Application of gas liquid chromatography for studies in Terpenoids

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INTRODUCTION

An organic chemist usually would follow, for purification and quality control, such well established methods as separation by repeated crystallisation, distillation, fractional distillation etc. However, these methods were not efficient enough to fulfil all the requirements of an organic chemist. Inspite of this, only very few workers showed interest in the development of the new methods for the separation technique.

It seems that the 'chromatographic technique' had its first beginning in the year 1850. It was conducted by a German dye chemist, Runge.[1] He observed that when solutions of dyestuffs are placed on a filter paper, they clearly separate into well defined zones. This technique was well known to dye-chemists during the nineteenth century.

However, a complete separation of the components was not rendered possible. Under the light of modern techniques, Runge's process may be called as 'frontal analysis'.

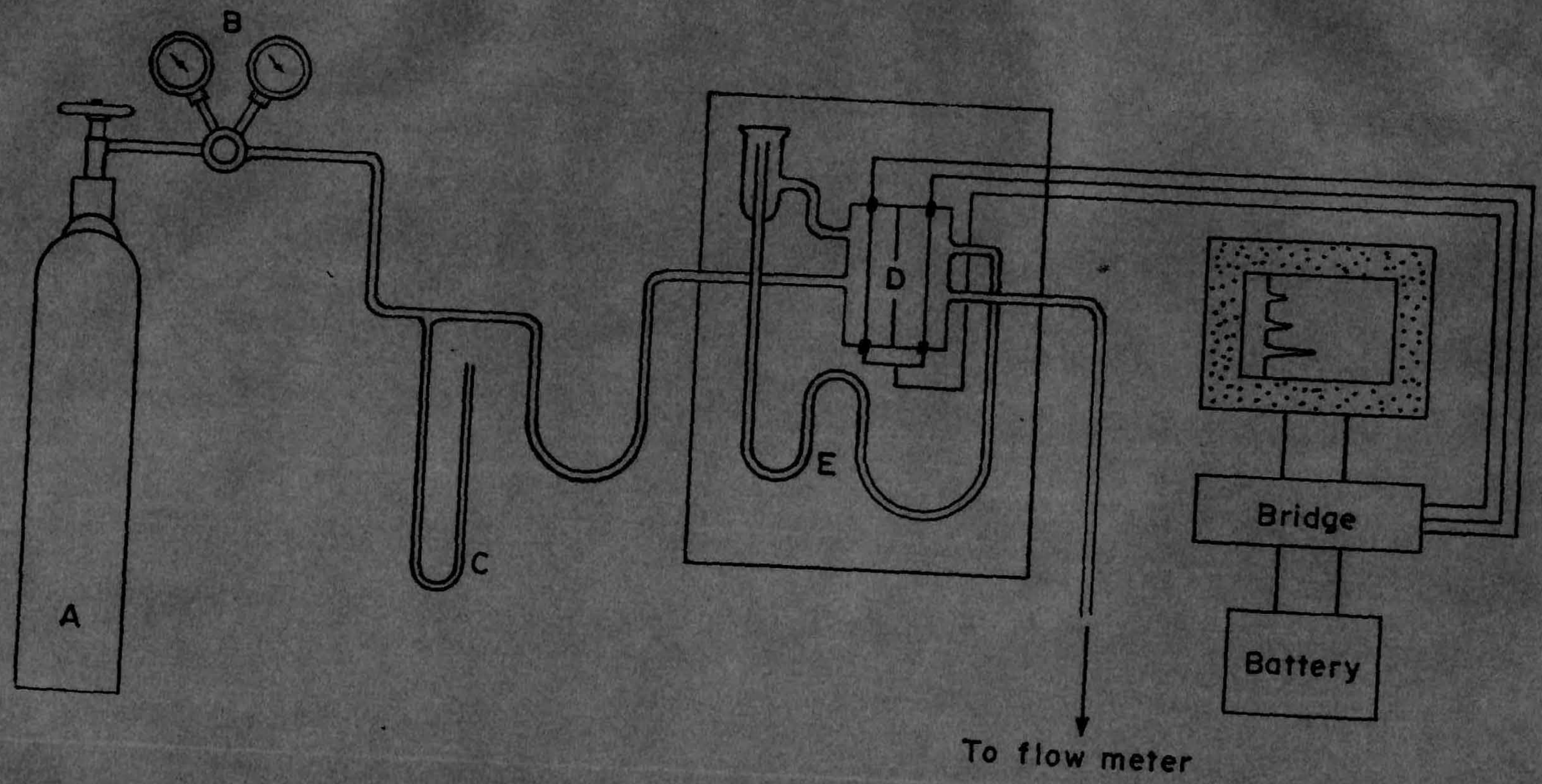
In 1906, Tswett[2] described a very typical process of separation. Now-a-days this process is called as 'liquid solid' elution chromatography. The most important achievement of Tswett's investigation was the use of pure solvents for developing chromatograms. With this technique, Tswett could separate the colouring pigments of the plant material. A few years later, he reported the separation of extracts of egg yolk. Subsequently, however, there was a fairly long gap during which no appreciable progress was made. Actually in the year 1931, Khun and Lederer furnished systematic refinements in this technique, enabling them to separate complex mixtures of terpenes and carotenoids.[3] Finally, Martin and Synge introduced (1941) the partition chromatography.[4] Almost simultaneously Tiselius[5] and Claesson[6] elaborated the three fundamental types of behaviour encountered during adsorption chromatography.

The technique of gas liquid chromatography, was introduced for the first time by James and Martin (1952)[7]. It has rendered possible, the separation and estimation of very small amounts of volatile substances, with a wide range of boiling points and has thus given a great impetus to many fields of research. This new technique has now firmly established itself as one of the most important methods for the analyses of gases and volatile compounds.

The gas liquid chromatography (GLC) process may be briefly described as follows. A typical, very high boiling and essentially non-volatile compound is impregnated on a suitable granular and inert material. This impregnated granular material in turn, is filled in a U-shaped column. This column has got an inlet through which some suitable inert carrier gas, such as nitrogen, hydrogen, helium etc., is passed, and then at the other end of the column, the carrier gas is vented out to the detecting device. The mixture of several components to be separated is injected inside, at the top of the column by some suitable injecting device. The components of the mixture, are carried over by the gas stream. The separation of the components takes place by the repeated equilibria between the moving gas phase and the high boiling organic compound, which is called a 'stationary phase'. Because of its negligible vapour pressure at the temperature of the column, the stationary phase is retained as such, while the volatile mixture passes out of the column. Thus one and the same column filling can be used repeatedly.

The gas liquid chromatography (GLC) is useful, both for separation as well as for identification. It is fairly rapid and more efficient than conventional fractional

FLOW SHEET



A	GAS CYLINDER
B	PRESSURE GAUGE
C	PRESSURE MANOMETER
D	DETECTOR
E	COLUMN

distillation technique and requires only a few milligrams or less of the sample. Its range of application seems to be very wide. Thus, organic compounds, boiling from several degrees below the temperature of ice, to say 300° to 400°, can be analysed both qualitatively and quantitatively. This technique has provided the modern analytical chemist, an instrument of great potentiality which has already simplified many complicated problems.

During the last few years, this technique has been developed to a very large extent and has made its prominent mark in almost each and every area of chemistry. Very large number of workers have contributed to this enormous development.

Initially, there were many obstacles, such as difficulties in handling the instrument at high temperatures, lack of highly sensitive detecting devices etc. But in due course of time, all these difficulties were overcome, and now it seems that this technique is sufficiently advanced to face complicated problems, frequently encountered in many branches of chemistry.

It is interesting to survey the development of this technique in its various aspects, such as, the design of analytical as well as preparative scale columns, the nature and the role of supporting material, various varieties of detectors and numerous varieties of stationary phases. The fundamental theories have been modified by many workers. This has rendered possible a better representation of the separation phenomenon that takes place in a typical GLC column.

The following description gives a broad outline of the several developments that have taken place during the last few years.

1. Packed columns:

Generally a column of 4 to 6 mm diameter and nearly 2 meters length is used for the analytical separations. But if the separations are difficult, then columns up to 4 to 5 meters length are also used. Glass-tubings, copper or brass tubings are used for this purpose. The use of stainless steel tubings, seems to be very safe because these do not possess any catalytic activity. Use of copper tubing has proved that the inner surface of such type of material may have some catalytic effect. Now-a-days enormously long columns (20 to 25 ft.) are being used for difficult separations. The classical example is the separation of the three xylene isomers.[8]

In discussing the final separation obtained by the combined effect of speed as well as resolution, R.P.W. Scott pointed out that a compromise can be made between the two. In this context, he has recommended the use of programmed flow rates.[9]

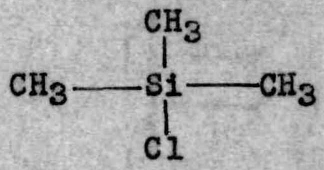
The most important aspect, which is always combined with 'column', happens to be the proper selection of supporting material. The behaviour of solid support in GLC columns has been systematically investigated by Theron Johns.[10] He observed that the corrected retention time is inversely proportional to the particle size of the supporting material. The particle size was also found to have a marked effect on both, the pressure drop of a column, as well as on the resolution. It was also found that the resolution does increase as particle size is decreased, but the actual rate of increase drops off as the particle size decreases. So, when efficiency of resolution and pressure drop of the column are both considered, it indicates that a particle size of 30 to 70 mesh furnishes a good compromise.

Crushed fire brick powder or 'celite' type supports have been extensively used, right from the beginning of this technique. The effect of adsorption, surface area, packing density etc. have been studied by previous workers.[11-15] An interesting observation has been made by Baker, Lee and Wall.[16] They found that the percentage of stationary phase is a very critical factor for supports having fewer fine pores.

However, modern trends in the case of supporting material, are to use 'fluorine' containing polymer supports. J.J. Kirkland has found that 'Teflon-6'[17] is comparable to 'Chromosorb W' as a solid-support.

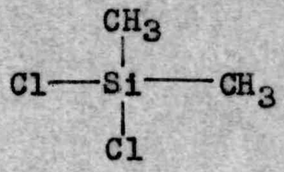
The adsorption effects have been mainly lessened by using several silanising agents, such as:

(i) Trimethyl chlorosilane



$$CH_3-\underset{\displaystyle Cl}{\overset{\displaystyle CH_3}{Si}}-CH_3$$

(ii) Dimethyl dichlorosilane



$$Cl-\underset{\displaystyle Cl}{\overset{\displaystyle CH_3}{Si}}-CH_3$$

(iii) Hexamethyl disilazane $(CH_3)_3-Si-NH-Si-(CH_3)_3$

According to Kirkland, dimethyl dichlorosilane seems to be the best one of the type.[17] A novel type of supporting material has been introduced by the same author. These are glass beads, modified with thin layers of finely divided diatomaceous earth. It is claimed that HETP values of 0.8 mm have been obtained by using such type of supporting material.[18]

2. Capillary columns:

The use of columns with very small diameter was already predicted by Martin.[19] Golay elaborated the necessary theoretical aspects of capillary columns.[20] The use of such a column attained a very high practical value when it was coupled with highly sensitive ionisation detectors. The most important factor, for capillary columns, happens to be the high number of theoretical plates.

Warren Averill[21] has described the practical hints for the preparation of capillary columns. He has discussed the difficulties and also the temperature limitations of a typical stationary phase which is used in capillary columns. He has mentioned that the phase, which is sufficiently stable at 200° in packed columns, will be stable upto 150° when used in a capillary column. The reproducibility of sample splitter is also discussed.

The exact comparison of the efficiency of a packed column and a capillary column can be made only after a critical comparative evaluation of the two types of the columns. Necessary theoretical treatment has been given by J.H. Purnell who has discussed the effect of dead volume on calculation of theoretical plates. This factor assumes vital importance in the case of capillary columns.[22,23]

3. Detectors

With regard to detector devices, the thermal conductivity cell still represents the most widely used detector. Several designs have been described for the construction of thermal conductivity cells. Recently[24] several attempts have been made towards eliminating flow sensitivity by screening the heated elements. It is claimed that some of the thermal conductivity cells can be used above 300°.[25,26]

Gas density balance was used by James and Martin.[27] This type of detector is a network of channels in a metal block, that compares the relative densities of the measuring and reference gas using a differential thermocouple anemometer sensing device. This net work of channels is mechanically equivalent to a Wheatstone's bridge circuit. However, both the above mentioned types of detectors have their limitations. This is where the ionisation detector has proved to be the most useful. In general, all these detectors employ changes in the electrical conductance of the effluent gas brought about by various methods of ionisation. Here, very large changes in electrical conductance can be realised and high signal to noise ratios can be readily achieved. Lovelock or Mc William and Dewar ionisation detectors are now commercially available.[28] Such type of detectors are specially suitable for capillary columns. Only minute quantities of samples are sufficient for analysis.

In the field of this detector development most of the workers feel a need for adequate comparison between the various types of detectors available today. The most notable attempt has been made by Dimbat _et al_.[29] who described the sensitivity parameter by the following equation.

$$S = (\text{ml x mv per mg.}) = \frac{A \times C_1 \times C_2 \times C_3}{W}$$

where S = sensitivity parameter.
A = peak area; sq. cm.
C_1= recorder sensitivity, mv per cm. of chart
C_2= chart speed, min per cm.
C_3= flow rate at exit of column, ml per min. corrected to column temperature and atmospheric pressure.
W = weight of sample, mg.

Very useful comparison data have been given by I.G. Young.[30] From this data, it can be obviously seen that ionisation detectors are several times more sensitive than the thermal conductivity detectors.

Very recently, a new trend has started dominating this area. The use of fast scan mass spectrometry with capillary columns was illustrated by Dorsey <u>et al</u>.[31] With this technique they could trace out the presence of several acetals in a typical sample of fruit volatiles.[32] Roy[33] Teranishi <u>et al</u>. calculated the column efficiencies and found that there was no significant loss by operating one end of the capillary column under the mass spectrometer vacuum. Ranger Ryhage[34] illustrated that a mass spectrometer can serve as a good detector for effluents coming out from high temperature GLC column.

4. Development of the theory

The fundamental theories have been developed through the efforts of many workers such as van Deemter,[35] Martin, Synge and others. A theoretical plate treatment is described by Martin and Synge.[36] They defined theoretical plate as a section of the column in which the vapour leaving the section has the composition that would be in equilibrium with the average concentration of liquid solute within the section. In the process of passing through one theoretical plate, the equivalent of one equilibrium between the liquid phase and vapour phase occurs. So the overall separation achieved with a typical column always depends on the two factors, (i) separation per plate, and (ii) number of plates. The separation effected by one equilibrium is dependent on the nature of the stationary phase as well as the nature of the components to be separated. The method of calculation of the number of theorotical plates is represented by the formula $n = 16 \times (\frac{t_R}{\Delta t})^2$

where: (i) n = number of theoretical plates.

(ii) t_R = retention time for solute

(iii) t = band width.

The height equivalent to a theoretical plate is calculated by dividing the column length by the number of plates.

The rate theory of van Deemter[35] is useful for considering the effects of several column and operational parameters.

This equation is shown below:

$$\text{HETP} = 2\lambda d_p + \frac{2\gamma D_{gas}}{u} + \frac{8\pi^2}{2(1+k)^2} \frac{d^2 f}{D\ liq.} u$$

$$\text{"} = A + B/u + Cu$$

where A = multiple path effect
B = molecular diffusion
C = resistance to mass transfer.

During the last few years, this theory has been modified considerably. It is substantially established that at least two gas phase mass transfer terms are necessary in addition to the previously accepted van Deemter equation.

In the year 1961 Jones reintroduced the van Deemter gas phase mass transfer term.[37] Very recently a number of papers have been published which deal with such type of modifications to the original theory.[38-40]

Applications of gas chromatography

The technique of gas chromatography has helped many branches of chemistry. The examples are too numerous to be mentioned in a brief introduction. Therefore, a general description of some of the important points is furnished below.

Fatty acids and esters have been analysed with great care and accuracy.[41-44] Steroids have been analysed at fairly low temperatures. The analyses were rendered possible by using low loaded columns. Thus recently, vanden Heuvel *et al*. analysed, bile acids, sex hormones and adrenal cortical steroids. For this purpose, they used column, containing SE-30 stationary phase to the extent of 0.75% only.[45,46]

In the case of amino acids, the modern trend is to convert them into some suitable volatile derivatives and then analyse on GLC columns.[47-49]

There are some cases where GLC analysis had helped to certain extent for the study of reaction mechanism.[50] For example, Summerbell et al. have utilised this technique for the study of iodine replacements in diiodides. The analysis of metals has been effected by the separation of organo-metallic complexes. With the proper modifications in this field, it can be expected that GLC should help in routine analysis of metals and alloys. Very recently Nestor L. Soulages has described a gas chromatographic method for the determination of lead alkyls.[51]

Some workers have tried the possibility of separating optically active compounds. For example, Casanova and Corey separated (±) camphor.[52] For this they followed the route of ketal formation with D(-) -2,3-butanediol. A ten feet column of tricyano-ethoxy propane as a stationary phase was found to be very useful for the separation of ketals. They also observed that only partial separation could be effected on columns containing silicone oil, silicone rubber, tricresyl phosphate, carbowax, dinonyl phthalate etc.

Factors responsible for separation

The final separation obtained on a typical GLC column, happens to be the result of several factors. Broadly, they can be classified into two parts. The part one covers the variable parameters of a typical column: (i) length, (ii) diameter, (iii) inlet and outlet pressure ratio, (iv) the nature of the supporting material, (v) the mesh size of the supporting particles, (vi) the temperature of the column, (vii) the flow rate of the carrier gas, etc.

The part two, which is responsible for the final separation, happens to be the nature of the stationary phase. Consequently, significant change in the degree of separation can be achieved by changing the nature of the stationary phase.

Importance of stationary phases

The importance of the nature of the stationary phase has been stressed by many workers. The general requirements of a typical stationary phase, are briefly discussed.

When a certain organic or organometallic compound has to be used as a stationary phase, it should have the following properties.

It should be stable at the temperature of column. Here it is worthwhile to mention that the stability of a stationary phase has to be judged in situ. That means, it should be stable, when it is in close contact with supporting material. A simple vapour pressure determination data may be some times misleading. For example, it has been found that dinonyl phthalate has got low vapour pressure in a temperature range of 120-150°. But actually, it has been observed that columns, prepared with this stationary phase, do not have a long life at a temperature of 140° to 150°.[67]

Dimethyl formamide, dimethyl sulpholane, ethyl acetoacetate and other polar compounds have been used as stationary phases at sufficiently low temperatures. They have proved to be very useful for the separation of the mixtures of saturates and unsaturates. However, above a certain temperature range their vapour pressure becomes so high that these compounds cannot be used as stationary phases.

Silver nitrate in glycols

Similarly, at low temperatures, solutions of silver nitrate in some suitable organic solvents have been used and they have proved to be superselective. A mixture of 1-methyl cyclohexene, 3-methyl cyclohexene, 4-methyl cyclohexene and methylene cyclohexane was completely resolved on such columns at 30°.[53] Isomeric C_8 and C_9 cyclohexenes and p-menthenes are completely resolved with such type of stationary phases.[54] Finally it was observed that silver nitrate-ethylene glycol and silver nitrate-glycerin columns can be used up to a temperature range of 30° to 65°.[55]

From the discussion, it is clear that at comparatively high temperatures highly polar stationary phases cannot be used for a long time, because of their volatility. But one can use different types of stationary phases.

Liquid paraffins $- CH_3 - (CH_2)_n - CH_3$

It is commonly used in a range of room temperature to nearly 100°. The maximum usable temperature depends upon the grade of paraffin oil.

A.I.M. Keulemans used this stationary phase at a temperature of 0°.[56] The sample analysed was a mixture of C_3 and C_4 hydrocarbons. It was observed that the separation of all the components was not possible.

James and Martin have used different types of stationary phases for the separation of hydrocarbons.[57] They have used a temperature ranging from 60° to nearly 200°. The nature of the stationary phase was also different in each case. n-Octadecane was a nonpolar stationary phase. Benzyl diphenyl may be looked upon as a medium polarity stationary phase. However, their exhaustive studies revealed that no single stationary phase will be efficient to separate all the components.

Esters:

Amongst all the medium polarity stationary phases, the ester type phases happen to be the most versatile and useful for different types of separations. Esters of phthalic acid have been extensively used. Dinonyl phthalate, dibutyl phthalate, didecyl phthalate are the prominent members of this series. These phases are used over a wide range of temperatures.[58,59]

Pollard and Hardy used dinonyl phthalate and dibutyl phthalate for the separation of halogenated compounds within a temperature range of 25° to 60°.[60] It was also observed that such types of stationary phases do have some interactions with the samples.

Zubuk and Conner used didecyl phthalate at 110°, 130°, 150° for the separation of terpenic hydrocarbons.[61] Eventually, they observed that slight acidity of these stationary phases may cause some acid catalysed rearrangements of the thermolabile terpenic hydrocarbons.

James and Martin used dioctyl phthalate for the separation and identification of the methyl esters of saturated as well as unsaturated fatty acids ranging from formic acid to n-octadecanoic acid.[62] They made a very important observation that the less viscous dioethyl phthalate gave columns of higher efficiencies than the more viscous paraffinic phases, so that whatever that is lost by fall in separation factor, is gained in greater zone sharpness. They also observed that it was not possible to cover the whole range of acids from C_1 to C_{18} by operating the column at only one temperature.

Daniel E. Martire, has used dinonyl phthalate for studying the aspects of thermodynamics of solutions.[63]

Tricresyl phosphate

Tricresyl phosphate is also being used very widely as a medium polarity stationary phases. Haslam and Jaffs used this stationary phase for the analyses of monoterpenic hydrocarbons.[64] These investigations were in turn used for the examination of commercial varieties of dipentene samples. It was found that those pairs of monoterpenic hydrocarbons, which could not be separated on dodecyl phthalate, were separated on tricresyl phosphate and vice versa. Once again, these columns were used at 100° to 105° only.

Herout *et al.* used tricresyl phosphate at 136° for the evaluation of ground unglazed tile as a supporting material.[65] By changing the percentage of tricresyl phosphate in a stepwise manner they were able to construct columns giving about 2300 no. of theoretical plates. A mixture of α-pinene, myrcene, Δ^3-carene, limonene and p-cymene was completely separated on this column.

Liberty and Cartoni also carried out a systematic examination of the monoterpenes occurring in the essential oils[66] and they have recorded the retention volume data for eleven hydrocarbons with tricresyl phosphate and silicone fluid DC 550, as the stationary phases. These phases have been used at100°.

Poly glycols

Totally different series of stationary phases are available in the range of moderate temperatures (130-170°). These compounds happen to be the various varieties of polyethylene glycols (Molecular weights ranging from 100 to 6000; higher values are described in the literature).[67] The lower molecular weight members are very soluble in water compared to the higher members which are less soluble.

Polyethylene glycols with end groups

$$ROCH_2CH_2 - (OCH_2CH_2)_n - OCH_2CH_2OR'$$

This group offers possibilities of many varieties, because the chain length, as well as the end groups, can be changed at will.

Polypropylene glycols

These types of glycols are also available. They are not so soluble in water.

An exhaustive study of these polymers has been carried out by E.R. Adlard.[67] A neat approach to the investigation of such type of polymers, has furnished very useful information. In the beginning, polyethylene glycols of various molecular weights were studied. Then the necessary temperature, required to attain 1 mm vapour pressure, was recorded in each case. It was found that within a temperature range of 110° to 130°, all the polyglycols attained a vapour pressure of 1 mm. It was supposed that within this temperature range, this stationary phase is fairly stable for a long time. A column was prepared as usual by using polyethylene glycol-400 as a stationary phase. Then a mixture of alcohols and ketones was run over this column at 100°; column was then run for 400 hours and again the same mixture of alcohols and ketones was run on the column. It was found that both the chromatograms were almost identical, indicating that the stationary phase must be fairly stable during these two runs. From this it was concluded that, this stationary phase is stable at 100° for extremely long time. It was found that polyethylene glycol type of polymers are highly selective for aromatic hydrocarbons.[67]

Halogenated esters

Halogenated esters have been used by Langer and others.[68] These are tetrahalophthalate esters. They are usually found to be selective for meta and para isomers, including the xylenes. These stationary phases were found to be stable up to 100° to 120°. At high temperature it seems that they lose their selective property.

From the above discussion, it is clear that highly polar and selective stationary phases can be used at low temperatures to attain the desired separation. The number of stationary phases that can be used at moderate temperatures, say between 90° to 140° or even up to 180° is very large. But comparatively polar or selective type stationary phases are few. Many workers have reported the temperature limitations of stationary phases.[69,70] Such type of temperature dependence data have been compiled by previous workers.[71] These data, especially regarding the maximum possible usable temperature for a typical stationary phase, have been based on a very large number of experimental evidences. However, a glance at these tables shows that as the temperature range goes on increasing, the number of stationary phases, goes on decreasing. This indicates that in practice there are only few stationary phases which can be used in a temperature range of 200° to 300° or higher.

Silicone grease and Apiezone grease are the most important members of these series. Analysis of extremely high boiling compounds like fluoroesters of camphoric acids, high molecular weight fatty acid esters, alcohols, amines, is rendered possible by the use of silicone grease or apiezone grease. The versatility of silicone grease at this temperature range is already described by the previous workers.[72] But unfortunately, both of these stationary phases lack the 'polarity' factor. As a result, whatever separations we may get from these stationary phases are mainly based on boiling point differences only.

Rohrschneider has studied the polarity of a number of liquid phases. The values, compiled by him, were relative ones,[73] being based on the value of 'oxidipropionitrile' as '100' and squalane as '0'.

According to this scale, the relative polarity of silicone oil is found to be '7' only.

However, it is soon realised that a medium polarity stationary phase with certain favourable characteristics is absolutely necessary. It should be fairly stable within a temperature range of 40 to 240°. It should have good resolving power. In this context polyester type of plasticisers were introduced as stationary phases. They proved to be excellent for analytical work. The most important beneficial factors of these ester type plasticisers are as follows.

1. They can be used over a wide range of temperature.
2. They are viscous liquids or low melting solids.
3. They can be easily synthesised.
4. They can be made fairly stable by proper chemical reactions.
5. They can be blended with other stationary phases without any difficulty, and
6. They are found to be fairly stable upto 200-240°. If not, they can be further purified and they can be made stable at the above mentioned temperatures.

Previous workers have used polyester stationary phases in various ways. Their results are being briefly discussed. They studied only limited number of polyesters. It was found to be rather difficult to compare the relative retention time data obtained by one group of workers with those obtained by other groups. Moreover, if at all such type of comparison has to be done with two observations, then it is absolutely necessary that all the column variables should be taken into consideration. Unfortunately, such observations were rarely available in the literature. Therefore only a limited analogy is given here.

In the year 1959, Lipsky, Landowne and Godet furnished a strikingly remarkable method of performing analyses of fatty acid esters by using several varieties of polyester type plasticisers.[74] Previous observations presented by other workers gave an idea that Apiezone or Silicone type of stationary phases are not sufficiently good for the separation of fatty acid esters.[75-77] Many difficulties were encountered when such type of analyses were carried out. Although some columns consisting of celite -545 coated with 'Apiezone' grease as a stationary phase gave efficiencies of nearly 3000 plates, the separation of each fraction of the very commonly occurring series of C_{18} acid esters was not complete at all. Some very typical separations as linolenate from linoleate were found to be very difficult and many a times there was considerable overlapping. Moreover when total mixtures of fatty acid esters were run on such type of columns, it was found that the retention time of esters upto C_{20} chain length was of the order of nearly 3 to 4 hours. It is worthwhile to note that almost all the optimal conditions were chosen during these analyses.

A preliminary observation by the same workers[74] gave them new concept that polyester type stationary phases can be used very beneficially for such type of analyses. Therefore, a number of well designed experiments were carried out by them. They used adipate polyesters of (i) neopentyl glycol, (ii) butylene glycol, (iii) diethylene glycol, (iv) diethylene glycol partially cross linked with penta-erythritol. It is worthwhile noting that the molecular weights were not the same in all cases. They also used succinate and sebacate polyesters of diethylene glycol. In this case also the molecular weights were fairly different. For comparison they used 'Apiezone' column as a reference.

Apiezone column was run at a temperature of 196° for the separation of a mixture of mono and diunsaturated acid esters of C_{12} to C_{18} acids. This column furnished nearly 4000 plates. However, it was found difficult to separate linoleate and oleate. The use of longer columns did not help much in this respect.

Then a column of sebacate polyester of diethylene glycol was used at 204°. On this column, it was found that retention times were lesser when compared to 'Apiezone' columns. Moreover it was found that the unsaturated acid esters are retarded and came out from the column after the corresponding saturated esters. Here linoleate and linolenate esters were separated completely. But stearate and oleate esters were not resolved.

For further studies, another polyester synthesised from neopentyl glycol and adipic acid was subjected for further examination at 195°. But this stationary phase also failed to separate the saturated and the mono-unsaturated ester mixture.

From these observations, it was felt by these workers that more polar stationary phases may separate such type of mixtures.

Therefore, a polyester of adipic acid and butylene glycol was used and fortunately some separation of stearate from oleate was noted. Similarly when adipate polyester of diethylene glycol was used, the separation of important mono-, di-, and tri- unsaturated C_{18} acid esters from stearate was found to be complete.

When the same polyester was cross linked with pentaerythritol, it was found that it could easily resolve the C_{18} acid ester mixture completely.

In the year 1961, Lipsky and Landowne presented a detailed study of the steroids with GLC technique.[78] They used several varieties of stationary phases, differing widely in polarity and chemical composition. In order to have a clear picture of the effect of the nature of the stationary phase on the degree of resolution, they had chosen a number of closely related steroids as standard samples.

For general comparison, these workers used a variety of polar and nonpolar stationary phases as follows: (1) polybutene, (2) bis-(x-tolyl-p-tolyl) ether, (3) Ucon - HB-50-2000 - a polyalkylene glycol. A standard mixture of steroids was analysed on all the three stationary phases. The performance of the nonpolar stationary phases, was found to be inferior to those observed on polar liquids. The polyalkylene glycol gave somewhat better separation. However, columns prepared with this material were not stable for a long time. But when polyester type stationary phases were used, extremely good separation was rendered possible. Polyesters used were neopentyl succinate, adipate and sebacate. All of them gave fairly rapid and good separation. The retention times where also low which resulted in sharp peaks for the corresponding components. The number of theoretical plates per foot was also fairly high.

When C21 steroids were analysed on the above mentioned columns, it was found that the separation was quite satisfactory. A complete separation of steroidal ketones, pregnanolone, pregnane-3-20-dione and allopregnane-3-20-dione was obtained in about 100 minutes only. Similarly it was found that a satisfactory separation of C27 steroids can also be obtained by the polyester type stationary phases.

The above mentioned discussion has clearly pointed out that 'polyester' type substrates are very suitable for the analyses of complicated mixtures. Some previous workers have already used such type of substrates for the separation of terpenoids. They observed that these stationary phases are far better than the conventional stationary phases.

However, literature survey indicated that a systematic approach was necessary to appreciate the performance of 'polyester' type stationary phases. A thorough investigation of such type of substrates has therefore been carried out and the results presented in three different parts of this thesis.

Since our interest specially concerned the essential oils and terpenoids, the work carried out so far, regarding their analyses and estimation using gas chromatography is being briefly discussed here.

GLC technique as applied to the study of essential oils

Fairly large number of workers have utilised this technique for the study of essential oils. Some of the important results which point out the speciality of this technique are discussed below.

Heartwood constituents were studied by Jarl Runeberg.[79] The neutral oil was initially fractionated using suitable vacuum, and the fractions having boiling range of the sesquiterpenic hydrocarbons were screened on GLC column. A Pye Argon Chromatograph was used and the stationary phase was a mixture of 2,4-dinitrophenyl-2-naphthyl ether (15%) and dibenzyl pyridine (0.75%), impregnated on Silocel C_{22} firebrick. Infrared and gas chromatographic analyses showed the presence of α-cedrene, thujopsene and cuparene.

