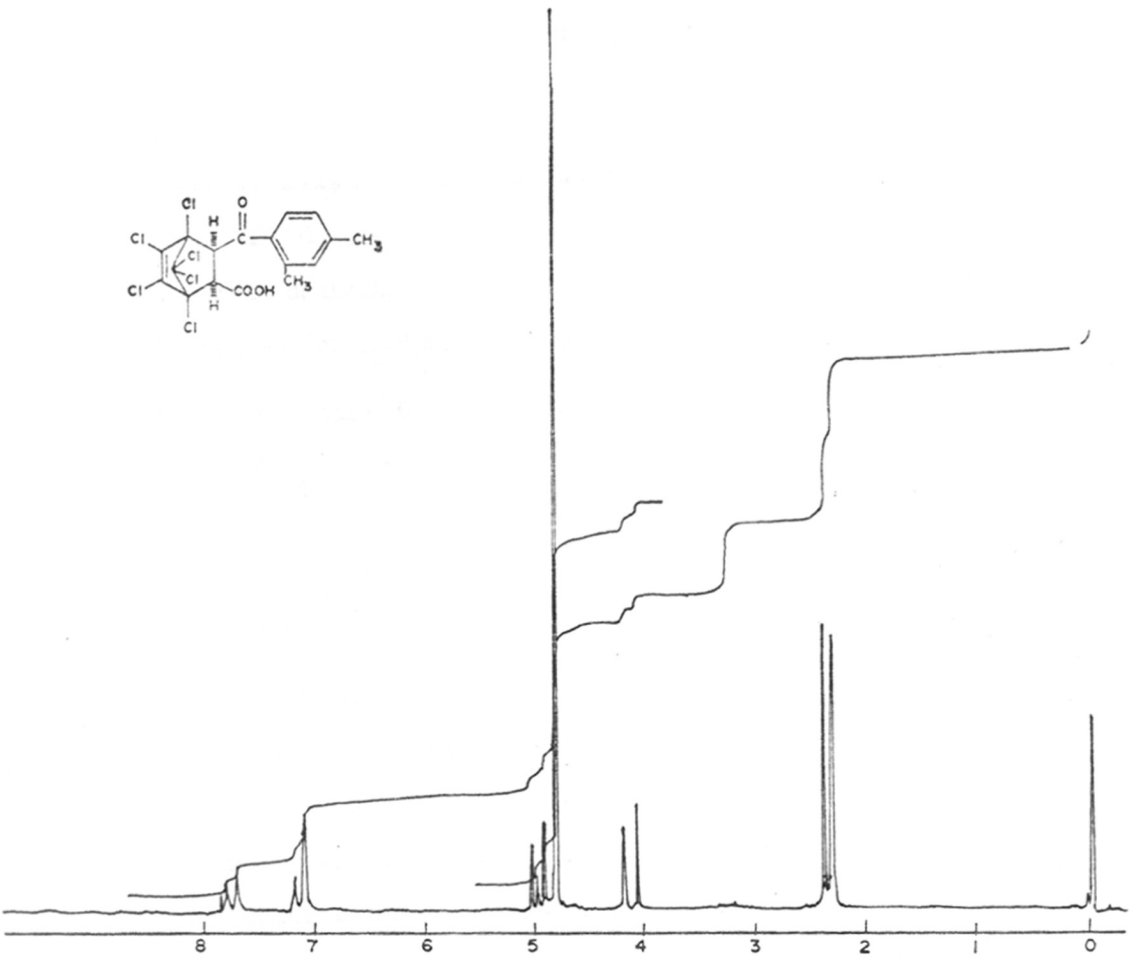
Recrystgallised from aqueous methanol.

M.p. 213°C.

Elemental analysis Found: C,36.77; H, 1.28; Cl, 50.62

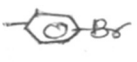
Calculated for $C_{15}H_7Cl_7O_3$, C, 37.23; H, 1.45; Cl, 51.40

IR: 3045 (acidic -OH) 1725 (acidic -C=O), 1685 (C=O), 1685 (C=O), 1590 (C=C).



FIG(4.2) 5(2,4-DIMETHYL BENZOYL) 1,2,3,4,7,7-HEXACHLORO BICYCLO(2·2·1) HEPT-2-ENE-6-CARBOXYLIC ACID

PMR: δ 4.33 (1H, d, J=10.27, Hz, C_5-H), 4.89 (1H, s, -COOH), 5.06 (1H, d, J=10.27 Hz, C_6-H), 7.60 (2H, d, J=9.10 Hz, Ar-H), 8.01 (2H, d, J=8.81 Hz, Ar-H).

G_7 : R = 

Recrystallised from aqueous methanol.

M.p. 221°C.

Elemental analysis Found: C, 33.98; H, 1.46; Br, 14.87; Cl,39.58.

Calculated for $C_{15}H_7BrCl_6O_3$ C,34.09; H, 1.33; Br, 15.15; Cl, 40.34.

IR: 3050 (acidic -OH), 1705 (acidic -C=O), 1690 (>C=O conjugated), 1590 (C=C).

PMR: δ 3.28 (1H, s, -COOH), 4.30 (1H, d, J=10 Hz, C_5-H), 5.0 (1H, d, J=10 Hz, C_6-H), 7.72 and 7.92 (2H each, d each J=7.5 Hz each, Ar-H).

Series H: Compound (XXIV) 5-Benzoyl, 1,2,3,4,7,7-hexachloro bicyclo (2.2.1) hept-2-ene, 6-carboxylic acid, alkyl ester

4.49 gms (0.01 mole) of G_1 were dissolved in 100 ml of n=propanol. 1.5 ml of conc. H_2SO_4 was added slowly and the mixture was refluxed for 9 hours. The reaction mixture was then poured in excess water and neutralised by $NaHCO_3$. The chloroform extract (50 ml x 2) of this solution was dried over anhydrous Na_2SO_4 and chloroform was subsequently evaporated to get H_1 in 70% yield. H_2 and H_3 were synthesised similarly using n-butanol and iso-butanol respectively.

H_1 R = $-CH_2CH_2CH_3$

Recrystallised from pet.ether (60-80°C).

M.p. 88°C.

Elemental analysis Found: C, 43.77; H, 2.58; Cl, 42.51.

Calculated for $C_{18}H_{14}Cl_6O_3$ C, 43.99; H, 2.85; Cl, 43.38.

IR: 1745 ($-C\langle^{O}_{O}$), 1690 ($-C\langle^{O}_{OH}$), 1600 ($\rangle C=C\langle$).

PMR: δ 0.80 (3H, t, J=7.04 Hz, primary methyl), 1.28 (2H, m, $-CH_2-$), 3.80 (2H, t, J=6.4 Hz, $-OCH_2-$), 4.12 (1H, d, J=10 Hz, C_2-H), 5.0 (1H, d, J=10 Hz, C_3-H), 7.64 (5H, m, Ar-H).

H_2 R = $-CH_2CH_2CH_2CH_3$

Recrystallised from pet.ether (60-80°C).

M.p. 100°C.

Elemental analysis Found: C, 44.53; H, 3.03; Cl, 41.68.

Calculated for $C_{19}H_{16}Cl_6O_3$ C, 45.14; H, 3.17; Cl, 42.18.

IR: 1730 ($-C\langle^{O}_{O}$), 1690 ($-C\langle^{O}_{OH}$), 1600 ($\rangle C=C\langle$).

PMR: δ 0.80 (3H, t, J = 6.4 Hz, primary methyl), 1.20 (4H, m, $-CH_2CH_2-CH_3$), 3.88 (2H, t, J=6.4 Hz, $-CO-OCH_2-$), 4.20 (1H, d, J=9.6 Hz, C_5-H), 4.96 (1H, d, J=9.6 Hz, C_6-H), 7.70 (5H, m, Ar-H).

H_3 R = $-CH_2CH\langle^{CH_3}_{CH_3}$

Recrystallised from pet.ether (60-80°C).

M.p. 110°C.

Elemental analysis Found: C, 45.01; H, 3.00; Cl, 41.78

Calculated for $C_{19}H_{16}Cl_6O_3$ C,45.14; H, 3.17; Cl, 42.18.

IR: 1740 ($-C\langle^{O}_{O}$), 1685 ($-C\langle^{O}_{ph}$), 1600 ($>C=C<$).

PMR: δ 0.76 (6H, d, J=6.4 Hz, two methyls), 1.72 (1H, m, $CH\langle^{CH_3}_{CH_3}$), 3.60 (2H, m, $-COOCH_2-$), 4.08 (1H, d, J=10.24, C_5-H), 4.88 (1H, d, J=10.24 Hz, C_6-H), 6.64 (5H, m, Ar-H). Fig. (13).

Series I: Compound (XXVI) N-Phenyl-5-benzoyl 1,2,3,4,7,7, hexachloro bicyclo(2·2·1) hept-2-ene, 6-carboxamide

4.49 gms (0.01 mole) of G_1 were dissolved in 50 ml of dry xylene. To this solution 1.83 gms (0.02 mole) of aniline were added[43] and mixture was refluxed with stirring for 1½ hours under nitrogen atmosphere. Xylene was removed by steam distillation. Reaction mixture was treated with (1:1) HCl and the benzene extract of this solution was dried over anhydrous Na_2SO_4 and the benzene was evaporated to get I_1, I_2, I_3 and I_4 were synthesised using p-toluidine, p-n-butyl aniline and p-anisidine, respectively by the same procedure.

I_1 : R = H

Recrystallised from aqueous methanol.

M.p. decomposed at 205°C.

Elemental analysis Found: C, 47.88; H, 2.19; Cl, 40.03; N, 2.30.

Calculated for $C_{21}H_{13}Cl_6NO_2$ C,48.09; H, 2.48; Cl, 40.65; N, 2.73.

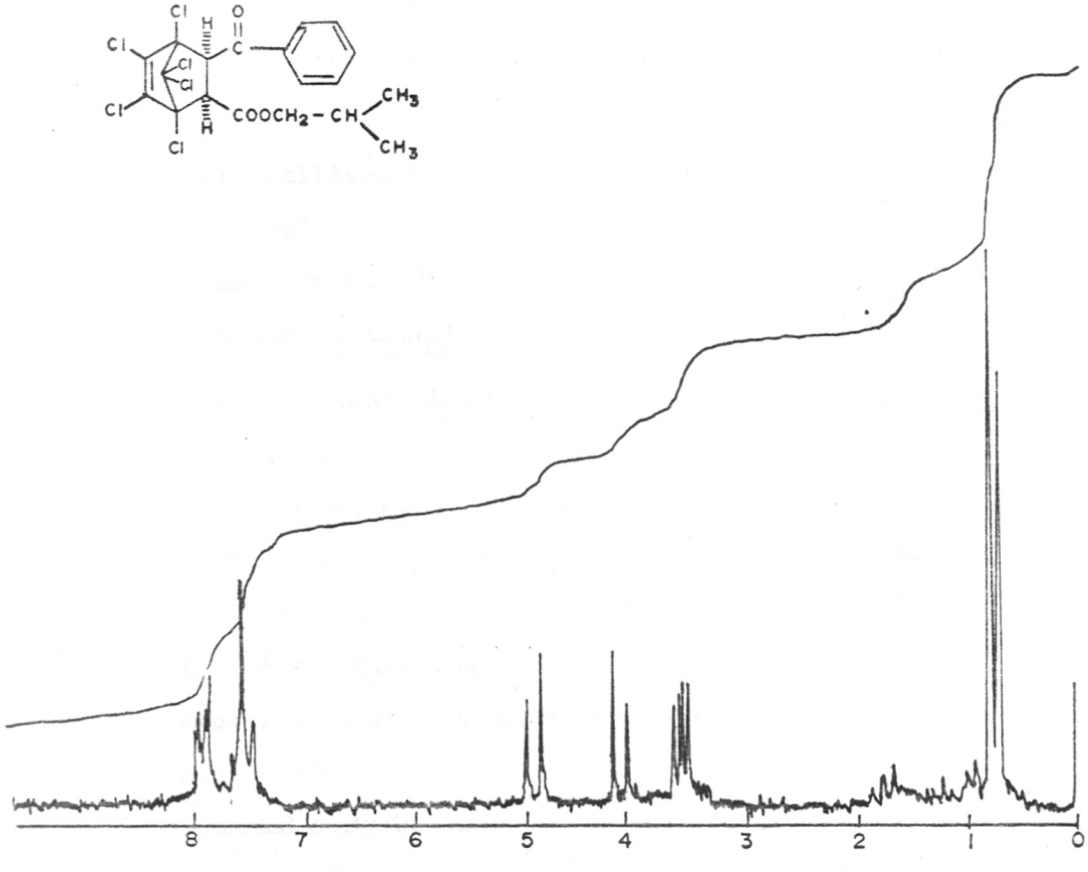


FIG (13) 6-(2-METHYL PROPYL) 5-BENZOYL 1,2,3,4,7,7-HEXACHLORO BICYCLO(2·2·1) HEPT -2-ENE 6-CARBOXYLIC ACID

IR: 3420 (-NH), 1685 (-C^{O}), 1600 (C_5).

PMR: δ 3.40 (1H, s, -NH), 3.80 (1H, d, J=7.5 Hz, C_5-H), 3.90 (1H, d, J=7.5 Hz, C_6-H), 6.88 (5H, m, Ar-H from ring b), 7.40 (5H, s, Ar-H from ring a).

I_2 R = $-CH_3$

Recrystallised from aqueous methanol.

M.p. 196°C.

Elemental analysis Found: C, 48.78; H, 2.59; Cl, 38.94; N, 2.32.

Calculated for $C_{22}H_{15}Cl_6NO_2$ C,49.06; H, 2.79; Cl, 39.59; N, 2.60.

IR: 3420 (-NH), 1680 (C=0), 1610 (C=C).

PMR: δ 2.28 (3H, s, Ar-CH_3), 3.48 (1H, s, C$^{O}_{NH}$), 3.80 (1H, d, J=7.5 Hz, C_5-H), 4.0 (1H, J=7.5 Hz, C_6-H), 6.81 and 7.10 (2H each, d each, J=7.5 Hz each, Ar-H from ring b), 7.48 (5H, s, Ar-H from ring a).

I_3 R = $-CH_2CH_2CH_2CH_3$

Recrystallised from aqueous methanol.

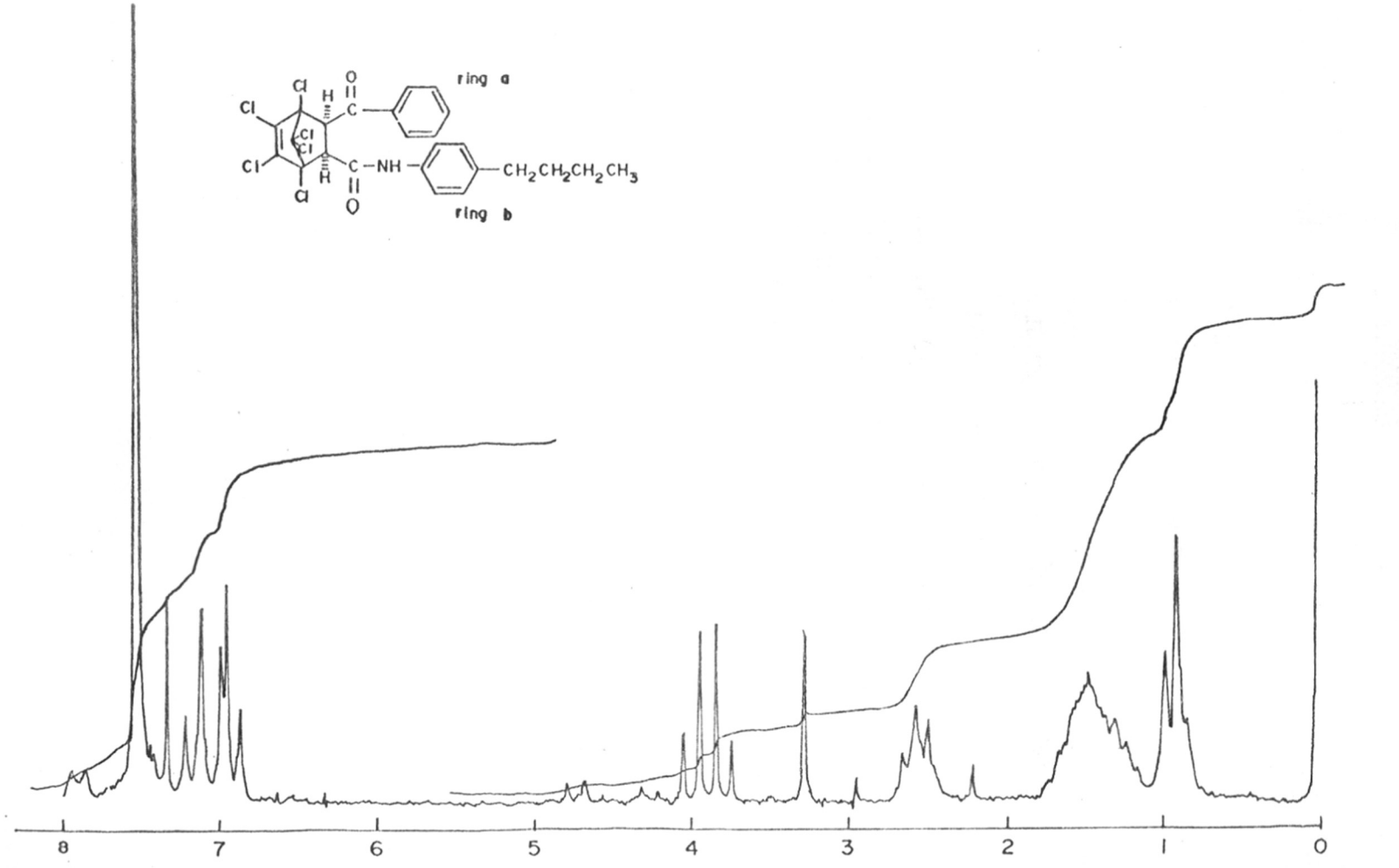
M.p. 125°C.

Elemental analysis Found: C, 51.12; H, 3.32; Cl, 35.53; N, 2.28

Calculated for $C_{25}H_{21}Cl_6NO_2$ C,51.72; H, 3.62; Cl, 36.73; N, 2.41.

IR: 3360 (-NH), 1690 (C=0), 1600 (C=C).

PMR: δ 0.92 (3H, t, J=6 Hz, primary methyl), 1.45 (4H, m, $-CH_2CH_2CH_2CH_3$), 2.50 (2H, t, J=7 Hz, benzylic $-CH_2$) 3.29 (1H, s, C$^{O}_{NH}$), 3.80 (1H, d, J=8 Hz, C_5-H), 4.0 (1H, d, J=8 Hz, C_6-H), 6.92 and 7.20 (2H each, d each, J=8 Hz each Ar-H from ring b), 7.53 (5H, s, Ar-H from ring a) Fig. (14).



FIG(14) N-(4-n-BUTYL PHENYL), 5-BENZOYL 1,2,3,4,7,7-HEXACHLORO BICYCLO (2·2·1) HEPT-2-ENE -6-CARBOXAMIDE

$\underline{I_4}$ R = $-OCH_3$

Recrystallised from aqueous methanol.

M.p. decomposed at 212°C

Elemental analysis Found: C, 47.46;H,2.58; Cl, 38.45; N, 2.28.

Calculated for $C_{22}H_{15}Cl_6NO_3$ C, 47.64;H, 2.70; Cl, 38.45; N, 2.53.

IR: 3420 (-NH), 1680 (>C=O), 1605 (C=C), 1250 (Ar-O-CH_3).

PMR: δ 3.20 (1H, s, -NH), 3.78 (1H, d, J=6Hz, C_5-H),
3.85 (3H, s, $-OCH_3$), 3.90 (1H, d, J=8 Hz, C_6-H),
6.78 (4H, m, Ar-H from ring b), 7.45 (5H, s, Ar-H from ring a).

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CHAPTER - III

Reverse Phase High Performance Liquid Chromatography

S U M M A R Y

Liquid chromatography has been received a tremendous boost with the development of High Performance Liquid Chromatography (HPLC). This instrumental technique enables the use of small diameter and high performance chromatography stationary phases. The back pressures generated by the use of such packings are overcome by employing high pressure constant flow solvent delivery systems. The efficiency is enhanced by the on-line sensitive detection devices.

Revesse phase HPLC (RPHPLC) employes a polar mobile phase (usually water with an organic modifier) and a non-polar stationary phase. With the introduction of chemically bonded stationary phase materials RPHPLC is almost exclusively carried out using these column packings. RPHPLC affords simultaneous analysis of polar, non-polar and ionic substances.

Retention in RPHPLC is the result of net lipophilicity of the compound in the mobile phase. The lipophilicity may be measured in terms of number of carbon atoms for a series of compounds. In RPHPLC, the retention is directly proportional to the number of carbon atoms present for a homologous series of compounds. This relationship is not valid for the compounds with different functional groups. In an

An attempt to find a suitable relation between retentions of such compounds and the number of carbon atoms, difference in selectivities on two columns with different polarities was estimated (Δx). These Δx values were then correlated with the number of carbon atoms and well defined relation was obtained for all the series of compounds under investigation.

In order to correlate insecticidal properties of the compounds, synthesis of which have been described in the Chapter II, with their chromatographic behaviour, instrumental liquid chromatography technique was selected as the method of choice. High Performance Liquid Chromatography, High performance Liquid Chromatography, more popularly known as'HPLC', was then employed in reverse phase mode for recording retention patterns of these compounds in series wise manner. The importance of Partition Coefficient in Quantitative Structure Activity Relationships (QSAR) has been explained in the Chapter I. The octanol-water partitioning system generally employed in QSAR has been thought to be similar to the lipid membrane with surrounding aqueous medium and earlier chromatography studies were primarily aimed at finding relation between retention in chromatogaphy and partition coefficient in octanol-water system. The present dissertation, however, correlates the chromatography selectivity, towards the test compounds, directly with their insecticidal activity. This view has recently been vindicated by studies of Horvath[1(a)] which led him to argue that the reverse phase chromatography stationary phase resembles more with the living lipid membrane than 1-octanol. The reverse phase chromatography may thus

provide better realistic situation for QSAR studies.

3.1 HPLC - Historical Background

The term HPLC has been variantly referred to explain many modern instrumental LIQUID Chromatography techniques - High Performance Liquid Chromatography, High Pressure Liquid Chromatography, High Speed Liquid Chromatography etc. In this dissertation the term HPLC has been used to describe essentially Instrumental Liquid Chromatography using high pressure pump systems and high performance column packing.

Over the past decade, HPLC has emerged as one of the most prominent tool of chemical and biochemical analysis. The rapid growth of HPLC technique may be attributed not only to the advances in both the instrumentation and column technology in Liquid Chromatography, but also to the need for a micro-analytical method that has the advantages of Gas Chromatography and is applicable to non-volatile substances. The high precision of modern HPLC technique, that embodies sophisticated microprocessor technology for control and data handling and the high efficiency of microparticulate packed columns, offers a formidable combination for rapid and efficient separations and quantitative analysis of submicrogram quantities at high convenience.

In the early 1960's Giddings[1] showed that the theoretical frame work developed for Gas Liquid Chromatogrpphy applied equally well to liquid chromatography. Earlier, Spackman, Stein and Moore[2] had introduced an automated amino acdd analysis system. Pioneering work of Hamilton[3] on Fundamental theory of HPLC and the work of C.D. Scott[4] on High Pressure Ion-Exchange Chromatography contributed to the further developments in HPLC. The potential advantages of modern HPLC were presented in the Fifth International Symposium on Advances in Chromatography[5] in 1969. Between 1967 to 1969 Kirkland[6], Huber[7] and Horvath[8] described first HPLC units. In the mid 1960's M/s. Waters Associates and J.C. Moore[9] introduced Gel Permeation Chromatography. By operating at high pressures (upto 5000psi) these instruments overcame the effect of higher liquid viscosities, relative to gas viscosities, and yielded analysis times comparable with Gas Liquid Chromatography. By late 1960's a number of workers were induced to conduct active research into what was to become modern HPLC and their combindd efforts led to 1969 breakthrough[5]. Since then tremendous activity has been aimed at the development of better equipment and columns for use in HPLC.

3.2 Classic Versus Modern Liquid Chromatography

The liquid column chromatography, which was developed before 1969, is generally termed as 'Classic Column Liquid Chromatography' and the recently developed technique of HPLC as 'Modern Column Liquid Chromatography'.

In classic Column Liquid Chromatography, the following features are predominant-

i) Columns with particle sizes of 100 μm or greater, are packed by the user, employed only once and discarded.

ii) Sample application is skill dependent and time consuming.

iii) Mobile phase flow is accomplished by gravity.

iv) Sample detection is afforded by collecting and analysing fractions manually.

v) Analysis time may be typically several hours.

In contract to Classic Column Chromatography, Modern Column Chromatography has following salient attributes -

i) Columns with particle sizes from 3 to 50 μm are generally commercially available as prepacked and tested columns and used for hundreds and possibly a thousand injections with due considerations.

ii) The use of syringes or injections valves enable rapid, easy and precise sample injection.

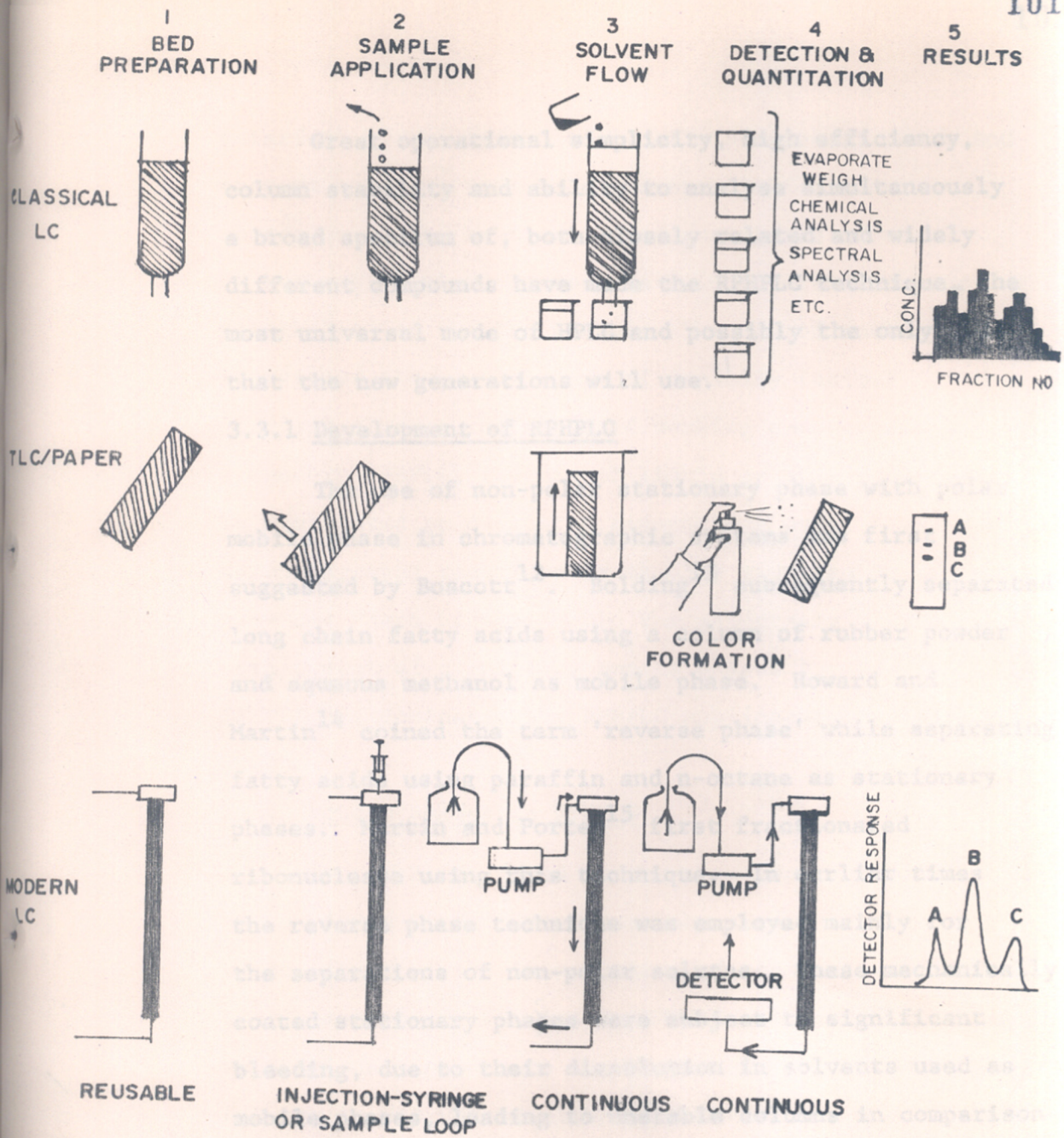
iii) Pumps, capable of operating at high pressure, are employed to obtain precise mobile phase flows of higher magnitudes.

iv) Sample detection is continuous by sensitive detection systems.

v) The analysis time is typically less than one hour, or in certain cases minutes, with resolution superior to that obtained over Classic Column Chromatography (Fig. 1)[10].

3.3 Reverse phase HPLC (RPHLC)

In the recent times, RPHLC has emerged as the most popular and general HPLC technique, so much so that today more than 80% of HPLC separations are performed using this technique with the field still expanding rapidly. The reverse phase mode of HPLC uses a non-polar stationary phase and a polar mobile phase in contrast to normal phase HPLC (NPHPLC) which employes a polar stationary phase with a non-polar mobile phase. The RPHPLC is ideally suited for separations of non-polar as well as moderately polar compounds. Solutes not readily separated by normal phase liquid-liquid chromatography or liquid-solid chromatography can easily be resolved by reverse phase liquid chromatography. Polar and ionic compounds can also be separated by making use of secondary equilibria such as ion suppression, ion-paining (ion-association) and ligand exchange etc.



DIFFERENT FORMS OF LIQUID CHROMATOGRAPHY

FIG. I.

Great operational simplicity, high efficiency, column stability and ability to analyse simultaneously a broad spectrum of, both closely related and widely different compounds have made the RPHPLC technique, the most universal mode of HPLC and possibly the only mode that the new generations will use.[11]

3.3.1 Development of RPHPLC

The use of non-polar stationary phase with polar mobile phase in chromatographic systems was first suggested by Boscott[12]. Bolding[13] subsequently separated long chain fatty acids using a column of rubber powder and aqueous methanol as mobile phase. Howard and Martin[14] coined the term 'reverse phase' while separating fatty acids using paraffin and n-octane as stationary phases. Martin and Porter[15] first fractionated ribonuclease using this technique. In earlier times the reverse phase technique was employed mainly for the separations of non-polar solutes. These mechanically coated stationary phases were subject to significant bleeding, due to their dissolution in solvents used as mobile phases, leading to unstable columns in comparison with similarly coated columns in Gas-Liquid Chromatography. The solvent flow rates were also restricted to small values due to the fear of excessive bleeding or stripping of the stationary phase. The weak forces at the

interface of the mechanically held stationary phase and support material were held responsible for this phenomenon. The heat generated during the movement of the mobile phase across the column further enhanced the miscibility of the two phases. The use of such systems restricted the useful range of capacity factors, gradient elution and elevated working temperatures. In modern RPHPLC, the use of liquid as stationary phase is restricted to dedicated separations only. This chromatography technique is usually referred to as 'Reverse Phase Liquid-Liquid Chromatography'. The development of chemically bonded phases for use in RPHPLC has dominated the RPHPLC applications in recent times.

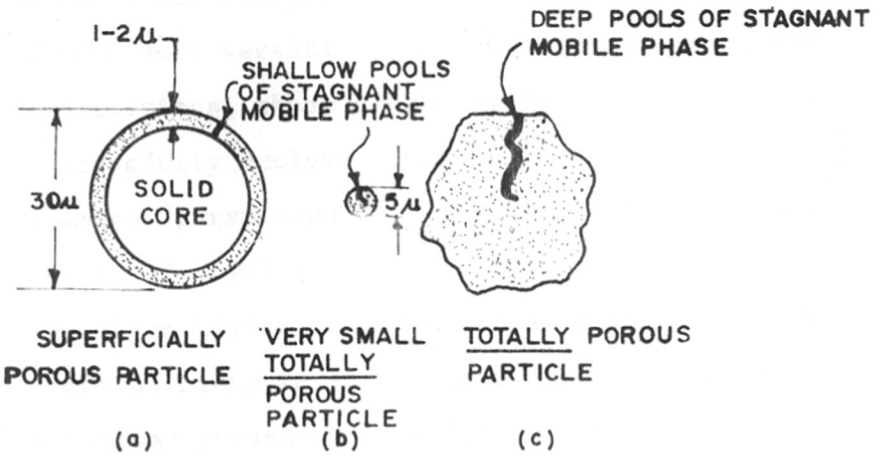
3.3.2 Bonded phases in RPHPLC

The problems faced in the practice of reverse phase Liquid-Liquid Chromatography were solved to a great extent by the introduction of chemically bonded stationary phases for use in RPHPLC. Chemical attachment of stationary phases to the supports was first described for use in Gas Chromatography by Abel[16] et. al. Subsequently Stewart and Perry[17] suggested that similar packings would augment the versatality and the life time of Liquid Chromatography Columns. The chemical bonding of stationary phases was popularised by Halasz and Sebastian[148].

Pellicular packing materials for HPLC were developed by Horvath[8] and Kirkland[6]. These pellicular packings have range of 30 to 40 μm and consist of a solid core and a thin porous outer shell (retention layer of stationry phase). These packings exhibit rapid solute mass transfer, good efficiency and relatively easy packing procedures. However, due to their small surface area, they have limited sample capacity.

In 1972, Majors[19] and Hartwick[20] popularised microparticulate packings. These packings were totally porous with the range of 5 to 10 μm. The chemically bonded microparticulate packings exhibit increased rate of mass transfer resulting from small interparticle spaces and short pore depths. Due to the increased surface area the loading capacity of these columns is greater than that of pellicular packing columns (Fig. 2)[21].

In the case of bonded phases for RPHPLC the attractive forces between the solute molecules, stationary phase and mobile phase are weak[22]. This results in weak surface energies, ultimately leading to great operational simplicity. Analyses are rapid and reequilibration times, during solvent change over, are short. Uusually only 5 to 10 column volumes are enough for reequilibration which is considerably less than in NPHPLC.



TYPES OF PARTICLES FOR HPLC

FIG. 2

As compared with reverse phase liquid-liquid chromatography systems, the chemically bonded stationary phases can be employed in much wider range of gradient elution and elevated temperatures, thereby making RPHPLC more versatile. Apart from chromatographic applications, these RPHPLC systems have also been successfully employed in QSAR for obtaining physico chemical parameters[23-26] and for predicting molecular nature of complex pharmaceuticals and antimetabolites[27]. The major limitation of chemically bonded reverse phase packings is their restricted pH range (pH 2 to 8). Sometimes presence of surface silanol groups (≡Si-OH) due to incomplete coverage of silicasurface, leads to the peak tailing, slow mass transfer and non-linearity of sorption isotherms. However, many chemically bonded reverse phase packings which are free from these defects to a great extent are now commecially available.

3.3.3 Types of chemically bonded phases for RPHPLC

Silica is generally used as phase material for chemically bonded reverse phase packings. Suitable organic moieties are chemically bonded to silica surface at the silanol sites[18,28,29].