Analysis of lemon oil was carried out by R.A. Bernhard.[80] The early attempts were not so successful. For example, only five distinct peaks were obtained with the old GLC technique. Subsequently, the same author[81] used LAC-4-R777 and LAC-2-R446 as stationary phases in conjunction with a sensitive detector and as many as twenty two peaks were recorded. It was also found that there were ten small peaks near the start of the chromatogram. These minor constituents escaped detection by the old analytical methods. At the same time, it is interesting to note that the total analysis was over within only 40 to 50 minutes.

The technique of 'programmed temperature' GLC is being used extensively now-a-days. The neutral leaf oil of the creeping junipers from Prairies was studied by Couchman and Rudloff.[82] By using the linear programmed temperature technique, they were able to find out as many as 42 components, a few could not be properly identified. Duplicate runs of the sample of the oil, rendered possible the percentage data determination of almost all the components. Amongst the 42 components, it is worthwhile to note that the percentage composition of some of the components was very low. For example, linalool and citronellol were present to the extent of 0.80% only, while camphene and β-phellandrene were present to the extent of 0.20%.

Similarly, the chemical composition of the volatile oil of the leaves of Savin juniper was studied by the same author.[83] Once again a programmed temperature GLC technique was used. The presence of as many as 37 components was recorded. Many sesquiterpenic oxygenated compounds were found to occur in traces. Such type of trace analysis can be rendered possible by GLC technique only. Previous workers, who studied the same oil, could detect and isolate only those components which were present in large proportion.

'Dipentene' samples were carefully analysed by Haslam and Jeffs.[64] They could identify 8 to 10 components in those samples.

The old methods of estimation of monoterpenic alcohols were investigated by Holness.[84] He investigated the method of determination of citronellol in admixture with geraniol. First, the conventional method of hot formylation was used on the assumption that boiling anhydrous formic acid converts citronellol to its formate and geraniol to hydrocarbons. It was supposed that the ester value of the formylated material could be used to determine the exact percentage of citronellol in the presence of geraniol. Actually, it was proved by the

GLC analyses of the reaction products that several by-products are formed. The percentage of these by-products depends upon the experimental conditions. Thus it was proved that from citronellol some glycol diformate is always produced and this causes high results at high concentration of acid. With geraniol, hydrocarbons are definitely formed, but simultaneously some other reaction products are also obtained, some of which have an ester value. The hot formylation thus always shows higher apparent content of citronellol than actually present in the mixture.

Now-a-days characterisation of essential oils as such, is often being carried out by GLC analyses. As a matter of fact the composition of an essential oil is influenced by several factors, such as the genetic origin of the plant from which it is extracted, and the process which is followed for the extraction.

In order to trace out the geographical origin of a typical essential oil sample, Smith and Levi have used GLC technique.[85] They have examined two varieties of peppermint oil. These are Mentha piperita and Mentha arvensis. Initially they found the percentage composition of the several components which are present in these two varieties of oils. From this data, the ratios of the constituents were calculated. The ratio of percentage composition of limonene/cineole was very useful and informative. Values ranging from 0.2 to 0.7 were characteristic for Mentha piperita variety, and values greater than 2 were characteristic for Mentha arvensis oils. Obviously one variety can be easily differentiated from the other. In this way they have shown that the ratios of such type of suitably chosen constituents give a very valuable idea about the origin and variety of the oil. They have also described the experimental procedure for the detection of common adulterents in these two oils.

These observations illustrate the utility of the GLC technique for the study of essential oils. Since the National Chemical Laboratory has been one of the leading centres of research on essential oils in India, we were interested in developing stationary phases, which will be helpful in characteristing and estimating the constituents present in the large number of essential oils examined by us. In this context, we have systematically synthesised a series of polyesters and studied them in details for their solvent capabilities in GLC columns for the separation of terpenoids and related products.

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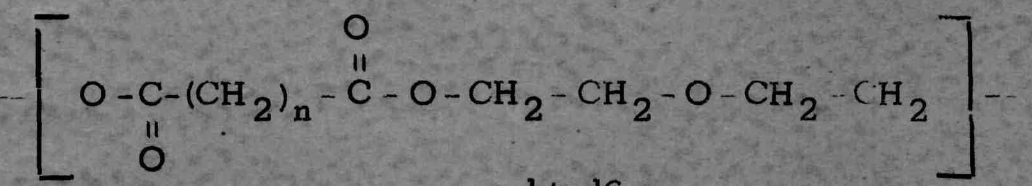
PART I

EVALUATION OF POLYESTERS DERIVED FROM 1, ω - DICARBOXYLIC ACIDS AND DIETHYLENE GLYCOL



$$--\left[O-\underset{\underset{O}{\|}}{C}-(CH_2)_n-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_2-O-CH_2-CH_2\right]--$$

n = 1 to 16

SUMMARY

In recent years, 'gas liquid chromatographic technique' has been widely used for the essential oils. Polyesters, the medium polarity substrates, are most suitable for such type of analyses. Previous workers studied a few polyester as substrates in GLC columns. Thorough study of these observations of previous workers, gave us only a broad idea about the nature and solvent capability of 'polyester type' stationary phase.

Therefore systematic study of 'polyester type stationary phases, was taken up by us. Polyesters from different dicarboxylic acids, $HOOC-(CH_2)_n-COOH$, ranging from malonic acid, $(n=1)$, to hexadecane-1,16-dicarboxylic acid, were synthesised and studied as substrates in GLC column. Diethylene glycol was used as the glycolic fragment for all the polyesters. Several terpenic and a few non-terpenic compounds were chosen as standard samples.

All the column variable parameters were maintained constant, so that a direct comparison of retention time data was rendered possible.

Thus, it has been found that if t_R^x is the retention time in minutes and x is the number of methylene groups, present in the acidic component of the polyester, and if t_R^y be the retention time on another polyester containing 'y' methylene groups, then $\frac{t_R^x - t_R^y}{x-y}=c$ (1), where 'c' is a constant for a particular compound at a particular temperature and is the individual contribution of a single methylene group. This relationship seems to be an integral characteristic of the number of methylene groups, between the two carboxylic groups and is valid for non-polar terpenic and other hydrocarbons. These observations have been used for the separation of monoterpenic oxygenated compounds and sesquiterpenic hydrocarbons. Several reasons for the deviations from this linearity relationship are also discussed. It seems that the long chain dicarboxylic acid-diethylene glycol polyester would be very useful in 'capillary columns'.

Next, the study of polyesters, was extended over a wide range of temperature. The ratio of the specific retention volumes on two different polyesters for a typical hydrocarbon is nearly equal to the number of methylene groups of the acidic fragment of the polyester.

It has been also found that the molecular weight plays a less important part than the number of methylene groups present in the acidic fragment of the polyester.

When the retention time was divided by the actual weight of the stationary phase used and then by the number of methylene groups, which may be considered as contribution per gram per methylene group, the values were nearly constant.

In this chapter, we are describing some of the results of our systematic examination of polyesters as stationary phases, in gas liquid chromatography.

General approach

A careful survey of the previously published literature, gave us a broad idea about the chemical composition of a typical polyester and its effects as a substrate, on separating capacity. But it was not possible to assess the exact nature and resolving power of the polyester type stationary phases. The difficulties which were encountered in such type of comparative evaluation are very obvious.

In order to judge the exact solvent capability of a typical organic compound, the other column variable parameters must be kept constant. Then only the exact role played by the stationary phase can be evaluated. If both the functions, column parameters, as well as, the stationary phase are changed simultaneously, then it becomes difficult to judge as to what is really the responsible factor for the final separation phenomenon. It was therefore decided that the column parameters must be kept constant and the stationary phase be changed.

Modification of the Apparatus

The apparatus which we had at our disposal was a 'Griffin and George MKII VPC Apparatus'. In the original apparatus, the flow system of the carrier gas is run by using a speedivac vacuum pump. The inlet and the outlet pressure can be controlled by the needle valves supplied. At the end of the flow path, there is a trap that can be closed and opened with a stop-cock. Sample vapours go on condensing here, resulting in the necessity of frequent cleaning.

It was a common difficulty that needle valves used to get chocked up and required frequent cleaning. It was also found difficult to adjust the exact inlet and outlet pressure ratios to get the required flow rate. Sometimes the pressure drops across the column were so high, that even with apparently efficient columns, satisfactory separations could not be effected.

A few changes were therefore made in the flow path system of the carrier gas. The inlet pressure manometer was removed and in turn a simple U shaped manometer was connected by strong rubber tubings. Thus it was possible to record the inlet pressure of the carrier gas. The condensing trap, at the flow path was also removed and the end of the outlet connected to a soap bubble flowmeter. The vaccum pump was discarded.

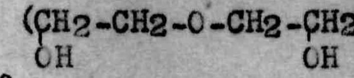
The rubber cap had to be fitted tightly by putting sturdy copper wire windings. With this flow path system, the carrier gas flow rate can be changed from very low values to as high as 10 litres per hour.

Whenever it was suspected that a typical sample contained high boiling components, the analysis could be conveniently performed by using high- flow rates. It was also possible to use hydrogen as the carrier gas.

However, it is interesting to mention the data collected on the same point by other workers. It seems that the detector sensitivity increases at low pressures. Locke and Brandt performed several experiments and found that no decrease in injection temperature can be gained through lowering the inlet pressure.[1] However, it has been pointed out that the rate of solute (sample vapour) movement is dependent only upon the velocity of the carrier gas and column temperature. A slight decrease in the efficiency can be predicted because of increased gas phase diffusion.

In the case of our work, we have found that a pressurised system is extremely convenient. Therefore, the same system has been used throughout this work.

The dicarboxylic acids and the glycol

In this part, we are describing the results of evaluation of a large number of polyesters which were synthesised by us. The glycol part in all the cases was diethylene glycol  $(\underset{OH}{CH_2}-CH_2-O-CH_2-\underset{OH}{CH_2})$. The acids were 1,ω-dicarboxylic acids.

$HOOC-(CH_2)_n-COOH$, carrying varying number of methylene groups, from malonic acid, in which n=1; to hexadecane-1,16-dicarboxylic acid where n = 16. Some of these acids, particularly those having large number of methylene groups, are comparatively rare chemicals which are not easily available to many. In our case, however, we were very fortunate. We had in this laboratory a long standing project for the synthesis of macrocyclic musk compounds for which long chain 1,ω-dicarboxylic acids are the basic raw materials. These acids were therefore available with us in large quantities which rendered the present investigation easy for us.

EXPERIMENTAL

(I) The dicarboxylic acids

(1) Malonic acid: HOOC-(CH_2)-COOH.

It was available from the laboratory stock. Its purity was checked by melting point determination, m.p. 133°; lit. 135°.

(2) Succinic acid: HOOC-$(CH_2)_2$-COOH.

It was available from the laboratory stock. Its purity was tested by melting point determination, m.p. 184-185°; lit. 185°.

(3) Glutaric acid: HOOC-$(CH_2)_3$-COOH.

It was prepared by the oxidation of cyclopentanone. When its diethyl ester was tested by GLC analysis, it showed the presence of a second component to the extent of 5%. Most probably it was diethyl succinate which is known to be formed. However, the acid was used as such, m.p. 93-94°; lit.96°.

(4) Adipic acid: HOOC-$(CH_2)_4$-COOH.

It was available from the laboratory stock. Its purity was tested by melting point determination, m.p. 150°; lit. 150°.

(5) Azelaic acid: $HOOC-(CH_2)_7-COOH$.

It was available from the laboratory stock. Its purity was checked by melting point determination, m.p. 105-106°; lit. 107°.

(6) Sebacic acid: $HOOC-(CH_2)_8-COOH$

It was available from the laboratory stock. Its purity was tested by melting point determination, m.p. 132-133°; lit. 133°.

(7) Undecane dioic acid

It was available from the laboratory stock. Its purity was tested by melting point determination, m.p. 109-110°; lit. 110°.

(8) Brassylic acid: $HOOC-(CH_2)_{11}-COOH$.

It was synthesised by the following procedure. Ethyl erucate was oxidised in acetone solution by $KMnO_4$. The temperature was maintained below zero degree. The product was worked up as usual. The low boiling fraction was distilled out. The remaining residue was half ester of brassylic acid. It was further esterified to diester, which was distilled under vacuum and finally saponified. Brassylic acid was obtained in pure state after two crystallisationsfrom alcohol, m.p. 110-113°; lit. 113°.

(9) Tridecane-1,13-dicarboxylic acid: $HOOC-(CH_2)_{13}-COOH$.

It was available from the laboratory stock. It was crystallised three times for purification, m.p. 112-115°; lit. 114-115°.

(10) Tetradecane-1,14-dicarboxylic acid $HOOC-(CH_2)_{14}-COOH$.

It was available from the laboratory stock, m.p. 122-123°; lit. 124°.

(11) Pentadecane-1,15-dicarboxylic acid $HOOC-(CH_2)_{15}-COOH$.

It was obtained from the laboratory stock. It was crystallised three times and then used, m.p. 116-118°; lit. 118°.

(12) Hexadecane-1,16-dicarboxylic acid

This acid was obtained by the oxidation of ω-hydroxy stearic acid ($HO-CH_2-(CH_2)_{16}-COOH$). Jones' reagent was used for this purpose, m.p. 123°; lit. 124°.

II. Diethylene glycol

It was purified by distillation and stored in a stoppered bottle. The same sample was used throughout this synthesis.

III. Diglycerol

It was prepared by the dehydration of glycerol, as described in the literature.

Preparation of polyester.

Broadly, there are two methods for the preparation of polyesters. In one case a cross-linking agent is used and in the other case a cross-linking agent is not used. In the present work, the cross-linking agent is diglycer~~ed~~ol used to the extent of 1 to 2%.

For the preparation of the polyesters, the method described by Craig and Murty has been followed essentially, with minor modifications.[2]

Diethylene glycol and the corresponding dicarboxylic acids were placed in molar proportions in a round-bottom flask and to this p-toluene sulphonic acid (0.2 g) and the required quantity of diglycerol were added. This mixture was heated carefully at 135-140°(bath/2 mm. for 4 hours. The resulting viscous material was dissolved in methylene chloride or chloroform. The solution was washed repeatedly with water, dried over anhydrous sodium sulphate, the solvent removed and the residue dried to a constant weight under vacuum at the room temperature. The polyesters from different dicarboxylic acids were parepared under almost identical conditions. In the case of malonic acid polyester preparation (n=1), instead of heating directly at 135° (bath), the mixture was initially heated slowly below 100° (bath) for 1 hour and then at the usual temperature of 135-140° (bath) for 4 hours.

Polyesters up to azelaic acid (n=7) are viscous liquids, while higher polyesters are low melting solids.

In order to have a better idea, regarding the extent of polymerisation, the neutralisation equivalents were determined. The structure of the monomeric units and neutralisation equivalents are given in Table I.

Solid support

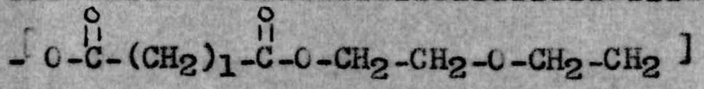
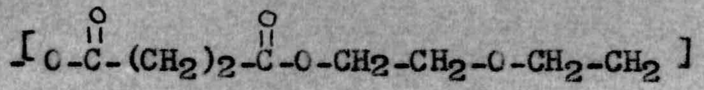
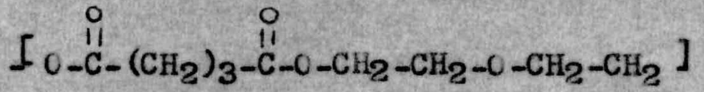
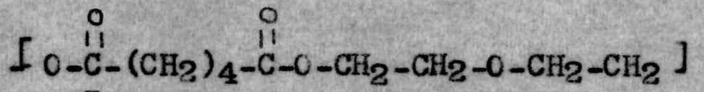
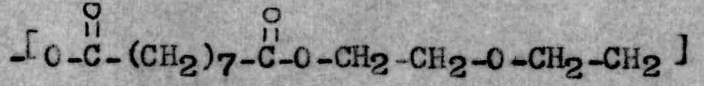
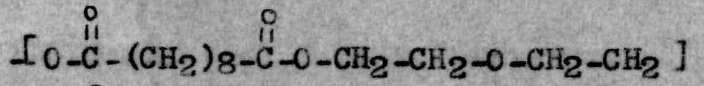
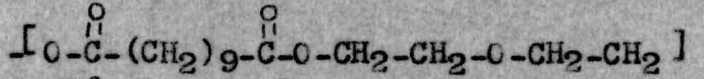
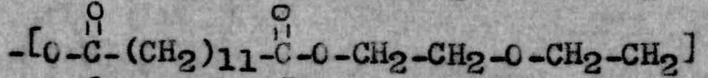
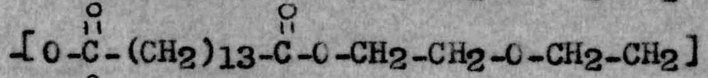
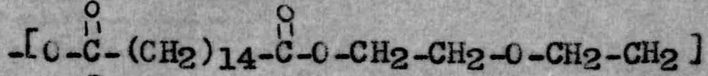
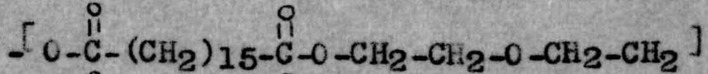
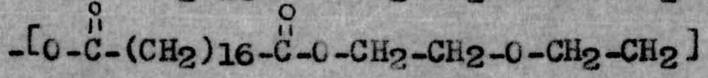
Indian fire-brick powder was used throughout these experiments. These bricks are used for the walls of furnace.

Initially, a few columns were run, where this supporting material was not washed at all. Such columns, showed very strong tendency towards the adsorption of sample vapours.

Therefore the following method was adopted for the purification of the various screen fractions of this supporting material.

TABLE 1

Molecular weights of the polyesters as determined by end group titration.

Structure of the monomeric unit	No. of methylene groups in the acidic fragment.	Molecular weights
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_1-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	1	545, 567
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_2-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	2	1246, 1253
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_3-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	3	344, 336
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_4-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	4	1129, 1180
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_7-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	7	399, 398
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_8-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	8	2166, 2143
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_9-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	9	684, 698
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_{11}-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	11	968, 947
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_{13}-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	13	646, 633
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_{14}-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	14	953, 936
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_{15}-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	15	866, 875
 $-[O-\overset{O}{\overset{\|}{C}}-(CH_2)_{16}-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-O-CH_2-CH_2]$	16	8 - -

Fire-brick powder, was repeatedly washed with concentrated hydrochloric acid and finally with water. It was then treated with hot potassium hydroxide solution(8%), and kept overnight as such. The material was washed thoroughly with distilled water till neutral. The product thus obtained was dried in a muffle furnace at about 500°.

Finally, the material was sieved again and the required screen fractions were collected. Each of these fractions, was again heated, nearly at 200° for several hours, cooled in a desiccator and stored in air-tight containers.

The last two steps regarding dehydration and storage were absolutely essential for getting reproducible results.

The packing density of such type of supporting material was found to be dependent upon (i) the mesh size of the particles, and (ii) the process of packing.

Impregnation and filling

The required quantities of supporting material and the polyester were weighed upto a milligram, the polyester was dissolved in methylene chloride or chloroform, applied to the support and the solvent was carefully removed on a water bath at 60°. When the evaporation was complete, the whole mass was taken in a shallow glass vessel and heated in an oven at 110° for several hours to a constant weight. The resulting impregnated fire-brick powder was clearly free flowing. In this way all the polyesters were impregnated on the fire-brick support.

Column filling

If the column dimensions are changed, the pressure drop across the column, also changes. One and the same column was therefore used throughout these experiments. Every time, the same weight of column filling was taken, so that the weight of the stationary phase was the same in all the cases. Then the column was ~~h~~tested for the flow rate and inlet pressure. If the inlet pressure was too high, the column material was repacked and glass wool plugs were slightly loosened. This adjustment gave the required flow rate at desired inlet pressure.

Column conditioning

The process of column conditioning does have some effect on the final retention time data. This will be discussed in the discussion part. The process which was followed, is described below.

During the process of conditioning, the column temperature was maintained at least 15° higher than that of the required one. Flow rate of the carrier gas, hydrogen, was nearly 6 to 7 ~~times~~ litres per hour. This process was continued for 4 to 6 hours. Within this time interval, the recorder recorded a stable base line. In some of the cases, nearly 10 hours conditioning period was necessary to get a steady base-line. The difference in the time interval required to stabilise two different columns, indirectly suggests that percentage of low boiling material must not be the same in all the cases.

Recording the retention time data

Retention times were recorded with the help of a stop-watch. The stop-watch was started when the air peak reached its maxima and stopped when the actual chromatographic peak reached its maxima. At regular intervals, internal standard samples were reinjected to check their retention times. Several concordent readings were recorded. In this way all the retention time data were collected.

In order to compare the retention time or retention volume data from column to column, all the column variables were kept constant.

(1) Column length - 6 ft.

(2) Weight of the column liquid: 2.95 g. (± 0.005).

(3) Carrier gas flow rate(hydrogen): 4 lit/hr.

(4) Temperature of the column: (i) 80°; (ii) 100°;
(iii) 120° & (iv) 162°.

(5) Bridge current: 150 m.A.

(6) Inlet pressure: (i) 125 ± 3 mm. at 80°
(ii)135 ± 3 mm. at 100°
(iii)143 ± 3 mm. at 120°
(iv)185 ± 4 mm. at 162°.

(7) Sample size: 1 ~~ml~~ µl (with the help of Hamilton syringe, 10 µl capacity).

The following chart (Chart I) gives the structures and the other physical properties of the compounds which are used as standard samples during the present investigation.

CHART I

MONOTERPENIC HYDROCARBONS

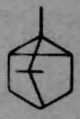
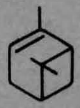
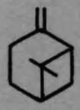
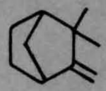
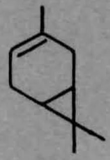
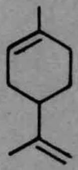
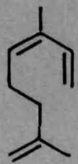
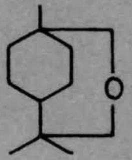
No.	NAME OF THE COMPOUND	STRUCTURE	m.p°C.	b.p°C.
1	TRICYCLENE		66°	153°
2	α-PINENE		–	156°
3	β-PINENE		–	162°
4	CAMPHENE		–	159°
5	Δ^3-CARENE		–	169°/705mm
6	LIMONENE		–	174°
7	OCIMENE		–	81°/30mm
8	1,8-CINEOLE		–	175°
9	p-CYMENE		–	177°

CHART I (contd.)

MONOTERPENIC OXYGENATED COMPOUNDS

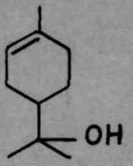
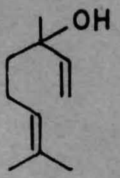
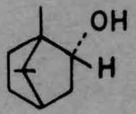
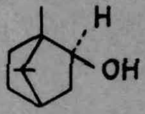
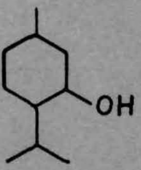
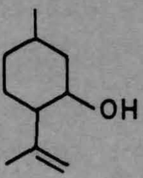
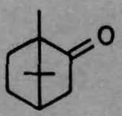
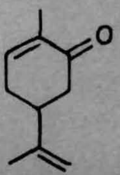
No.	NAME OF THE COMPOUND	STRUCTURE	m.p.°C	b.p.°C
1	α-TERPINEOL		–	104°/15mm
2	LINALOOL		–	199°
3	BORNEOL		208°	–
4	ISOBORNEOL		217°	–
5	MENTHOL		–	98°/10mm
6	ISOPULEGOL		–	94°/14mm
7	CAMPHOR		179°	–
8	CARVONE		–	230°

CHART I (contd.)

MONOTERPENIC OXYGENATED COMPOUNDS

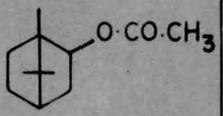
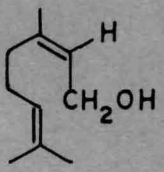
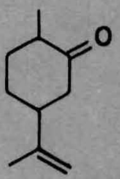
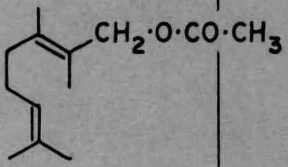
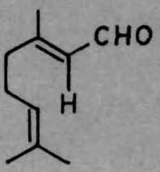
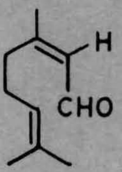
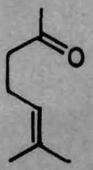
No.	NAME OF THE COMPOUND	STRUCTURE	m.p. °C.	b.p. °C.
9	BORNYL ACETATE		-	106°/15mm.
10	NEROL.		-	125°/25mm
11	DIHYDROCARVONE		-	75°/3mm
12	GERANYL ACETATE		-	120°/17mm.
13	CITRAL a		-	117°-118°/ 20mm.
14	CITRAL b		-	118°-119°/ 20mm
15	METHYL HEPTENONE		-	108°/20mm

CHART I (contd.)

SESQUITERPENIC HYDROCARBONS

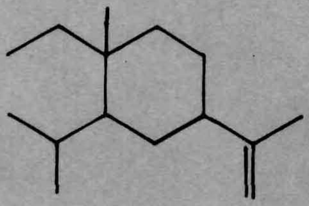
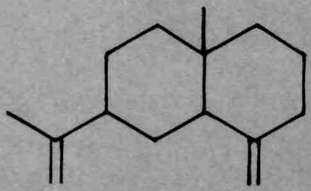
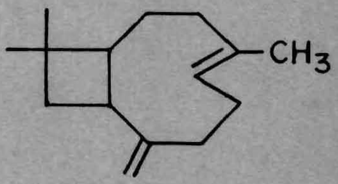
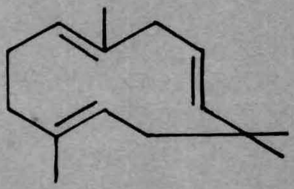
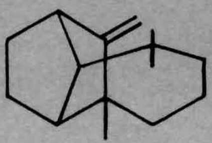
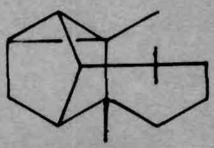
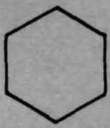
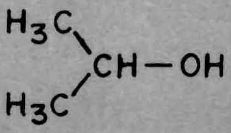
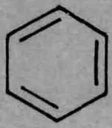
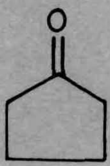
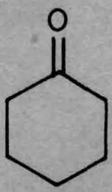
No.	NAME	STRUCTURE	M. P. °C	B. P. °C
1	TETRAHYDRO ELEMENE		–	–
2	β-SELINENE		–	136°/17mm.
3	CARYOPHYLLENE		–	113°/8mm.
4	HUMULENE		–	127°/12mm.
5	LONGIFOLENE		–	–
6	LONGICYCLENE		–	–

CHART I (contd.)

NON TERPENIC COMPOUNDS

No.	NAME	STRUCTURE	M. P. °C.	B. P. °C.
1	CYCLOHEXANE		–	80°
2	METHYL ALCOHOL	CH_3-OH	–	65°
3	ETHYL ALCOHOL	CH_3-CH_2-OH	–	78°
4	ISOPROPYL ALCOHOL	 $(H_3C)_2CH-OH$	–	82°
5	BENZENE		–	80°
6	CYCLOPENTANONE		–	131°
7	CYCLOHEXANONE		–	156°

Results and discussion

Monoterpenic hydrocarbons have a boiling range of 150° to 180°. The retention times of monoterpenic hydrocarbons, 1:8-cineole, cyclohexane, benzene, toluene, p-cymene, cyclopentanone, cyclohexanone, methyl alcohol, ethyl alcohol and isopropyl alcohol were determined under identical conditions. The results are presented in Table 2 (a, b, c) for the temperatures 80°, 100° and 120° respectively. The operations were carried out at different temperatures, 80°, 100° and 120° to get good resolution of the different compounds. While examining the behaviour of monoterpenes on polyesters of higher dicarboxylic acids ($n>7$), low temperature ranges (80-100°) were avoided, as the retention time was inordinately long to be of any practical value. Only the temperature of 120° was used.

For sesquiterpenic hydrocarbons and monoterpenic oxygenated compounds, only the temperature of 162° was chosen and the results are shown in Table 3. This high temperature was necessary, because of the high boiling nature of the above mentioned compounds.