These bonded phases may be classified into four major groups, according to the type of bond between the silica support and the organic moiety bonded to the surface:

i) Ester phases $\rightarrow$ Si-OR

ii) Amino phases $\rightarrow$ $Si-NR_2$

iii) Carbon phases $\rightarrow$ $Si-CR_3$

iv) Siloxane phases $\rightarrow$ $Si-O-Si-CR_3$

The chemically bonded reverse phase stationary phases may also be classified according to their structures as 'monomeric' and 'polymeric' phases.

i) Monomeric (Brush type or bristle structure phase)

The monomeric phases exhibit formation of monomolecular organic layer on the surface of supporting silica particles. Thus, single organic molecule is attached to single surface site. Steric hindrance during the grafting process of these monomeric phases leaves non-bonded silanol sites, resulting in the presence of free silanol groups. The efficiency of monomeric layer packings is much greater than that of polymeric. Phases, primarily due to rapid mass transfer,

ii) Polymeric

The polymeric phases are formed by a layer structure with properties similar to those of a liquid. These phases exhibit slow solute mass transfer, slow equilibration and high coverage of the silica surface[28,30].

Though the silica based chemically bonded reverse phase packings are most commonly used in RPHPLC, some non-silica based phases have also been used for specific

applications, e.g. styrene divinyl benzene copolymers and modified carbon black. The co-polymers possess wider usable pH range (pH 1 to 12) than that of silica based bonded phases. Though they exhibit physical durability[31] these phases undergo variations when solvents are changed, from organic to aqueous, limiting their applications. The modified carbon black phases are made by modifying carbon black by benzene pyrolysis[32]. These phases have been used for separation of solutes differing slightly in molecular weights but belonging to the same homologous series. They, however, lack from high efficiency and stability.

3.3.4 Mobile phases in RPHPLC

The mobile phase generally employed in RPHPLC consists of water and an organic modifier for neutral compounds. For basic and acidic substances, an aqueous buffer added with suitable organic modifier is used. If secondary equilibrium is desired, as in reverse phase ion-pairing chromatography, couterions of varying degree of polarity are also added to the mobile phase. The eluting strength of polar solvents and/or their mixtures commonly used in RPHPLC separations, is reversed[33], compared to Snyder's eluotropic series for alumina[23]. Solvent purity assumes paramount importance due to highly sensitive detection devices

used in modern HPLC units. A new grade, known as 'HPLC grade solvents', has been introduced for the use in this technique. A solvent of this grade is specially distilled and has high purity and specific spectral and chemical characteristics. The organic modifiers typically used are acetonitrile, methanol and tetrahedrofuran. Acetone, dioxane, ethanol, isopropanol, etc. are other rather uncommon RPHPLC organic solvents. Acetonitrile and methanol have highest eluting power or strength in RPHPLC. Physical properties, like viscocity, surface tension and dielectroic constant, determine the overall solvent selectivity change as a function of composition[23,34-36].

3.3.5 Separation mechanisms of RPHPLC

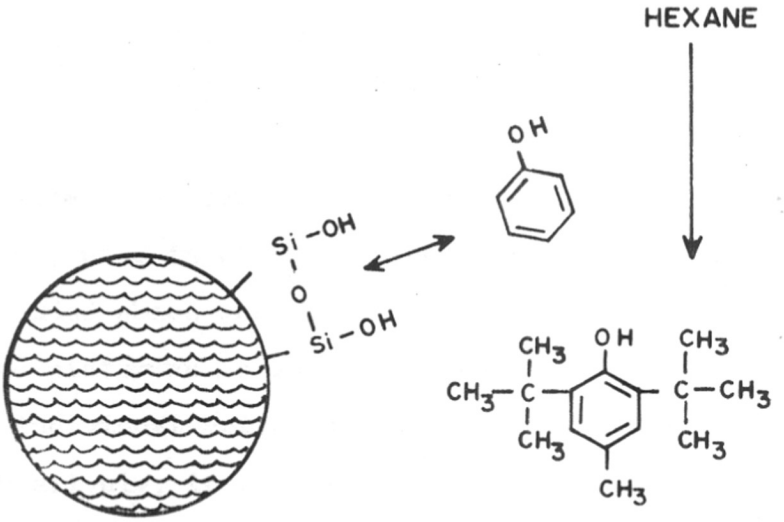
The separation mechanism of chemically bonded reverse phase packings has been a subject of continuing controversy. Interaction between the solute and polar stationary phase is the predominant factor in determining retention in NPHPLC. On the other hand, with non-polar phases in RPHPLC the solvent effects determine the selectivity. Although the nature of the organic group controls the selecstivity with respect to the solute functional groups[37], the separation of closely related solutes is governed by solution phenomena in the mobile phase[38].

Several workers[29,39-41] consider the separation mechanism of chemically bonded RPHPLC packings to be a mixture of adsorption and partition. However, Pryde[39] opines that neither term explains the separation mechanisms correctly. A comprehensive review[42] on this subject is available in the literature.

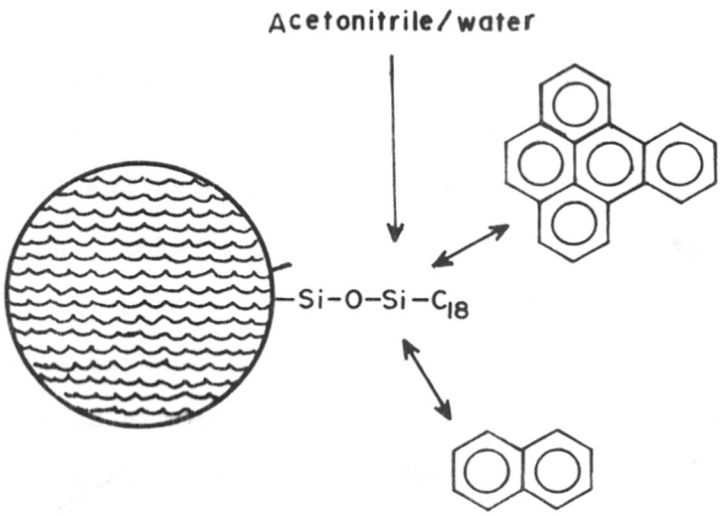
Comparative separation characteristics of NPHPLC and RPHPLC are depicted in the Fig.(3).

As is evident from this figure, the adsorption of phenol and a hindered phenol on to silica, from the mobile phase hexane, is determined by the polarisibility of the molecules, resulting in retardation of movement of phenol. The polarisibility is related to the dispersion or London forces. These effects hold true for NPHPLC where column packing is polar and carrier is less polar.

The chemically bonded RPHPLC packing is nonpolar and when used in combination with a polar mixture of acetonitrile and water as mobile phase, does not reject Naphthalene and Benzopyrine samples. However, the carrier being very polar does reject them. This forces the samples into the packing. The actual mechanisms are involved in solubilities, surface tensions, Van der Wall's forces and thermodynamics.



SEPARATION IN NPLC



SEPARATION IN RPHPLC

FIG.(3)

The exact surface topology of the bonded phases, commonly used in RPHPLC, is not yet fully understood. The bonded phase anchored to the surface of the support can be pictured as an alkyl brush[43] or molecular fur[23] with individual bristles making up the active surface. Alternatively, it may also be envisioned as a hydrocarbonaceous sheath made up of recumbent alkyl chains, aggregating to reduce contact area with the polar solvent[44]. The later liquid like structure is assumed to be composed of liquid droplet clusters permitting a three dimensional interaction with solutes by allowing the inercalation of solute molecules between the clustered alkyl chains[41].

It has been thought improbable in the case of non-cross linked monomeric bonded supports that 'brush type' will behave like a bulk liquid, since the average distance between the hydrocarbon chains is large and the bonded groups are deprived of some translational and relational decrease of freedom compared to the non-bonded state[42]. However, Lochmuller et al.[41] considered that isolated alkyl bristles could be energetically unfavourable and that hydrophopic association of bonded ligands is likely to occur in polar aqueous organic solvents. The presence of large negative entropy was considered to be a result of hydrophopic association of alkanes at low concentrations[44].

Chemically bonded polymeric phases are generally considered to act as an inert gel which contains small pools of mobile phase of different thermodynamic activity from the interstial liquid[28,45]. The predominant separation mechanism on these phases is considerdd to be the partition, although adsorption may also occur to some extent. This is primarily due to the differences in solute solubilities between the stationary and mobile phases. This is exemplified in increasing capacity factory (k') values with increased surface coverage, keeping surface area constant. These phases do not have typical properties of either a partitioning liquid or a chemical adsorbent[45,46].

The old adage 'like dissolves like' or 'like adsorbs like' actually becomes very involved when trying to explain separation phenomenon of RPHPLC.

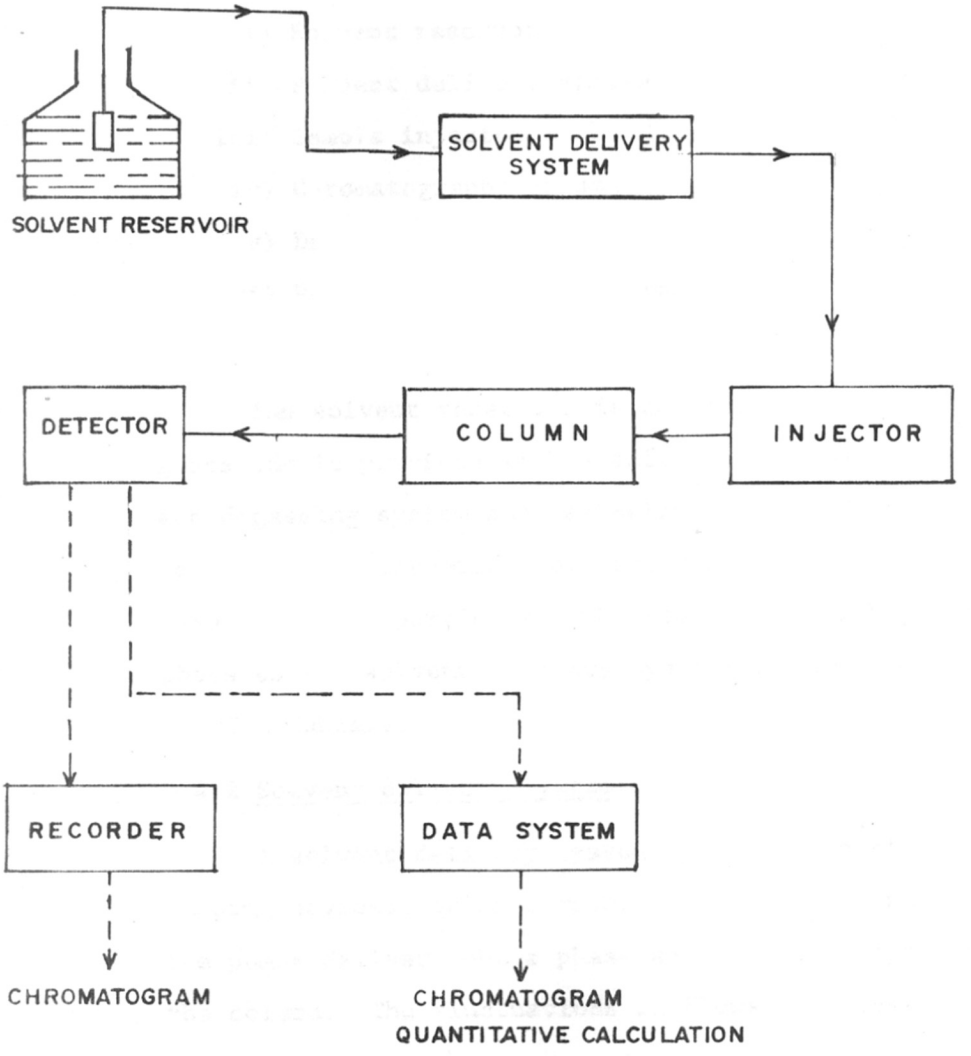
In summary -

i) The greater the lipophilic character of a compound the larger its retention on column;

ii) The smaller the lipophilic character of the mobile phase, the larger the retention of solute on to the column.

3.4 Instrumentation of HPLC

The instrumental liquid chromatography systems are available in many configurations depending upon specific applications. The basic features common to any HPLC unit are as follows : Fig. (4).



INSTRUMENTATION SCHEMATIC

FIG. (4)

i) Solvent reservoir

ii) Solvent delivery system

iii) Sample injector

iv) Chromatography column

v) Detector

vi) Recorder and data systems

3.4.1 Solvent reservoir

The solvent reservoir is generally made up of glass and is provided with a suitable stirring arrangement and degassing system such as Helium purge. The reservoir is protected from contaminations by atmosphoric gases and dust particles. The reservoir supplies mobile phase to the solvent delivery system through S.S. 316 or PTFE tubings.

3.4.2 Solvent delivery system

A solvent delivery system typically consists of pumping devices, pulse dampners and solvent mixing chamber. The pumps deliver mobile phase at prescribed rate into the column. The fluctuations in flows and pressure due to various mechanical reasons are taken care of by pulse dampners. Solvents delivered from different pump heads or, in the case of gradient operation, different reservoirs are thourhgly mixed in the mixing chambers. The main function of solvent delivery system is to deliver a smooth and constant flow of mobile phase

to the column, via injector, at the present flow rate and pressure. Many types of pumps are incorporated in HPLC units primarily depending upon applications.

3.4.3 Sample injector

At one end the injector is connected to the solvent delivery system and at the other end to the column inlet. Sample loops with switching valves typically constitute the injecting system in modern HPLC units. A sample can be injected either under pressure with flow or at atmospheric pressure without flow, depending upon the configuration. The injector acts as the entry point for solutes into the HPLC system.

3.4.4 Column

The column provides chromatographic bed for separation of sample components. Suitable stationary phase is packed into the columns with microfilters at both ends to hold the stationary phase in place and probibit entry of particulate matter on to the column. The column also acts as a guide to the passing solvent stream. Though S.S. 316 is preferred material of construction, HDPE has also been employed in certain models. Whenever necessary a constant temperature environment is provided for the column system.

3.4.5 Detector

HPLC technique demands a high sensitivity detection device to continuously monitor column eluant and generate signals for recording devices to trace chromatograms. Many physico-chemical properties are made use of in designing

detection systems to meet stringent demands of various applications. The detectors are generally classified by their functional principles. Two of the most commonly used detector types in HPLC are bulk property detectors and solute property detectors.

i) Bulk property detectors

A property which is common to both sample and mobile phase is differentially measured by bulk property detectors. The most widely used of these detectors, is Differential Refractometer. Difference between refractive indices of mobile phase and the mixture of mobile phase and solute molecules eluting from column generates peaks on the chromatogram.

ii) Solute property detectors

This type of detectors makes use of property which is specific to the solute molecules only e.g. Absorbance detectors (UV and fluorescence). UV detector is more popular. The mobile phase used in this det ector system is transparent at the selected wave-length whereas the solute absorbs as and when it elutes from the column causing a peak on the chromatogram.

Bulk property detectors like Differential Refractometer cannot be used for gradient operation where the solvent composition changes continuously. On the other hand, the solute property detectors like Absorbance Detector, are insensitive to changes in Gradient Elution Chromatography.

3.4.6 Recorder and data systems

The function of a recorder in instrumental liquid chromatography is to trace signals generated by detectors evolving a chromatogram. When only the mobile phase flows through the detector system, the recorder traces straight line; as soon as the detector senses presence

of solute, it transmits electronic signals to the recorder, causing Guassian curve trace known as 'peak'. The areas of these triangular peaks are proportional to the amount of solute passing through the detector and, hence, in the sample matrix. Data systems provide additional chromatographic informations like retention times, percent composition and other chromatographic parameters data pertaining to specific applications.

3.5 Chromatography

Reverse phase HPLC data was collected for the compounds described in the preceeding chapter using two organic modifiers in the mobile phase. Attempts to correlate the retention parameters in RPHPLC and lipoplilicity factor viz. number of carbon atoms in a molecule met with success and good correlations could be established between these two values.

3.5.1 Choice of stationary phase

The principal aim of the present work is to explore a possible relationship between retentions in RPHPLC and insecticidal activity. RPHPLC retentions have been successfully correlated with the number of carbon atoms for compounds in a series. Similarly, the biological activity has also been known to be a function of the number of carbon atoms for compounds of a series.

It is, therefore, proposed to compare the retentions in RPHPLC first with the number of carbon atoms and then with activity.

Linearally proportional relationship of retentions in RPHPLC with the number of carbon atoms for a series of compounds, failed to accommodate structurally different molecules. This point may be explained further as follows:

If two solutes with the same number of carbon atoms but different functional groups were chromatographed under identical conditions, then their retentions will reflect their polarity. A stationary phase like octadecyl bonded μBondapak - C_{18} will yield lower retention for polar molecule and higher retention for lipophilic functionalities.

A series consisting of such compounds did not produce a good relationship between RPHPLC retention and number of carbon atoms.

In an effort to generate retention parameters which will be proportional to the number of carbon atoms of a solute the concept of taking difference of selectivities of two columns in a given solvent system was introduced. The two column packings required for this purpose should be of different polarity. As the retention in RPHPLC was the result of net lipophilicity

of the solute molecule in a given mobile phase, the two columns were expected to produce different retentions for the same solute, e.g. in the case of a compound with a polar functional group, like hydroxyl group, octadecyl bonded phase will exhibit lower retention than alkylnitrile bonded stationary phase for the same solvent system. This difference was essentially due the polarity or hydrophobicity of the molecule under consideration. The difference in selectivities of the two columns was expected to indicate net retention of the said molecule in RPHPLC for the specified mobile phase and column packings. This difference was then successfully correlated with the number of carbon atoms for series of compounds. These values were also further related with activity of these compounds.

Two packing materials chosen by us were μBondapak-C_{18} and μBondapak -CN. Their general structures might be written as follows:

μBondapak - C_{18} : ≡Si - $(CH_2)_{17}CH_3$

μBondapak - CN : ≡Si - $(CH_2)_3CN$

The totally porous silica (μporasil) was used as base material for both these packings. The μBondopak-C_{18} packing was prepared by attaching an alkyl octadecyl group to silica surface at 10% by

weight level. A propionitrile group was chemically bonded to silanol sites at 9% by weight to prepare μBondapak -CN.

A μBondapak-C_{18} column packing was considered to be the best lipophilic commercially available stationary phase. The presence of cyano group imparts an intermediate polarity to the μBondapak-CN column packings. Thus, combination of one non-polar and one moderately polar column was investigated by keeping the nature of organic modifier constant.

Use of two columns of differing polarity with the same organic modifier presented chromatographic problems. The same composition of organic modifier and water could not be used for both columns. The amount of organic solvent in the mobile phase was adjusted so as to observe the '4R' rule of retention for the reference compound. The retentions of test compounds were then normalised to that of the reference compound to compensate for differences appearing due to the change in composition of organic modifier. α-Endosulfan was used as the reference standard for activity studies. The same compound was also taken as the reference standard in all the chromatography calculations and studies.

3.5.2 HPLC system used for RPHPLC

Retention times were recorded on a liquid chromatography unit supplied by M/s. Waters Associates,

USA with following modular configuration.

i) Solvent delivery system = M 6000 A pump with high pressure solvent mixing chamber capable to deliver 0.0 to 9.9 ml/min flow upto 6000 psi pressure.

ii) Injector - U 6 K - Universal Liquid chromatographic injector

iii) Detectors - a) M 440 Absorbance Detector at 254 nm
b) R 401 Differential Rectractometer

iv) Recorder - M 730 Data Module

The two column packings were also procured from M/s. Waters Associates as prepacked columns. The μBondapak- C_{18} was supplied as HDPE column cartridge to be used with RCM -100 Radical Compression Module. μBondapak - CN was available prepacked SS. column. The differences in dimensions were taken care of by taking the retentions relative to the reference. Particle size of both the packings was 10 μm.

Methanol and acetonitrile, possessing the highest eluting power in RPHPLC, were used as organic modifiers in conjunction with water to make the mobile phases for the present chromatography studies. Both these solvents as well as water were specially purified to make them HPLC grade and then used. Mobile phase flow rates were typically between 2 to 4 ml/min.

The relative retention concept takes care of change in flow rate for two columns, if any. All compounds of a series and Endosulfan were chromatographed at the same flow rate for a particular column and mobile phase combination.

The compositions of mobile phase used for two columns were as follows:

i) μBondapak - C_{18}

Methanol-water 80: 20 v/v (for series A,B,C,F,H & I)
90:10 v/v (for series D)

Acetonitrile-water 70:30 v/v (for series A,B,C,D,F,H & I)

ii) μBondapak - CN - for series A,B,C,D,F and H

Methanol-water 50:50 v/v

Acetonitrile-water 40:60 v/v

3.5.3 - Terminology and calculations

i) Capacity factor K'

$$K' = \frac{t_R - to}{to}$$

where, t_R = Retention time of the test compound

to = Retention time of the unretained compound (Acetonitrile)

to = 1.00 min for methanol water and acetonitrile, water on μBondapak -C_{18} (flow 3 ml/min) for series A,B,C,D,F,H,I

to = 1.50 min for methanol:water on μBondapak -CN (flow 2 ml/min) - for series A,B,C,D,F,H

to = 1.00 min. for acetonitrile:water on μBondapak-CN (flow 3 ml/min) - for A,B,D and F series

to = 1.5 min for acetonitrile:water on μBondapak -CN (flow 2 ml/min) for C and H series]

ii) Selectivity ratio (Relative capacity factor)

$$\alpha = \frac{K' \text{compound}}{K' \text{ Endosulfan}}$$

iii) Difference in selecstivities of two columns for a particular organic modifier added to water to prepare mobile phase.

$$\Delta\alpha = \alpha C_{18} - \alpha CN$$

where, αC_{18} and αCN are relative capacity factors (α) for a solute on μBondapak -C_{18} and μBondapak -CN columns respectively using the same organic modifier.

iv) Catm. = Number of carbon atoms in the dissimilar portion of the molecule of a compound in a given series.

3.5.4 Results and Discussions

Chromatographic data (Table No.III-1, III-2 and III-3) collected on the two columns was processed keeping the nature of an organic modifier constant. Thus, two sets of results were available - one each for methanol and acetonitrile. The capacity factor (K') values were normalisdd with that of reference standard α-Endosulfan and the resulting α and $\Delta\alpha$ values were plotted against Catm.

Table - III (1)

		Methanol - klater µBondapak-C18		Methanol-water µBondapak-CN		Acetonitrile-klater µBondapak-C18		µBondapak-CN	
S.No.	*Compd.No.	t_R min	$K'_{C_{18}}$	t_R min	K'_{CN}	t_R min	$K'_{C_{18}}$	t_R min.	K_{CN}
1	2	3	4	5	6	7	8	9	10
	α-Endosulfan	6.00	5.00	4.20	1.80	6.30	5.30	5.10	4.10
1	A_1	4.50	3.50	4.20	1.80	5.00	4.00	4.70	3.70
2	A_2	5.50	4.50	5.10	2.40	6.90	5.90	6.60	4.60
3	A_3	14.20	13.20	9.70	5.47	15.30	14.30	10.00	9.00
4	A_4	4.30	3.30	6.80	3.53	4.00	3.00	5.80	4.80
5	A_5	-	-	-	-	40.50	39.50	12.50	11.50
	α-Endosulfan	6.90	5.90	4.10	1.73	6.00	5.00	4.60	3.60
6	B_1	7.10	6.10	6.00	3.00	7.40	6.40	7.00	6.00
7	B_2	9.90	8.90	6.80	3.53	9.90	8.90	8.10	7.10
8	B_3	12.90	11.90	7.70	4.13	13.30	12.30	9.80	8.80
9	B_4	15.10	14.10	7.90	4.27	14.50	13.50	9.70	8.70
10	B_5	9.80	8.80	7.70	4.13	10.90	9.90	9.50	8.50
11	B_6	11.10	10.10	8.20	4.47	12.00	11.00	10.30	9.30
	α-Endosulfan	6.30	5.30	4.50	2.00	5.90	4.90	7.10	3.73
12	C_1	3.60	2.60	5.50	2.67	5.20	3.20	9.00	5.00
13	C_2	4.50	3.50	6.30	3.20	5.30	4.30	10.10	5.73
14	C_3	11.20	10.20	10.60	6.02	13.00	12.00	17.60	10.73

cont...

1	2	3	4	5	6	7	8	9	10
15.	C_4	3.40	2.40	5.60	2.73	3.80	2.80	8.40	4.60
16.	C_5	3.50	2.50	5.30	2.53	3.80	2.80	8.00	4.33
17.	C_6	5.10	4.10	5.20	2.47	5.70	4.70	8.30	4.53
18.	C_7	3.20	2.20	3.30	1.20	3.10	2.10	4.90	2.27
19.	∝-Endosulfan	2.70	1.79	4.90	2.27	6.30	5.20	5.10	4.10
20.	D_1	5.40	4.40	3.90	1.60	32.50	31.50	3.00	2.00
20.	D_2	6.20	5.20	4.10	1.73	39.40	38.40	3.10	2.10
21.	D_3	7.00	6.00	4.40	1.93	46.60	45.60	3.40	2.40
22.	D_4	3.50	2.50	6.50	3.33	16.20	15.20	2.30	1.30
23.	D_5	5.30	4.30	3.90	1.60	30.30	29.30	2.90	1.90
24.	D_6	2.10	1.10	4.70	2.13	4.60	3.60	4.30	3.30
25.	D_7	2.30	1.30	4.90	2.27	5.90	4.90	4.90	3.90
	∝-Endosulfan	6.00	5.00	4.20	1.80	5.90	4.90	4.70	3.70
26.	F_1	3.90	2.90	4.00	1.67	4.40	3.40	4.20	3.20
27.	F_2	5.10	4.10	4.50	2.00	5.90	4.90	4.90	3.90
28.	F_3	5.10	4.10	4.60	2.07	5.90	4.90	4.60	3.60
29.	F_4	7.00	6.00	5.40	2.60	8.10	7.10	6.10	5.10
30.	F_5	6.80	5.80	5.20	2.47	8.00	7.00	6.00	5.00
31.	F_6	9.50	8.50	6.60	3.40	11.10	10.10	7.50	6.50
32.	F_7	13.30	12.30	8.00	4.33	15.20	14.20	9.00	8.00

cont....

1	2	3	4	5	6	7	8	9	10
	∝ -Endosulfan	6.10	5.10	4.50	2.00	6.00	5.00	4.60	2.07
33.	H_1	6.40	5.40	7.20	3.80	9.50	8.50	9.30	5.20
34.	H_2	8.50	7.50	8.60	4.73	11.90	10.90	11.10	6.40
35.	H_3	8.40	7.40	8.80	4.87	11.50	10.50	12.30	7.20
	∝ -Endosulfan	6.10	5.10	-	=	6.20	5.20	-	-
36.	I_1	~~3310~~ 3.10	2.10	-	-	3.80	2.80	-	-
37.	I_2	3.70	2.70	-	-	4.40	3.40	-	-
38.	I_3	7.20	6.20	-	-	8.80	7.80	-	-
39.	I_4	~~2270~~ 2.70	1.70	-	-	3.50	2.50	-	-

Designation of compounds as per Chapter II, Table No.II-1

Table III - (2)

Compd. No.	Methanol-water: Bondapak-C_{18}	Methanol-water: Bondapak-CN	Methanol-water: $\Delta\alpha$	Acetonitrile:water: Bondapak-C_{18}	Acetonitrile:water: Bondapak-CN	Acetonitrile:water: $\Delta\alpha$	Catm
1	2	3	4	5	6	7	8
α-Endosulfan	1.00	1.00	0.00	1.00	1.00	0.00	2
A_1	0.70	1.00	-0.30	0.75	0.90	-0.15	3
A_2	0.90	1.33	-0.43	1.11	1.12	-0.01	4
A_3	2.64	3.04	-0.40	2.70	2.20	0.50	9
A_4	0.66	1.96	-1.30	0.57	1.17	-0.60	8
A_5	-	-	-	7.45	2.80	4.65	9
B_1	1.03	1.73	-0.70	1.28	1.67	-0.39	7
B_2	1.51	2.04	-0.53	1.78	1.97	-0.19	8
B_3	2.02	2.39	-0.37	2.46	2.44	0.02	9
B_4	2.39	2.47	-0.08	2.70	2.42	0.28	9
B_5	1.49	2.39	-0.90	1.98	2.36	-0.38	7
B_6	1.71	2.58	-0.87	2.20	2.58	-0.38	7
C_1	0.49	1.34	-0.85	0.65	1.34	-0.69	7
C_2	0.66	1.60	-0.94	0.88	1.54	-0.66	8
C_3	1.92	3.04	-1.12	2.45	2.88	-0.43	11
C_4	0.45	1.37	-0.92	0.57	1.23	-0.66	8
C_5	0.47	1.27	-0.80	0.57	1.16	-0.59	8
C_6	0.77	1.24	-0.47	0.96	1.21	-0.25	7
C_7	0.42	0.60	-0.18	0.43	0.61	-0.18	3
D_1	2.59	0.70	1.89	6.06	0.49	5.57	3
D_2	3.06	0.76	2.30	7.38	0.51	6.87	4
D_3	3.53	0.85	2.68	8.77	0.59	8.18	5
D_4	1.47	1.47	0.00	2.92	0.32	2.60	4
D_5	2.53	0.70	1.83	5.63	0.46	5.17	5
D_6	0.65	0.94	-0.29	0.69	0.80	-0.11	4
D_7	0.76	1.00	-0.24	0.94	0.95	-0.01	5

cont.....

Table III - (2)

1	2	3	4	5	6	7	8
F_1	0.58	0.93	-0.35	0.69	0.86	-0.17	2
F_2	0.82	1.11	-0.29	1.00	1.05	-0.05	3
F_3	0.82	1.15	-0.33	1.00	0.97	0.03	3
F_4	1.20	1.44	-0.24	1.45	1.38	0.07	4
F_5	1.16	1.37	-0.21	1.43	1.35	0.08	4
F_6	1.70	1.89	-0.19	2.06	1.76	0.30	5
F_7	2.46	2.41	+0.05	2.90	2.16	0.74	6
H_1	1.06	1.90	-0.84	1.70	2.51	-0.81	10
H_2	1.47	2.37	-0.90	2.18	3.09	-0.91	11
H_3	1.45	2.44	-0.99	~~3.18~~ 2.10	~~3.38~~ 3.48	-1.38	11

Designation of compounds as per Chapter II, Table II-1

Series A [Fig. (5)]

Three members of this series A_1, A_2 and A_3 were hydrocarbonaceous in nature. A_4 possessed a hydroxyl group and A_5 incorporated two chlorinated bicyclo moieties. A_5 did not elute in aqueous methanol on both the columns.

Methanol - water

μBondapak-CN produced a better linear relationship between α and Catm, than μBondapak C_{18}. In the graph of Catm νs $\Delta\alpha$, A_3 was exception to the pattern developed by others.

Acetonitrile - water

For this solvent system as well μBondapak -CN proved to be better than μBondapak-C_{18}. A_5 though observing linear relation in Catm. versus α was found to be far away in Catm. versus $\Delta\alpha$ graph. The other four members indicated reasonably good pattern.

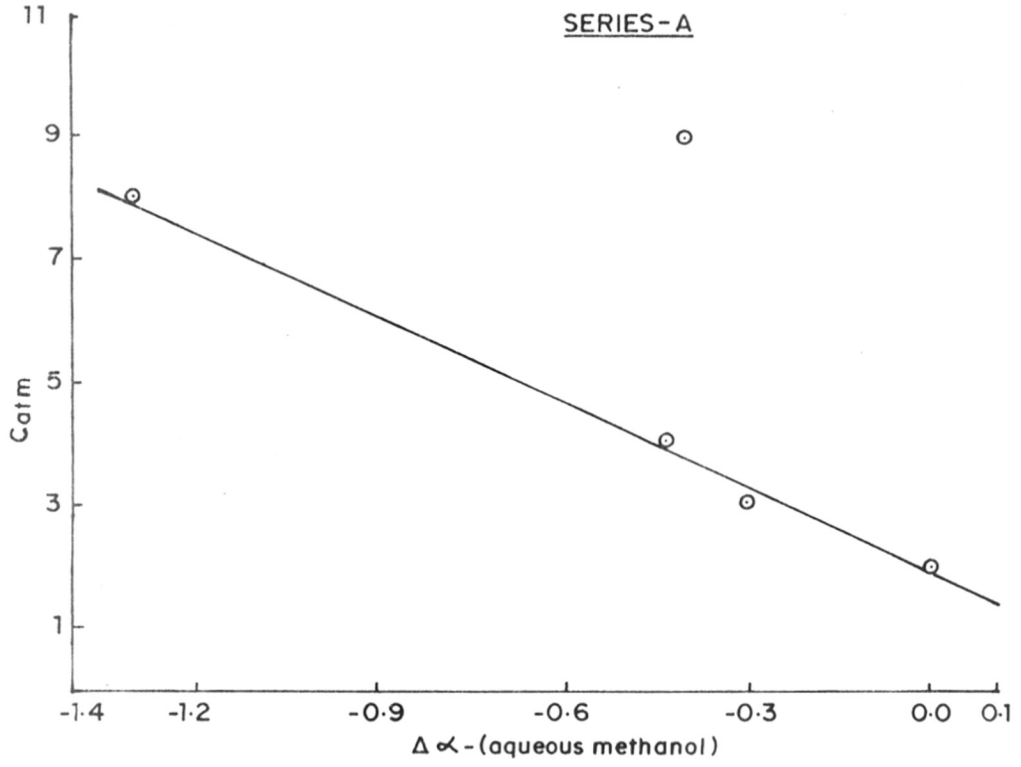
Thus for this series, acetonitrile was found to be better than methanol for $\Delta\alpha$ values.

Series B [Fig. (6)]

B_5 and B_6 characterised by the presence of chlorine and bromine respectively, were different than other members of this series.