A glance at the above mentioned tables shows that the retention times of monoterpenic hydrocarbons follow a clear arithmetical pattern up to brassylic acid-

Table 2a

Retention time in minutes at 80°

Compound	Number of methylene groups in the acidic fragment of the polyester. $(CH_2)_1$	$(CH_2)_2$	$(CH_2)_3$	$(CH_2)_4$	$(CH_2)_7$
α-Pinene	1.38	2.73	3.92	5.40	9.67
β-Pinene	2.52	4.83	7.27	9.73	16.90
Δ^3-Carene	3.18	6.18	9.02	12.87	22.03
Limonene	4.57	8.83	13.12	17.83	30.90
Ocimene	6.71	12.53	18.55	24.80	41.73
Tricyclene	1.25	2.40	3.63	4.92	9.13
Camphene	1.73	3.70	5.63	7.50	13.13
Benzene	1.61	2.50	3.10	3.55	4.48
Toluene	2.83	4.52	5.80	7.00	9.35
p-Cymene	9.60	15.70	22.80	29.90	45.30
Cyclohexane	0.20	0.36	0.56	0.77	1.23
Cyclohexanone	26.60	34.00	39.10	41.00	47.70
Cyclopentanone	15.80	20.60	22.00	21.80	23.50
Methyl alcohol	1.77	2.22	2.63	2.36	2.25
Ethyl alcohol	1.85	2.72	3.32	3.03	3.26
Isopropyl alcohol	1.56	2.40	3.20	2.94	3.45
1:8-Cineole	7.60	12.33	18.90	22.10	42.10

Table 2b

Retention time in minutes at 100°

Compound	Number of methylene groups in the acidic fragment of the polyester.							
	$(CH_2)_1$	$(CH_2)_2$	$(CH_2)_3$	$(CH_2)_4$	$(CH_2)_7$	$(CH_2)_8$	$(CH_2)_9$	$(CH_2)_{11}$
α-Pinene	0.75	1.43	2.18	2.75	5.26	6.04	6.73	8.82
β-Pinene	1.34	2.53	4.01	5.13	8.66	9.90	10.92	14.50
Δ^3-Carene	1.70	3.07	4.85	6.66	10.75	12.30	13.58	17.80
Limonene	2.28	4.30	6.71	8.50	14.65	16.30	17.38	24.00
Ocimene	3.08	5.53	8.30	11.10	18.54	20.20	21.50	27.50
Tricyclene	0.70	1.30	2.11	2.73	4.93	5.46	6.52	8.34
Camphene	1.23	1.93	3.17	4.03	7.10	7.82	8.60	11.53
Benzene	0.89	1.40	1.83	1.95	2.60	2.68	2.35	2.81
Toluene	1.48	2.30	3.18	3.81	4.90	5.30	4.75	5.90
p-Cymene	4.25	7.02	10.70	13.40	20.80	22.80	23.10	29.60
Cyclohexane	0.13	0.27	0.38	0.45	0.78	0.92	0.95	1.26
Cyclohexanone	11.40	17.60	18.80	18.80	20.8	21.40	20.6	22.2
Cyclopentanone	8.40	10.1	10.50	10.40	11.50	11.40	10.3	11.5
Methyl-alcohol	0.97	1.23	1.48	1.27	1.32	1.15	1.01	1.05
Ethyl-alcohol	1.00	1.39	1.80	1.55	1.70	1.53	1.47	1.46
Isopropyl-alcohol	0.80	1.32	1.70	1.53	1.80	1.53	1.67	1.70
1:8-Cineole	3.35	5.67	9.4	10.6	18.2	19.6	22.60	25.5

Table 2c

Retention time in minutes at 120°

Compound	Number of methylene groups in the acidic fragment of the polyester							
	$(CH_2)_8$	$(CH_2)_9$	$(CH_2)_{11}$	$(CH_2)_{13}$	$(CH_2)_{14}$	$(CH_2)_{15}$	$(CH_2)_{16}$	Apiezon
α-Pinene	3.45	3.80	4.60	4.75	4.85	5.23	5.75	9.8
β-Pinene	5.43	5.90	7.45	7.30	7.33	8.0	9.00	11.3
Δ^3-Carene	6.63	7.20	8.75	8.80	8.66	9.63	10.70	16.4
Limonene	8.30	9.10	11.30	11.10	11.33	12.50	13.40	19.4
Ocimene	9.75	10.50	12.90	12.50	13.45	13.36	15.03	18.1
Tricyclene	3.30	3.70	4.48	4.48	4.80	4. 95	5.58	9.5
Camphene	4.46	4.90	5.93	5.95	6.55	6.50	7.58	11.8
Benzene	1.66	1.50	1.80	1.60	1.55	1.64	1.80	1.50
Toluene	3.47	2.80	3.86	3.10	3.15	3.20	3.60	3.30
p-Cymene	11.65	11.80	14.53	13.90	13.45	14.3	16.60	18.1
Cyclohexane	0.62	0.63	0.80	0.76	0.83	0.87	0.95	1.60
Cyclohexanone	11.50	11.10	12.2	10.90	11.0	10.63	11.1	6.40
Cyclopentanone	6.10	5.80	6.30	5.60	5.50	5.33	5.60	-
Methyl-alcohol	0.66	0.64	0.60	0.54	0.55	0.52	0.50	1.1
Ethyl-alcohol	0.90	0.86	0.86	0.78	0.74	0.74	0.70	1.5
Isopropyl alcohol	0.93	0.96	0.95	0.87	0.90	0.87	0.80	1.6
1:8-Cineole	9.75	11.50	12.76	12.6	13.55	13.40	14.10	19.8

Table 3

Retention time in minutes at 162°

Compound	Number of methylene groups in the acidic fragment of the polyester:		
	$(CH_2)_2$	$(CH_2)_7$	$(CH_2)_{11}$
Longifolene	3.61	12.30	20.30
Caryophyllene	3.60	13.30	20.70
Humulene	5.50	17.66	26.95
Nerol	10.16	18.75	19.30
Terpineol	7. 75	15.20	16.56
Camphor	5.40	9.25	10.30
Borneol	7.70	14.20	16.16
Linalool	3.40	7.07	7.70

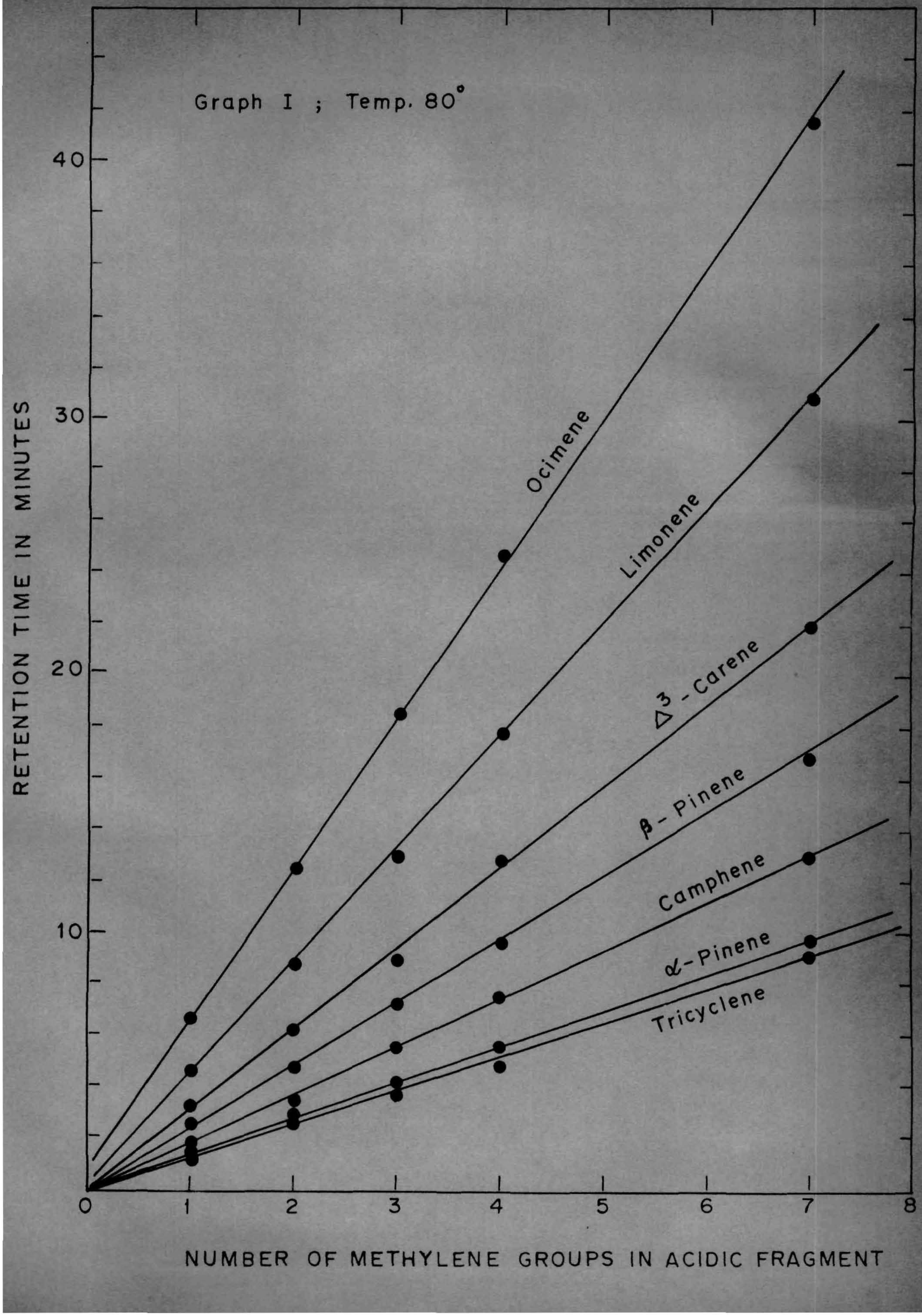
diethylene glycol polyester. When the graph of the number of methylene groups against the retention time is plotted, good straight lines are obtained.

These are shown in graphs I and II.

This typical arithmetical pattern of retention times has been further indicated in Table 4. Here the average ratios of the retention time for seven monoterpenes on different polyesters have been recorded against the expected values. It can be seen from the table that the observed and the calculated values agree extremely well. We must take into consideration that such type of ratios reflect the behaviour of seven monoterpenic hydrocarbons - differing in structural features as an aggregate. Even then these figures agree extremely well to the expected values. This may indicate that structural differences do not have any vital effect on the retention times on these polyesters. The effect if any will not be pronounced. The order of emergence of all these compounds remains the same throughout the series of polyesters.

The retention times follow an arithmetical pattern.

Thus it has been found that if t_R^x is the retention time in minutes and x is the number of methylene groups present in the acidic components of the polyester, and if t_R^y be the retention time on another polyester containing y


Graph I ; Temp. 80°
RETENTION TIME IN MINUTES
40
30
20
10
0
1
2
3
4
5
6
7
8
NUMBER OF METHYLENE GROUPS IN ACIDIC FRAGMENT
Ocimene
Limonene
Δ3 - Carene
β - Pinene
Camphene
α-Pinene
Tricyclene

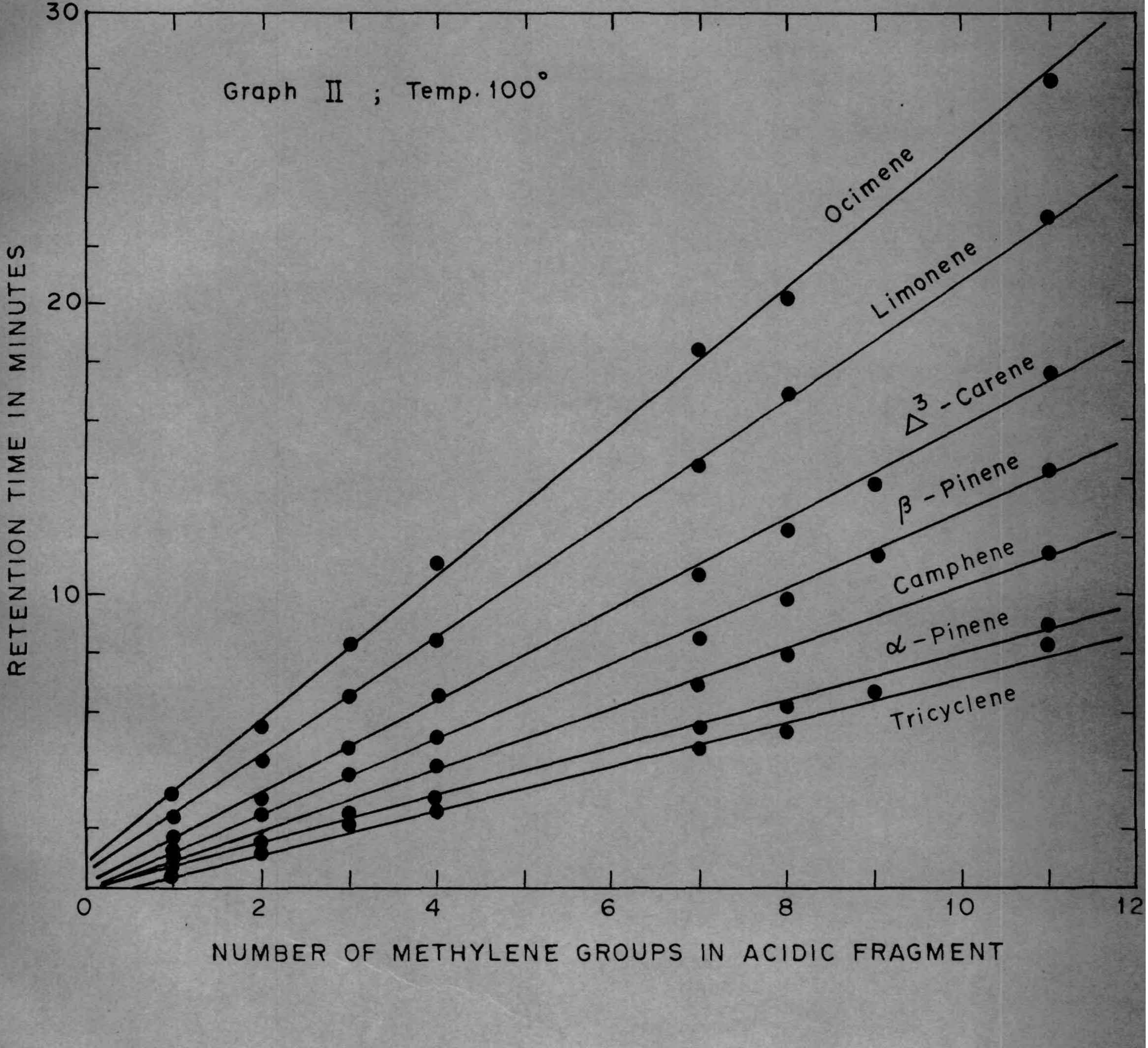

Graph II ; Temp. 100°
Ocimene
Limonene
Δ^3 - Carene
β - Pinene
Camphene
α - Pinene
Tricyclene
RETENTION TIME IN MINUTES
30
20
10
0
2
4
6
8
10
12
NUMBER OF METHYLENE GROUPS IN ACIDIC FRAGMENT

Table 4

Relationship between the ratio of the number of methylene groups and the ratio of the retention times on the respective substrates.

Ratio of the no. of methylene groups	Average ratio of observed retention time.	Calculated ratio	Error (%)
9/2	4.40	4.50	2.23
9/3	2.82	3.00	6.00
9/4	2.17	2.25	4.47
9/7	1.24	1.29	3.55
9/8	1.10	1.13	2.65
8/7	1.12	1.16	3.57
8/4	1.96	2.00	2.00
8/3	2.53	2.66	4.89
8/2	3.96	4.0	1.00
7/4	1.73	1.75	1.37
7/3	2.24	2.33	3.98
7/2	3.54	3.50	1.17
4/3	1.31	1.33	1.90
4/2	2.05	2.00	2.70
4/1	3.81	4.00	4.87
3/2	1.54	1.50	2.30
3/1	2.90	3.00	3.20
2/1	1.90	2.00	5.00

methylene groups, then: $$\frac{t_R^x - t_R^y}{x - y} = C \text{------} (I)$$

where 'c' is a constant for a particular compound at a particular temperature and is the individual contribution of a single methylene group.

This relationship seems to be an integral characteristic of the number of methylene (CH_2) groups between the two carboxylic groups and is valid for non-polar terpenic and other hydrocarbons and is nearly so for aromatic hydrocarbons.

The large difference in retention times observed for the higher polyester stationary phases (n = 9 to 16) would render them specially useful for preparative separation.

The values of the constant 'c' have been calculated according to the formula (1) and tabulated in Tables (5a) and (5b) for the two temperatures, 80° and 100°. The constant nature of the value of 'c' is apparent from these tables.

When such type of polyesters were studied by other workers, they studied only limited number of the esters. Craig and Rudloff felt that the number of methylene groups situated between the two carboxyl groups of the dicarboxylic

Table 5a (Temp. 80°)

Evaluation of C at 80°

$t_R^x - t_R^y$	x-y	C	$t_R^x - t_R^y$	x-y	C
α-Pinene			**β-Pinene**		
8.29	7-1=6	1.38	14.38	7-1=6	2.39
6.94	7-2=5	1.39	12.07	7-2=5	2.41
5.75	7-3=4	1.44	9.63	7-3=4	2.40
4.27	7-4=3	1.42	7.17	7-4=3	2.39
Δ³-Carene			**Limonene**		
18.85	7-1=6	3.14	26.33	7-1=6	4.39
15.65	7-2=5	3.13	22.07	7-2=5	4.41
13.01	7-3=4	3.25	17.78	7-3=4	4.44
9.16	7-4=3	3.05	13.07	7-4=3	4.36
Ocimene			**Tricyclene**		
35.02	7-1=6	5.84	7.88	7-1=6	1.31
29.20	7-2=5	5.84	6.73	7-2=5	1.35
23.18	7-3=4	5.79	5.50	7-3=4	1.37
16.93	7-4=3	5.64	4.21	7-4=3	1.40
Camphene			**Cyclohexane**		
11.4	7-1=6	1.90	1.03	7-1=6	0.17
9.43	7-2=5	1.89	0.97	7-2=5	0.19
7.50	7-3=4	1.87	0.67	7-3=4	0.17
5.63	7-4=3	1.88	0.46	7-4=3	0.15

Table 5b (Temp. 100°)

Evaluation of C at 100°

$t_R^x - t_R^y$	x-y	C	$t_R^x - t_R^y$	x-y	C
α-Pinene			**β-Pinene**		
5.98	9-1=8	0.75	9.6	9-1=8	1.20
5.3	9-2=7	0.76	8.49	9-2=7	1.21
4.55	9-3=6	0.76	6.91	9-3=6	1.15
3.98	9-4=5	0.79	5.79	9-4=5	1.16
Δ^3-Carene			**Limonene**		
11.88	9-1=8	1.49	15.1	9-1=8	1.90
10.51	9-2=7	1.50	13.08	9-2=7	1.87
8.73	9-3=6	1.46	10.67	9-3=6	1.78
6.92	9-4=5	1.40	8.88	9-4=5	1.78
Ocimene			**Tricyclene**		
18.42	9-1=8	2.30	5.82	9-1=8	0.73
15.97	9-2=7	2.28	5.22	9-2=7	0.75
13.20	9-3=6	2.20	4.41	9-3=6	0.74
10.4	9-4=5	2.08	3.79	9-4=5	0.76
Camphene			**Cyclohexane**		
7.37	0-1=8	0.92	0.82	9-1=8	0.103
6.67	9-2=7	0.95	0.68	9-2=7	0.097
5.44	9-3=6	0.91	0.57	9-3=6	0.095
4.57	9-4=5	0.92	0.50	9-4=5	0.100

acid, might play some significant role on the degree of separation.[3] Our present investigations have fully justified that possibility.

Such type of effect of the spacing of the methylene groups on the ester function can be appreciated up to where eleven methylene groups (brassylic acid) are involved, in the acidic fragment of the polyester. But beyond this range, it seems that the retention time is almost constant.

This may be explained as follows. In the case of higher dicarboxylic acids, the relative proportion of the oxygen function is greatly reduced, as a consequence of which the resulting polyester behaves increasingly like a hydrocarbon type stationary phase. However, when we compare the retention time data obtained on 'Apiezon column', we find that even the longest chain length dicarboxylic acid polyester differs from the same. It thus means that the longest chain length dicarboxylic acid-diethylene glycol polyester does possess its special resolving power compared to 'Apiezon'.

Relative Retention Time Data: The concept of relative retention time

The retention time of a certain organic compounds is very prone to temperature fluctuations of the column and slight fluctuations in the rate of flow of the carrier gas as well. Some other factors, such as the quantity of the sample etc. also effect the retention time. So it is customary to report the retention time with respect to some well known organic compound. For example, the retention time of α-pinene on a certain GLC column may be 10 minutes. Similarly, the retention time of p-cymene (corrected to the dead volume) on the same column may be 35 minutes. Then the relative retention time of p-cymene, with respect to α-pinene is 3.50. Most of the workers have realised that the 'relative retention time' values are accurately reproducible.[4]

Relative retention time data with respect to the observations carried out during the present series of investigations are given in Table 6. For comparison purposes, some figures collected by others are also given.

When we take into consideration the nature of the monomeric units of our polyesters given in Table 1, we find a stepwise addition of one methylene group. They are expected to affect the polarity of the resulting polyester. Naturally, the succinic acid-diethylene glycol polymer would be more polar than the brassylic acid$(CH_2)_{11}$ - diethylene glycol polymers. However, in the case of the

Table 6

Relative retention times of monoterpenic hydrocarbons and related compounds.

						Number of methylene groups in the acidic fragment of the polyester (Present Series)			
Stationary phase	Tricresyl phosphate*	D.C.Silicone oil*	Didecyl phthalate	LAC-2-R446 *	LAC-1-R296 *	$(CH_2)_2$	$(CH_2)_7$	$(CH_2)_{11}$	$(CH_2)_{16}$
Temperature	100°	100°	110°	100°	100°	100°	100°	120°	120°
α-Pinene	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
β-Pinene	1.76	1.40	1.52	1.67	1.70	1.77	1.65	1.62	1.57
Δ^3-Carene	1.97	1.90	1.77	-	-	2.15	2.04	1.90	1.86
Ocimene	-	-	-	-	-	3.87	3.53	2.80	2.61
Limonene	2.48	1.95	2.16	2.64	2.84	3.00	2.77	2.46	2.38
Tricyclene	-	-	-	-	-	0.91	0.93	0.97	0.97
Camphene	1.24	1.30	1.24	1.37	1.46	1.35	1.35	1.29	1.32
p-Cymene	3.40	2.42	2.46	4.01	4.59	4.9	3.97	3.02	2.88
1:8-Cineole	-	-	-	-	-	3.97	3.46	2.77	2.45
Cyclopentanone	-	-	-	-	-	7.10	2.19	1.37	0.97
Cyclohexanone	-	-	-	-	-	12.30	3.96	2.65	1.97

* Burchfield H.P. and Stross E.E.,
'Biochemical Applications of Gas Chromatography', p. 384 (1962).

polyesters, which are prepared from very long chain dicarboxylic acid and diethylene glycol, the rate of variation of the polarity factor loses its significance. As a result there should be some distinctive difference when we take into consideration the relative retention time data on say succinic acid-diethylene glycol column and brassylic acid-diethylene glycol column. This is easily borne out when relative retention time data on the above mentioned polyesters are considered. For example, we may take into consideration the relative retention time values for α-pinene and tricyclene. α-Pinene is bicyclic with one trisubstituted double bond, while tricyclene is tricyclic and does not possess any unsaturation. Therefore, such closely related pairs should show some difference in the relative retention time on a more polar column such as succinic acid-diethylene glycol. For example, the relative retention time of tricyclene with respect to α-pinene, is 0.909 on succinic acid-diethylene glycol polyester, but the same value comes to be 0.946, on brassylic acid-diethylene glycol polyester. This leads to an important observation that these two compounds are coming close together on brassylic acid-diethylene glycol polyester. Therefore their separation is easier on succinic acid-diethylene glycol polyester.

Now, these observations may safely be compared with those of other workers. When the polyesters were examined by earlier workers, they also found analogous results, which are described in short below.

Lipsky Landowne and Godet used several varieties of polyesters to analyse esters of fatty acids.[5] They found that sebacate polyester of diethylene glycol could resolve methyl ester of linoleic acid, which contains three double bonds. But this polyester failed to resolve methyl oleate and methyl stearate mixture. It is interesting to note that stearic acid does not contain any double bond, but oleic acid contains one. Obviously they could resolve this pair of methyl esters by using a more polar stationary phase. So they used adipate polyester of diethylene glycol and successfully separated the biologically important mono, di and tri unsaturated C_{18} acid esters from stearic acid ester.

Similarly the analysis of a standard mixture of methyl esters of saturated and unsaturated fatty acids from C_{12} to C_{22} was almost complete, by using the highly polar stationary phase - succinate polyester of diethylene glycol.

It is interesting to take into account the values of separation factors - α, given by them for a few stationary phases.

Stationary phase	'α' for stearate and oleate	Remarks
1. Apiezon	not resolved	not useful
2. Adipate polyester of diethylene glycol	1.08	requires very high number of theoretical plates.
3. Adipate polyester of diethylene glycol, partially cross-linked with pentaerythritol	1.12	the requirement of number of theoretical plates, is moderate.
4. Succinate polyester of diethylene glycol	1.17	best performance amongst all the four phases mentioned presently.

This critical difference, the values of 'α', is more pronounced on a polar stationary phase like succinate polyester of diethylene glycol and it is not pronounced on a nonpolar stationary phase.

A steady and stepwise change in the polarity of these polyesters has been further indicated by the following observations.

Relative retention times of 1:8-cineole and p-cymene, with respect to limonene have been calculated and presented in Table 7.

Table 7

Relative retention times of 1:8-cineole and p-cymene with respect to limonene.

	Number of methylene groups in the acidic fragments of the polyester:					
	Temperature 80°			Temperature 120°		
	$(CH_2)_1$	$(CH_2)_2$	$(CH_2)_7$	$(CH_2)_8$	$(CH_2)_{11}$	$(CH_2)_{16}$
1,8-Cineole	1.682	1.480	1.36	1.174	1.129	1.05
p-Cymene	2.10	1.885	1.466	1.404	1.286	1.239

These figures would show that for critical pairs like 1:8-cineole and limonene or p-cymene and limonene, the relative retention time data are in favour of short-chain dicarboxylic acid-diethylene glycol polyesters. But it must be remembered that 1:8-cineole contains one oxygen in the form of ether linkage and p-cymene is aromatic.

A steady decrease in a relative retention values, indicates that the arithmetical pattern of retention times is not strictly observed by aromatics and ether type oxygenated compounds. It is also interesting to find out the effect of these type of polyesters on the retention times and relative retention times of oxygenated compounds. It can be seen from the Table 8 that the total contribution to the retention times of oxygenated compounds is nearly '2'.

Table 8

Relative retention times of monoterpenic oxygenated compounds and sesquiterpenic hydrocarbons:
Temperature 162° ; α-Terpineol = 1.0

Compound	Number of methylene groups in the acidic fragment of the polyester		
	2	7	11
Longifolene	0.466	0.809	1.23
Caryophyllene	0.465	0.869	1.25
Humulene	0.710	1.116	1.63
Nerol	1.31	1.23	1.165
Terpineol	1.0	1.0	1.0
Camphor	0.487	0.607	0.623
Borneol	-	-	-
Linalool	0.465	0.465	0.465

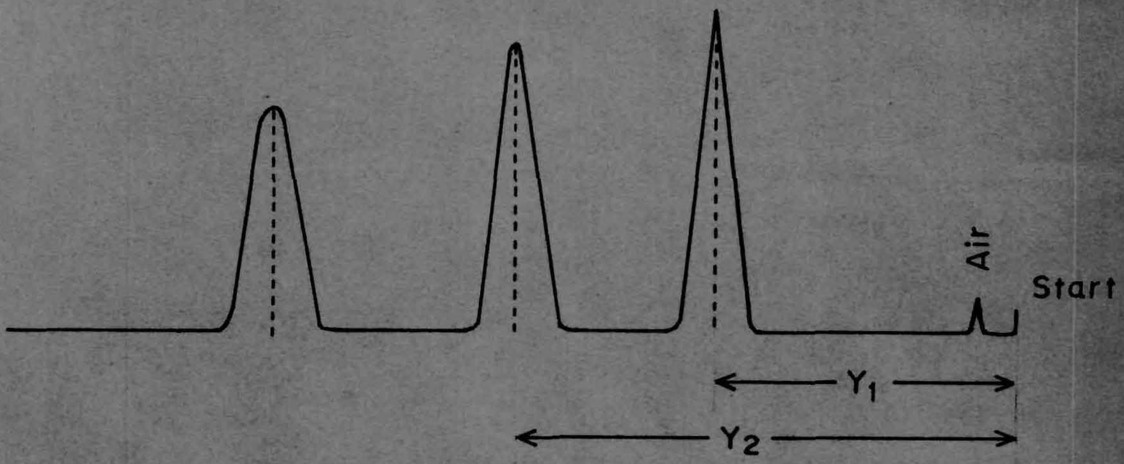
These observations are specially useful for the separation of the mixtures of monoterpenic oxygenated compounds and sesquiterpenic hydrocarbons as will be evident from the results described in the subsequent pages.

DIAGRAM No. 1

THE INTERRELATIONSHIP OF COLUMN EFFICIENCY AND RESOLVING POWER IN GAS CHROMATOGRAPHY [After Stephen S. Ober]

RESOLVING POWER = $Y_2 - Y_1 / \frac{1}{2} (Y_2 + Y_1)$

WHERE Y = RETENTION TIME (IN UNITS OF LENGTH)



Resolving power

Geometrical representation of 'resolving power' is shown in Diagram No. 1.

The concept of resolving power technique has been used and the resolving powers of two typical polyesters are noted in Table 9.

Table 9

Resolving powers for two typical polyesters
$n = 7$ and $n = 11$

No. of methylene groups in the acidic fragment of the polyester.	Compound pair	y_2-y_1 in mm.	$\frac{y_2+y_1}{2}$ in mm.	Resolving power $y_2 - y_1 / \frac{y_2 + y_1}{2}$
7	Caryophyllene-camphor	10.00	29.0	0.3448
7	Humulene-borneol	9.00	40.50	0.2222
11	Caryophyllene-camphor	26.00	40.00	0.6500
11	Humulene-borneol	28.00	55.00	0.5090

These data and the chromatogram clearly illustrate the utility of higher polyesters.

Bernhard has used polyester type substrates in capillary columns, for the separation of terpenic hydrocarbons.[6] He found that an adipic acid polyester column has a better separation factor as compared to 'Apiezone grease' or silicone grease columns, in spite of the low number of theoretical plates. So it can be expected that if these higher polyesters are used in capillary columns, they would furnish enormously high number of theoretical plates and still they would maintain their properties.

Now, the comparative evaluation of these polyesters is described under the light of data given in Table 8.

For the separation of nerol, a primary C_{10} alcohol and α-terpineol, a tertiary C_{10} alcohol, we require nearly 650 plates on succinic acid-diethylene glycol column. The same pair of alcohols can be separated on brassylic acid-diethylene glycol column, but we require nearly 1800 plates. Therefore it seems that it is easier to resolve polar compounds on polar substrate like succinic acid-diethylene glycol polyester. But it must be pointed out that many sesquiterpenic hydrocarbons would overlap. In actual practice it is beneficial to use longer chain dicarboxylic acid-diethylene glycol polyester. It is obvious that we require more efficient column.

Because of the high solubilities of the terpenic samples, the evaporation of the stationary phase, does not affect so heavily to the efficiency of the column. For example, columns of sebacic acid-diethylene glycol or brassylic acid-diethylene glycol have been used for a very long time, without any apparent loss in the efficiency.

Separation of the individual components of the terpenic oxygenated compounds is generally performed by using highly polar substrates. Polyethylene glycols have been extensively used for this purpose. Recently some new stationary phases have been developed. Sucrose acetate isobutyrate, hyprose etc. are the important members of them.[7,8] These stationary phases can resolve even an intricate mixture of monoterpenic oxygenated compounds. 'THEED', tetrahydroxy ethyl ethylenediamine is used nowadays.

Sesquiterpenic hydrocarbons and monoterpenic oxygenated compounds

The same column fillings were used for the collection of retention time data of sesquiterpenic hydrocarbons and monoterpenic oxygenated compounds. The polyesters chosen were (i) succinic acid-diethylene glycol polyester, (ii) azelaic acid-diethylene glycol polyester, and (iii) brassylic acid-diethylene glycol polyester.

The retention time data are presented in Table 3 and the relative retention time data in Table 8. It was difficult to collect the retention time data from the literature for the sesquiterpenic hydrocarbons. However, a general description is furnished below.

Rigby and Bethune studied several varieties of hop oil by gas liquid chromatography.[9] But they used silicone grease and Apiezone grease as the stationary phases, for the analyses of various varieties of oil. As many as thirteen components could be detected, but this analysis required nearly ~~4~~ 3 to 4 hours and later peaks were very broad.

Howard determined the composition of the essential oils of hops.[10] He also chromatographed the various fractions on Apiezon grease columns.

Similarly volatile oils of the leaves of black and white colorado spruce were investigated by Rudloff.[11] When GLC analyses were performed, it was found that high boiling monoterpenic alcohols overlap with sesquiterpenic hydrocarbons.

The usual way to separate these compounds by GLC technique is to use silicone or Apiezon type of stationary phases. But we find that the individual components of the hydrocarbon group as well as monoterpenic oxygenated compounds, are not separated well. But a broad cut can be had between the two species.

A glance at the retention time data, given in Table 3, shows that the retention times of hydrocarbons go on increasing as the number of methylene groups in the acidic fragment of the polyester are increased. But the retention times of the oxygenated compounds are increased by a factor of two only.

One more interesting application, though somewhat indirect, may be pointed out. In the symposium of 1957 ('Gas chromatography': Coates, Noebels and Fagerson(Editors), p.65, 1958) the point was discussed regarding the identification of substances by using them as GLC substrates. The investigation carried out above shows that 1,ω-dicarboxylic acids can be identified with considerable accuracy by preparing a polyester of unknown acid and running the column for some hydrocarbons under standard conditions.

Sources of deviation of the linearity factor

In the preceeding description, we have seen that an arithmetical relationship exists between the number of methylene groups in the acidic fragment of the polyester and the retention time of hydrocarbons. The various sources of deviation are described below.

1. The Nature of the polyester

It has been found that the polarity of the polyester goes on slowly decreasing. So the difference between the polarity of the two consecutive polyesters of dicarboxylic acids of more than C_{14} atoms will be comparatively very low, so that it is rendered difficult to differentiate between the two.

2. The most important of all the variable factors, the temperature of the column, affects the most, to the solubilities of the sample vapours in stationary phase.[12] The nature of the column packing, the size of the supporting material also have some influence on the retention volumes. Previous workers have reported that a change of 1° of the column temperature may give a difference of 3 to 5% to the final specific retention volume of a typical sample.[12] In the case of our instrument, used for the present work, it was possible to control the temperature up to 1° only. Therefore a slight deviation in the temperature of the oven may change the retention volume data to some extent.

3) The weight of the stationary phase in a GLC column also affects the final specific retention volume data.[4] Great care was taken on this point, so that in the present series of polyesters columns, the weight of the stationary phase was almost the same in the case of all the columns. But the same weight may not remain on the column after the process of conditioning is over. The rate of evaporation of all the polyesters may not be the same.

Another important factor is that the weight of stationary phase may not be sufficient enough to cover all the surface available on the supporting material. Thus the retention of solute vapours due to adsorption on the uncovered surface, may add to some extent to the lower polyester column.

Such type of study has been performed by previous workers.[13] Their remarks indicated that below a certain percentage of the stationary phase, the specific retention volumes, 'Vg' values - are not reproducible. Actually, when our fire-brick support was tested, it was found that it can take up sufficient load of stationary phases. These observations were performed by using succinic acid-diethylene glycol polyester. This suggested that when the weight of the stationary phase in a typical GLC column is slightly more than 3.20 g., the resulting chromatograms doznot show any tailings. But actually, we have used only 2.95 g., because the retention times on the long chain dicarboxylic acid-diethylene glycol polymers were too high to be of any practical value. But this lessening of the stationary phase might have affected our 'Vg' value data.

4) The physical nature of the polymer

It has been stated previously that the polyesters up to azelaic-acid diethylene glycol are viscous liquids while the rest of the polyesters are low melting solids. This may also give rise to some deviation from the arithmetical relationship. Even though, at a temperature of 80°, 100° and 120°, all the polyesters must be in liquid form, their densities and viscosities may be appreciably different. So in the case of higher dicarboxylic acid-diethylene glycol polyesters, their viscosities at the column temperature may be such that solute vapours are not fully dissolved in them, which may result in low retention times. But it is quite likely that the lower dicarboxylic acid-diethylene glycol polyesters are not so viscous at the same column temperature, which may result in better type of solution of sample vapours in the stationary phase. This may give rise to somewhat high retention times than expected.

= It is an established fact that the size of the sample has got a definite effect on the retention volume. In the present investigations, nearly 1 microlitre sample size was used. But the peaks coming out on a short-chain dicarboxylic acid diethylene glycol column, were very sharp because the retention times were very low. But the peaks obtained after a long time were rather broad so that the exact peaks maxima could not be judged accurately.

The solution for this 'peak broadening' is to use a preheater-block vapourisor, so that the sample vapours are introduced on the top of the column, as a 'plug'. But such type of device has been eliminated, because the retemtion time is changed if the block vapourisor is used, and then the retention time may not represent exactly the thermodynamical property of the stationary phase.

Harrison has studied the effect of flash heater temperature on retention volume.[14] He found that as the temperature of the flash heater goes on rising, the retention volume goes on decreasing. A few of the important observations are given below.

Variation of Retention Volume with Temperature

Column at 35°		Column at 77°	
Temp.	Ret.volume(ml) CH_2Cl_2	Temp.	Ret. volume(ml) CH_2Cl-CH_2Cl
30	340	70	260
60	320	95	240
80	310	120	235
100	310	168	235

Therefore it seems that a block vapourisor may not give the exact idea of the real retention volume.

6. Some efforts were directed towards the saponification values of the polymers, but no relationship, between the nature of the polymer, the constituents and the retention time was found.

Effect of modification in the preparation of polyester

After taking into consideration, the previous sources of error, the experimental procedure for the preparation of polyester, was slightly changed as follows:

(1)	Hexadecane-1,16-dicarboxylic acid $HOOC-(CH_2)_{16}-COOH$	6.297 g.
(2)	Diethylene glycol	2.651 g. ($\sim$25% more than theoretical)
(3)	Diglycerol	0.500 g.
(4)	p-Toluenesulphonic acid	0.300 g.

This mixture was heated at 140° at atmospheric pressure for 1 hour only and then at 140° (bath)/5 mm. for one hour only, then at 150° (bath)/2 mm. for one hour and finally at 170° (bath)/2 mm. for one hour.

The temperature of the bath was slowly raised as shown above, because it was thought that a temperature of 140° may not be sufficient for completing the polyester formation of $HOOC-(CH_2)_{16}-COOH$ and diethylene glycol. Diethylene glycol was also taken in slight excess, because it was thought that, this compound may slowly distill off, during the process of ester formation. The quantity of the catalyst was also increased.

Then the procedure for working up this polyester was also changed. The new method is described below.

The whole polyester mass was well powdered and treated with hot water, with vigorous stirring. The whole suspension was allowed to settle down. When this suspension cooled to room temperature, the supernatant aqueous layer was discarded. This step was repeated thrice. It was expected that the unreacted acid and diethylene glycol should wash out.

The water-washed polyester was dissolved in chloroform and this solution was again washed with sodium bicarbonate solution. At this stage again it was expected that the unreacted acid should wash out. The polymeric mass is expected to have some free end carboxylic groups. But this may be a weak acid due to its high molecular weight. Therefore it was felt that it will not react with weak alkali like sodium bicarbonate. But to be on the safe side, this was again washed with dilute hydrochloric acid. By this operation any sodium salt formed, should be converted to its original acidic form. Then finally it was repeatedly washed with water, till it was neutral.

Yield of the polyester was found to be 5.20 g.

This type of polyester was again impregnated on the same support. The weight of the stationary phase was also the same. This column filling was heated in air oven at 110° for nearly 4 to 5 hours. Then it was used for filling the column. The retention time data of such a column is given below.

Compound	Observed retention time (in minutes) on modified polyester	Expected retention time (in minutes)	
		On the basis of sebacic acid polyester.	On the basis of brassylic acid polyester.
α-Pinene	5.53	6.93	6.8
β-Pinene	8.55	10.9	11.1
Δ^3-Carene	9.9	13.1	12.9
Ocimene	14.2	19.9	19.0
Limonene	12.4	16.8	16.6

The last two columns give the retention times expected on the basis of sebacic acid-diethylene glycol polyester and brassylic acid-diethylene glycol polyester. It can be seen that there is a difference between observed and the expected retention times. Therefore it may be concluded that the retention times or in turn the solubilities of the sample vapours, could not be increased proportionately above a certain limit.

It is expected that such type of dicarboxylic acids and diethylene glycol may require a very long time for getting fully polymerised.

The same type of results were obtained in the case of other long chain dicarboxylic acid-diethylene glycol polymer, eventhough it was prepared according to the above mentioned modified procedure.

Effect of temperature

The arithmetical pattern of the retention times of hydrocarbons was found at the temperatures of 80°, 100° and 162°. On the basis of these interesting findings, it was felt necessary to examine the effect of temperature more closely.

The same column fillings were used for this investigation of temperature gradient. Retention time data were carefully recorded ~~b~~between 70° to 120° at an interval of 5° approximately. Not all the polyesters were used, but only succinic acid-diethylene glycol, adipic acid-diethylene glycol, azelaic acid-diethylene glycol and brassylic acid diethylene glycol polyesters were used for this aspect of the investigation. Reference samples were α-pinene, β-pinene, limonene and n-amyl acetate.

Specific retention volume data were calculated according to the standard procedure and used for the construction of graphs.[4]

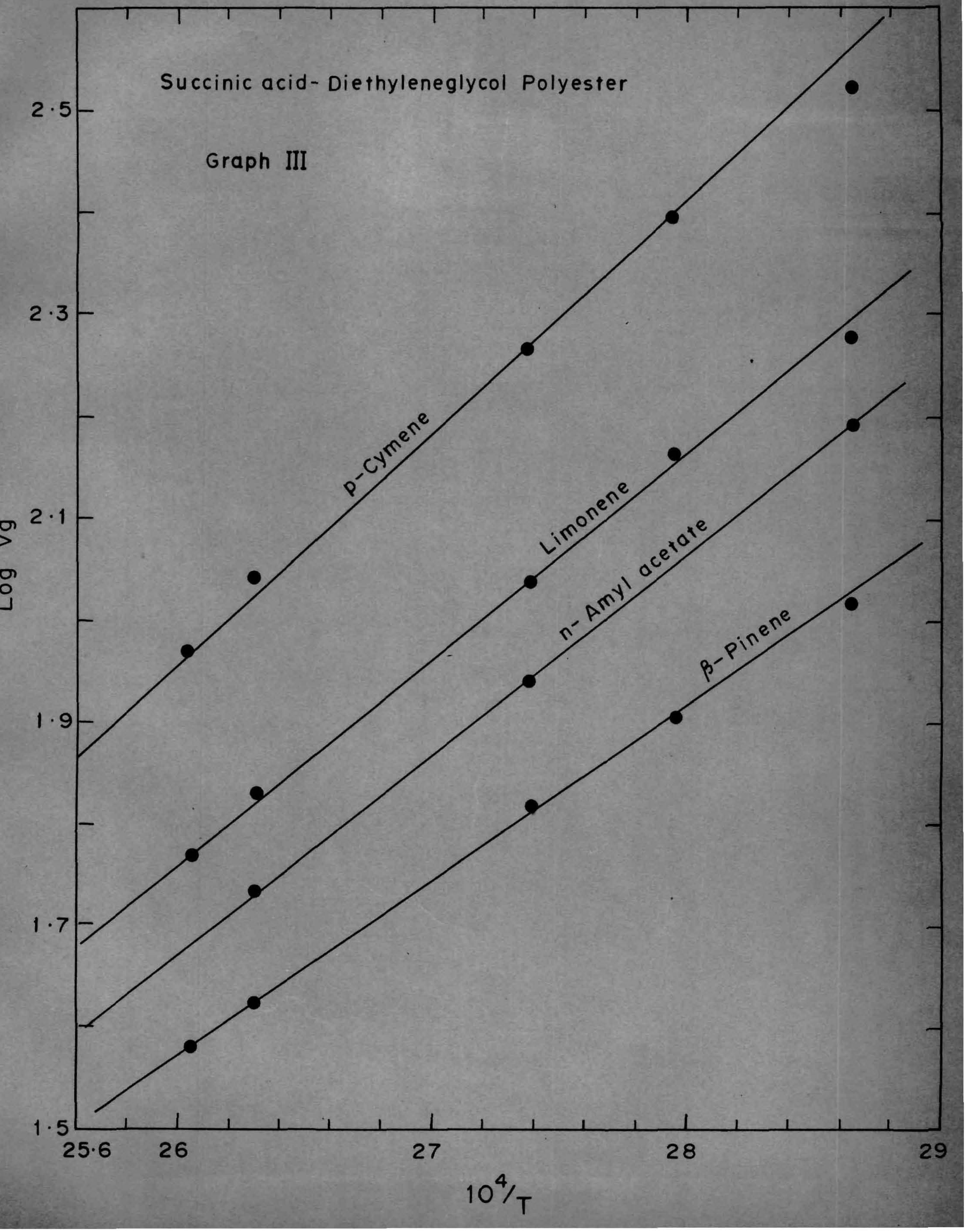
In order to find out whether the phenomenon of previously observed arithmetical relationship of retention times, holds good throughout this temperature range, the following procedure was adopted.

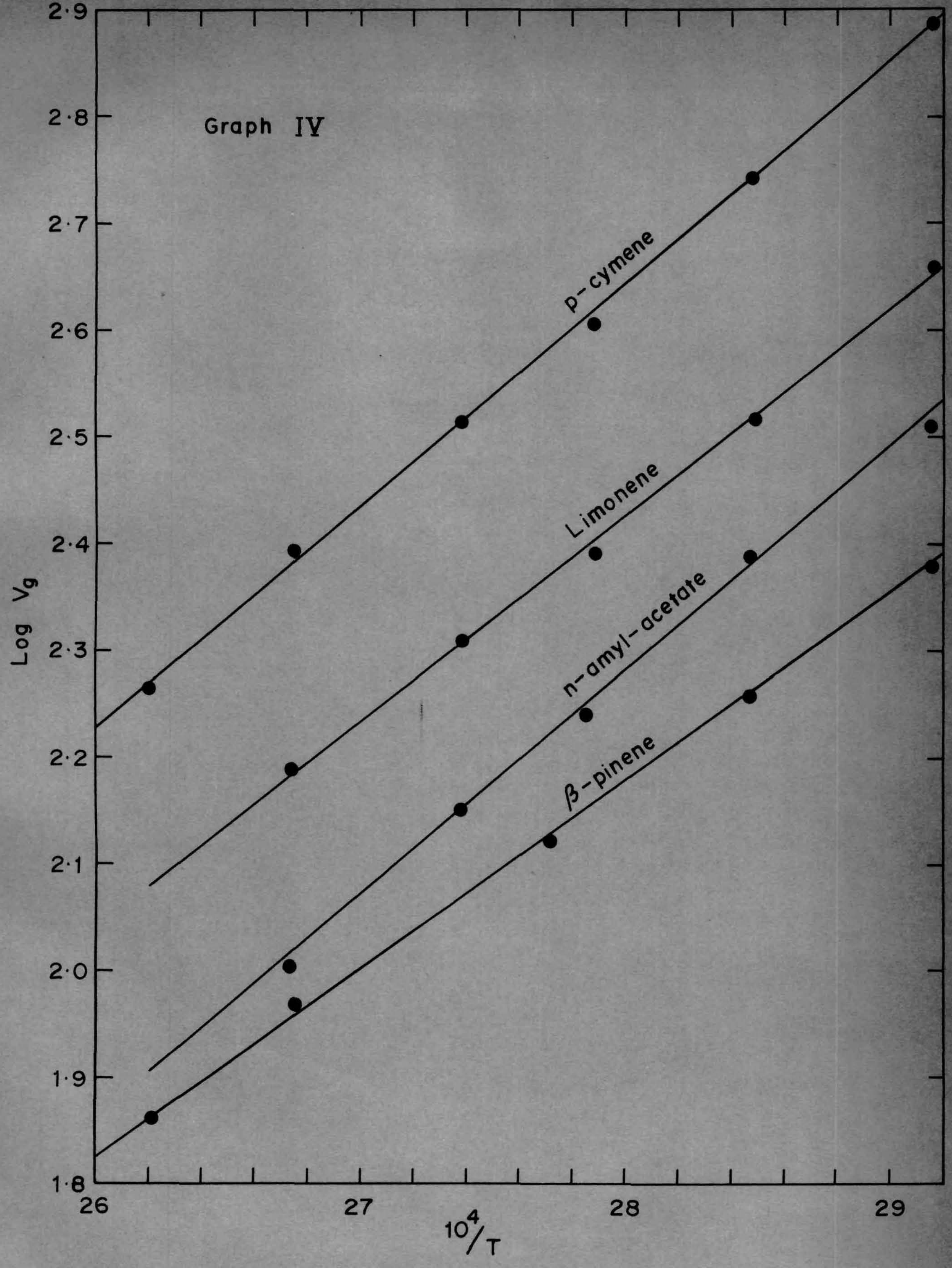
Graphs of log V_g Vs $^1/T$ were constructed in the case of all the four polyesters as shown in Graphs III to VI, and at different temperatures log V_g of the required compounds were read out from each graph. The difference in the log V_g values at the same temperature, would furnish the ratio for the two specific retention volumes. In this way the ratios for the two terpenic compounds, β-pinene and limonene have been calculated and presented in Table No.10. The maximum deviation in each case has also been calculated and given in the same table. Generally the deviation seems to be within the permissible experimental limits and is about 10% in an extreme-case. The possible sources of deviation have been discussed previously.

The behaviour of n-amyl acetate is found to be very interesting and useful. This compound contains two oxygen atoms in the form of a medium polar ester function,

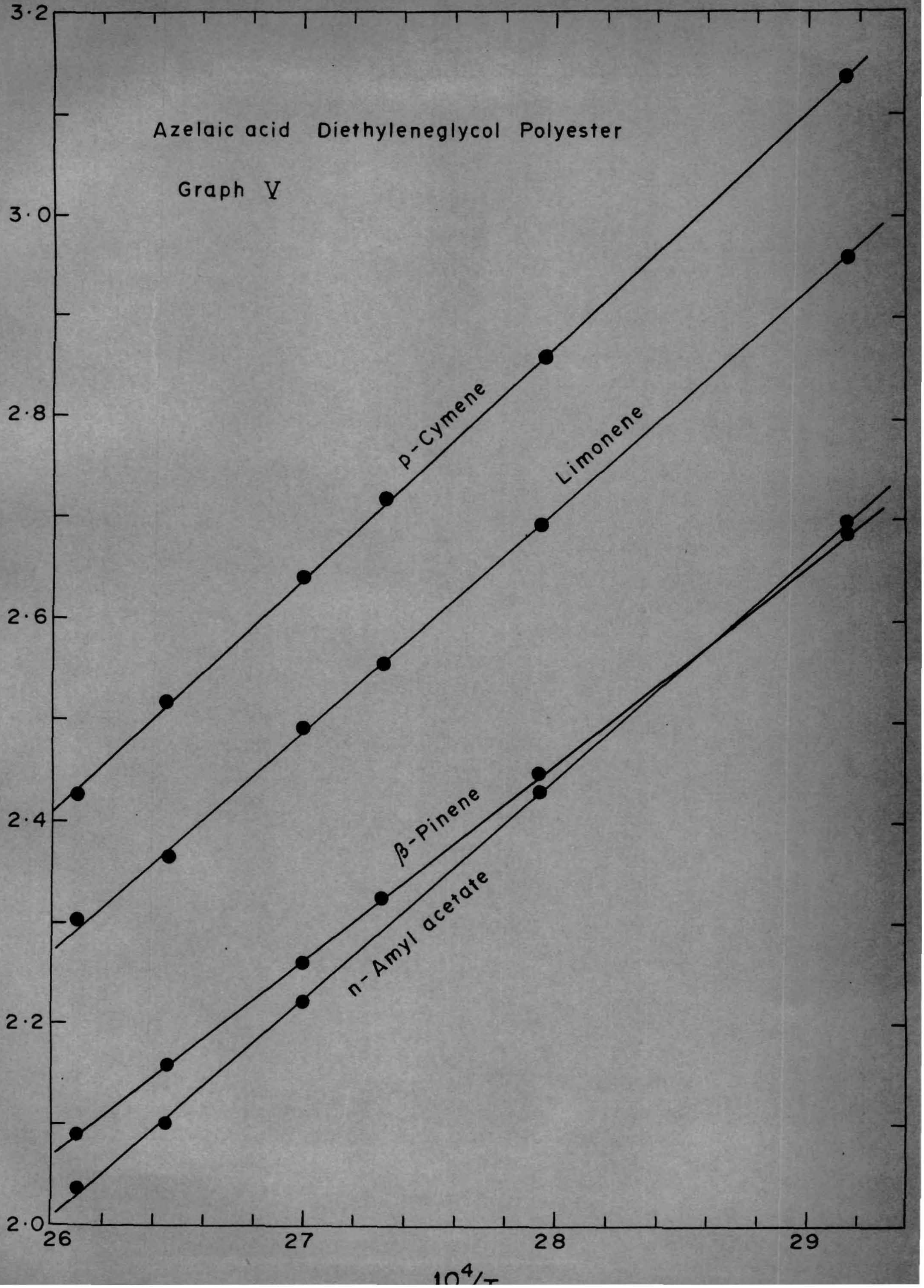
Table 10

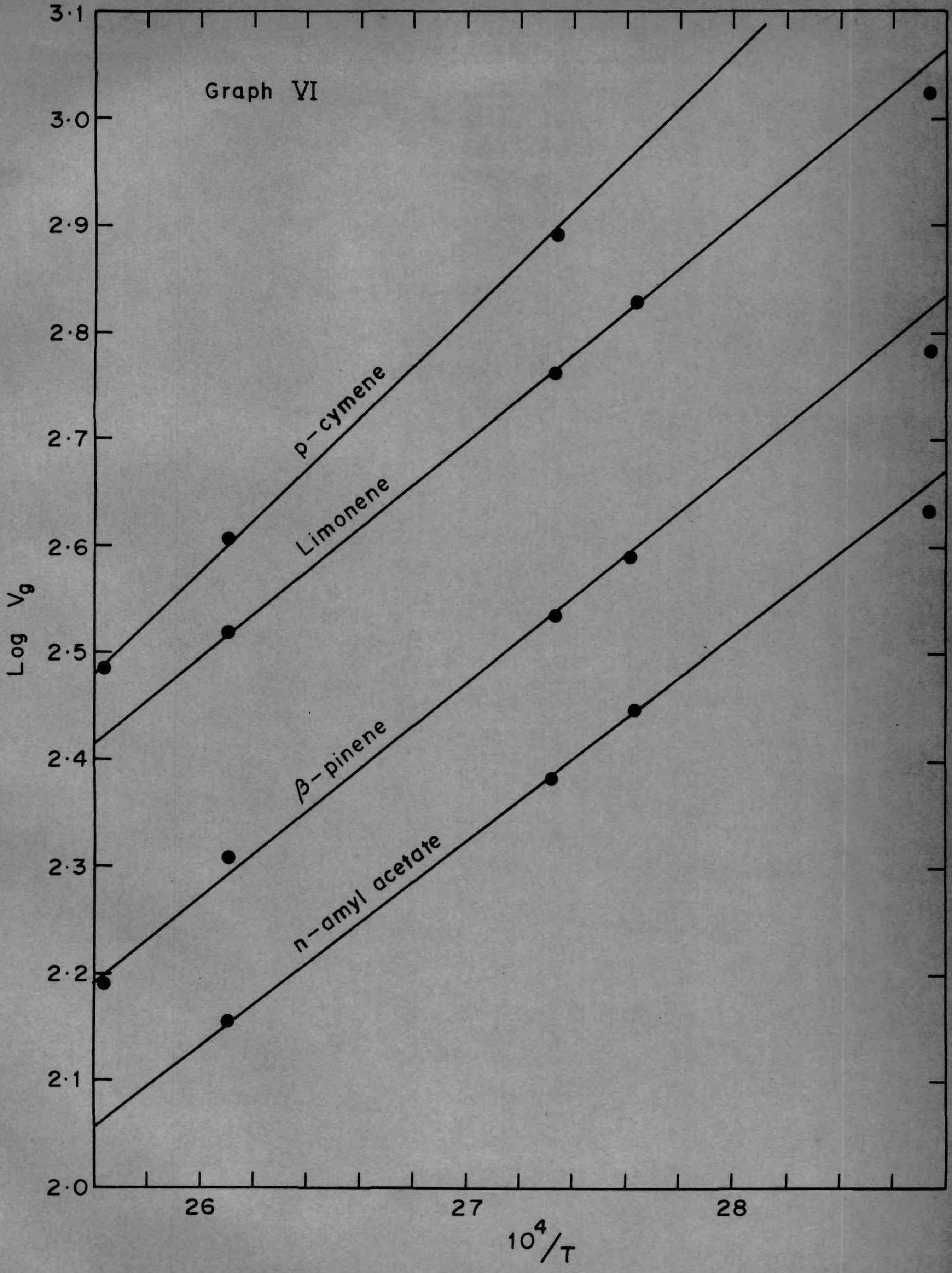
Ratio of the number of methylene groups.	Temperature $10^4/K$					Maximum deviation
	26.20	26.80	27.0	27.50	27.9	
Compound: β-Pinene:						
11/2 = 5.50	5.129	5.346	5.309	5.495	5.697	6.75
11/4 = 2.75	2.851	2.927	2.951	3.020	3.020	9.82
11/7 = 1.57	1.603	1.596	1.614	1.622	1.641	4.52
7/2 = 3.50	3.20	3.273	3.289	3.388	3.467	8.57
7/4 = 1.75	1.778	1.82	1.828	1.862	1.90	8.57
Compound: Limonene:						
11/2 = 5.50	5.433	5.383	5.559	5.249	5.129	6.75
11/4 = 2.75	2.851	2.825	2.917	2.950	2.884	6.05
11/7 = 1.57	1.641	1.589	1.648	1.585	1.514	4.97
7/2 = 3.50	3.311	3.388	3.296	3.311	3.888	5.82
7/4 = 1.75	1.738	1.778	1.770	1.862	1.90	8.57


Succinic acid- Diethyleneglycol Polyester
Graph III
p-Cymene
Limonene
n- Amyl acetate
β- Pinene
Log Vg
2·5
2·3
2·1
1·9
1·7
1·5
25·6
26
27
28
29
$10^4/T$



ADIPIC ACID DIETHYLENE GLYCOL POLYESTER.


Azelaic acid Diethyleneglycol Polyester
Graph V
Log Vg
3·2
3·0
2·8
2·6
2·4
2·2
2·0
26
27
28
29
p-Cymene
Limonene
β-Pinene
n-Amyl acetate
10⁴/T



BRASSYLIC ACID DIETHYLENE GLYCOL POLYESTER.

so that it could be taken as a distinctly different type of compound with respect to the terpenic hydrocarbons.

On succinic acid and diethylene glycol column, this compound emerges after β-pinene, even though the boiling point of β-pinene is several degrees higher than that of n-amyl acetate. This indicates that succinic acid-diethylene glycol polyester column is compar~~i~~atively more polar. The same is the case with adipic acid-diethylene glycol polyester. On this column also β-pinene ~~amd~~emerges before n-amyl acetate. But this is not the case with azelaic acid and diethylene glycol polyester. Here both the compounds almost overlap and on brassylic acid-diethylene glycol column, β-pinene emerges after n-amyl acetate. This in turn would indicate that brassylic acid-diethylene glycol column is comparatively less polar.

Analogous observations

Some interesting observations on this theme~~ne~~ have also been made by previous authors. E. Von Rudloff presented the data of retention times obtained on different dio~~b~~leates, and some of the important data are quoted below for direct comparison.[15]

Temperature 80° ; Standard sample -Limonene

Phase	Weight with the solid support.	Flow rate (ml / min.)	Retention time (min.)
Potassium oleate	10.9	78	10.9
Methyl oleate	10.5	94	75.0
1:3-Propane diol dioleate	10.0	81	66.5
1,4-Butane diol dioleate	10.5	127	33.9
1,6-Hexane diol dioleate	10.4	86	55.3
Diethylene glycol poly azelate	11.6	103	13.0

A glance at the above mentioned data shows that linearity relationship does not hold good in this case. For example in the case of column No.4 and 5 the weight of the column filling is nearly the same, but the flow rates are different. Even after applying correction factor for the difference in flow rates, we find that the retention time of limonene on 1:6-hexane diol-dioleate would not differ much than that on 1:4-butane diol dioleate. Apparently it may be due to the unsaturated centres in oleic acid, otherwise some indications of the linearity factor should have been observable from these data.

In a recent paper Petsev and Dimitrov studied the correlation between the structure of stationary phase and activity coefficients.[16] They have recorded retention volumes - V_g^t - of the aromatic hydrocarbons per gram of the stationary phase at different column temperatures. The stationary phases used were aliphatic esters of phthalic acid. These esters were:

i) Di-n-propyl phthalate
ii) Diallyl phthalate
iii) Di isopropyl phthalate
iv) Di-n-butyl phthalate
v) Di-sec-butyl phthalate
vi) Di-isobutyl phthalate
vii) Di-n-hexyl phthalate.
viii) Di-n-octyl phthalate.

It is very important to note that the number of carbon atoms in alcoholic fragment are changed stepwise. These authors also could observe the exist~~a~~ence of a linearity factor. They found that the activity coefficients decrease for one and the same solute with the increase in the number of carbon atoms in the normal alcohols which are used as alcoholic fragment of the phthalate ester. This change was practically linear. Obviously when a graph of activity coefficients of benzene and toluene Vs no. of carbon atoms in the chain of normal alcohols ~~were~~ was plotted, it gave straight lines.

Molecular weights and its effects

Uptill now, we have studied the behaviour of the several varieties of polyesters. However, when neutralisation equivalents were determined, it was found that the neutralisation equivalents went on varying with respect to each polyester. Therefore, it was thought that the effect of molecular weights, may also have some significant effect on the retention times and consequently on degree of separation.

For this purpose, columns were constructed as follows. In the previous case, the weight of the column liquid was 2.95 g., but in the present study the weight of the column liquid was adjusted in such a way that each column contained 1/500 mole of the stationary phase. Molecular weights were determined by neutralisation equivalents method as shown previously and the columns were run under identical conditions. α-Pinene, β-pinene, Δ^3-carene and limonene were chosen as standard samples. Retention time data were recorded as previously.

It is easier to visualise the effect of molecular weight when we take into consideration the actual phenomenon that takes place inside a typical GLC column.

When a typical sample is introduced in the GLC column, it vapourises and these vapours are dissolved in the stationary phase. It is here where the interactions between the solute and solvent molecules, take place as outlined. The difference between the escaping tendency of the two components in the stationary phase is determined by the forces and energy of interactions between the solute molecules and solvent molecules. The van der Walls forces are looked upon as a sum of all the 3 forces.

Almost all the ester type of stationary phases are looked upon as medium polar substrates. Dinonyl phthalate, dibutyl phthalate etc. are also treated as medium polar substrates.

A critical study of such type of esters has been carried out by previous workers.[17] They used a mixture of C_3 and C_4 hydrocarbons as standard mixture. Several stationary phases were tried. They ranged from transformer oil - a nonpolar phase, to highly polar liquid like dimethyl formamide. Since they differed in molecular weights (ranging from 418 to 73), their molar concentration also differed to a large degree.

When the chromatograms, obtained on dinonyl phthalate and dibutyl phthalate are compared, we do find some significant change. The molecules of dibutyl phthalate are comparatively smaller than dinonyl phthalate. Therefore, the molecules of solvent and solute are closer together. So, the comparatively polar compounds - olefins - are selectively retarded. Butene-1 and isobutene are well separated from n-butane.

Similarly when we compare the separations obtained on ethyl acetoacetate and dimethyl formamide, we find that dimethyl formamide gives better separation. This is due to the fact that its molar density is higher, so the number of stages are also higher.

In the present investigation, the molecular weights of the polyesters are also sufficiently different. So a low molecular weight stationary phase should furnish comparatively more number of stages of induction. Therefore, the columns are filled in such a way that each column contained 1/500 mole of each polyester. These columns were conditioned at 80° for 1.5 hr. The retention times were recorded as usual and results are tabulated in Table 11.

The retention times were not identical but widely different, indicating that molar proportion was not the deciding factor. This in turn indicates that molecular weights of such type of polyesters do not have any significant effect on retention time or degree of separation.

Table 11

Compound	Number of methylene groups	Molecular weight	Weight of the liquid.	Retention time	Contribution per gram, per methylene group
α-Pinene	2	1249	2.448	2.03	0.4146
	3	340	0.676	0.99	0.4879
	4	1154	2.266	3.75	0.4138
	7	398	0.791	2.55	0.4605
β-Pinene	2	1249	2.448	3.60	0.7352
	3	340	0.676	1.55	0.7640
	4	1154	2.266	6.63	0.7306
	7	398	0.791	4.08	0.7369
Δ^3-Carene	2	1249	2.448	4.56	0.9313
	3	340	0.676	1.99	0.9811
	4	1154	2.266	8.50	0.9380
	7	398	0.791	5.31	0.9590
Limonene	2	1249	2.448	6.55	1.338
	3	340	0.676	2.80	1.380
	4	1154	2.266	11.93	1.316
	7	398	0.791	7.18	1.296
Camphene	2	1249	2.448	2.81	0.5738
	3	340	0.676	1.35	0.6655
	4	1154	2.266	5.33	0.5882
	7	398	0.791	3.28	0.5294

A somewhat similar type of investigation, regarding the effect of molecular weight of polyethylene glycols, has been carried out.[18] Adlard used polyethylene glycols (molecular weights ranging from 200 to 1000) as stationary phases, and then homologous series of hydrocarbons, ketones, esters, alcohols etc. were run. When the graph of log (V^oR/g) against methylene (CH_2) units for paraffins and aromatics on various polyethylene glycols is plotted, it is found that no substantial increase in resolution has been effected by increasing the molecular weight of the stationary phase. However, it is worthwhile noting that retention volumes of paraffins have increased. The retention volumes of alcohols have decreased by increasing molecular weight of polyethylene glycol. It is also important that when a column of polyethylene glycol is being run, the molecular weight of the stationary phase remains constant.