Methanol - water

A good linear relationship could be observed between Catm. and α with the exception of B_5 and B_6


SERIES-A
11
9
7
5
3
1
Catm
-1·4
-1·2
-0·9
-0·6
-0·3
0·0
0·1
Δ ∝-(aqueous methanol)

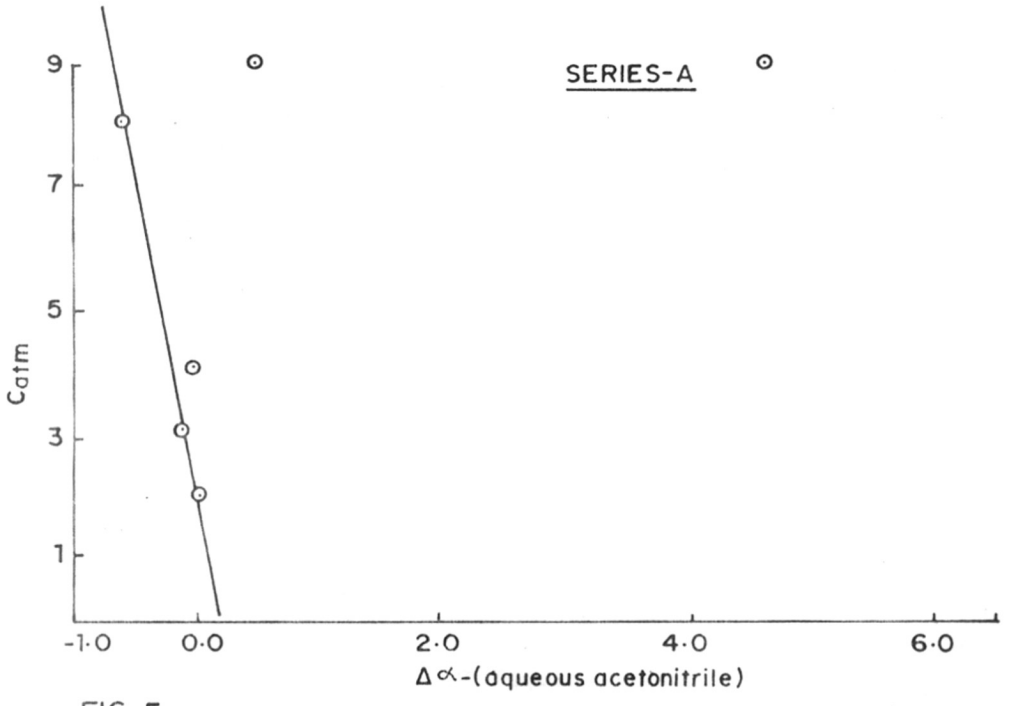
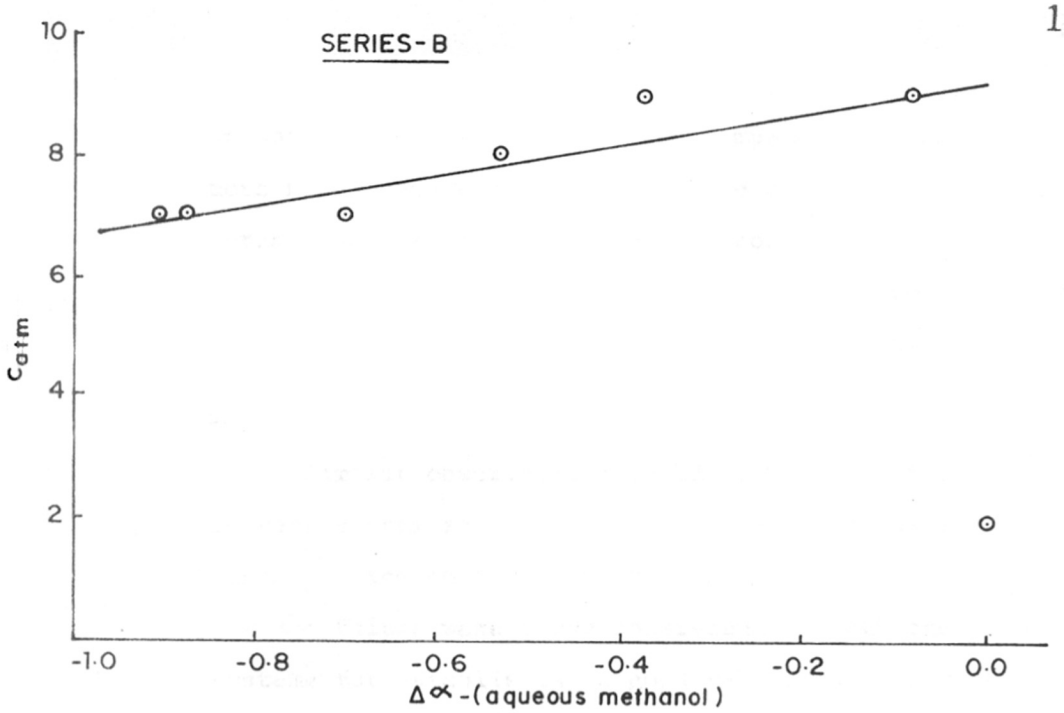

SERIES-A
9
7
5
3
1
Catm
-1·0
0·0
2·0
4·0
6·0
Δ ∝-(aqueous acetonitrile)

FIG. 5


SERIES-B
10
8
6
4
2
C_{atm}
-1·0
-0·8
-0·6
-0·4
-0·2
0·0
Δ∝-(aqueous methanol)

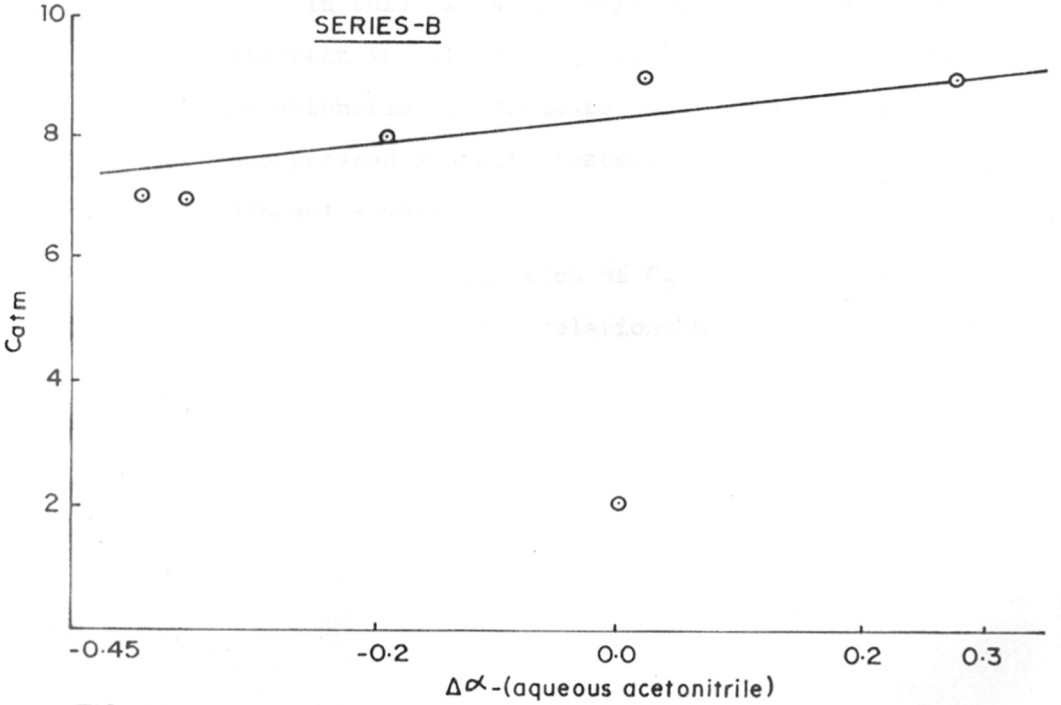

SERIES-B
10
8
6
4
2
C_{atm}
-0·45
-0·2
0·0
0·2
0·3
Δ∝-(aqueous acetonitrile)

FIG. 6

on both the columns. These two compounds exhibited more retentions than B_1 on both the columns though all three possessed same number of carbon atoms. When $\Delta\alpha$ values were compared with Catm. a linear relationship could be obtained for all the compounds of this series.

Acetonitrile - water

Similar observations could be made for this solvent system as well. B_5 and B_6 appeared as the straight line on the Catm. versus $\Delta\alpha$ plots.

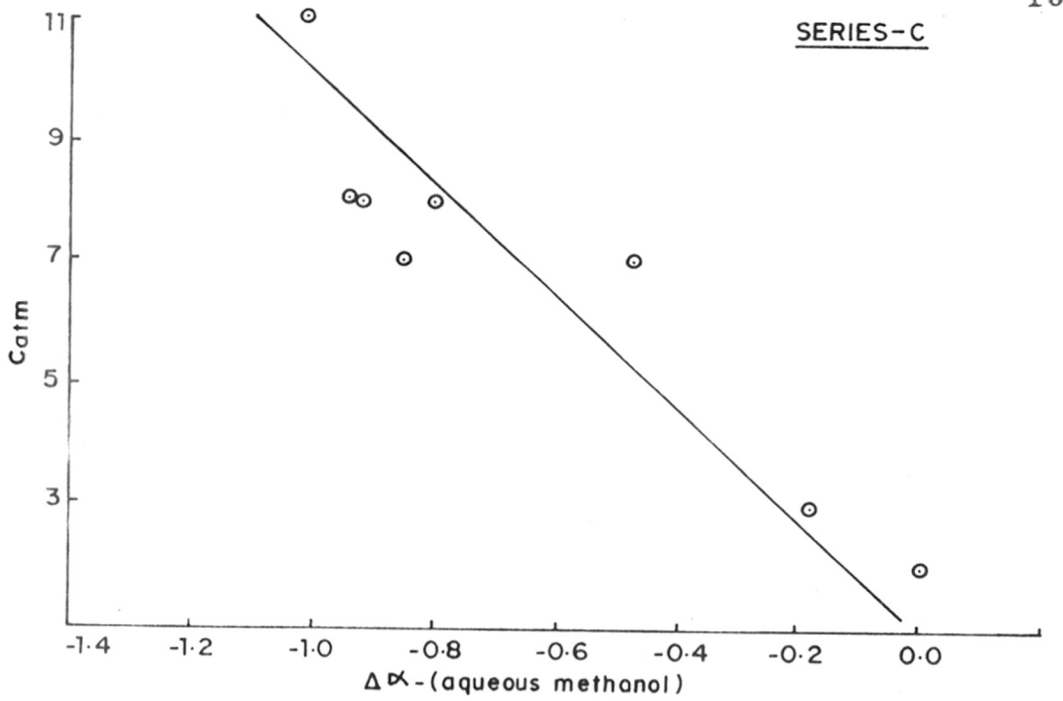
$\Delta\alpha$ Values were found consistent in both the solvent systems for establishing a good relationship with Catm.

Series C [Fig. (7)]

In this series as well as two compounds were different in nature. C_6 and C_7 possessed cyclohexyl and aliphatic substituents, while all others incorporated aromatic systems.

Methanol - water

With the exception of C_7 all other compounds established a linear relationship between α and Catm. on both the columns. When Catm versus $\Delta\alpha$ graph was examined even C_7 was found to follow the general pattern.


SERIES-C
11
9
7
5
3
Catm
-1·4
-1·2
-1·0
-0·8
-0·6
-0·4
-0·2
0·0
Δ∝-(aqueous methanol)

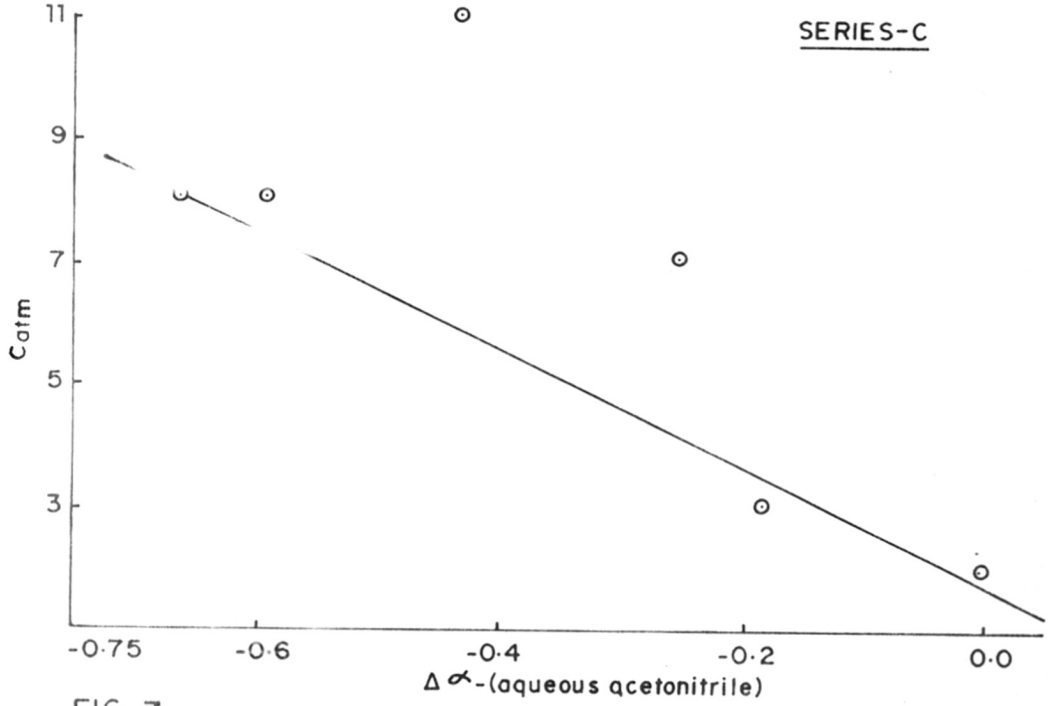

SERIES-C
11
9
7
5
3
Catm
-0·75
-0·6
-0·4
-0·2
0·0
Δ∝-(aqueous acetonitrile)

FIG. 7.

Acetonitrile - water

The observations for α were similar on both the columns as in the case of aqueous methanol. The Catm versus $\Delta\alpha$ graph was however found to be different. Though C_7 could still be found on the straight line C_3 and C_6 were exceptions.

Aqueous methanol was, therefore, the right solvent for this series.

Series- D

Three compounds of this series D_1, D_2 and D_3 could be considered to constitute a homologous series. D_4 possessed two hydroxyl groups, while D_5 contained one hydroxyl group and an ether linkage. D_6 and D_7 were mono ethers with no free hydroxyl groups. The behaviour of these compounds was characteristic to their structures.

Methanol - water

D_1, D_2 and D_3 exhibited linear relationship between Catm and α on both the columns. D_6 and D_7 were retained more on the Bondapak-CN. The $\Delta\alpha$ values did not help much in establishing a good relationship with all the members. The first three compounds still exhibited a linear relationship. Only D_5 could be seen to fall much closer.

Acetonitrile - water

The basic patterns were essentially similar to the ones obtained for methanol water for both α and $\Delta\alpha$

Series - F [Fig. (8)]

This series could be regarded as a truly homologous series. Linear relationships were obtained not only between Catm and α but with $\Delta\alpha$ as well for both solvents and columns. Only F_7 was exceptional in all the cases.

Series H

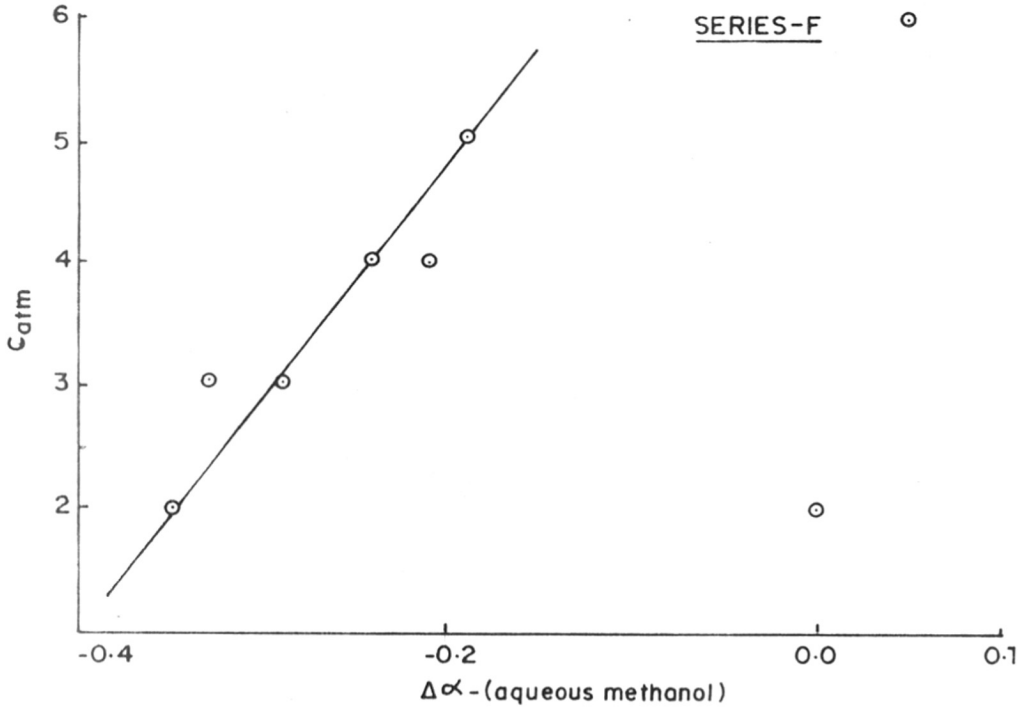
Observations in this series were essentially similar with those of series F for both the solvent systems and columns.

Series I

μBondapak - CN column irrevocably held some members of this series while decomposing others in both the solvent systems. The same compounds were chromatographed well on μBondapak - C_{18} in both solvent systems. Comparison was not, therefore, possible.

3.5.7 Series E and Series G

Chromatography of series E and G could not be carried out under the chromatographic conditions employed for the compounds of the other series.


SERIES-F
6
5
4
3
2
C_{atm}
-0·4
-0·2
0·0
0·1
$\Delta\alpha$-(aqueous methanol)

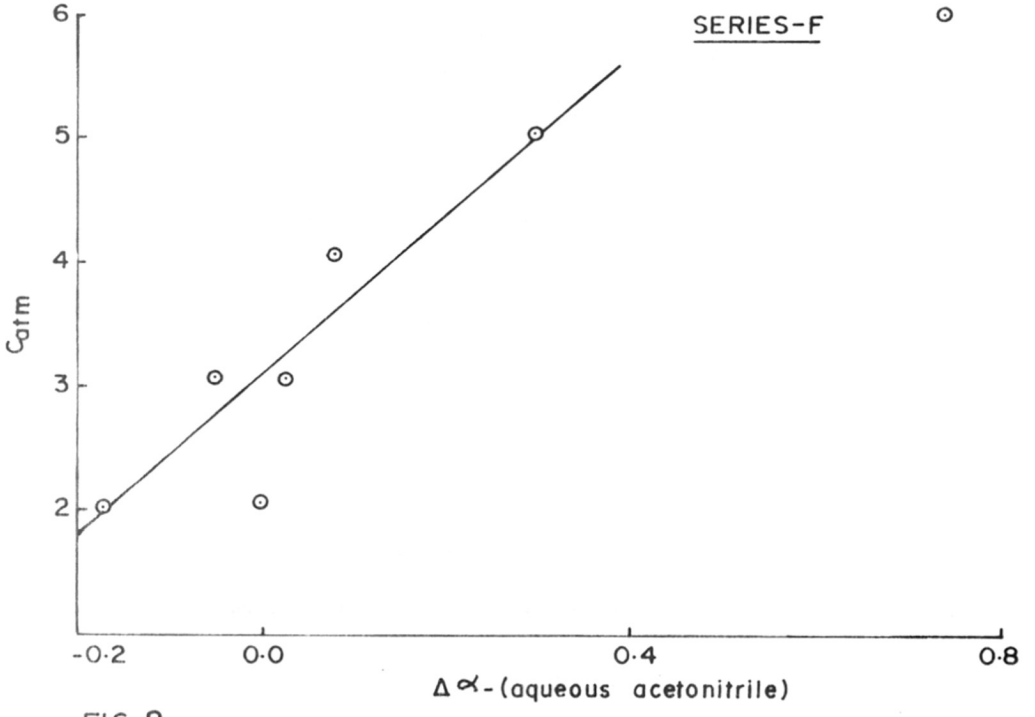

SERIES-F
6
5
4
3
2
C_{atm}
-0·2
0·0
0·4
0·8
$\Delta\alpha$-(aqueous acetonitrile)

FIG. 8

Series E

These are salts of compound (XVI) with various organic amines. They are water soluble and prone to high degree of dissociation into ionic species in both the mobile phases, used for RPHPLC in the preceeding sections. All attempts to preserve their original molecular forms in the mobile phase proved to be futile, despite changes in mobile phase compositions and introduction of buffers. The inevitable presence of water in RPHPLC mobile phase possibly rules out chromatogaphy of these compounds under present circumstances.