Previous workers have studied the effect of conditioning a polyester column at different temperatures.[19] A typical column of polypropylene glycol-sebacate was slowly conditioned, at fixed temperature and flow rate. The vapours coming out from the effluents were condensed and IR spectra were recorded. It was found that water is the main constituent in the condensates. This in turn indicates, that free hydroxyl and carboxyl groups go on condensing together as the column is being conditioned. So the molecular weight may not be constant as long as the conditioning is not over. Lipsky, Landowne[5] and Godet studied various polyesters whose molecular weights were ranging from 3000 to 30,000. But no significant effect was observed. The above mentioned observations, indicate that molar proportion was not the deciding factor. However, when the retention time was divided by the actual weight of the stationary phase and then by the number of methylene groups, which may be considered as contribution per gram per methylene group, the values were nearly constant. These values are tabulated in Table 11 under 'contribution per gram per methylene group'.

This is in agreement with the linearity factor observed previously, though it may be worth remembering that in the earlier investigations, equal weights of stationary phases were used.

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Previous observations have already given us a clear idea about the nature of the polyester and its effect on the retention times and degree of separation. Obviously the actual chromatograms, drawn under various conditions would represent a clear picture of the present investigations. Therefore some of the important chromatograms are presented here.

Not all the polyesters were used for this purpose, but only three polyesters were used. They are as follows:

1. Succinic acid-diethylene glycol polyester.
2. Sebacic acid-diethylene glycol polyester.
3. Hexadecane-1,16-dicarboxylic acid-diethylene glycol polyester.

The selective choice for these polyesters happens to be one, highly polar, phase No.2 is having medium polarity and phase No.3 seems to be least polar.

Celite 545, 120-150 mesh size, was used for this purpose. It was thoroughly purified as described in literature.

Description of the chromatograms

It would be difficult to describe all the chromatograms individually. Therefore the following procedure is followed.

Chromatograms at 120°

The following experimental conditions were maintained for the resolution of compounds at this temperature.

(I) Temperature 120° (± 1°)

(II) Supporting material - Celite 545, 120-150 mesh size.

(III) Bridge current 150 mA.

(IV) Sample size 1 to 5 μl as per requirements.

(V) Stationary phases:

1. Succinic acid-diethylene glycol polyester -20%
2. Sebacic acid-diethylene glycol polyester -15%
3. Hexadecane-1,16-dicarboxylic acid-diethylene glycol polyester - 15%

(VI) Flow rate - 9 secs./10 cc. of hydrogen(recorded by soap bubbler).

Note: The total weight of the fillings was not the same in the cases of the three columns. Therefore, the actual weight of the stationary phase was not the same for the three columns. Inlet pressure, required to get the expected flow rate was also not the same.

Compound pair: 1,8-Cineole, cyclohexanone

Fig. 1

Stationary phase: Succinic acid-diethylene glycol polyester.

1,8-Cineole contains only one oxygen atom in the form of a non-polar ether linkage. But cyclohexanone contains one carbonyl group which is fairly polar one. This polar group suffers heavy interactions with this polar stationary phase. Therefore, cyclohexanone emerges after the less polar compound, 1,8-cineole, even though the boiling of 1,8-cineole happens to be higher by nearly 20°. This observation may be looked upon as an example of boiling point reversal. Exactly opposite results are obtained on 'Apiezon column'.

Fig. 2.

Stationary phase: Sebacic acid-diethylene glycol polyester.

In this case of stationary phase, we find that the separation takes place in form of partially separated two peaks. Relative retention time is found out to be 1.20. However, the order of emergence remains the same as is the case with succinic acid-diethylene glycol polymer.

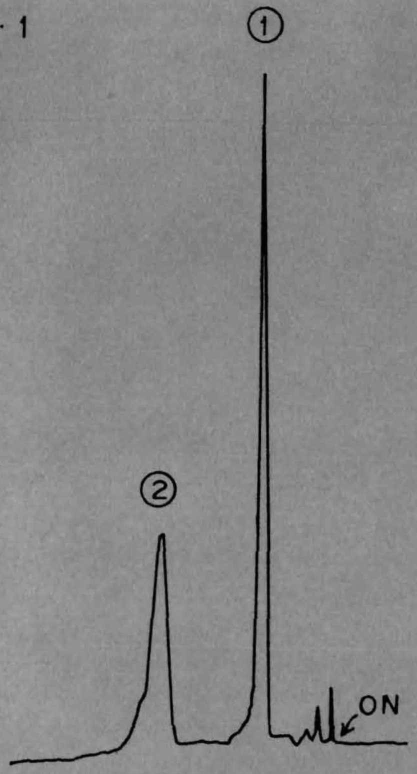
Fig. 3.

Stationary phase: Hexedecane-1,16-dicarboxylic acid-diethylene glycol polyester.

This polymer contains sixteen methylene groups in its acidic fragment. Naturally the solubility of non-polar compound 1:8-cineole, is increased to a very large extent, while this is not the case with polar compound-cyclohexanone. At once we find that the order of emergency is reversed, as compared to that observed on the previous two stationary phases. The relative retention time value comes out to be 1.22 which is better than 1.20 that is obtained on sebacic acid-diethylene glycol polymer.

Therefore the resulting chromatogram comes out in the form of two well separated bands. Note that the same pair is very nicely separated on succinic acid-diethylene glycol polymer but the order of emergence is reversed. Also it may be noted that the bands are fairly sharp.

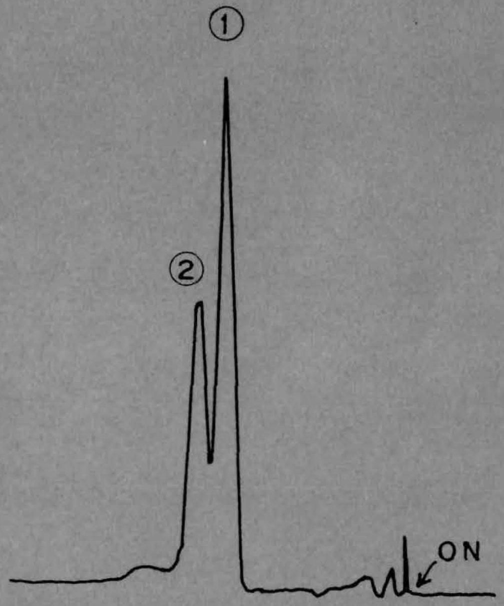
FIG·1



PHASE: SUCCINIC ACID DIETHYLENE GLYCOL POLYESTER

① 1,8-CINEOLE
② CYCLOHEXANONE

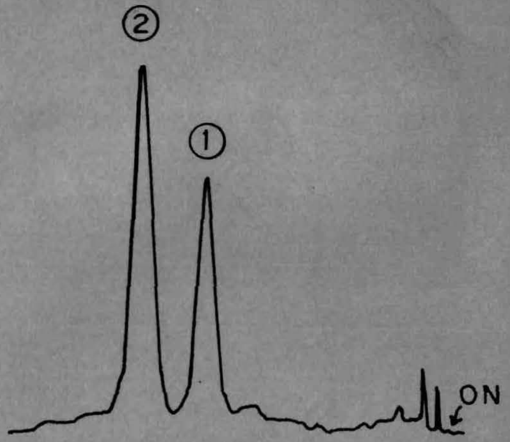
FIG·2



PHASE: SEBACIC ACID DIETHYLENE GLYCOL POLYESTER

① 1,8-CINEOLE
② CYCLOHEXANONE

FIG·3



PHASE: HEXADECANE-1,16-DICARBOXYLIC ACID DIETHYLENE GLYCOL POLYESTER

① CYCLOHEXANONE
② 1,8-CINEOLE

Compound pair: Limonene, cyclohexanone.

Fig. 4

Stationary phase: Succinic acid diethylene glycol polyester.

Because of the polar character of this stationary phase, cyclohexanone emergers after limonene, eventhough its boiling points is lower by nearly 20° compared to that of limonene. Relative retention time is found out to be 3.0. The retention time of limonene is fairly low, so the band corresponding to this compound is sharp one.

Fig. 5.

Stationary phase: Sebacic acid-diethylene glycol.

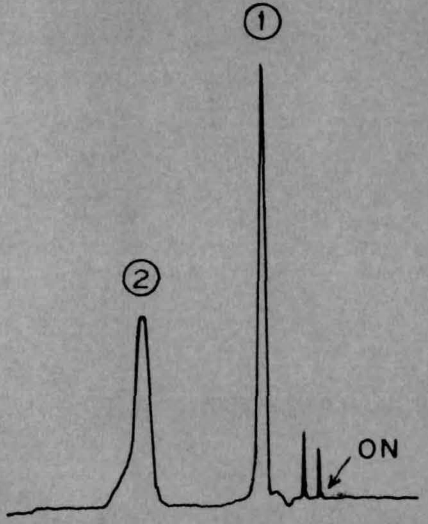
This polyester contains sebacic acid as the acidic fragment having eight methylene groups. Therefore the solubility of non-polar compound limonene has increased. The resulting chromatogram comes out in the form of two peaks, which are sufficiently separated apart. But the order of emergence is the same as is the case with succinic acid-diethylene glycol polyester.

Fig. 6.

Stationary phase: Hexadecane-1,16-dicarboxylic acid-
diethylene glycol polyester.

Because of the comparatively non-polar character of this stationary phase, the separation of the two components takes place according to the boiling points. Limonene - a nonpolar and high boiling compound emerges after cyclohexanone.

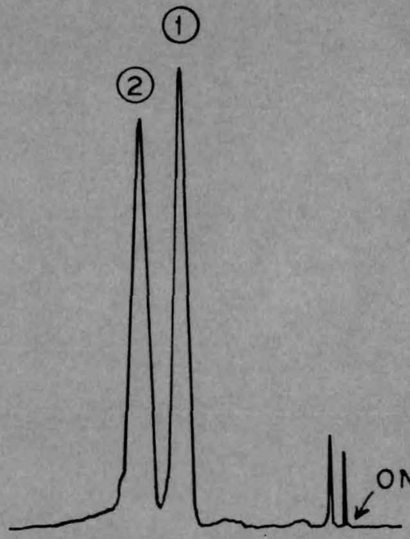
FIG·4



PHASE: SUCCINIC ACID DIETHYLENE GLYCOL POLYESTER

① LIMONENE
② CYCLOHEXANONE

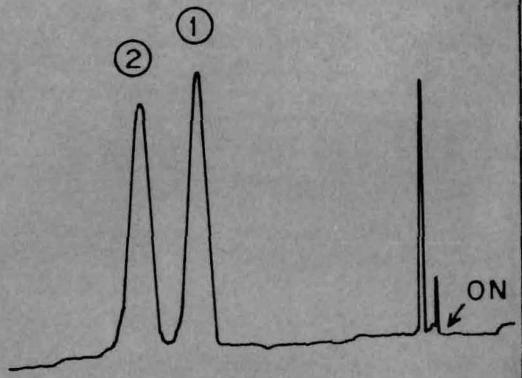
FIG·5



PHASE: SEBACIC ACID DIETHYLENE GLYCOL POLYESTER

① LIMONENE
② CYCLOHEXANONE

FIG·6



PHASE: HEXADECANE-1,16-DICARBOXYLIC ACID DIETHYLENE GLYCOL POLYESTER

① CYCLOHEXANONE
② LIMONENE

<u>Compound pair: Cyclohexanone, p-cymene</u>

Fig. 7

Stationary phase: Succinic acid-diethylene glycol polyester.

Cyclohexanone - a polar compound - emerges after the corresponding non-polar compound p-cymene. Peaks are sharp. Relative retention time is found out to be 1.78. Once again, this observation may serve as an example of 'boiling point reversal'.

Fig. 8

Stationary phase: Sebacic acid-diethylene glycol polyester.

The solubility of p-cymene has increased to a large extent, compared to that of cyclohexanone. Therefore, the two peaks come very close together. The chromatogram is nearly a single peak. However, careful observation reveals a shoulder at the right hand side of the peak.

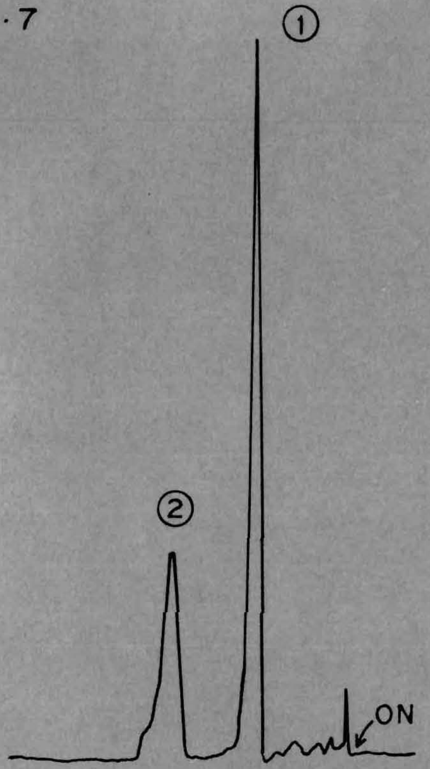
Fig. 9

Stationary phase: Hexadecane-1,16-dicarboxylic acid-diethylene glycol polyester.

This stationary phase contains sixteen methylene groups in the acidic fragment. Therefore the solubility of a nonpolar compound p-cymene is increased to a very large extent. p-Cymene emerges after cyclohexanone. This order of emergence is in accordance with the boiling points of the two compounds.

Relative retention time is found out to be 1.41. The two bands are found to be well separated.

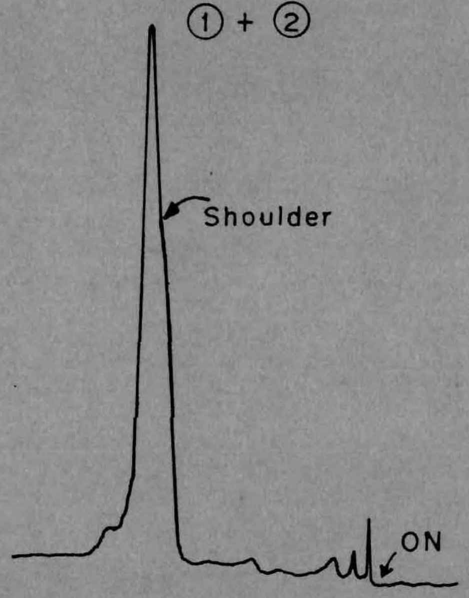
FIG·7



PHASE: SUCCINIC ACID DIETHYLENE GLYCOL POLYESTER

① p-CYMENE
② CYCLOHEXANONE

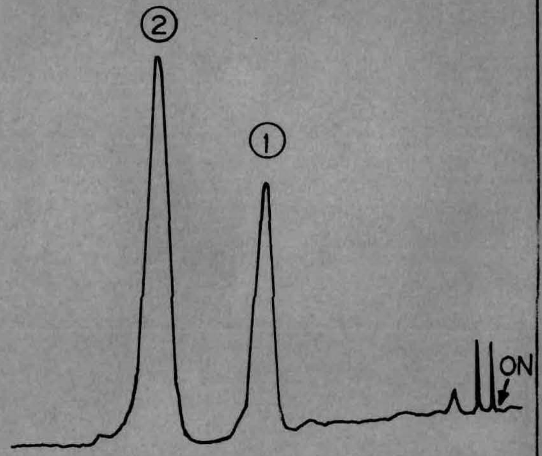
FIG·8



PHASE: SEBACIC ACID DIETHYLENE GLYCOL POLYESTER

COMPONENTS OVERLAPPING

FIG·9



PHASE: HEXADECANE-1,16-DICARBOXYLIC ACID DIETHYLENE GLYCOL POLYESTER

① CYCLOHEXANONE
② p-CYMENE

Compound pair: 1,8-cineole, limonene

Fig. 10

Stationary phase: Succinic acid-diethylene glycol polyester.

The solubility of hydrocarbon-limonene - is far less compared to 1:8-cineole. Therefore they are well separated. The 'specific retention' volumes are also quite low, compared to those obtained on other polyesters. Therefore, the bands are very sharp. Relative retention time is found out to be 1.230.

Fig. 11

Stationary phase: Sebacic acid-diethylene glycol polyester.

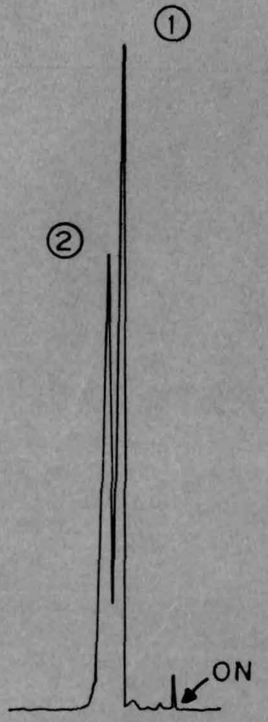
This stationary phase contains sebacic acid as the acidic fragment of the polyester. This acid contains eight methylene groups. The solubilities of hydrocarbon or similar type of non-polar compounds are found to be very high, while the solubility of oxygenated compound, 1,8-cineole walso increases to some extent. The resulting chromatogram is - two closely spaced bands. Partial separation can be easily marked at the top of the peak. RRT: 1.143.

Fig. 12.

Stationary phase: Hexadecane-1,16-dicarboxylic acid-diethylene glycol polyester.

The acidic fragment contains as high as sixteen methylene groups. The solubility of hydrocarbon - limonene - is found to be very high. Also the solubility of 1,8-cineole reaches its saturation point. The resulting chromatogram happens to be almost a single band. Relative retention time is found out 1.04.

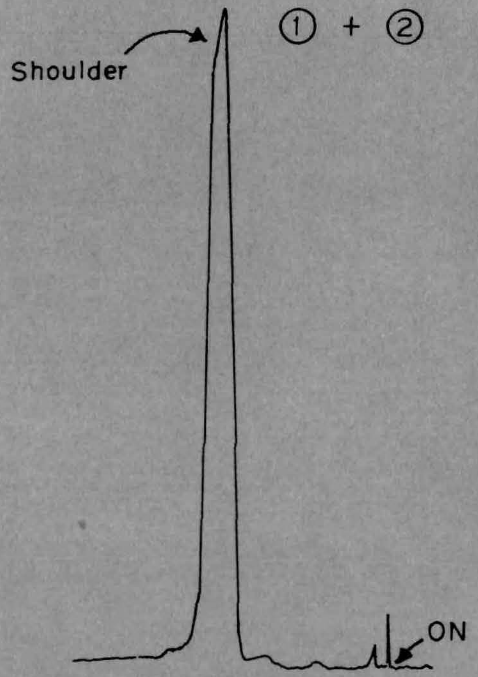
FIG·10



PHASE: SUCCINIC ACID DIETHYLENE GLYCOL POLYESTER

① LIMONENE
② CINEOLE

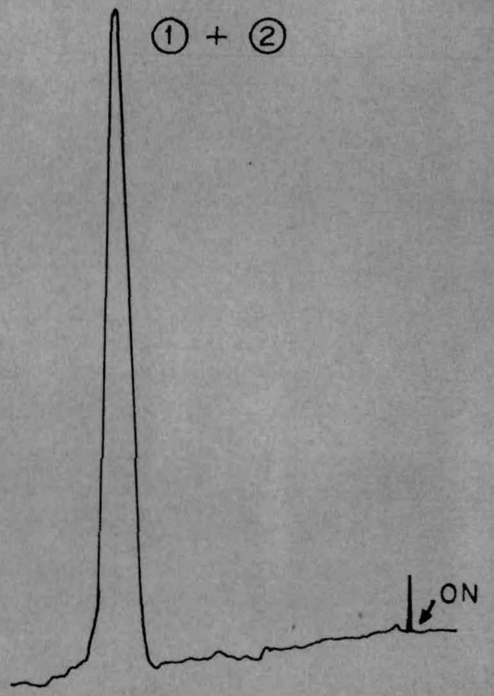
FIG·11



PHASE: SEBACIC ACID DIETHYLENE GLYCOL POLYESTER

COMPONENTS OVERLAP

FIG·12



PHASE: HEXADECANE-1,16-DICARBOXYLIC ACID DIETHYLENE GLYCOL POLYESTER

COMPONENTS OVERLAP

Chromatograms at a
Temperature range of 180-190°.

Set No.	Abbreviation	Experimental conditions.
(I)	A	i) Temperature 179-180° ii) Carrier gas flow rate: 10 secs/10 c.c. hydrogen iii) Stationary phase: 20% succinic acid-diethylene glycol polyester on 120-150 mesh celite.
(II)	B	i) Temperature 184-185° ii) Carrier gas flow rate: 8.5 sec./10 c.c., hydrogen. iii) Stationary phase: 15% sebacic acid - diethylene glycol polyester on 120-150 mesh celite.
(III)	C	i) Temperature 186-187° ii) Carrier gas flow rate: 6 sec./10 c.c. hydrogen iii) Stationary phase: 15% sebacic acid-diethylene glycol polyester on 120-150 mesh celite.
(IV)	D	i) Temperature 182-183° ii) Carrier gas flow rate: 7 sec./10 c.c. hydrogen. iii) Stationary phase. 15% Hexadecane-1,16-dicarbonylic acid diethylene glycol polyester on 120-150 mesh celite.

Chromatograms of monoterpenic oxygenated compounds and sesquiterpenic hydrocarbons.

Compound pair: Camphor and borneol

Fig. 13 Set of conditions - A

Camphor is a C_{10} ketone, but borneol is C_{10} secondary alcohol. The separation of the two compounds furnishes a general idea about the nature of the stationary phase.

Under the present set of conditions, borneol emerges after camphor and its relative retention time with respect to camphor, is 1.352.

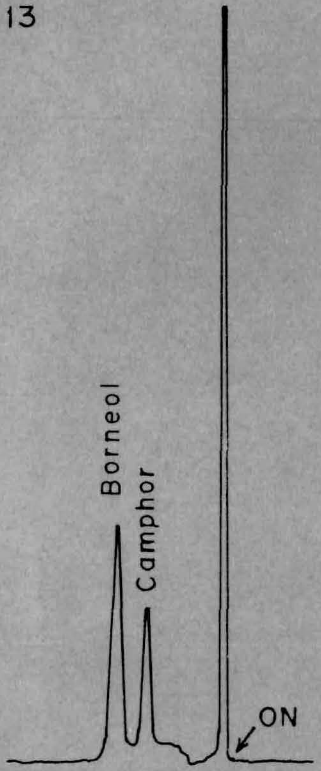
Fig. 14 Set of conditions - B

Sebacic acid-diethylene glycol happens to be the stationary phase. The order of emergence remains the same. Relative retention time of borneol with respect to camphor, is found to be 1.393. This figure seems to be somewhat better than the previous one.

Fig. 15 Set of conditions D

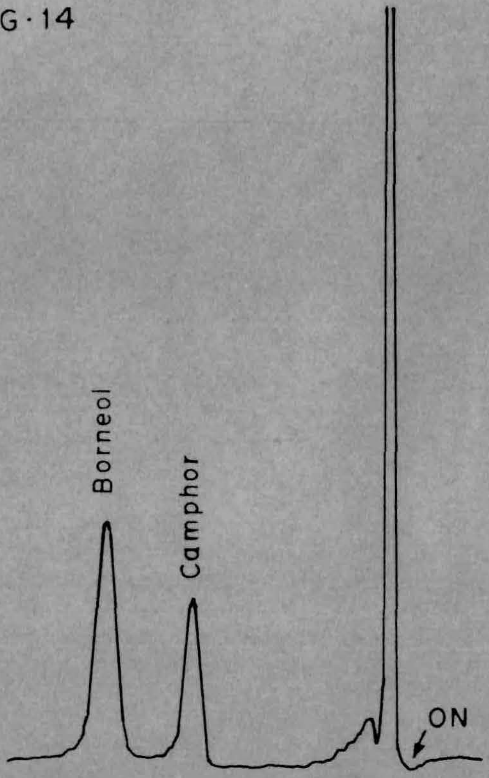
In this case also, the relative retention time of borneol with respect to camphor, is 1.39. This is just the same as the last value obtained.

FIG. 13



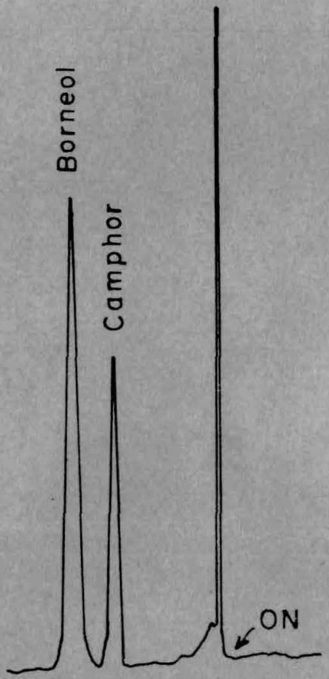
PHASE: SUCCINIC ACID DIETHYLENE GLYCOL POLYESTER

FIG. 14



PHASE: SEBACIC ACID DIETHYLENE GLYCOL POLYESTER

FIG. 15



PHASE: HEXADECANE-1,16-DICARBOXYLIC ACID DIETHYLENE GLYCOL POLYESTER

Compound pair: Carvone and Dihydrocarvone

Fig. 16 Set of conditions A

Carvone contains a conjugated ketonic function and a methylenic double bond. Dihydrocarvone did not contain the double bond that was in conjugation with the carbonyl group. In this case, the relative retention time of carvone with respect to dihydrocarvone was found out to be 1.55.

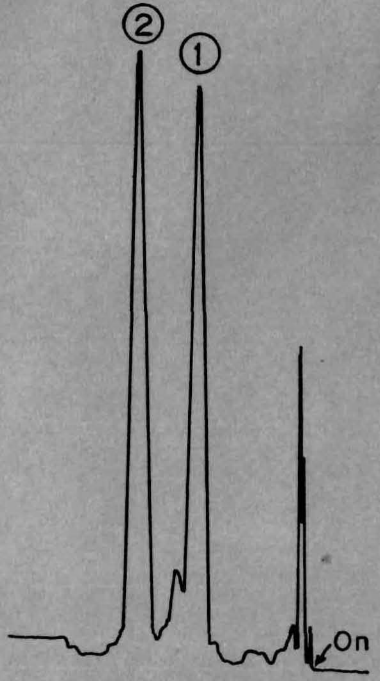
Fig. 17 Set of conditions B

The two above mentioned compounds, when resolved under this conditions, recorded a relative retention time value of 1.41.

Fig. 18 Set of conditions D

In this case, the relative retention time of carvone with respect to dihydrocarvone was observed to be 1.35. This figure is lower than the figure, 1.55, obtained on succinic acid-diethylene glycol polyester. These observations indicate that the succinic acid-diethylene glycol polyester is more polar than the other two polyesters.

FIG. 16.

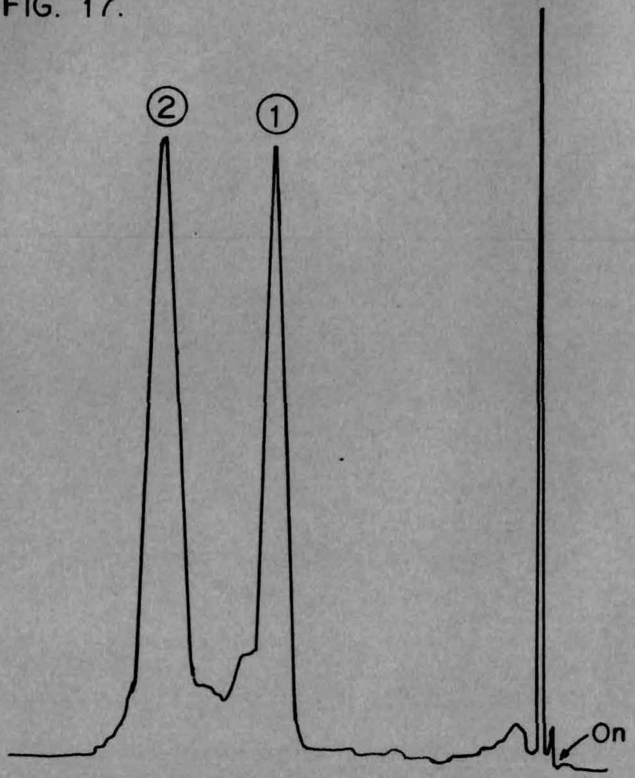


Phase: Succinic acid diethylene glycol polyester.

① Dihydrocarvone.

② Carvone.

FIG. 17.

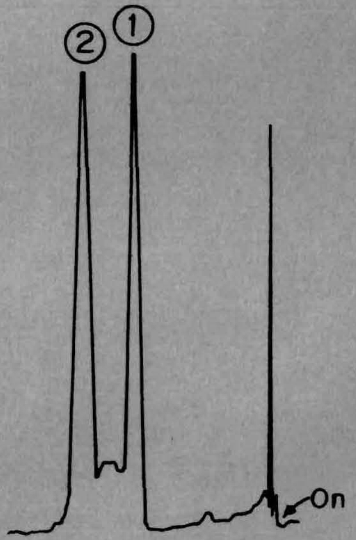


Phase: Sebacic acid diethylene glycol polyester.

① Dihydrocarvone.

② Carvone.

FIG. 18.



Phase: Hexadecane-1,16 dicarboxylic acid diethylene glycol polyester.

① Dihydrocarvone.

② Carvone.

Sample: Longicyclene, longifolene, humulene.

Fig. 19 Set of conditions A.

The separation of all the components can be seen in this figure. The separation of longicyclene and longifolene is somewhat incomplete, but the separation of longifolene and humulene is nearly complete.

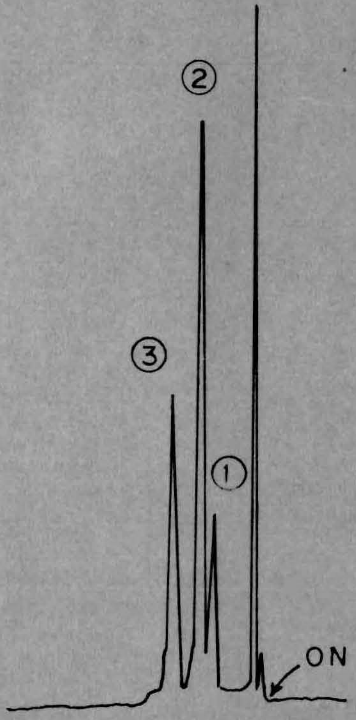
Fig. 20 Set of conditions B.

In this case, nearly the same type of separation is obtained. The relative retention time of longifolene with respect to longicyclene, is found out to be 1.305.

Fig. 21 Set of conditions D.

Under this set of conditions, relative retention times of longifolene and humulene with respect to longicyclene are found out to be 1.23 and 1.54 respectively. These values when compared to those obtained in Fig. 19, would indicate a slight loss. But the total number of theoretical plates obtained with this set of conditions happens to be very high. Therefore there is no loss in the final separation.

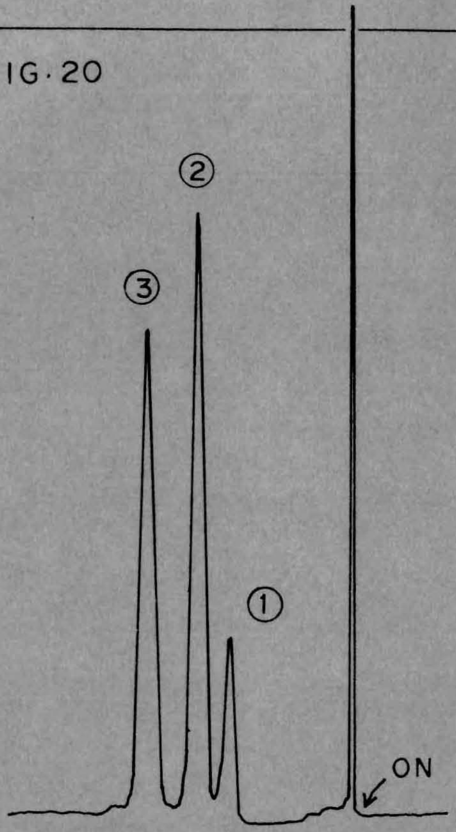
FIG·19



PHASE: SUCCINIC ACID DIETHYLENE GLYCOL POLYESTER

① LONGICYCLENE
② LONGIFOLENE
③ HUMULENE

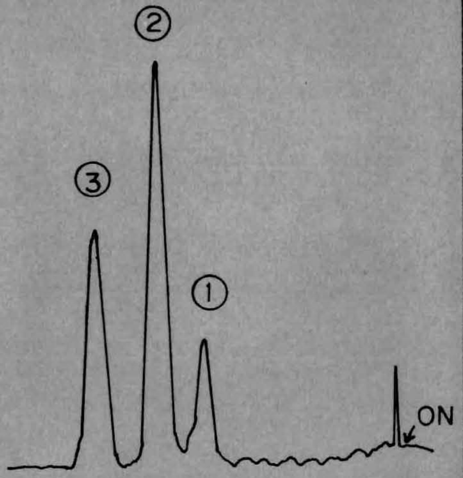
FIG·20



PHASE: SEBACIC ACID DIETHYLENE GLYCOL POLYESTER

① LONGICYCLENE
② LONGIFOLENE
③ HUMULENE

FIG·21



PHASE: HEXADECANE-1,16-DICARBOXYLIC ACID DIETHYLENE GLYCOL POLYESTER

① LONGICYCLENE
② LONGIFOLENE
③ HUMULENE

Compound pair: Borneol and humulene.

Fig. 22 Set of conditions A.

Borneol happens to be monoterpenic secondary alcohol and humulene is a C_{15} hydrocarbons. Obviously, borneol emerges after humulene. Relative retention time of borneol with respect to humulene, is found to be 1.29.

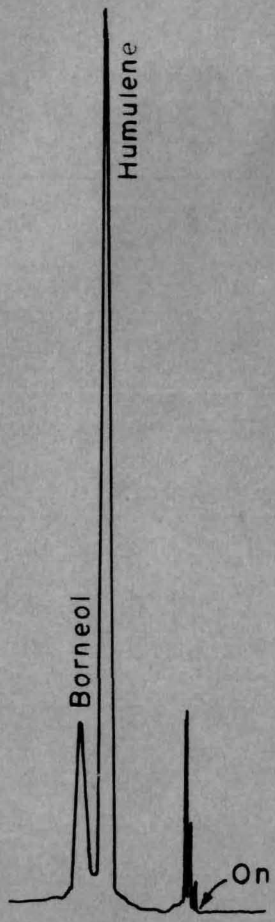
Fig. 23 Set of conditions C.

Due to the influence of the nature of the stationary phase, we find that the order of emergence is reversed. Humulene emerges after borneol. The relative retention time of humulene with respect to borneol, happens to be 1.414. Separation seems to be almost complete.

Fig. 24 Set of conditions D.

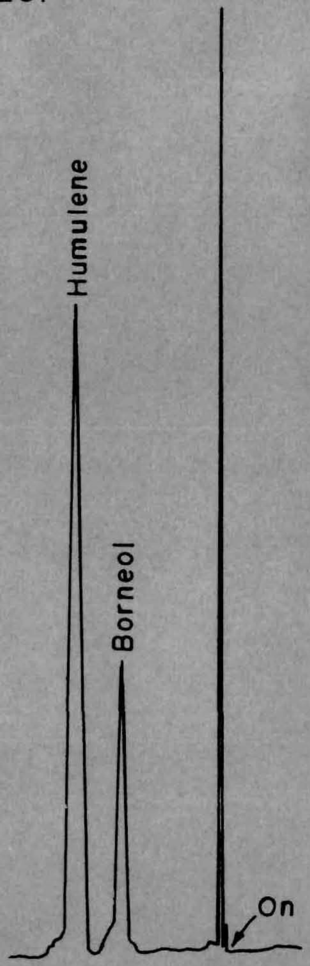
In this case, the stationary phase contains sixteen methylene groups in its acidic fragment. Therefore the retention time of humulene is found to be very high; and the relative retention time of humulene with respect to borneol rises up to 1.928.

FIG. 22.



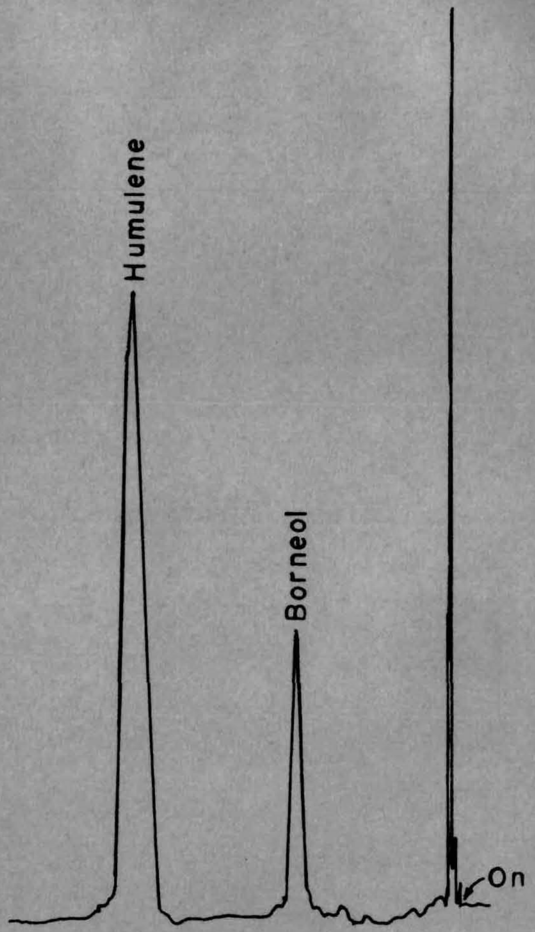
Phase: Succinic acid diethylene glycol polyester.

FIG. 23.



Phase: Sebacic acid diethylene glycol polyester.

FIG. 24



Phase: Hexadecane-1,16-dicarboxylic acid diethylene glycol polyester.

Compound pair: Geranyl acetate and humulene

Fig. 25 Set of conditions A.

When this mixture is resolved on this column, humulene emerges before geranyl acetate. Relative retention time of geranyl acetate, with respect to humulene, is found to be 1.33.

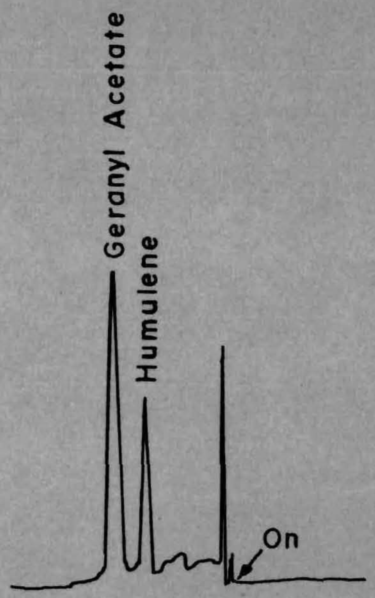
Fig. 26 Set of conditions B.

Sebacic acid-diethylene glycol polyester happens to be the stationary phase. Therefore the order of emergence is reversed. Humulene emerges after geranyl acetate. Relative retention time of humulene, with respect to geranyl acetate comes out to be 1.22.

Fig. 27 Set of conditions D.

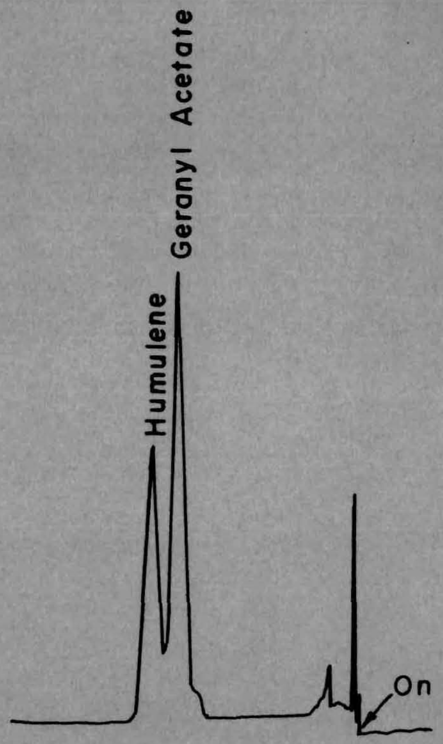
This stationary phase has got a very high solubilities for hydrocarbon type nonpolar compounds. Therefore the two components are stretched wide apart. Relative retention time of humulene, with respect to geranyl acetate is found to be 1.44.

FIG. 25.



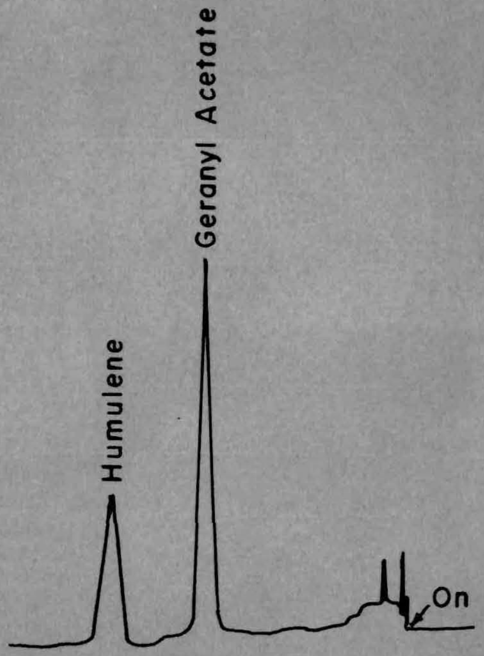
Phase: Succinic acid diethylene glycol polyester

FIG. 26.



Phase: Sebacic acid diethylene glycol polyester.

FIG. 27.



Phase: Hexadecane-1,16-dicarboxylic acid diethylene glycol polyester.

Compound pair: Carvone and cyclopentadecane

Fig. 28 Set of conditions C.

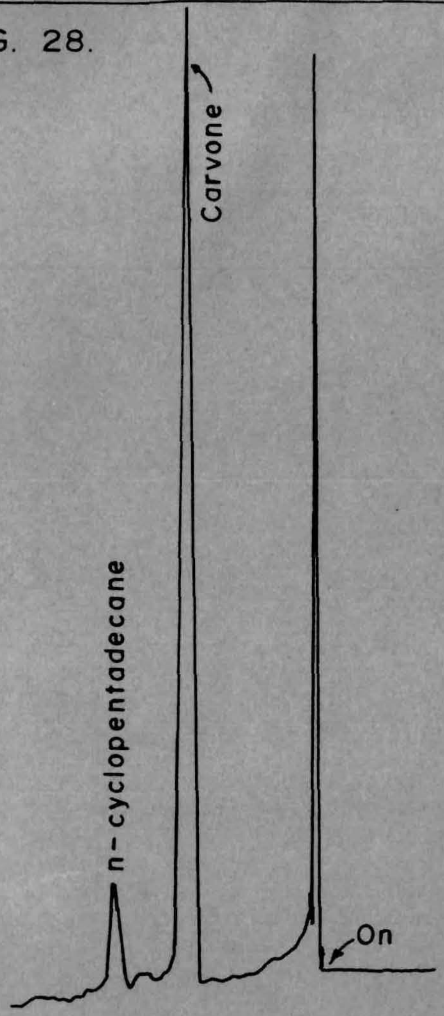
These two compounds were chosen because of the fact that both of them had very high retention times.

These two compounds are well separated under these conditions. Therefore the presence of the other component can be easily marked out.

Fig. 29 Set of conditions D.

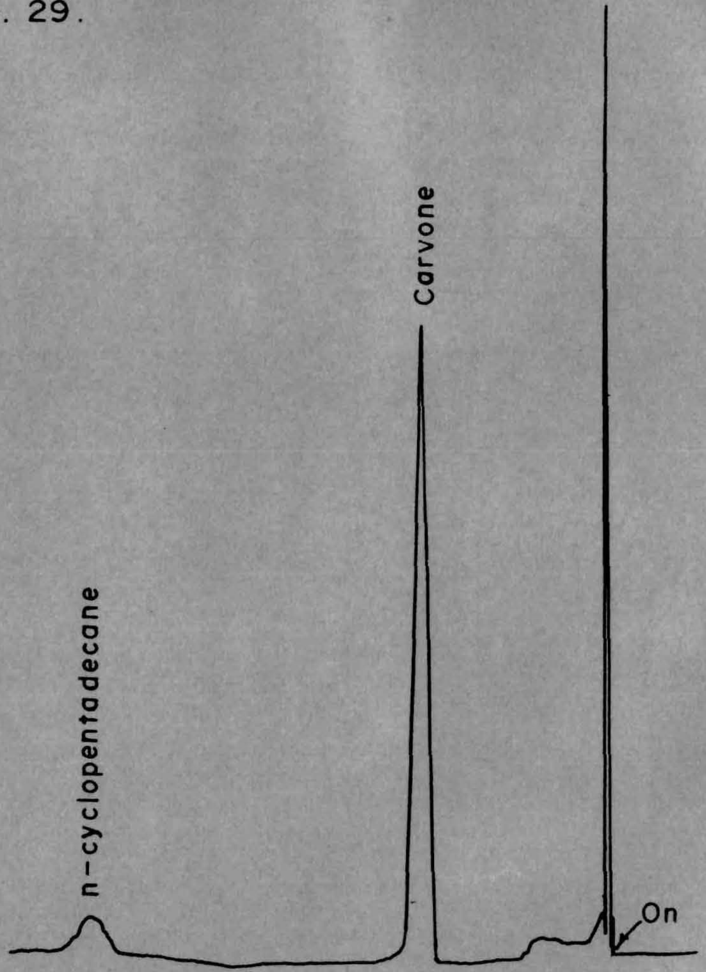
The stationary phase, used in this case has sixteen methylene groups in the acidic fragment of the polyester. Therefore the peak corresponding to the hydrocarbon- cyclopentadecane, is far away from the peak that corresponds to carvone. However, the peak emerging after a long retention time is broad and flat-topped.

FIG. 28.



Phase: Sebacic acid diethylene glycol polyester.

FIG. 29.



Phase: Hexadecane-1,16-dicarboxylic acid diethylene glycol polyester.

Compound pair: Lithio-ethylenediamine
isomerisation product of himachalene

Fig. 30 Set of conditions A.

Previous observations have already shown that succinic acid-diethylene glycol polyester is a polar stationary phase compared to other polyesters. Its polar character is again revealed out by the present observations. The two hydrocarbons are well separated. Relative retention time of the second hydrocarbon with respect to first one, happens to be 2.638.

Fig. 31 Set of conditions B.

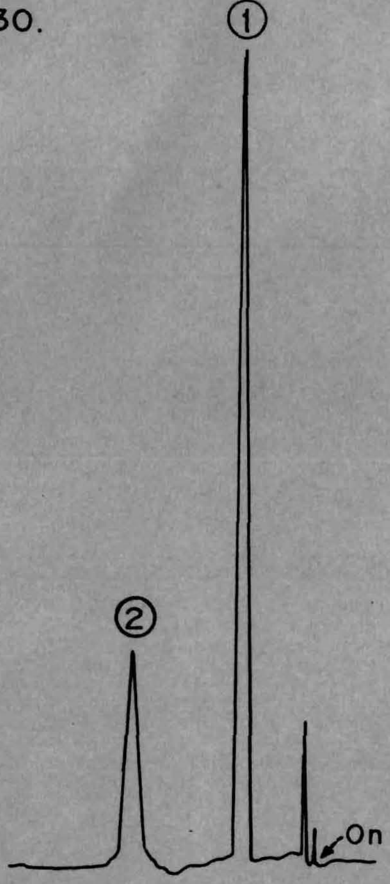
In this case, the relative retention time comes out to be 2.05.

Fig. 32 Set of conditions D.

When the same mixture of hydrocarbons is chromatographed under these conditions, we find that the relative retention time comes out to be 1.91. This value is fairly low when compared to 2.638. This fall in the value of relative retention time, once again indicated that the polyester derived from a long chain dicarboxylic acid and diethylene glycol, is relatively non-polar.

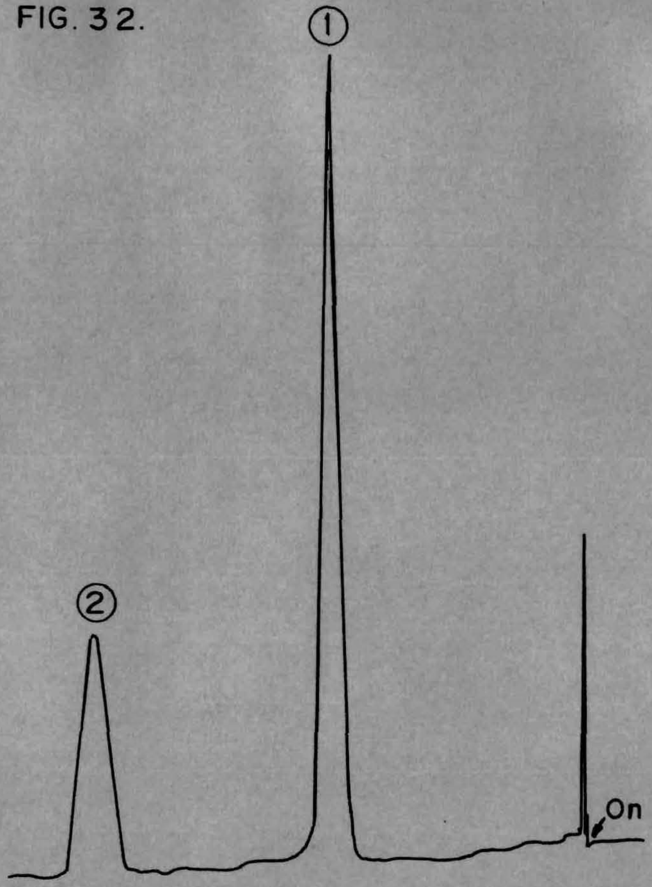
However, this long chain dicarboxylic acid-diethylene glycol polyester, furnishes ~~many~~ large number of theoretical plates, so the final separation is not effected much.

FIG. 30.



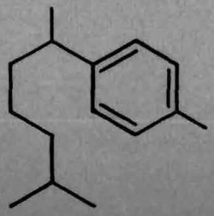
Phase: Succinic acid diethylene glycol polyester.

FIG. 32.



Phase: Hexadecane-1,16-dicarboxylic acid diethylene glycol polyester.

①



②

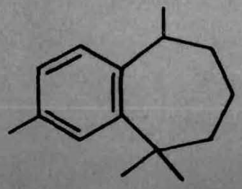
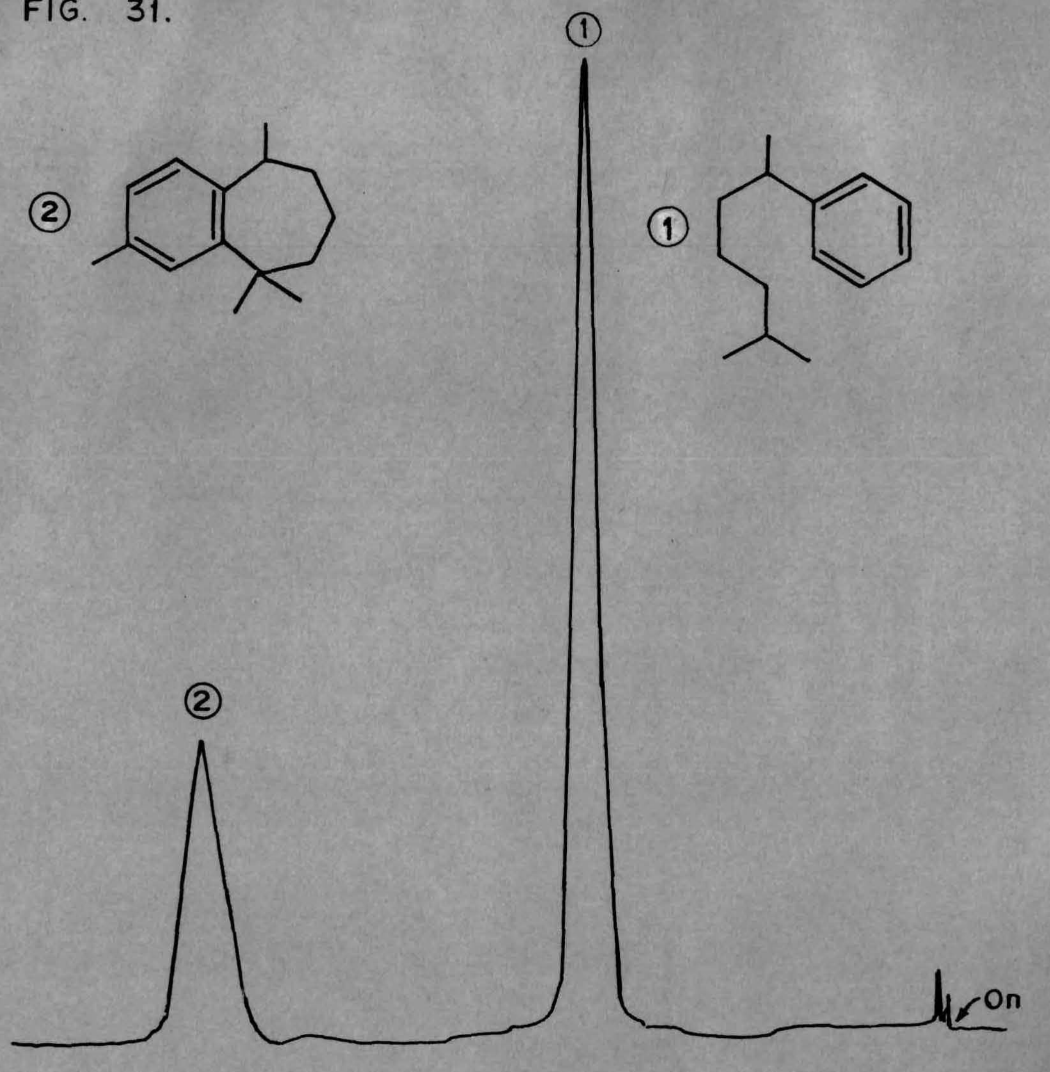


FIG. 31.



Phase: Sebacic acid diethylene glycol polyester.

Sample: Mixture of linalool, camphor, borneol,
caryophyllene and humulene.

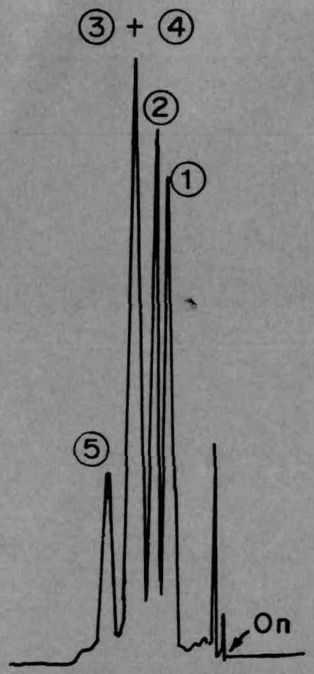
Fig. 33. Set of conditions A.

On this column, it is possible to separate all the components except borneol and caryophyllene, so only 4 peaks are obtained, instead of 5 peaks.

Fig. 34. Set of conditions C.

On this column, a partial separation of caryophyllene and borneol is rendered possible. This is possible only because the stationary phase contains sebacic acid as the acidic fragment of the polyester.

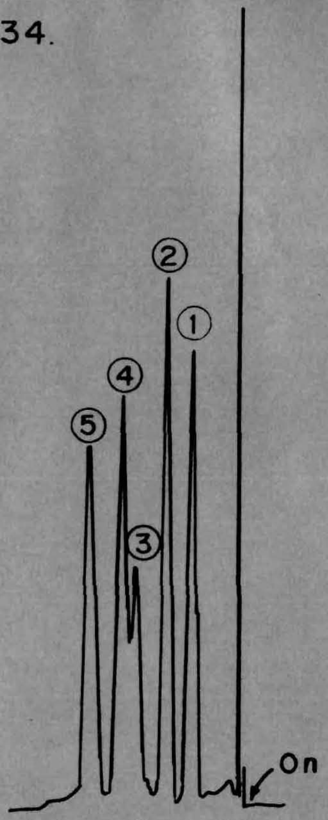
FIG. 33.



Phase: Succinic acid diethylene glycol polyester.

① Linalool.
② Camphor.
③ Borneol.
④ Caryophyllene.
⑤ Humulene.

FIG. 34.



Phase: Sebacic acid diethylene glycol polyester.

① Linalool.
② Camphor.
③ Borneol.
④ Caryophyllene.
⑤ Humulene.

PART II

EVALUATION OF POLYESTERS DERIVED FROM DIFFERENT DICARBOXYLIC ACIDS AND 1, ω - GLYCOLS

SUMMARY

Polyesters from different 1, ω-glycols, ranging from ethylene glycol to 1,15-pentadecanediol, with adipic acid were prepared and evaluated as substrates for gas-liquid chromatography. Several terpenic and a few non-terpenic compounds were analysed. It was observed that the retention time is a characteristic of the number of methylene groups in the glycolic fragment of the polyester and, as before, follows a linear relationship, which starts deviating at 1,10-decanediol. The effect on retention times by using ethylene glycol and changing the acid fragment was also studied. Similarly, the effect on retention times by using 1,5-pentanediol (which has the same number of polyvalent atoms in the chain unit as diethylene glycol) and changing the acidic fragment was also examined. From the above mentioned observations, it was clear that the retention times of hydrocarbon-type compounds may be enormously increased by using a polyester derived from very-long-chain dicarboxylic acid and long-chain 1, ω-glycol. However, the retention times of hydrocarbons reach a certain saturation point above which their solubilities do not increase appreciably. Finally, the effect of dead volume of the column was discussed with

respect to the case of long-chain as well as short-chain polyesters. Some of the stationary phases described in the part are likely to be very useful for capillary columns and for preparative gas-liquid chromatography.

In the last chapter, a systematic study of the polyesters was presented. These polyesters were derived from 1,ω-dicarboxylic acids and diethylene glycol. It was also found that, when all the column parameters were normalised, the retention times of hydrocarbons follow a clear arithmetical pattern. However, such type of pattern is not followed by oxygenated compounds.

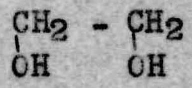
It is therefore thought worthwhile to study whether the same relationship would exist in the case of polymers derived from 1,ω-glycols and, some suitable acids.

This chapter describes a detailed study of the above mentioned problems. In order to have a clearer idea about this pattern of arithmetical relationship, some other related factors have also been investigated and the results are included in this chapter.

EXPERIMENTAL

A series of polyesters were synthesised from adipic acid (constant) and 1,ω-glycols, $HO-CH_2-(CH_2)_n-CH_2-OH$, ranging from ethylene glycol($n=0$) to pentadecane-1,15-diol ($n=13$). The sources of all the glycols are described below.

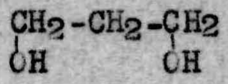
1. Ethylene glycol,



$$\underset{OH}{CH_2}-\underset{OH}{CH_2}$$

It was commercially available and was purified by careful fractionation. It gave a single peak, when it was analysed by GLC.

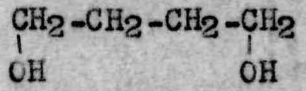
2. 1,3-Propane diol:



$$\underset{OH}{CH_2}-CH_2-\underset{OH}{CH_2}$$

It was commercially available. It was purified by fractionation. It was 95% to 96% pure as tested by GLC analysis.

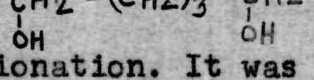
3. Butane-1,4-diol:



$$\underset{OH}{CH_2}-CH_2-CH_2-\underset{OH}{CH_2}$$

It was purified by fractionation. It was about 95% pure as shown by GLC analysis.

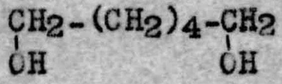
4. Pentane-1,5-diol



$$\underset{OH}{CH_2}-(CH_2)_3-\underset{OH}{CH_2}$$

It was purified by fractionation. It was about 94% pure as shown by GLC analysis.

5. Hexane-1,6-diol

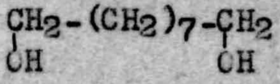


$\underset{\mathrm{OH}}{\underset{|}{CH_2}}-(CH_2)_4-\underset{\mathrm{OH}}{\underset{|}{CH_2}}$

It was not easily available. It was therefore synthesised during these investigations.

Pure adipic acid was esterified to its diethyl ester. The diethyl adipate was carefully distilled under vacuum. This distilled products was reduced with lithium aluminium hydride as usual. The resulting hexane-1,6-diol was slowly distilled under vacuum. The distilled product at once solidified, m.p. 41° (lit. 42°). It recorded a single peak as shown by GLC analysis.

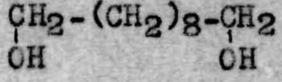
6. Nonane-1,9-diol:



$\underset{\mathrm{OH}}{\underset{|}{CH_2}}-(CH_2)_7-\underset{\mathrm{OH}}{\underset{|}{CH_2}}$

It was prepared by the lithium aluminium hydride reduction of the diethyl ester of azelaic acid, m.p. 42-43°; (lit. 42-45°). It gave a single peak on GLC analysis.

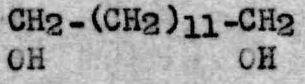
7. Decane-1,10-diol:



$\underset{\mathrm{OH}}{\underset{|}{CH_2}}-(CH_2)_8-\underset{\mathrm{OH}}{\underset{|}{CH_2}}$

It was also prepared by the lithium aluminium hydride reduction of the diethyl ester of sebacic acid, m.p. 73° (lit. 73-75°).

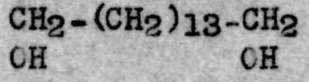
8. Tridecane-1,13-diol:



$\underset{OH}{CH_2}-(CH_2)_{11}-\underset{OH}{CH_2}$

It was prepared by the lithium aluminium hydride reduction of the diethyl ester of brassylic acid. However, this glycol did contain traces of second component, as shown by GLC analysis. It was used as such, m.p. 74-76° (lit. 76°).

9. Pentadecane-1,15-diol:



$\underset{OH}{CH_2}-(CH_2)_{13}-\underset{OH}{CH_2}$

It was also obtained by the lithium aluminium hydride reduction of the diethyl ester of pentadecane dicarboxylic acid, m.p. 87° (lit. 88°).

The other series of polyesters studied were as follows:

(1) Ethylene glycol (constant glycolic fragment), and
  (i) Succinic acid
  (ii) Adipic acid
  (iii) Sebacic acid.

(2) Pentane-1,5-diol (constant glycolic fragment), and
  (i) Succinic acid
  (ii) Adipic acid
  (iii) Sebacic acid.

(3) Hexadecane-1,16-dicarboxylic acid(constant), and
  (i) Butane-1,4-diol
  (ii) Decane-1,10-diol
  (iii) Pentadecane-1,15-diol.

The sources of the corresponding acids have been already described in the previous chapter.