Series G [Table-III-3]

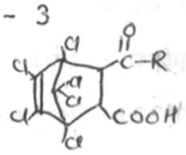
Presence of free carboxylic acid group and water solubility presented chrpmatograpby problems for the compounds of thiis series. The ion exchange chromatography could have been the method of analysis. However, intention to use the same RPHPLC colum, viz. Bondapak-C_{18}, employed for other series led to the use of a newly developdd technique known as'Ion-Pairing' of 'Paired Ion' chromatography for this series.

Ion Paaring Chromatography (IPC)[47]

On RP column packing separations are achieved because of different degrees of attraction of the sample for a non-polar stationary phase and polar mobile

Table III - 3

Series G



μBondapak C_{18}

Solvent system

$CH_3CN:H_2O$: Tetrabutyl ammonium ~~hydroxide~~: ~~H_3PO_4:NH_4OH~~

70:30:~~1~~ v/v : ~~(pH 4.33):(pH 7.50)~~

to = 0.9 min. flow 3 ml/min

S.No.	* Compd.No.	t_R min.	K'	Insecticidal activity against mosquito
1	G_1	3.40	2.78	0
2	G_2	5.50	5.11	0
3	G_3	7.20	7.00	0
4	G_4	6.10	5.78	0
5	G_5	2.60	1.89	0
6	G_6	4.80	4.33	0
7	G_7	5.26	4.78	0

* Designation of compounds as per Chaper II - Table II - 1

phase. By its very nature, RPHPLC has been most useful for non-polar, non-ionic compounds. To separate ionic compounds by reverse phase, it is necessary that the ionic compound should behave as non-ionic species with some non-polar lipophilic characteristics.

For many ionic compounds following equilibrium exists:

non-ionic $\rightleftharpoons$ ionic

It is possible to drive the equilibrium to the non-ionic side for weak acids and bases by adjustment of pH with addition of buffers. This technique is known as 'Ion-Suppression'. Strong acids and bases, however, cannot be separated by ion suppression as they remain ionic in the working pH range (2 to 8) of RPHPLC packings and are either poorely retained or eluted as skewed peaks.

Attempt to chromatograph compounds of series G by ion suppression method was done at pH 4. However, most of the compounds were found to decompose under chromatographic conditions.

In IPC a large organic counterion is added to the mobile phase to form a reversible ion-pair complex with the ionised sample. This complex behaves as an electrically neutral and non-polar lipophilic compound. The extent to which the ionised sample and the counter-ion form an ion-pair complex, affects the degree to which retention is increased. By adjusting the pH,

so that the sample is present in its ionic form, and choosing a strongly ionic counterion, with a very lipophilic group attached, the situation may be represented by the following equilibrium:

$$Sample^{+}- + Counterion^{\mp} \rightleftharpoons [Sample^{\pm}- Counterion^{\mp}]^{\circ} \text{ pair}$$

The actual mechanism of IPC is much more complex and is still in dispute.

The lipophilicity of the ion-pair formed depends upon the nature of sample and counter-ion involved. The more lipophilic ion-pair complex is, the greater will be its attraction for and retention on the non-polar stationary phase.

Thus, for basic samples an acidic pH (typically 3.5 to 4.0) with alkyl sulphonates as counter-ions is usually employed. A quarternary ammonium salt buffered to the pH 7.5 is used as the mobile phase for the acidic samples. The choice and use of organic modifiers follow the same rules in ~~this case~~ as other RPHPLC separations, now that resulting species is non-ionic and lipophilic.

Generally in IPC, the ion-suppression also plays its role and the versatility of IPC increases enabling analysis of mixtures of both weak and strong acids and bases.

Tetrabutyl ammonium phosphate, buffered to pH 7.5 with diammonium hydrogen phosphate buffer, was used as couner-ion in the mobile phase of acetonitrile-water for the separation of series G constituents. The same μBondapak-C_{18} column packing, which was earlier used for other series, was used as stationary phases. Acetonitrile-water (70:30) was used as mobile phase solvent. The graph of Catm versus α indicated that the IPC technique had indeed been successful. The compounds G_1 to G_4 formed a straight line graph. Halogenated compounds G_6 and G_7 did not fall on the line. Compound G_5 with a hydroxyl group, predictably, had low retention. It was not possible to collect similar data on μBondapak-CN column. Hence $\Delta\alpha$ values could not be obtained for this series.

3.6 Conclusions

in RPHPLC, retentions have been shown to be directly proportional to the number of carbon atoms in compounds of homologous series. This relationship no longer remained linear when different substituents were introduced. This fact was evident from the graphs of Catm versus α. Thus, in series A, the compound A_4 and in Series B two compounds B_5 and B_6 did not exhibit the linear relation as other members of their respective series. This behaviour was attributed to different

lipophilicities of these compounds. The proposed parameter, $\Delta\alpha$ appeared to take care of these differences to various extents in different series. Thus A_4 could be found on straight line in the graph of Catm versus $\Delta\alpha$ in both the mobile phases. Similarly, B_5 and B_6 also exhibited linear relationship between Catm and $\Delta\alpha$ and formed part of the straight line as others. At the same time, it was not possible for this concept to adequately compensate for all the structural differences. Thus D_4 and F_7 did not indicate linear relationship between Catm and $\Delta\alpha$ in many of the solvent systems. The concept of $\Delta\alpha$ was primarily developed for correlation with insecticidal activity (Chapter IV). The attempts for establishing a relation between $\Delta\alpha$ and Catm also met with success. The $\Delta\alpha$ concept appeared to take care of substitutions and linear relations could be obtained between Catm and $\Delta\alpha$ in many cases.

The ensuing observations highlighted the role of the mobile phases as well. The polarity, and also its nature, differs from methanol to acetonitrile. This difference could probably be linked with the observance of linear relationship in a particular solvent system only. Thus, in the case of Series-A, acetonitrile proved to produce better relations between Catm and $\Delta\alpha$ then methanol. But for Series-D, it was methanol that

brought D_5 nearer to the main pattern. The role of the solvent was also apparent in the case of Series C. The aqueous methanol was better solvent than acetonitrile water. For Series-B and F good relations could be observed for both the solvent systems.

In general the newly introduced $\Delta\alpha$ concept was found to work atleast in one of the two solvent systems in every case. Though, choie of the organic modifier is entirely empirical at this stage it may be possible in future to choose the correct solvent system as more and more data will be available.

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CHAPTER - IV

Chromatoraphy and Insecticidal Activity

SUMMARY

Applications of RPHPLC in the field of QSAR have been mostly to determine physicochemical parameters. The chromatographically estimated partition coefficients were generally related with other organic-aqueous partition coefficient values. Attempts were also made to correlate the capacity factor (K') values with these coefficients. Direct correlation of activity and chromatographic retention values was not always successful.

The reason for the failure of direct correlation was considered to be the use of single RPHPLC column packing. It was, therefore, proposed that if two columns with different polarity were employed to determine chromatographic partition values, then their difference would take care of the drawbacks encountered in the use of single column system.

When this hypothesis was applied for the series of compounds in the present studies, the difference values (ΔP) indeed nicely correlated with the insecticidal activity. As the ΔP values approached that of the reference standard the activity also found to increase.

This newly introduced concept was applicable equally well for all the four series of compounds. For the homologous series the pattern was found to be parabolic in nature.

This ΔP concept does not need any recourse to octanol-water partition coefficient and may be correlated with activity directly.

Reverse Phase High Performance Liquid Chromatography data presented in Chapter III for the compounds described in Chapter II, have been presently used to correlate the insecticidal activities of these compounds with their selectivity in the chromatography systems. HPLC itself is a newly introduced technique and is only about 16 years old. The reverse phase chromatography, though practised for a longer period of time, has history in HPLC of hardly a decade or so. The use of RPHPLC is essentially a new field and background information is scanty. The application of RPHPLC for the computation of physicochemical parameters is also very recent. In certain cases biological activity of a compound has been successfully correlated with its functional groups and to its liophilicity as estimated by reverse phase chromatography. The present dissertation attempts to find a relation between molecular lipophilicity obtained as a function of retention time in RPHPLC and insecticidal activity without recourse to octanol-water partition coefficient.

4.1 Partition phenomenon, HPLC and biological activity

Eversince 1899[1,2], when the first report appeared establishing correlationship of organic-aqueous partitioning with biological activity, many organic phases[3] have been employed to determine partition

coefficients of chemical compounds. In Quantitative Structure Activity Relationship (QSAR) studies octanol-water partition coefficients (P) have been an accepted model for lipophilicity[4,5]. A term was developed (π) as means for calculating the contribution of substituents to P[6]. This term may be defined as follows[7,8]:

$\pi = \log (P/Po)$ P = Partition coefficients of substituted compound

Po = Partition coefficients of unsubstituted compound

In practice, steric hindrances and electronic effects interfere in the determination of P for many systems[9,10]. Further, the established "shake-flask" method to measure partition coefficients is time consuming and required larger amounts of samples. This method also incorporates errors due to the presence of impurities in the compound under investigation, its detectibility in the analytical techniques used, its dissociation and decomposition and, sometimes, stable emulsion formation. Compounds of high or low partition pose difficulties in measurement of P by this method[1].

Partition chromatography was explored as an alternative technique for measuring lipophilicity. Chromatography is widely applied in the Hansch approach to QSAR based on a linear free energy relationship[11]. An analogous approach to the evaluation of substituent

contributions to chromatographic retentions, Quantitative Structure Retention Relationships, has recently been proposed by Horvath[12].

Most of the papers published in Chromatography in QSAR concern hydrophobicity changes. That part of the free energy change which can be attributed to hdrophobic bonding usually gives the major contribution to the biological response compared with the electronic and steric terms. The hydrophobic interaction in a chromatographic process can be separated from other types of intermolecular bonding. At first, the partition coefficient was considered as a measure of ability of a given compound to penetrate through biological membranes. In other words, it was considered as a measure of the probability for a given molecule to reach its site of action in a given time interval. Another mechanistic interpretation of meaning of 'partitioning properties' is based on the concept of the hydrophobic bond between the compound and the hydrophobic protein receptor[13].

The Fig. (1)[14] aptly depicts association between polar solvent and non-polar ligand at the surface of the stationary phase in Reverse Phase Chromatography. The binding is considered essentially due to solvent effects. The association of two species is facilitated by the decrease in molecular surface area exposed to the solvent

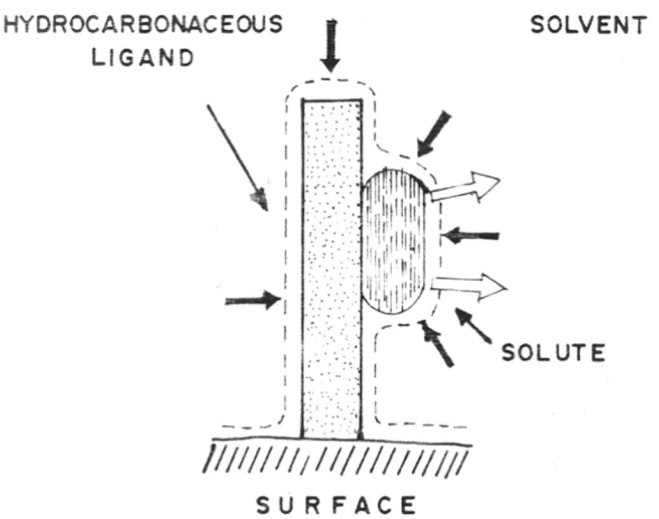
upon complex formation, as indicated by the 'solid' arrows. Attractive interactions with the solvent, symbolised by 'open' arrows, have countervailing effect. The difference between the two effects determines solute retention. Polar functions in the solute tend to enhance interaction with polar solvents reducing binding. Binding is stronger when the contact area in the complex is greater and/or surface tension of the solvent is higher. Due to its hydrocarbonaceous ligands and polar solvents, the RPHPLC system has been considered to be similar to the lipid membrane and extracellular fluid system.

Organic phase (octanol, silicone oil etc.) impregnated paper chromatography[15] and TLC[16,17] were in use in Quantitative Structure Retention Relationship Studies. The terms R_M and ΔR_M were derived to parallel log P and π and to directly correlate with biological activity[16].

$$R_M = \log\left(\frac{1}{RF} - 1\right) \quad \text{and} \quad \Delta R_M = RMx - RM_H$$

where, RMx and RM_H are the RM's of substituted and unsubstituted compounds, respectively.

Of the above two chromatography systems, TLC was more reproducible and simpler to use. It did not require quantitative analysis of the solute. Impure samples with smaller quantities could also be used.



CHROMATOGRAPHIC INTERACTIONS IN RPHPLC
FIG. 1.

TLC expanded the range of log P values that could be determined as compared to the 'shake flask' technique[16].

A semiempirical approach which allows the prediction of capacity factor, (K'), for a given chromatographic systems in Reverse Phase Chromatography, from octanol-water partition coefficient, may be illustrated by following equation[18]:

$$\log K'X = C_1 \log P \text{ oct. } X + C_2$$

where, P oct. X = octanol-water partition coefficient, C_1 and C_2 are the slope and Y intercept of the straight line that defines the straight line.

This correlation is based on the premise that partition of a compound between an organic-aqueous mobile phase and a non-polar bonded stationary phase is approximated by octanol-water partition coefficients. This relationship is inherently limited, since a separate equation must be derived for any given column - mobile phase combination.

By using the above equation a number of workers have investigated RPHPLC as means for determining lipophilicity by attempting to correlate cpacity factor (K'), which is essentially a retention time, parameter, with P_{oct}[19-21]..

The HPLC method possesses all the advantages of TLC plus added usefulness of accuracy and ease of

controlling experimental conditions. A broader range of lipophilicity may be estimated by RPHPLC as compared with 'shake flask' technique and RPTLC. Attempts have been made to directly correlate capacity factor values in HPLC with biological activity[19,22,23] with limited success.

4.2 Present work

RPHPLC is ideally suited for determinations of liphophilicity and partition coefficients. The first consideration towards calculating liphophilicity of a series of compounds by HPLC is the nature of stationary phase used in the column. This stationary phase replaces the organic phase 'shake-flask' method, thus necessitating use of RPHPLC. In RPHPLC the column packing comprises of two components - siliceous solid support and organic stationary phase. Both are capable of interaction with the solute in a given chromatographic system. With the advent of 'chemically bonded' RPHPLC packings, which produce stable columns, the lipophilicity determinations have been increasingly carried out on these non-polar bonded phases. However, it has been argued[21] that alkyl bonded phases will produce inferior correlation. This argument is based on the fact that these phases lack the polar character of octanol.

Adsorptive interactions with the support also prevail to some extent. However, the same arguments are used by some workers[18,20] to explain why superior correlations can be obtained with bonded phases.

Due to the differences between the nature of packing in RPHPLC and the organic phase in 'shake flask' technique, several researchers[20,23,24] have tried to duplicate 'shake-flask conditions by using octanol impregnated stationary phase. Unger, et al.[21] used an octanol-coated octadecyl bonded stationary phase and octanol saturated buffered mobile phase. The HPLC data was nicely correlated with log P and with biological activity. However, use of octanol presents problems in HPLC, primarily due to its viscosity, immiscibility and objectionable odour.

Some researchers have used chemically bonded stationary phases alone. The newer breed of RPHPLC column packings are stable and readily available as prepacked columns with assured efficiency. The major drawback of these packings was residual silanol sites present. McCall[18] had further deactivated the RPHPLC packings to remove active silanol groups. However, now many manufacturers have introduced in the market RPHPLC column packings which have been completely deactivated and, hence, are free from the above defect. We, in our studies, have used the most popular and assured quality

RPHPLC packings from M/s. Water Associaties, which have been universally acclaimed as the best reverse phase stationary phase materials, viz. μ Bondapak-C_{18} and μ Bondapak-CN. No further treatment was given to any of these prepacked columns and were used as such, after ensuring their efficiency as per manufacturer's instructions.

Baker[22] had drawn up a retention index as an universal scale for lipophilicity. The intention in drawing up such an index was to directly compare retention time data of different columns and mobile phases by using 2-keto alkanes as retention references compounds. This concept failed because 2-keto alkane standards could not account for all the interactions with the column and mobile phase that would be experienced by molecules bearing the full range of possible functional groups. This could be easily seen in Baker's work[25] itself, where inversion of elution order for various drugs occured with changing methanol content in the mobile phase.

Considering the above background information, it was decided to construct retention indices limited to every series for a particular stationary and mobile phase combination. The α-isomer of commercially available insecticide Endosulfan, was used as the standard reference for constructing these indices.