Preparation of the polyesters:

The preparation of the polyesters of the corresponding glycols and acids was carried out, as described in the last chapter with some modifications.

In the previous series, we have used a temperature of 135-140°. A few preliminary experiments showed that this range of temperature was rather low. Therefore, the temperature of ester formation in the present case was maintained at 145-150° throughout. At this temperature range, the starting mixture of the corresponding acid and the glycol at once became homogeneous and the process of ester formation was found to be satisfactory.

The resulting mass was dissolved in methylene chloride and wherever necessary, chloroform was used. This solution was washed repeatedly with water, dried (sodium sulphate), the solvent removed and the residue dried to constant weight.

Polyester derived from ethylene glycol and adipic acid is a viscous liquid. One that has been synthesised from adipic acid and butane-1,4-diol is a semisolid. The rest of the polyesters obtained from pentane-1,5-diol onwards upto pentadecane-1,15-diol and adipic acid are all low melting solids.

The other polyesters derived, either from hexadecane-1,16-dicarboxylic acid and the corresponding glycols, or from sebacic acid and the corresponding glycols, are all low melting solids.

The approximate molecular weights were obtained by the end group titrations in alcoholic solutions. Some of the polyesters were not fairly soluble, their titrations were rendered difficult. The results of these observations are recorded in Table 1.

In order to have consistency in the case of retention time data recorded for 1,ω-dicarboxylic acid and diethylene glycol polymers and the present polymers, the following samples were chosen as standard samples.

Monoterpenic hydrocarbons

(i) α-Pinene, (ii) β-pinene, (iii) Δ^3-carene, (iv) limonene, (v) tricyclene, (vi) camphene, (vii) 1:8-cineole, and (viii) p-cymene.

Nonterpenic samples:

(i) Cyclohexane, (ii) methyl alcohol, (iii) ethyl alcohol.

Table 1

Molecular weights of the polyesters as determined by the end group titrations.

Components of the polyester		Molecular weights
Acid	Glycol	
Adipic acid	Ethylene glycol	433, 420
""	1,3-Propane diol	442, 446
""	1,4-Butane diol	1021, 978
""	1,5-Pentanediol	-
""	1,6-Hexane diol	7563, 6730
""	1,9-Nonane diol	insoluble
""	1,10-Decanediol	""
""	1,13-Tridecanediol	""
""	1,15-Pentadecanediol	""
Succinic acid	Ethyleneglycol	333, 286
Adipic acid	"	433, 420
Sebacic acid	"	1138, 1008
Hexadecane-1,16-dicarboxylic acid	1,4-Butanediol	insoluble
""	1,10-Decanediol	""
""	1,15-Pentadecanediol	""
Succinic acid	1,5-Pentanediol	""
Adipic acid	"	""
Sebacic acid	"	""

Monoterpenic oxygenated compounds

(i) α-Terpineol, (ii) linalool, (iii) l-borneol, (iv) isoborneol, (v) menthol, (vi) isopulegol, (vii) camphor, (viii) carvone, and (ix) bornyl acetate.

Sesquiterpenic hydrocarbons

(i) Tetrahydroelemene, (ii) β-selinene, (iii) caryophyllene, (iv) humulene, (v) longifolene, (vi) longicyclene, and (vii) cyclopentadecane.

All these samples were nearly 95% pure as found by GLC analyses.

Impregnation and filling

Indian fire-brick support (60-80 mesh) was used throughout these experiments. The required quantity of the polyester and fire-brick powder was weighed up to a milligram. The polyester was dissolved in methylene chloride or chloroform as required and then it was impregnated upon the fire-brick support by slurry method. The solvent was slowly removed on water a bath. The impregnated material was dried at 110-120° for several hours to a constant weight. Thus the material was ready for filling the column.

In the case of present investigation also, one and the same column was used in order to have uniformity. When the study of one column filling was over, it was removed from the column, the column was washed repeatedly and then dried by passing hot air for some time. Great care was taken so that there would not be any chance of contamination of the previous column filling with the next one.

Every time column was tested for inlet pressure and the corresponding flow rate of the carrier gas. If the expected flow rate was not obtained with the required inlet pressure, then the column was repacked. Thus it was rendered possible to adjust the expected flow rate with desired inlet pressure (within the limits of ± 3 mm.).

In the previous chapter, it has been shown that if the column variables are normalised properly, a direct comparison of the retention time data is rendered possible. So the following column parameters were maintained constant.

1. Column length 6 ft.
2. Weight of the column ~~required~~ liquid 1.98 g. (± 0.005)
3. Carrier gas flow rate(hydrogen) 4 lit./hr.
4. Inlet pressure (i) 125 (± 3 mm) at 80°
   (ii) 180 (± 4 mm) at 163°
5. Bridge current: 150 mA
6. Sample size: 1 μl (by the help of Hamilton syringe, 10 μl capacity).

In the previous series, it was observed that sometimes the retention times were too high. Therefore in the present investigations, the weight of the column liquid was reduced to 1.98 g., against 2.95 g. which was the case with the previous series. However, some columns were run with still lesser weight of the polyester, but the retention times were found to be too low. Therefore the weight of the column liquid was maintained at 1.98 g. throughout these experiments. Once again the retention times were recorded with the help of a stop-watch, as described in previous chapter.

RESULTS AND DISCUSSION

Monoterpenic hydrocarbons and the other low boiling nonterpenic samples were run at 80° only and the retention time data are presented in Table 2.

Monoterpenic oxygenated compounds and sesquiterpenic hydrocarbons were run at 163-165°. The results are presented in Table 3.

A glance at these two tables, once again furnishes a clear arithmetical relationship between the retention time of hydrocarbons and number of methylene groups in the glycolic fragment of the polyester. Once again the arith-

Table 2

Retention time in min. at 80°

Compound	Number of methylene groups in the glycolic fragment of the polyester. Acidic component - Adipic acid - constant.					
	2	3	4	5	6	9
α-Pinene	2.95	4.10	5.47	6.35	8.30	11.9
β-Pinene	5.05	7.23	9.50	10.75	14.35	19.5
Δ^3-Carene	6.70	9.08	12.10	13.86	18.10	25.5
Limonene	9.13	12.70	17.00	19.26	25.3	35.0
Tricyclene	2.838	3.93	5.10	5.75	8.20	10.6
Camphene	4.280	5.80	7.50	8.66	11.10	15.3
p-Cymene	15.25	20.0	26.0	28.40	37.0	41.3
Cyclohexane	0.366	0.460	0.66	0.666	1.00	1.40
Methyl alcohol	1.366	-	1.42	1.50	1.15	1.10
Ethyl alcohol	1.783	-	2.10	2.00	1.70	1.60
1:8-Cineole	13.70	-	22.1	24.0	27.6	33.9

Table 3

Retention time in min. at 163-64° Acidic component - Adipic acid - constant.

Compound	Silicone	Apiezone	Number of methylene groups in the glycolic fragment of the polyester.								
			2	3	4	5	6	9	10	13	15
α-Terpineol	4.30	4.80	7.55	9.20	9.20	10.60	-	12.00	-	12.40	11.75
Linalool	2.53	3.70	3.40	4.00	4.15	4.95	4.50	4.60	4.75	4.90	4.60
Borneol	3.95	9.20	6.75	8.75	8.60	9.70	10.40	10.93	11.66	11.45	10.75
Isoborneol	3.70	3.47	5.90	7.33	7.30	8.95	-	9.75	10.25	10.00	9.75
1-Menthol	3.86	9.33	6.20	7.00	7.15	8.17	8.80	9.66	9.86	9.75	10.00
Camphor	3.35	7.75	4.70	5.40	5.50	6.10	6.80	6.85	7.10	7.35	7.00
Carvone	5.37	14.10	10.00	11.57	11.50	12.80	13.60	15.10	14.80	13.80	13.50
Isopulegol	3.66	10.00	4.80	5.86	6.00	6.87	7.93	9.10	8.30	8.13	9.40
Bornylacetate	6.10	13.60	5.00	5.80	6.20	7.00	8.25	9.77	9.83	9.81	9.70
Tetrahydro-elemene	10.90	27.35	3.40	4.05	4.90	6.60	8.10	11.50	12.70	15.13	14.65
β-Selinene	16.13	45.50	8.00	9.63	11.35	15.00	17.80	23.90	26.13	29.56	28.75
Longifolene	11.65	33.00	4.66	5.53	6.93	8.70	10.80	15.60	16.00	19.16	18.60
Caryophyllene	12.66	34.00	5.40	6.00	7.43	9.60	10.90	15.90	16.90	19.26	19.10
Humulene	14.30	30.50	6.93	8.17	9.90	12.25	15.00	21.13	21.85	25.41	26.00
Longicyelene	10.33	32.80	3.50	4.40	5.26	6.75	9.00	11.80	13.00	14.15	15.40
Cyclopenta-decane	27.60	-	7.25	10.00	12.40	18.00	22.00	31.56	33.83	43.50	-

metical relationship

$$\frac{t_R^x - t_R^y}{x - y} = C \qquad (1)$$

found in the case of previous series of polyesters, was valid in the case of 1,ω-glycols-adipic acid polyesters series. The tabulated results are presented in Tables 4A and 4B. From the results, it is obvious that the linear relationship between the retention time and the number of methylene groups in the glycolic fragment, is valid in the present series as well. When the retention times of the monoterpenic and sesquiterpenic hydrocarbons are plotted against the number of methylene groups in the glycolic fragment of the polyester, good straight lines are obtained as shown in Graph I and Graph II.

Once again the arithmetical pattern was tested as previously. Here the average ratio of the retention time for six monoterpenes and six sesquiterpenes on different polyesters are shown against the expected values. These values are recorded in Table 5. In this case, it can be seen that the experimental and the expected values agree well, within the limits of experimental error, the maximum deviation being 9%.

Further careful examination of the retention time data shows that this linear relationship of the retention time and the number of methylene groups in the glycolic fragment, breaks at 1,10-decane diol and adipic acid

Table 4A

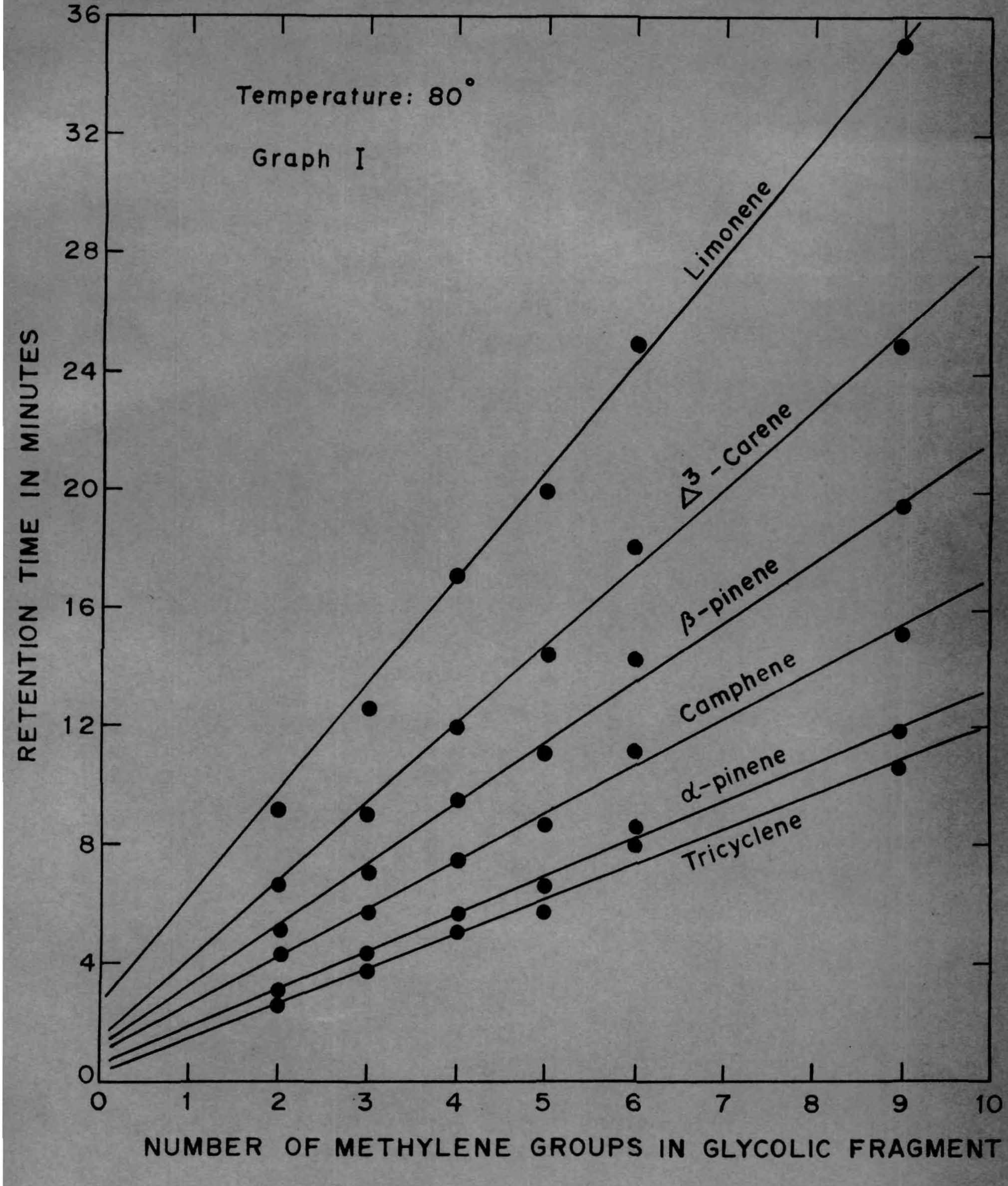
Evaluation of 'C' at 80°

$tR^x - tR^y$	x-y	C	tR^x-tR^y	x-y	C
α-Pinene			β-Pinene		
8.95	9-2=7	1.26	14.45	9-2=7	2-06
7.80	9-3=6	1.30	12.27	9-3=6	2.05
6.43	9-4=5	1.29	10.00	9-4=5	2.00
Δ^3-Carene			Limonene		
18.80	9-2=7	2.70	25.87	9-2=7	3.70
16.42	9-3=6	2.74	22.30	9-3=6	3.71
13.40	9-4=5	2.68	18.00	9-4=5	3.60
Tricyclene			Camphene		
7.77	9-2=7	1.11	11.02	9-2=7	1.57
6.67	9-3=6	1.11	9.50	9-3=6	1.58
5.50	9-4=5	1.10	7.80	9-4=5	1.56
Cyclohexane					
1.034	9-2=7	0.148			
0.940	9-3=6	0.157			
0.740	9-4=5	0.148			

Table 4B

Evaluation of 'C' at 163-64°

Tetrahydroelemene			β-Selinene		
9.30	10-2=8	1.16	18.15	10-2=8	2.27
8.65	10-3=7	1.23	16.52	10-3=7	2.36
7.80	10-4=6	1.30	14.80	10-4=6	2.46
6.11	10-5=5	1.22	11.14	10-5=5	2.23
Longifolene			Caryophyllene		
12.36	10-2=8	1.54	11.50	10-2=8	1.45
10.47	10-3=7	1.49	10.90	10-3=7	1.56
9.07	10-4=6	1.51	9.47	10-4=6	1.58
7.30	10-5=5	1.46	7.30	10-5=5	1.46
Humulene			Longicyclene		
14.92	10-2=8	1.87	9.50	10-2=8	1.33
13.68	10-3=7	1.95	8.60	10-3=7	1.23
11.95	10-4=6	1.99	7.74	10-4=6	1.29
9.63	10-5=5	1.92	6.25	10-5=5	1.25


Temperature: 80°
Graph I
Limonene
Δ^3 -Carene
β-pinene
Camphene
α-pinene
Tricyclene
RETENTION TIME IN MINUTES
0
4
8
12
16
20
24
28
32
36
0
1
2
3
4
5
6
7
8
9
10
NUMBER OF METHYLENE GROUPS IN GLYCOLIC FRAGMENT

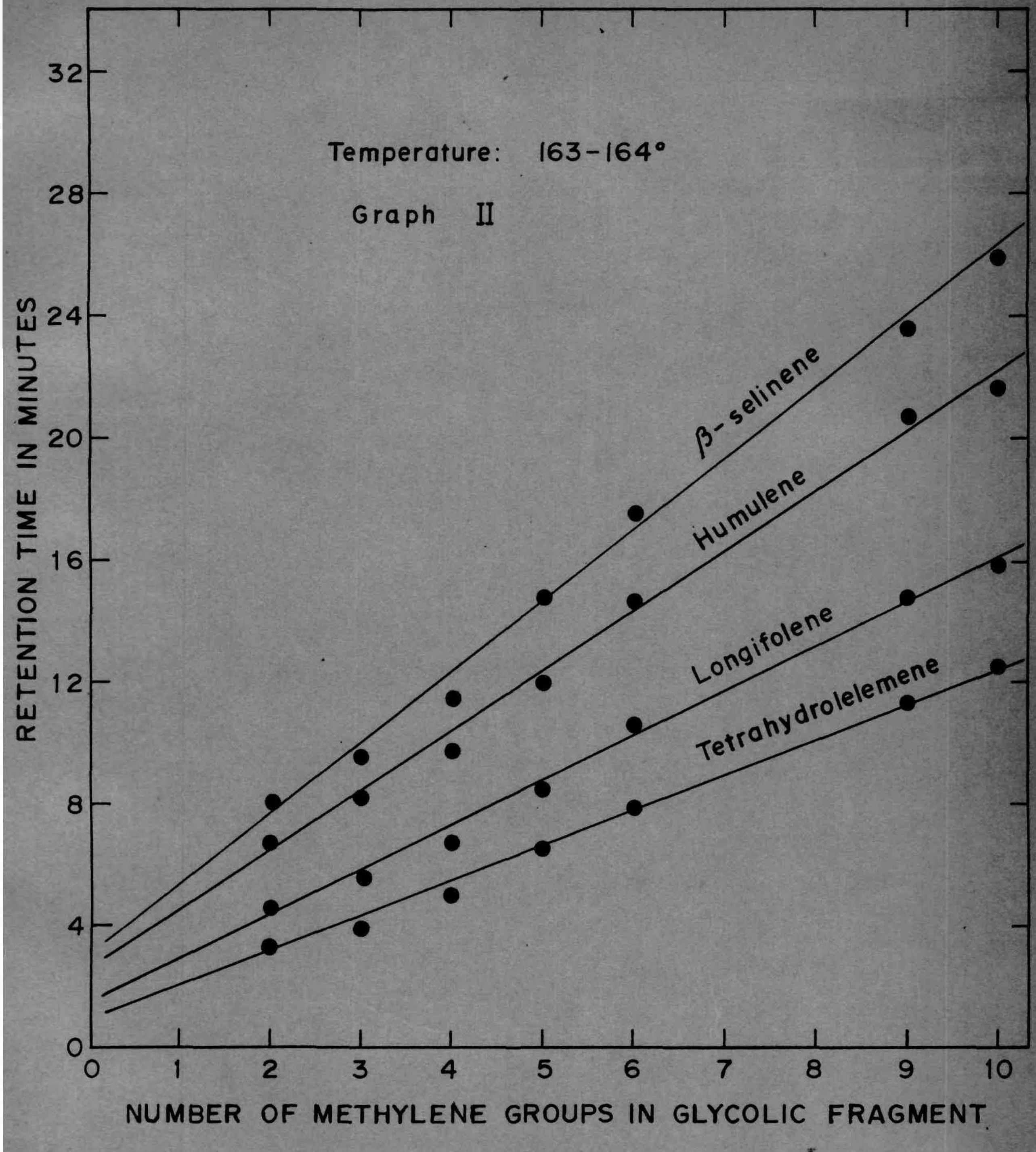

Temperature: 163-164°
Graph II
RETENTION TIME IN MINUTES
0
4
8
12
16
20
24
28
32
0
1
2
3
4
5
6
7
8
9
10
NUMBER OF METHYLENE GROUPS IN GLYCOLIC FRAGMENT
β-selinene
Humulene
Longifolene
Tetrahydrolelemene

Table 5

Relationship between the ratio of the number of methylene groups and the ratio of the retention times on the respective substrates.

Ratio of the number of methylene groups	Average ratio of observed retention times at 163°	Calculated ratio	% Error	Average ratio of observed retention times at 80°	Calculated ratio	% Error
10/4	2.36	2.50	5.6	-	-	-
10/6	1.50	1.66	9.6	-	-	-
9/4	2.19	2.25	3.0	2.09	2.26	7
9/6	1.42	1.50	5.0	1.37	1.70	8
6/3	1.91	2.0	5.0	1.99	2.0	<1
6/4	1.58	1.5	5.0	1.51	1.50	<1
4/3	1.21	1.33	9.0	1.31	1.33	2
3/2	-	-	-	1.39	1.50	7

polyester. There seems to be a striking similarity between this breakdown, and that in the previous series in which the polyesters were made by changing the length of the acidic fragment. In both the cases the linearity breaks down with ten or eleven methylene groups in the chain.

The above mentioned results gave an impetus to study this phenomenon in further details. Consequently several other polyesters were synthesised and studied:

(I) Ethylene glycol (constant), and
- (i) Succinic acid,
- (ii) Adipic acid
- (iii) Sebacic acid.

All the three polyesters were synthesised as previously and the retention time data, presented in Table 6. Also the average ratio of the retention times of C_{15} hydrocarbons and the corresponding methylene groups in the acidic fragment, have been calculated. The average ratio of the retention times of these hydrocarbons on sebacic acid (8 methylene groups) polyester and succinic acid polyester, shows that the ratio is 5.08 instead of 4. Similarly in the case of adipic acid and succinic acid polyesters, it is 2.42 instead of 2.0. However, strikingly in both the cases the values are nearly higher by about 25%,

Table 6

Compound	Number of methylene groups in the acidic fragment of the polyester. Glycolic fragment- ethylene glycol - constant.		
	8	4	2
α-Terpineol	3.80	7.55	1.066
Linalool	4.70	3.40	2.00
l-Borneol	10.70	6.75	4.40
Isoborneol		5.90	0.700
l-Menthol		5.20	3.20
Camphor	6.80	4.70	3.10
Carvone	14.10	10.0	6.85
Isopulegol	8.30	4.80	3.0
Bornyl acetate	8.60	5.0	2.83
Tetrahydroelemene	9.00	3.40	1.83
β-Selinene	19.83	8.0	3.90
Longifolene	11.66	4.66	2.20
Caryophyllene	12.10	5.40	2.50
Humulene	16.30	6.93	3.40
Longicyclene	9.60	-	2.20
Cyclopentadecane		7.25	3.50

as compared to the expected values. Also the ratio of the observed and the expected values remains nearly constant, but it is not in the form of multiple of unity.

This difference can be attributed to the difference in the nature of the glycolic fragment of the polyester in both the cases. Diethylene glycol has been used in the previous series and ethylene glycol is used in the present series. This probably indicates that only diethylene glycol having ethereal oxygen is responsible for the exact unitary nature of the arithmetical relationship in the polyester series prepared from 1,ω-dicarboxylic acids and diethylene glycol.

(II) Pentane-1,5-diol (constant), and
- (i) Succinic acid,
- (ii) Adipic acid,
- (iii) Sebacic acid.

Another series of polyesters were synthesised as shown above. As usual, the retention time data were recorded and presented in Table 7.

In this case, the average ratio of the retention times of the same sesquiterpenic hydrocarbons on sebacic acid-pentane-1,5-diol polyester and adipic acid-pentane-1,5-diol polyester comes out to be 1.45 instead of 2.

Table 7

Retention time in min. at 163-64°

Compound	Number of methylene groups in the acidic fragment of the polyester. Glycolic fragment -pentane,1,5-diol-const. 8	4	2
α-Terpineol	11.45	10.60	8.75
Linalool	5.23	4.95	3.90
1-Borneol	10.45	9.70	81.7
Isoborneol	8.66	8.95	7.17
1-Menthol	8.66	8.17	6.70
Camphor	6.40	6.10	5.40
Carvone	12.60	12.80	11.40
Isopulegol	8.50	6.87	5.60
Bornyl acetate	8.00	7.00	5.70
Tetrahydroelemene	9.56	6.60	4.40
β-Selinene	20.80	15.00	10.50
Longifolene	12.70	8.70	5.70
Caryophyllene	14.20	9.60	6.70
Humulene	18.00	12.25	8.70
Longicyelene	10.00	6.75	4.33
Cyclopentadecane	-	18.00	10.90

Similarly the average ratio of the retention times of the same hydrocarbons on adipic acid-pentane-1,5-diol polyester and succinic acid-pentane-1,5-diol polyester comes out to be 1.48 instead of 2. It is interesting to note that both these values are lower by nearly 26% as compared to the calculated value of 2.

When the retention time data on the above mentioned various series of polyesters, were carefully examined, it was thought that a typical polyester synthesised from a very long chain dicarboxylic acid and long chain 1,ω-glycol may furnish fantastic solubilities for hydrocarbon type samples. Therefore, it was found necessary to find out whether such type of phenomenon can be observed in practice. Therefore the following polyesters were prepared and the retention time data recorded and presented in Table 8.

Hexadecane-1,16-dicarboxylic acid(constant), and
(i) 1,4-Butane diol
(ii) 1,10-Decane diol
(iii) 1,15-Pentadecane diol.

In this case also retention time data reveal some useful results. Surprisingly, it is found that there is some saturation point in the case of retention times of

Table 8

Compound	Number of methylene groups in the glycolic fragment of the polyester. Acidic fragment - Hexadecane-1,16-dicarboxylic acid - constant. 4	10	15
α-Terpineol	17.60	4.00	4.10
Linalool	7.66	4.60	4.83
l-Borneol	15.60	10.70	10.90
Isoborneol	13.50	2.50	2.60
l-Menthol	14.20	10.00	9.80
Camphor	9.20	6.90	7.30
Carvone	18.10	12.70	13.83
Isopulegol	11.50	8.10	7.93
Bornyl acetate	13.60	10.20	10.30
Tetrahydroelemene	17.81	16.40	19.00
β-Selinene	34.50	32.50	37.50
Longifolene	22.00	22.30	24.10
Caryophyllene	23.10	20.50	22.83
Humulene	29.20	27.00	30.10
Longicyclene	18.00	18.50	19.50
Cyclopentadecane	-	-	-

hydrocarbons on these polyesters. While going from a polyester derived from butane-1,4-diol and hexadecane-1,16-dicarboxylic acid to a polyester derived from pentadecane-1,15-diol and the same dicarboxylic acid, it was observed that the retention times of hydrocarbons, recorded on both these polyesters are almost the same, being only slightly higher in the latter case.

Comparative evaluation of the polyesters:

Relative retention time data show that polyesters synthesised from the long chain 1,ω-glycols and adipic acid are having very high solubilities for sesquiterpenic hydrocarbons . These results can also be very conveniently used for the separation of monoterpenic oxygenated compounds and sesquiterpenic hydrocarbons.

The values of 'α' the relative volatility,'N' the number of theoretical plates and 's' the separation factor have been calculated for three typical pairs of monoterpenic hydrocarbons according to Purnell and presented in Table 9.

Thus a mixture of isopulegol and longifolene would require nearly 50,000 plates if it is to be resolved on a column of polyester synthesised from adipic acid and

Table 9

Components of the polyester	Compound pair	α	N	S
Adipic acid and ethylene glycol	Borneol and caryophyllene	1.250	1009	900
	Bornyl acetate and caryophyllene	1.08	7570	6560
	Isopulegol & longifolene	1.03	49840	42430
Adipic acid and pentadecane-1,15-diol	Borneol and caryphyllene	1.776	196.4	188.6
	Bornyl acetate and caryophyllene	1.969	154.8	148.6
	Isopulegol & longifolene	1.979	153.3	147.1
Hexadecane-1,16-dicarboxylic acid and pentadecane-1,15-diol	Borneol & caryophyllene	2.095	136.5	131.7
	Bornyl acetate and caryophyllene	2.217	123.7	119.5
	Isopulegol & longifolene	3.039	82.68	79.97
Silicone elastomer	Tetrahydroelemene and β-selinene	1.480	-	342.2
	Caryophyllene and humulene	1.129	-	2758
	Longifolene and longicyclene	1.128	-	2797
Adipic acid and pentadecane-1,15-diol	Tetrahydroelemene and β-selinene	1.963	-	149.5
	Caryophyllene & humulene	1.361	-	512.1
	Longifolene and longicyclene	1.208	-	1213

ethylene glycol, but the same mixture can be resolved by only 83 plates if a column of polyester synthesised from hexadecane-1,16-dicarboxylic acid and pentadecane-1,15-diol is used. Similar is the case with other pairs as presented in Table 9.

Such type of stationary phases prepared from either long chain 1,ω-glycols or long chain dicarboxylic acids, seem to give a very nice and distinctive cut between the oxygenated compounds and hydrocarbons. At the same time these phases will resolve the individual components as well. From Table 9, it is clear that the separation of tetrahydroelemene and β-selinene would require nearly 343 plates on silicone column, but the same mixture can be separated by only 150 plates if it is chromatographed on a polyester synthesised from adipic acid and pentadecane-1,15-diol. Similar is the case with other pairs of hydrocarbons.

In the year 1956, A.J.P. Martin[1] predicted the use of very small diameter columns for gas chromatography. Golay reported the use of capillary columns coated[2] on inside walls with the stationary phase. Such type of columns apparently furnish enormously high number of theoretical plates. Zlatkis and Kaufman have reported a column having a million theoretical plates.[3]

Thus calculation of 'N' the number of theoretical plates in the case of capillary columns used to give very high values, but the same degree of separation was easily attainable by packed columns also. This anomaly was clearly explained by Purnell.[4] The general equation given by him is as follows:

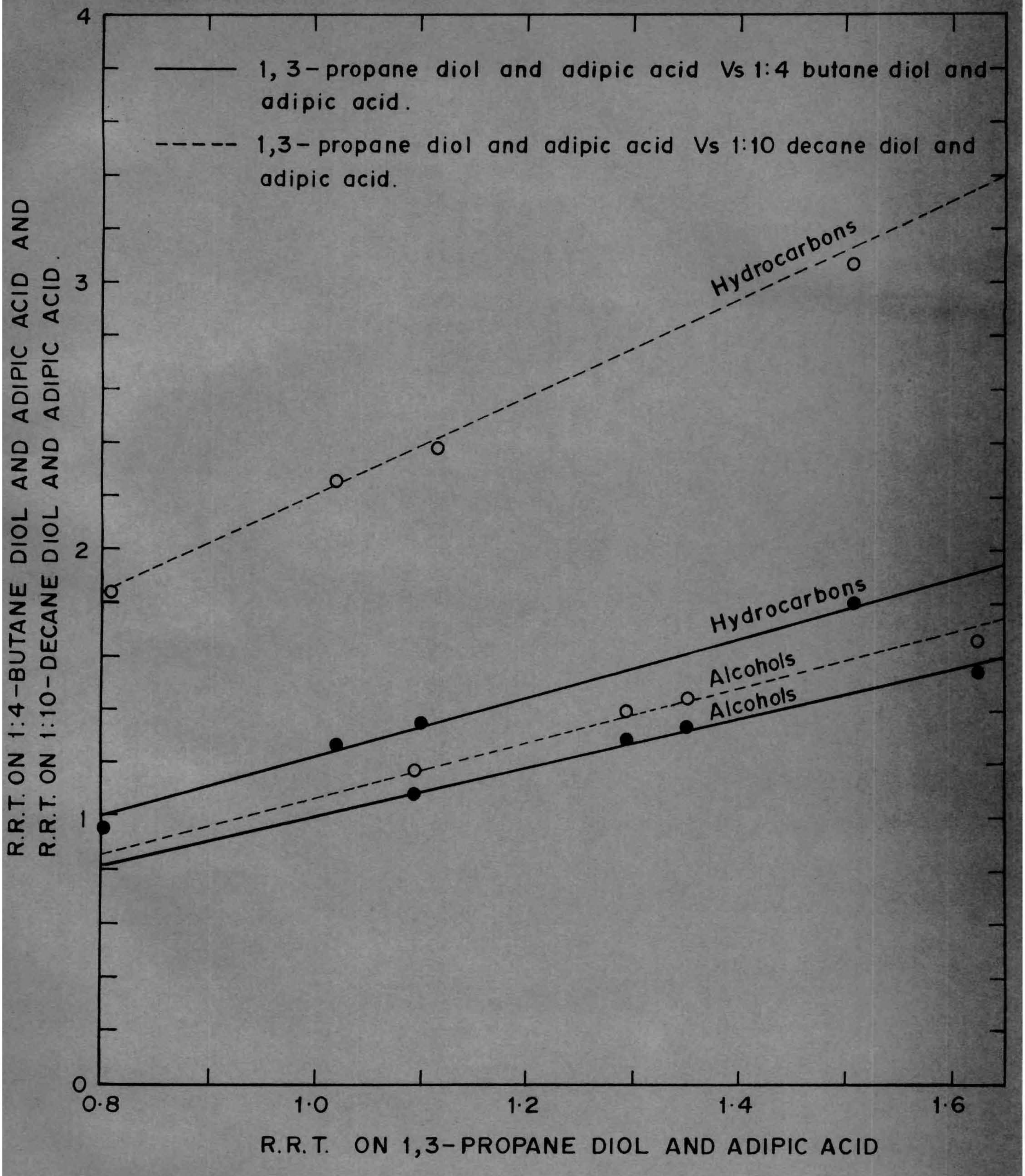
$$N=S\left[(1+2\left(\frac{Vd}{VR}\right)+\left(\frac{Vd}{VR}\right)^2\right] \quad (4)$$

In this equation full account is made for the dead volume of the column, which becomes significant when the retention volume is too low compared to the dead volume.