The retention indices were constructed on two different polarity columns, viz. μ Bondapak-C_{18} and μBondapak-CN. As has already been argued in the Chapter III, the retention of a compound in RPHPLC is the result of its net lipophilicity in a given solvent system. The lipophilicity of the solute, when measured as a function of the number of carbon atoms in the dissimilar portion of the compound under investigation, in a given series, was then found to be linearly proportional to the $\Delta\alpha$ values (Chapter III).

The retention parameter, K' in a particular column-solvent combination failed to elicit any definitive pattern with respect to the insecticidal activity. It was, therefore, considered to calculate P values by the following equation[27]:

$$P = K' \div \left(\frac{Vs}{Vm}\right)$$

where P = Chromatography partition coefficient

Vs = Volume of the stationary phase

Vm = volume of the mobile phase within the column

As already indicated this P value cannot be easily correlated with P of octanol-water partitioning. There is a specific solvent composition for a given column when these two values will coincide[27]. The compounds which we have studied are new compounds and their octanol-

water partition coefficients are not available. Direct correlation of chromatographic P values with octanol-water P values was not, therefore, possible. P now denotes chromatographically calculated values in all further discussions, unless stated otherwise.

An index of P values was created for every series using α-Endosulfan as reference standard.

The retention of a solute on μBondapak-C_{18}, which is an alkyl bonded stationary phase, is primarily due to its lipophilicity. The μBondapak-C_{18} is considered to lack the polarity of octanol in 'shake flask' method. Therefore, P values calculated from K' figures may be considered to suffer from certain drawbacks. The μBondapak-CN column was selected to overcome the difficulties encountered with the use of μBondapak-C_{18} alone. The μBondapak-CN possesses an alkyl nitrile group, imparting an intermediate polarity characteristic. A solute with a polar functional group, like hydroxyl group, will have different selectivities on these two columns with the same nature of solvent systems. It will be retained more on μBondapak-CN than on μBondapak-C_{18}. P values calculated from K' on these columns will also incorporate same drawbacks.

In order to remove anomalies created by the use of single RPHPLC column, combination of two columns of different polarity, compensating for functional differences

amongst members of a series of compounds, was considered essential. The μBondapak-C_{18} and μBondapak-CN have served well for this purpose for the series of compounds in our studies.

The difference between two P values (ΔP) obtained from these two columns in a given solvent system was correlated with insecticidal activity. Here again α-Endosulfan was introduced as the reference standard to generate indices of ΔP values.

In developing a lipophilicity constant, our method does not attempt to duplicate P in octanol-water partitioning but rather develops a new constant which may serve as a pointer towards possible insecticidal activity.

It has been possible to establish a definite relation between this new constant ΔP and the insecticidal activity of a compound. Instead of attempting any universal relationship it has been proposed to restrict the application of this hypothesis to particular series only. As has been observed in the section 4.3, the nearer the ΔP value of a compound towards that of α-Endosulfan, the more is its insecticidal activity. Thus, by calculating P values, from retentions on two different polarity columns, in a solvent system, it is possible to predict the extent of probable insecticidal activity

of a compound in a series. Eventhough the actual ratio of methanol and acetonitrile to water is different for μBondapak-C_{18} and μBondapak-CN columns, the basic characteristic of the mobile phase is considered to be the same. The changes in the organic modifier composition were effected to observe 4R rule of chromatographic retention. The difference in actual composition was considered to be nullified by normalising K' values of compounds with that of Endosulfan. Thus, two ΔP values were generated for every compound- one each for aqueous-methanol and aqueous-acetonitrile- using two column packings viz. μ Bondapak-C_{18} and μBondapak-CN. Both these values have good correlations with the insecticidal activity of a series.

4.3 Results and Discussions

Chromatographic retention values were correlated with insecticidal activity for four series of compounds viz. series A,B,C and F. The insecticidal activity was discussed in Chapter II and chromatography methods employed were described in Chapter III. Presently the retention values were used to calculate the partition coefficients for the various compounds which were then correlated with activity along with α and $\Delta\alpha$ values.

4.3.1 Terminology and Definitions

i) Partition coefficient (Distribution constant)

$$P = K' \div \left(\frac{Vs}{Vm}\right)$$

where, K' = capacity factor

Vs = Volume of the stationary phase

Vm = Volume of the mobile phase within the column

K' values obtained for test compounds were normalised with that of Endosulfan, which was used as the reference standard. This value was represented by

$$\alpha = \frac{K' \text{ compound}}{K' \text{ Endosulfan}}$$

This α value was then employed in the place of K' in the above equation which could then be written as,

$$P = \alpha \div \left(\frac{Vs}{Vm}\right)$$

Use of the relative value α was necessitated due to the different amount of organic modifier (methanol and acetonitrile) present in the mobile phases for the two columns used viz. μ-Bondapak-C_{18} and μ-Bondapak-CN. As the difference in selectivitees of these two columns formed the basis of the present studies, it was considered necessary to opt for the concept of relative retention. As the α-endosulfan was used as the reference for activity studies the same compound was selected for chromatography reference as well.

ii) $\Delta\rho = P_{C_{18}} - P_{CN}$

where,

$P_{C_{18}}$ = Partition coefficient of a compound on μ Bondapak C_{18} column for a particular organic modifier

P_{CN} = Partition coefficient of the same compound on μBondapak -CN column using the same organic modifier

iii) Insecticidal Activity (I.A.) was expressed in terms of percent mortality at 5 ppm.

iv) α and $\Delta\alpha$ bore the same meanings as defined in Cahpter III

4.3.2 Calculations

In order to determine P, it was necessary to estimate Vs and Vm for both the columns. The two columns had different dimensions. The μ Bondapak C_{18} packing was available in a HDPE cartridge fitted into Radial compression module (RCM-100). The cartridge has following geometry:

I.D. = 8 mm and Length = 100 mm

The μ Bandapak -CN packing was packed in S.S. 316 column system. The dimensions were - I.D. = 3.9 mm and Length = 300 mm.

The volume of the mobile phase within the column (V_m) was taken to be equal to the void volume. The void volume was calculated from the retention time of a non-retained compound. The difference between the total volume of the chromatography column and the

void volume was considered as the volume of the stationary phase (Vs). Thus, following two values were obtained -

μ Bondapak - C_{18} : Vs/Vm = 0.67

μ Bondapak - CN : Vs/Vm = 0.20

These values were then subsequently used for calculating P.

4.3.3 Correlation

The compounds used in the present studies were newly synthesised and tested for their insecticidal activities. This inherently restricted the scope of correlation studies to the limited range of activities obtained.

Three indices were constructed using two retention parameters (α and $\Delta\alpha$) and partition coefficient ($\Delta\rho$) for every series of compounds. Attempts were then made to correlate the insecticidal activities with these indices. While comparing data, nature of the organic modifier was kept constant. Thus, two sets were examined: One for methanol-water and the other for acetonitrile-water.

The P values were obtained by the division of α by chromatography system constant (Vs/Vm) and, hence, behaviourial patterns of α and P with respect to the insecticidal activity were found to be similar for

individual series. On the analogy of $\Delta\alpha$ introduced in the preceeding chapter, concept of Δp was mooted. A definite relation could be observed between this Δp and activity. The concept of P for comparison with activity was introduced primarily due to ill-defined patterns obtained in the graphs of α and $\Delta\alpha$ versus activity.

Thus for each series eight graphs were plotted - four each for two organic modifiers.

4.3.3.1 α and activity

Attempts to correlate α values with insecticidal activities of compounds met with partial success.

In the case of series A the μBondapak -CN solumn was found to be better than μBondapak-C_{18} in both solvent systems. It was observed that as α values approached unity the activity increased.

Similar observations could be made for series B as well. However, in this case definable patterns were visible in all the four solvent and column combinations.

Only aqueous methanol and μBondapak-CN column system produced a good relation between α and activity for compounds of the series C. Other three combinations failed to indicate any useful information.

Series F could be considered as a true homologous series.. Only μBondapak-CN column and aqueous methanol combination produced a well defined pattern. Compounds with more activity had values nearer to that of Endosulfan. Further, acetonitrile-water and μBondapak-CN column combination also indicated similar but somewhat obscure relationship.

Thus, in the case of α only certain solvent-column combinations appeared to afford proportional relationships with activity of compounds.

4.3.3.2 Difference in selectiviteis between two column packings ($\Delta\alpha$) and insecticidal activity

In an effort to derive a chromatography parameter which could be finitely related with activity of compounds under investigation the differences in selectivities between the two column packings, viz. μBondapak -C_{18} and μBondapak -CN, were plotted against the obserwed insecticidal activities. Thus, for every series, two graphs, one each for the two organic modifiers, were available for comparison.

For series A it was not possible to obtain a definitive pattern for $\Delta\alpha$ versus insecticidal activity in the case of aqueous acetonitrile. However, with methanol-water it was observed that as the values approached zero the activity was found to be more.

Similarly, while methanol-water yielded a proportional relationship between $\Delta\alpha$ and activity in the case of series B, the acetonitrile-water mobile phase failed to produce any definitive results. The pattern of graph of $\Delta\alpha$ versus I.A. for aqueous methanol for this series was the same as obtained for series A.

Acetonitrile-water appeared to be more suitable solvent system for the compounds of series C. A good pattern could be seen in the plot of $\Delta\alpha$ versus I.A.^{2} in this solvent system as against the confusing picture obtained for aqueous methanol.

For the homologous series F, good relationship between $\Delta\alpha$ and activity was obtained in aqueous methanol. It should, however, be noted that F_5 exhibiting maximum activity was found to be away from the main pattern. No such observations were evident for acetonitrile-water mobile phase and overall picture was found to be obscure.

It was apparent from above observations that the nature of organic modifier present in the mobile phase exercised its influence and in no case a good relationship could be obtained for both the solvent systems. Aqueous methanol was found to be better for series A,B and F, while for series C acetonitrile water proved to be suitable.

4.3.3.3 Difference in partition coefficients on two column packings ($\Delta\rho$) and insecticidal activity

The plots of $\Delta\rho$ versus I.A. for all the series under consideration were found to be consistent. Both the solvent systems produced similar and good proportional relationships (Fig. 2 to Fig. 5). As the $\Delta\rho$ values of compounds in a series approached that of Endosulfan the activity also increased. The advantage of $\Delta\rho$ over α and $\Delta\alpha$ was evident in the fact that the correlation could be obtained in both the solvent systems.

One more peculiar observation could be made from the graphs of $\Delta\rho$ V/S I.A. for various series with respect to the exceptions to the main patterns. Thus, A_5 in series A and C_6 and C_7 from series C did not fit into the relationships developed by other members of their respective series. All these three compounds were structurally different from other members. It might, therefore, be concluded that comparisons should be limited to structurally similar compounds. This further restricted relating $\Delta\rho$ values of one series with other series.

The series F yielded a distinctive pattern in addition to the one drawn according to those of other series. Parabolic relationships could be seen in both the solvent systems for this series with the maximum activity compound F_5 at the apex. This pattern pointed

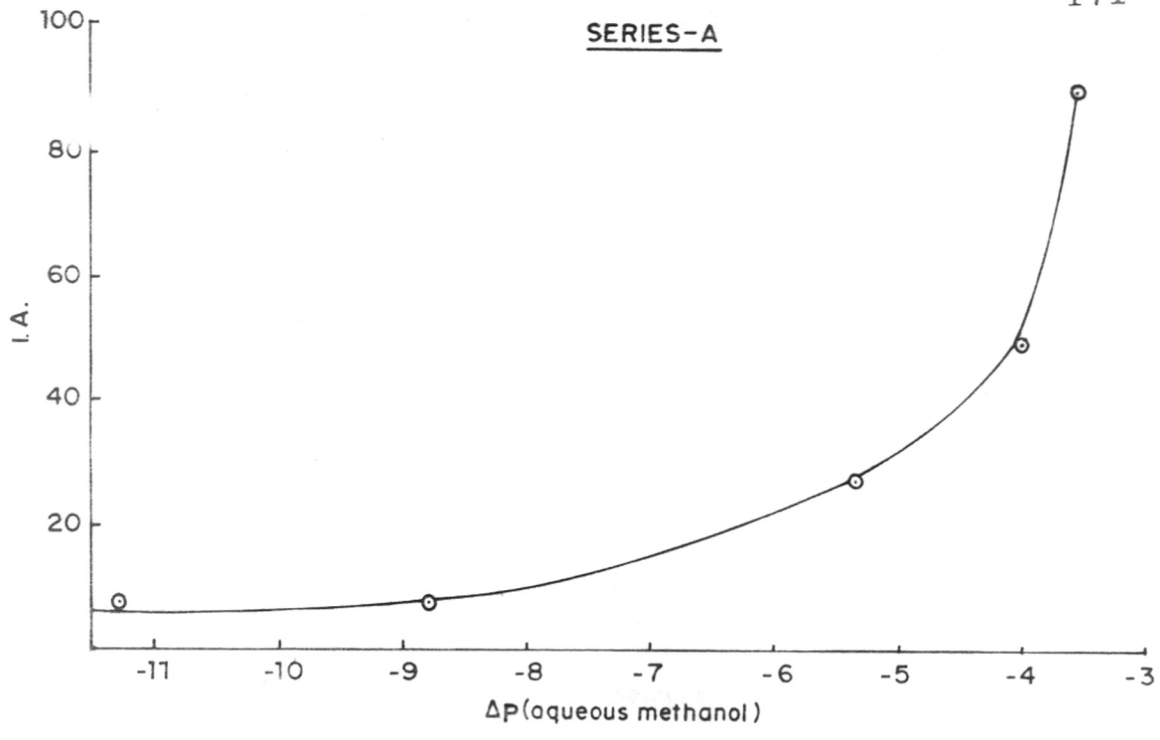
Table IV - (1)

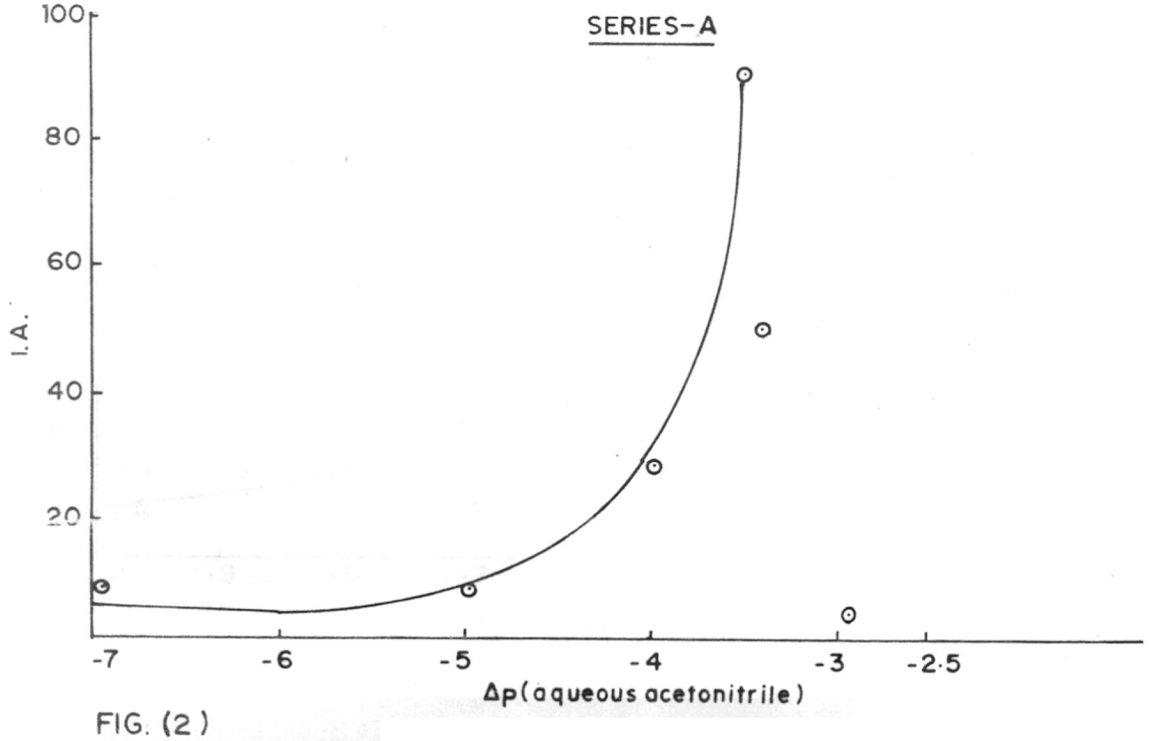
Compd. No.	Methanol-water			Acetonitrile:water			
	µBonda-pak-C_{18}	µBonda-pak CN		µBonda-pak-C_{18}	µBonda-pak-CN		I.A. Mosquito
	P	P	P	P	P	P P	
α-Endosulfan	1.49	55000	-23551	1.49	5.00	-3.51	90
A_1	1.04	5.00	-3.96	1.12	5.50	-3.38	50
A_2	1.34	6.65	-5.31	1.66	5.60	-3.94	27
A_3	3.94	15.20	-11.26	4.03	11.00	-6.97	7
A_4	0.99	9.80	-8.81	0.85	5.85	-5.00	7
A_5	-	-	-	11.12	11400	-2.88	4
B_1	1.54	8.65	-7.11	1.91	8.35	-6.44	14
B_2	2.25	10.20	-7.95	2.66	9.85	-7.19	334
B_3	3.01	11.95	-8.94	3.67	12.20	-9.53	10
B_4	3.57	12.35	-8.78	4.03	12.10	-8.07	37
B_5	2.22	11.95	-9.73	2.96	11.80	-8.84	14
B_6	2.55	12.90	-10.35	3.28	12.90	-9.62	7
C_1	0.73	6.70	-5.97	0.97	6.70	-5.73	8
C_2	0.99	8.00	-7.01	1.31	7.70	-6.39	10
C_3	2.87	15.20	-12.33	3.66	14.40	-10.74	14
C_4	0.67	6.85	-6.18	0.85	6.15	-5.30	15
C_5	0.70	6.35	-5.65	0.85	5.80	-4.95	7
C_6	1.15	6.20	-5.05	1.43	6.05	-5.62	0
C_7	0.63	3.00	-2.37	0.64	3.05	-2.41	0
F_1	0.87	4.65	-3.78	1.03	4.30	-3.27	14
F_2	1.22	5.55	-4.33	1.49	5.25	-3.76	16
F_3	1.22	5.75	-4.53	1.49	5.85	-3.36	24
F_4	1.79	7.20	-5.41	2.16	6.90	-4.74	10
F_5	1.73	6.85	-5.12	2.13	6.75	-4.62	97
F_6	2.54	9.45	-6.91	3.07	8.80	-5.73	44
F_7	3.67	12.05	-8.38	4.33	10.80	-6.47	0

Designation of compounds as per Chapter II, Table II-1
values from Chapter III, Table III-2

Table IV - (1)

Compd. No.	Methanol-water		Acetonitrile:water	
	Bondapak-C_{18} P	Bondapak CN P	Bondapak-C_{18}	Bondapak-CN


SERIES-A
100
80
60
40
20
I.A.
-11
-10
-9
-8
-7
-6
-5
-4
-3
Δp(aqueous methanol)


SERIES-A
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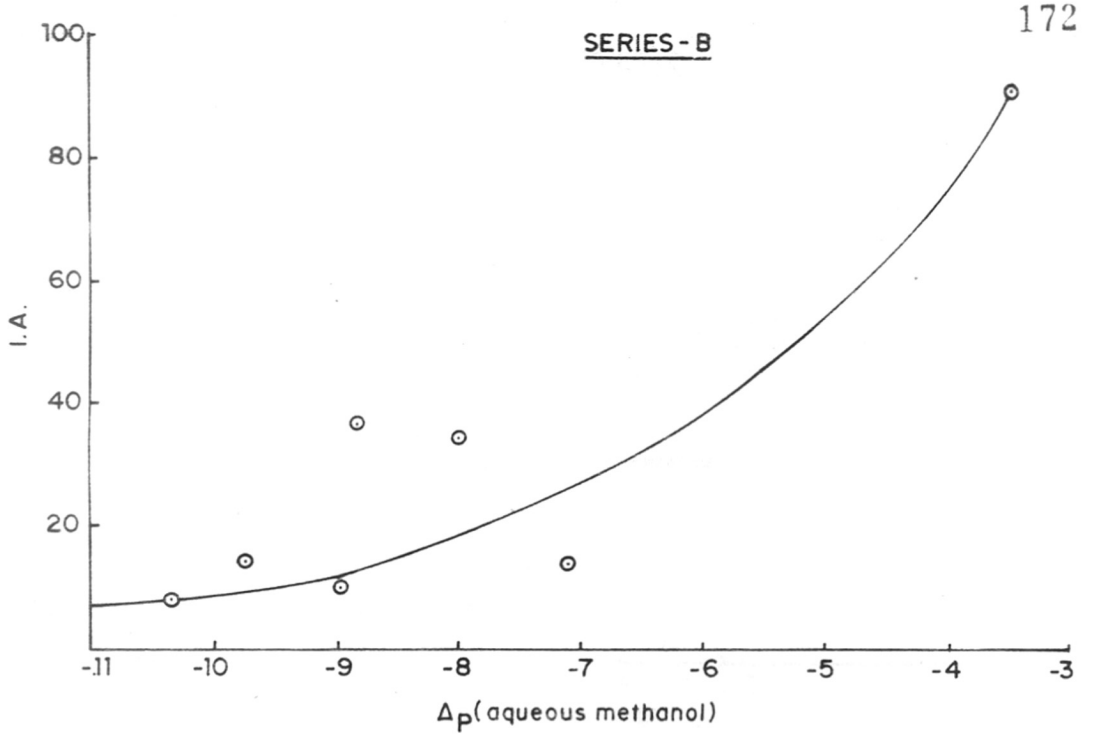
60
40
20
I.A.
-7
-6
-5
-4
-3
-2·5
Δp(aqueous acetonitrile)

FIG. (2)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I.A.
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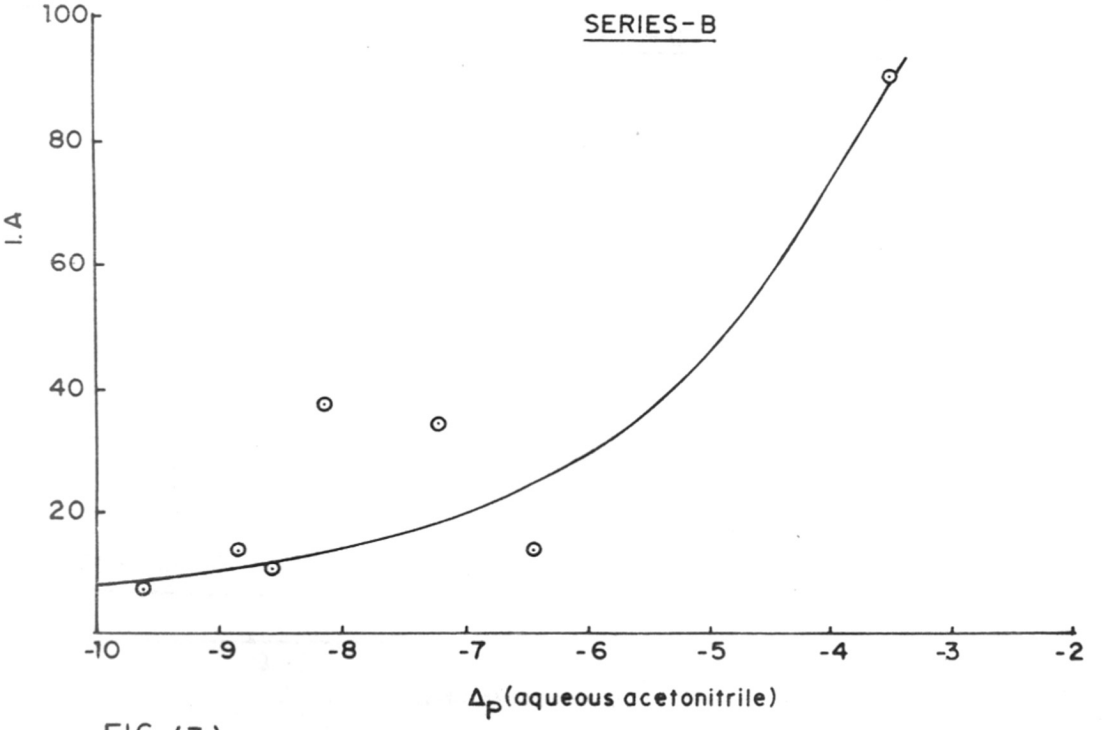
-10
-9
-8
-7
-6
-5
-4
-3
Δp(aqueous methanol)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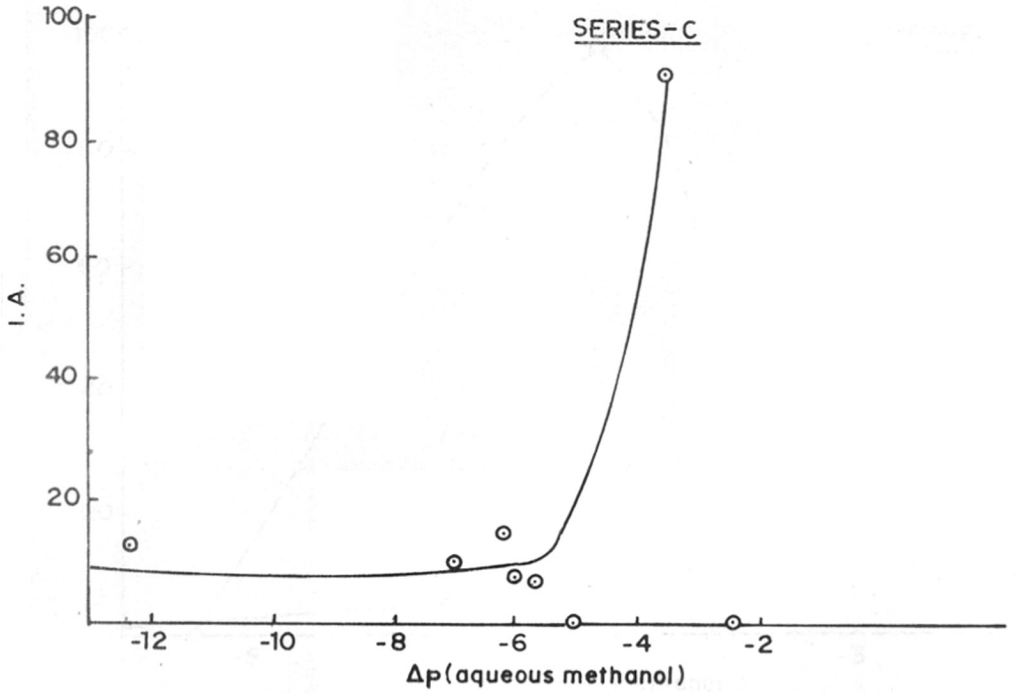
80
60
40
20
I.A
-10
-9
-8
-7
-6
-5
-4
-3
-2
Δp(aqueous acetonitrile)

FIG. (3)


SERIES-C
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I. A.
-12
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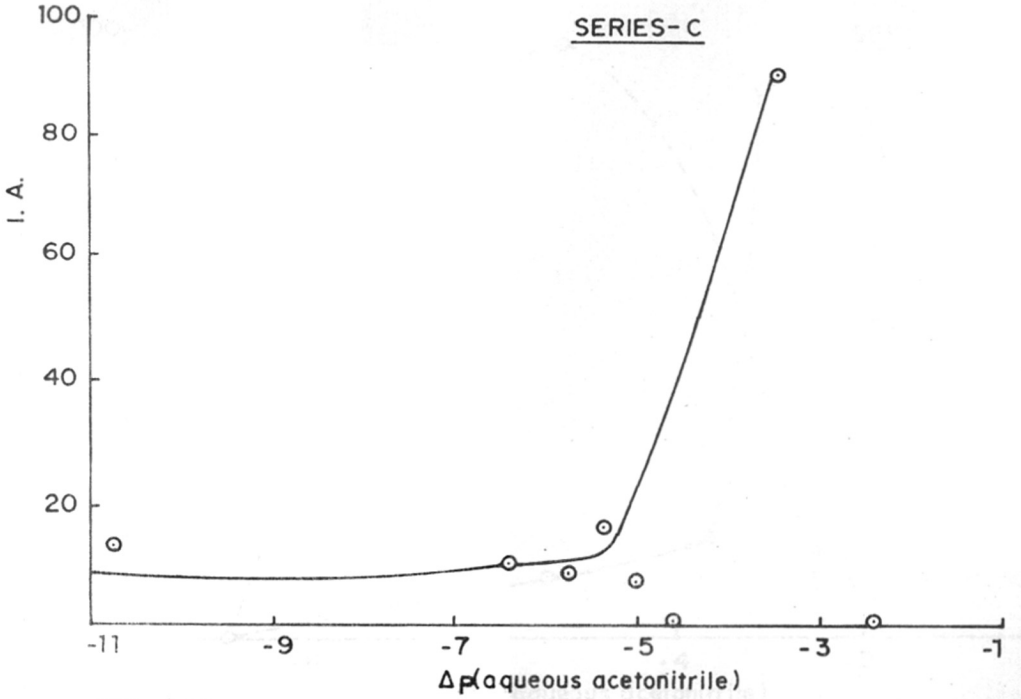
-6
-4
-2
Δp(aqueous methanol)


SERIES-C
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I. A.
-11
-9
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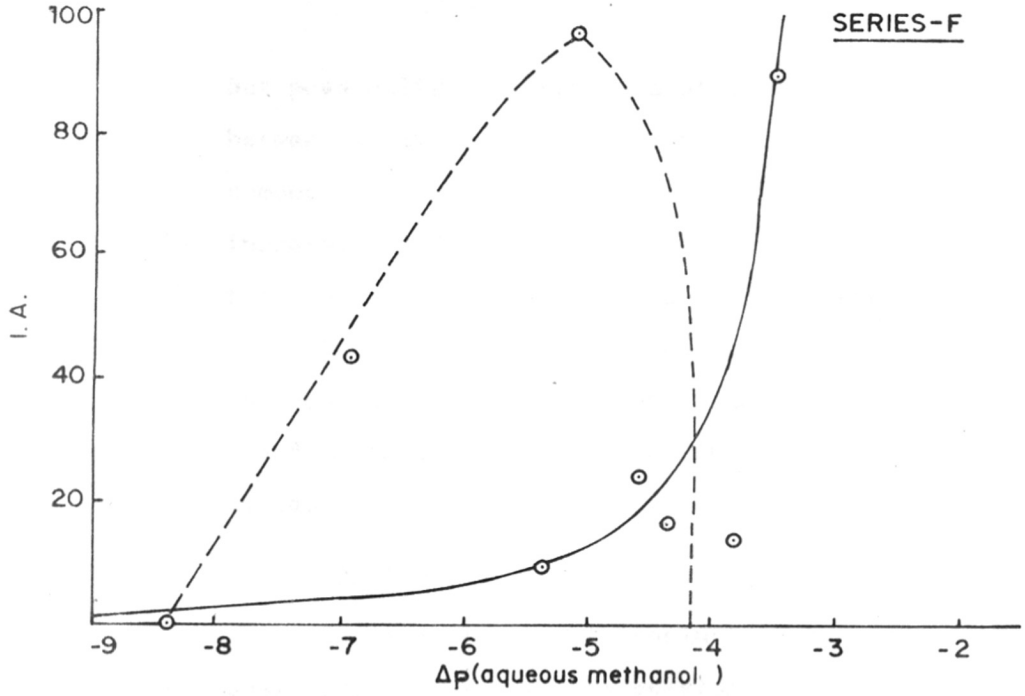
-3
-1
Δp(aqueous acetonitrile)

FIG. (4)


SERIES-F
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60
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I. A.
-9
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-7
-6
-5
-4
-3
-2
Δp(aqueous methanol)

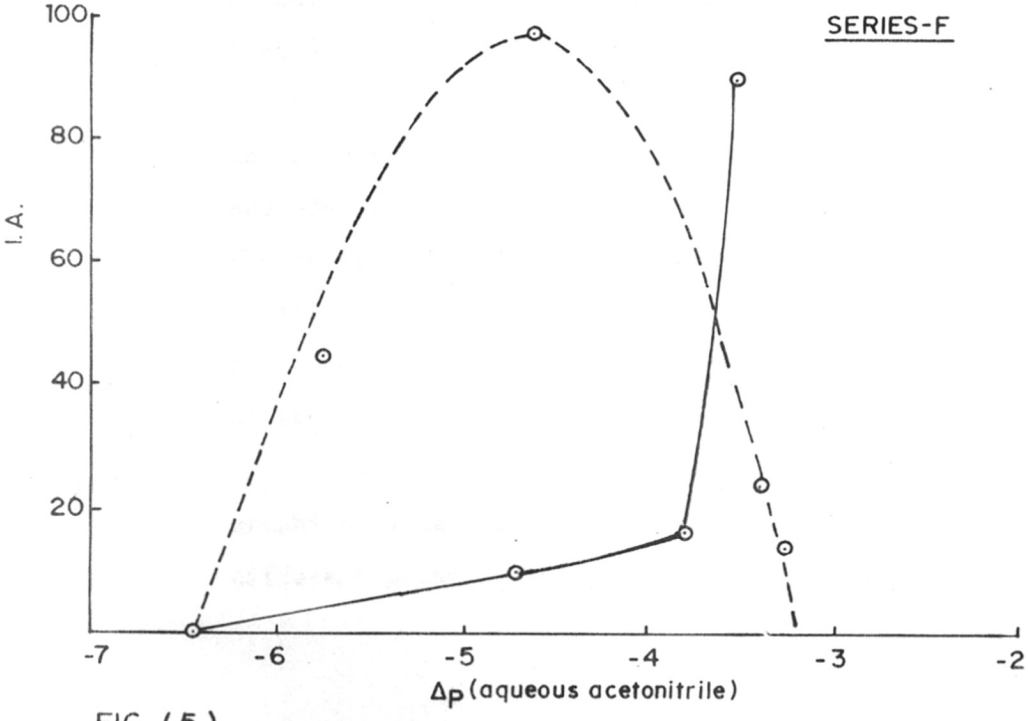
SERIES-F
100
80
60
40
20
I. A.
-7
-6
-5
-4
-3
-2
Δp (aqueous acetonitrile)

FIG. (5)

out possibility of existence of parabolic relationship between activity and Δp for homologus series of compounds, in which case the activity might go on increasing upto a certain level of substitution and then decline with any further additive substitution.

In its overall applicability the concept of Δp was found to be more successful than its predecessors α and $\Delta\alpha$ in all the cases and in both the solvent systems.

4.8.4 Conclusions

The most important conclusions that may be drawn from the ensuing discussions is that there exists a definite relationship between Δp and activity of compounds. This inference is drawn from the fact that a well defined pattern can be observed on the plots of I.A. versus Δp for both the solvent systems irrespective of the clarity of the graphs of I.A. $\forall \alpha$ and I.A. $\forall \alpha$. Further, this holds good for all the series investigated. The observance of good relationships in both the solvent systems affords choice of organic modifier depending upon solubilities of test compounds.

The presently proposed concept of chromatographically calculated partition coefficients on two different polarity columns and then taking their

difference signifies a major advance in quantitative retention activity relationship studies. Attempts to establish direct relationships with primary retention data and difference of selectivities ($\Delta\alpha$) with activity were not always successful. However, ΔP has produced good results in all cases. Thus, when an index of ΔP values is created for compounds of a series whose activities are known, it can serve as very useful guide to predict extent of activity of the untested compounds. Such an index will not only help in making necessary structural modifications in the synthesis schemes, but will also serve as a pointer when actual activity testing facilities are not immediately available and there is urgency to continue the synthetic work.

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