In the present series of polyesters, it can be seen from the retention time data that, while going from ethylene glycol-adipic acid polyester, to the last polyester, the retention times of oxygenated compounds are increased by nearly a factor of two and the retention times of hydrocarbons are increased by several fold. Consequently the effect of dead volume should be pronounced in the case of lower polyesters and negligible in the case of higher polyesters.

The values of 'N', 's' and 'α' calculated for typical pairs of compounds (Table 9) indicate that there is not much difference in the values of 'N' and 's' in

GRAPH III


1, 3-propane diol and adipic acid Vs 1:4 butane diol and adipic acid.
1,3-propane diol and adipic acid Vs 1:10 decane diol and adipic acid.
Hydrocarbons
Hydrocarbons
Alcohols
Alcohols
R.R.T. ON 1:4-BUTANE DIOL AND ADIPIC ACID AND
R.R.T. ON 1:10-DECANE DIOL AND ADIPIC ACID.
R.R.T. ON 1,3-PROPANE DIOL AND ADIPIC ACID
0
1
2
3
4
0·8
1·0
1·2
1·4
1·6

the case of long chain polyesters and this point would ~~be~~ strongly favour the use of these polyesters as stationary phases in the capillary columns.

When a graph of relative retention times (Graph III) (with respect to camphor) on 1,3-propane diol-adipic acid polyester is plotted against the relative retention times on 1,10-decane diol-adipic acid polyester, two distinct straight lines are obtained. One line represents the hydrocarbons and the other one represents the alcohols. As the number of methylene groups in the alcoholic fragment of the polyester goes on increasing these lines divert apart. This clearly indicates that these two groups of compounds will be separated on the polyesters synthesised from long chain glycols. If we examine relative retention time data obtained on ethylene glycol adipic acid polyester and 1,15-pentadecane diol-adipic acid polyester, we find that there is no much loss in the resolution of the individual components of the oxygenated compounds, but at the same time the hydrocarbon group is well separated also with no loss in the resolution of the individual components of the hydrocarbon group.

In the last chapter, it was pointed out that 1,ω-dicarboxylic acids can be identified with considerable accuracy by preparing a polyester of the unknown acid and running the column for some hydrocarbons under identical conditions.

Similarly, it may be pointed out in the present case, that 1,ω-glycols can also be identified with considerable accuracy by preparing a polyester of the unknown 1,ω-glycol and running the column for some hydrocarbons under identical conditions.

REFERENCES

1. Martin A.J.P., Gas Chromatography (ed. by D.H.Desty), p. 2 (1957).

2. Golay M.J.E., Gas Chromatography (ed. by Coats, Noebels and Fagerson), p.1 (1958).

3. Zlatkis A. and Kaufman H.R., Nature 184, 2010(1959).

4. Purnell J.H., J.Chem.Soc., 1268(1960).

Description of the chromatograms

Chromatograms at 120°.

The following experimental conditions were maintained for the resolution of compounds at this temperatures.

(I) Temperature 120° (± 1°).

(II) Supporting material - Celite 545,
120-150 mesh size.

(III) Bridge current 150 mA.

(IV) Sample size - 1 to 5 μl as per requirements.

(V) Stationary phases:
(i) Adipic acid-1,4-butane diol polyester;
(ii) Adipic acid-1,13-tridecane diol polyester.

(VI) Flow rate: 9 sec./10 c.c. of hydrogen.

Note: The total weight of the column filling was not the same in the case of the two columns mentioned above. The actual weight of the stationary phase, therefore, was not the same. Inlet pressure, required to obtain the required flow rate was also not the same.

Compound pair: 1,8-Cineole, cyclohexanone.

Fig.1

Stationary phase: Adipic acid-1,4-butane diol polyester.

Cyclohexanone emerges after the less polar compound, 1,8-cineole. The relative retention time of cyclohexanone with respect to 1,8-cineole comes out to be 1.43.

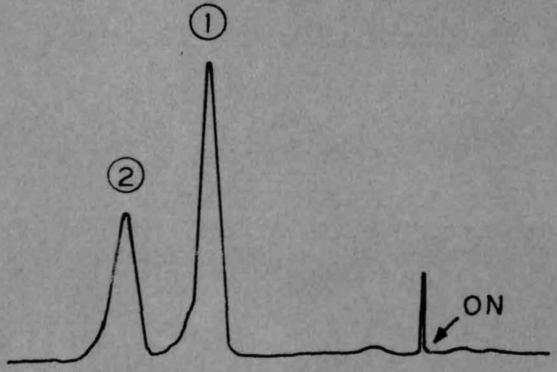
Fig. 2

Stationary phase: Adipic acid-1,13-tridecane diol polyester.

This stationary phase contains 1,13-tridecane diol as the glycolic fragment of the polyester, so that the resulting polyester becomes comparatively non-polar. Therefore, the separation takes place on boiling point basis only. 1,8-Cineole emerges after cyclohexanone. The relative retention time of 1,8-cineole with respect to cyclohexanone, is found to be 1.33.

TEMPERATURE: 120°

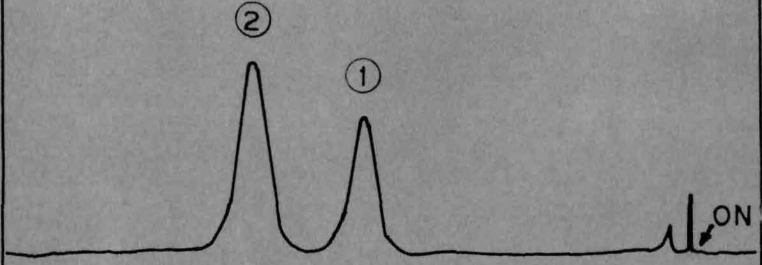
FIG·1



PHASE: ADIPIC ACID 1,4-BUTANE DIOL POLYESTER

① 1,8-CINEOLE
② CYCLOHEXANONE

FIG·2



PHASE: ADIPIC ACID 1,13-TRIDECANE DIOL POLYESTER

① CYCLOHEXANONE
② 1,8-CINEOLE

Compound pair: Limonene, cyclohexanone.

Fig. 3

Stationary phase: Adipic acid-1,4-butane diol polyester.

Because of the polar nature of this polyester, cyclohexanone emerges after limonene. Once again this is an example of 'boiling point reversal'. The relative retention time of cyclohexanone, with respect to limonene, comes out to be 1.74.

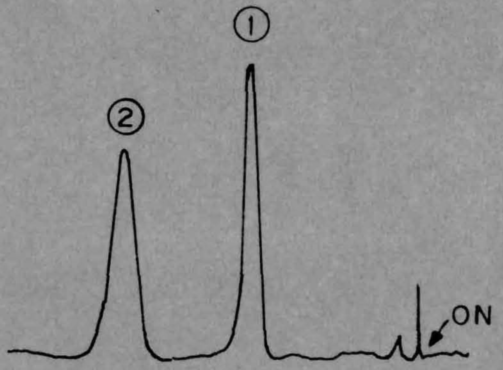
Fig. 4

Stationary phase: Adipic acid-1,13-tridecane diol polyester.

The separation takes place according to the boiling points only. Limonene emerges after cyclohexanone. The relative retention time of limonene with respect to cyclohexanone is found to be 1.34.

FIG·3

TEMPERATURE: 120°

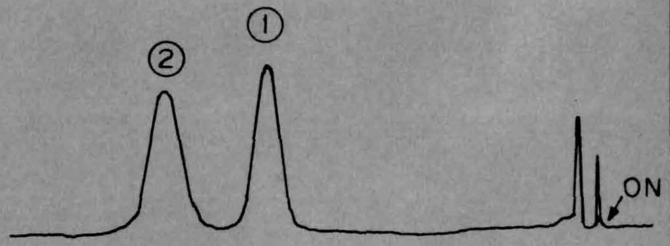


PHASE: ADIPIC ACID 1,4-BUTANE DIOL POLYESTER

① LIMONENE

② CYCLOHEXANONE

FIG·4



PHASE: ADIPIC ACID 1,13-TRIDECANE DIOL POLYESTER

① CYCLOHEXANONE

② LIMONENE

Compound pair: Cyclohexanone, p-cymene

Fig. 5

Stationary phase: Adipic acid-1,4-butane diol polyester.

Cyclohexanone - a polar compound emerges after the non-polar aromatic hydrocarbon p-cymene. Relative retention time of cyclohexanone with respect to p-cymene is found out to be 1.21.

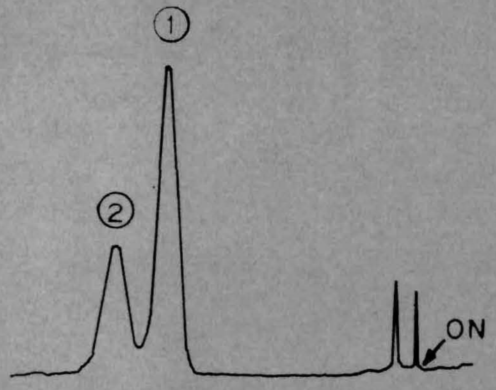
Fig. 6

Stationary phase: Adipic acid, 1,13-tridecane diol polyester.

This stationary phase happens to be non-polar. Therefore, the solubility of non-polar compound p-cymene, has increased and it emerges after cyclohexanone. The order of emergence is in accordance with the boiling points of the two compounds. The relative retention time of p-cymene with respect to cyclohexanone is found to be 1.56.

TEMPERATURE : 120°

FIG. 5

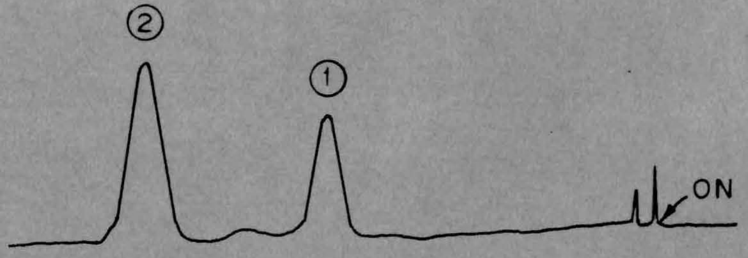


PHASE: ADIPIC ACID 1,4-BUTANE DIOL POLYESTER

① p-CYMENE

② CYCLOHEXANONE

FIG. 6



PHASE: ADIPIC ACID 1,13-TRIDECANE DIOL POLYESTER

① CYCLOHEXANONE

② p-CYMENE

Compound pair: 1,8-Cineole, limonene

Fig. 7

Stationary phase: Adipic acid, 1,4-butane diol polyester.

Because of the polar character of this stationary phase, 1,8-cineole emerges after limonene. Relative retention time of 1,8-cineole with respect to limonene comes to be 1.22.

Fig. 8

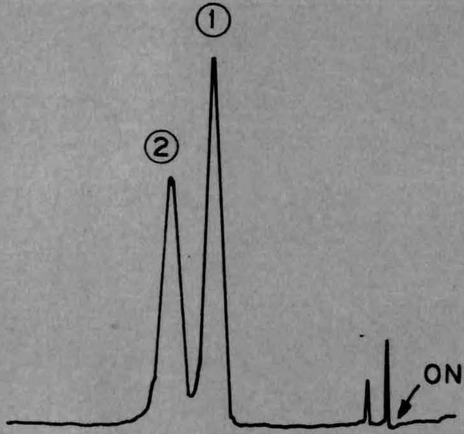
Stationary phase: Adipic acid-1,13-tridecane diol polyester.

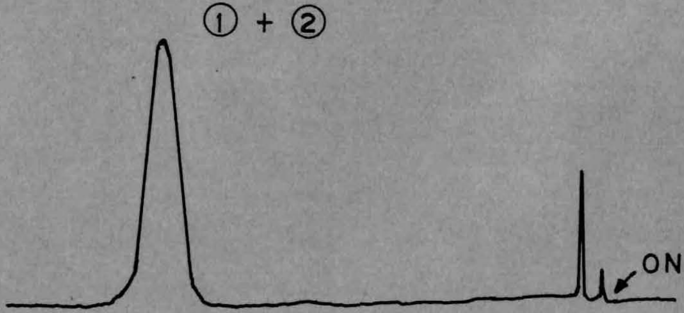
The two compounds are not resolved at all. The resulting chromatogram comes out to be a single peak only.

FIG. 7

FIG. 8

TEMPERATURE: 120°





PHASE: ADIPIC ACID 1,4 BUTANE DIOL POLYESTER

① LIMONENE

② 1,8-CINEOLE

PHASE: ADIPIC ACID 1,13-TRIDECANE DIOL POLYESTER

① + ② UNRESOLVED

Chromatograms at a temperature range of 180° to 196°.

It was found rather difficult to maintain all the column parameters constant. Therefore the following two different sets of conditions were maintained.

Set No.	Abbreviation	Experimental conditions
(I)	A	i) Temperature 184-186° ii) Stationary phase: 20%; Adipic acid-1,4-butane diol polyester on 120-150 mesh celite.
(II)	B	i) Temperature 194-195° ii) Stationary phase: Adipic acid-1,13-tridecane diol polyester.

Note: The carrier gas used for the present investigations, was hydrogen. The flow rate was adjusted in such a way that the retention times of the different samples were not too long. This adjustment gave good resolution of the different samples, used in the present investigation.

Compound pair: Humulene and Borneol

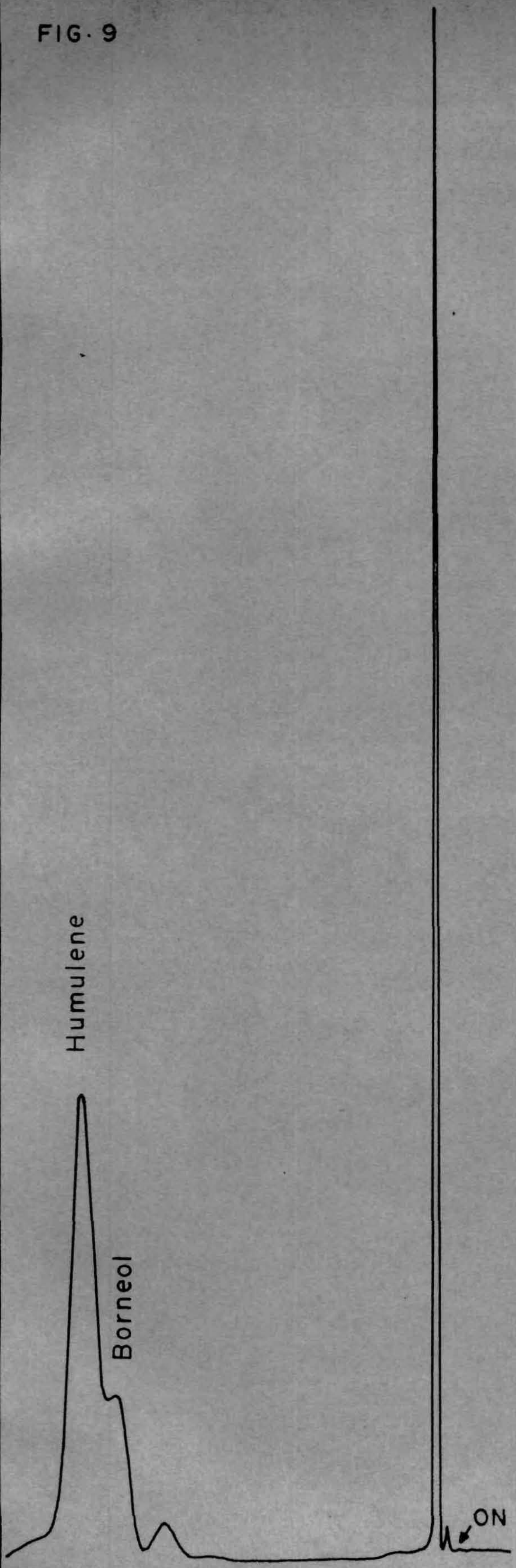
Fig. 9 Set of conditions A.

This stationary phase contains 1,4-butane diol as the glycolic fragment of the polyester. Therefore, these two compounds are not completely resolved, but a partial separation can be obtained. Relative retention time of humulene, with respect to borneol, is 1.14 only.

Fig. 10 Set of conditions B.

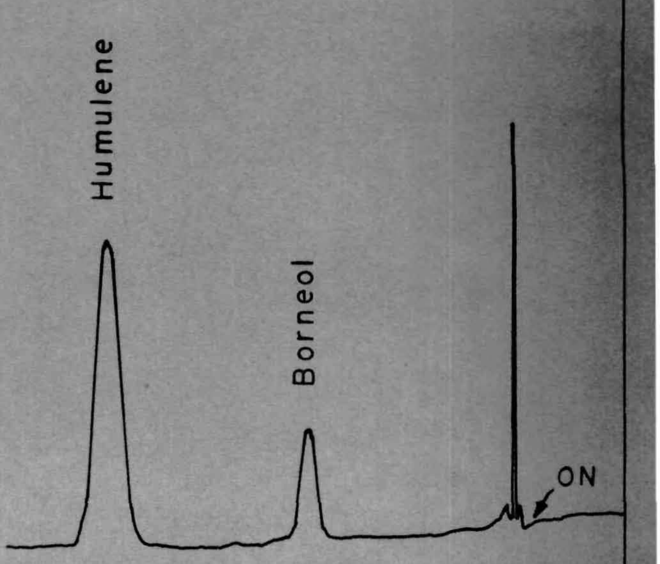
Because of the large number of methylene groups in the glycolic fragment of the polyester, the two compounds are separated wide apart. The relative retention time of humulene, with respect to borneol rises up 1.94.

FIG. 9



PHASE: ADIPIC ACID-1,4-BUTANE DIOL POLYESTER

FIG. 10



PHASE: ADIPIC ACID-1,13-TRIDECANE DIOL POLYESTER

<u>Compound pair</u>: <u>Carvone and dihydrocarvone</u>

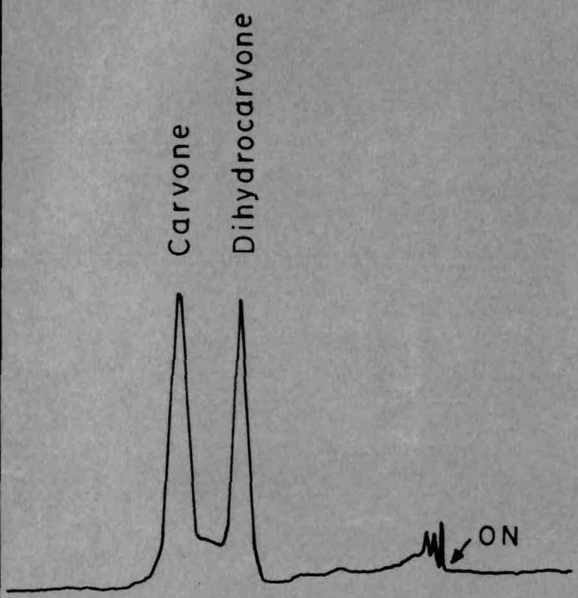
Fig. 11 Set of conditions B.

Carvone and dihydrocarvone are sufficiently separated apart. Relative retention time of carvone, with respect to dihydrocarvone, is 1.31 only.

Fig. 12 Set of conditions A.

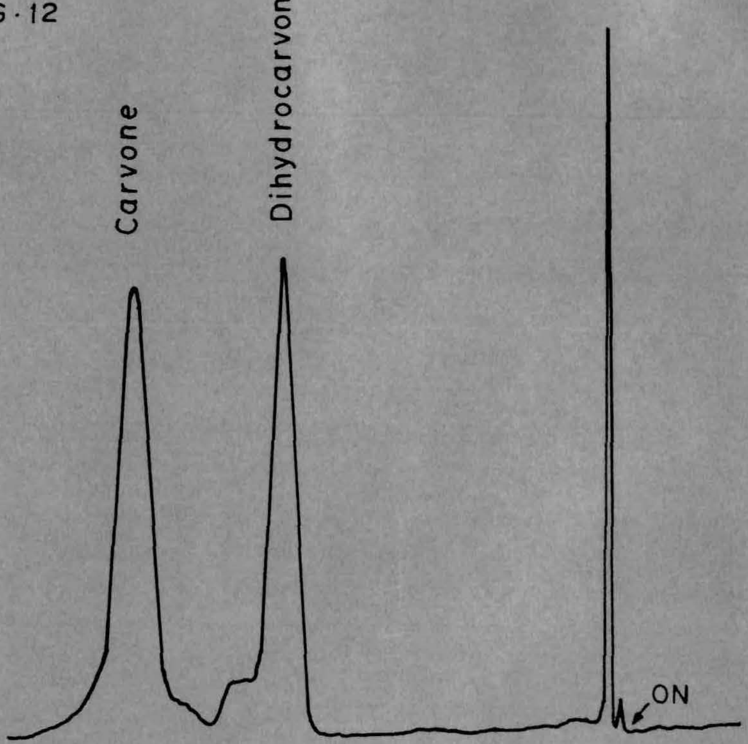
Since this stationary phase is sufficiently polar one, the two compounds are well separated. Relative retention time value rises up to 1.45.

FIG·11



PHASE: ADIPIC ACID 1,13-TRIDECANE DIOL POLYESTER

FIG·12



PHASE: ADIPIC ACID 1,4-BUTANE DIOL POLYESTER

<u>Compound pair:</u> <u>Lithio-ethylene-diamine</u>
Isomerisation product of himachalene.

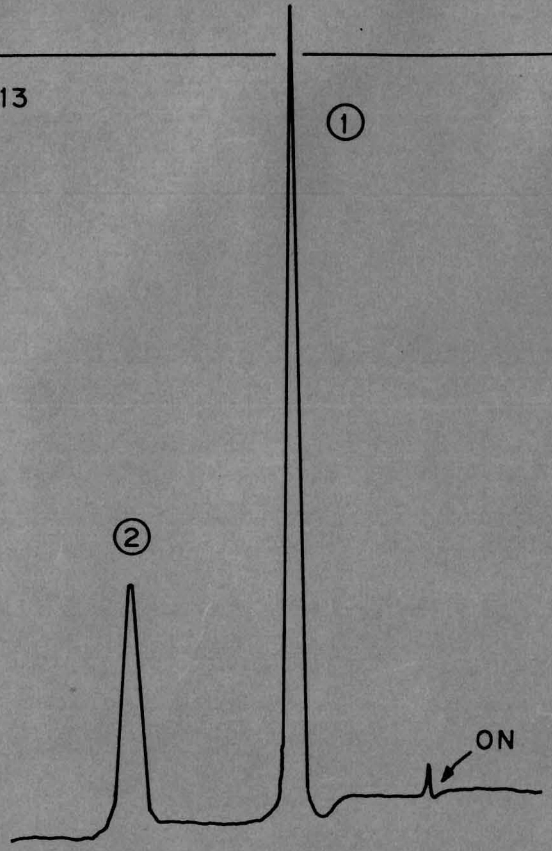
Fig. 13 Set of conditions A.

The stationary phase happens to be polar one, therefore the two hydrocarbons are well separated apart. The relative retention time value comes out to be 2.18. There is a time interval of nearly eight minutes between the two peaks.

Fig. 14 Set of conditions B.

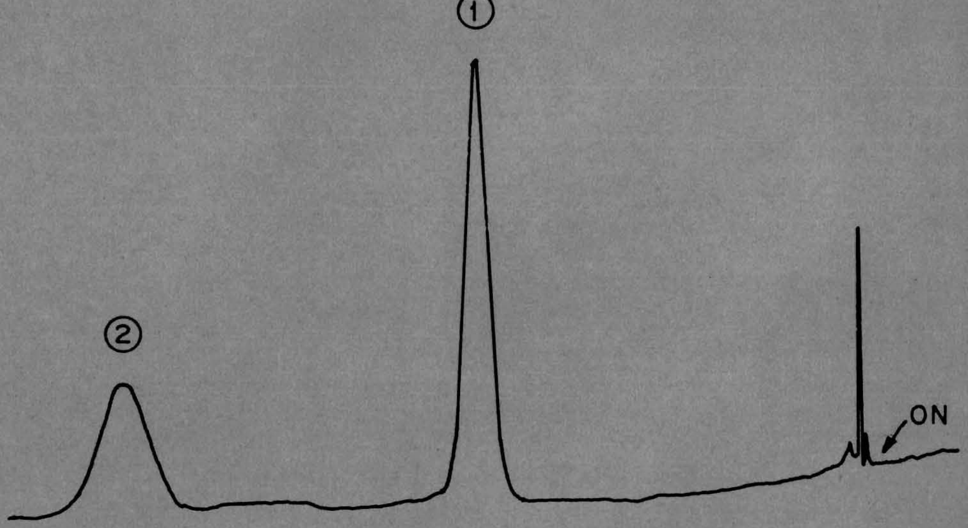
Because of the non-polar character of this stationary phase, the relative retention time of second hydrocarbon with respect to first one falls up to 1.88. But it is worthwhile to note that, the time interval between the two peaks rises up to eighteen minutes.

FIG. 13



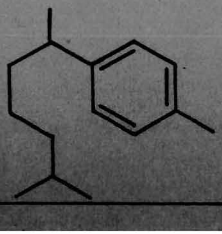
PHASE: ADIPIC ACID-1,4-BUTANE DIOL POLYESTER

FIG. 14

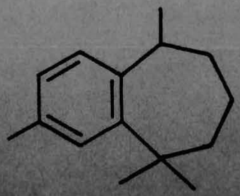


PHASE: ADIPIC ACID-1,13-TRIDECANE DIOL POLYESTER

①



②



Compound pair: Longicyclene, longifolene

Fig. 15 Set of conditions A.

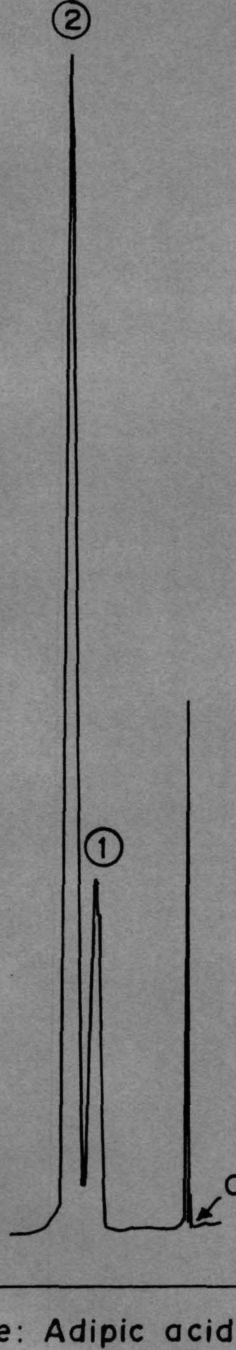
Longicyclene and longifolene are well separated. The relative retention time of longifolene, with respect to longicyclene is found to be 1.28.

Sample: Humulene, longifolene, longicyclene

Fig. 16 Set of conditions B.

The nature of the stationary phase is such that the solubilities of hydrocarbons are increased to a very large extent. Therefore the resulting peaks are somewhat broad compared to those seen in Fig. 15. The relative retention time of longifolene with respect to longicyclene, is 1.23.

FIG. 15.

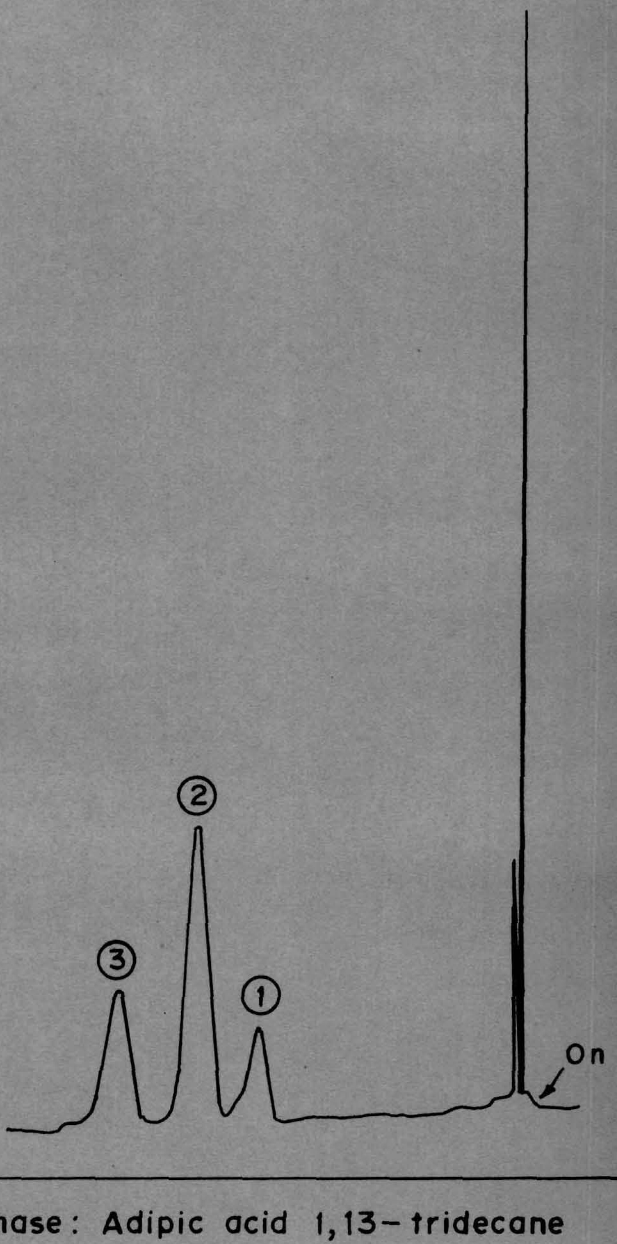


Phase: Adipic acid-1,4-butane diol polyester.

① Longicyclene

② Longifolene.

FIG. 16.



Phase: Adipic acid 1,13-tridecane diol polyester.

① Longicyclene

② Longifolene

③ Humulene.

Sample: α-Pinene, β-pinene, limonene

Fig. 17 Set of conditions B.

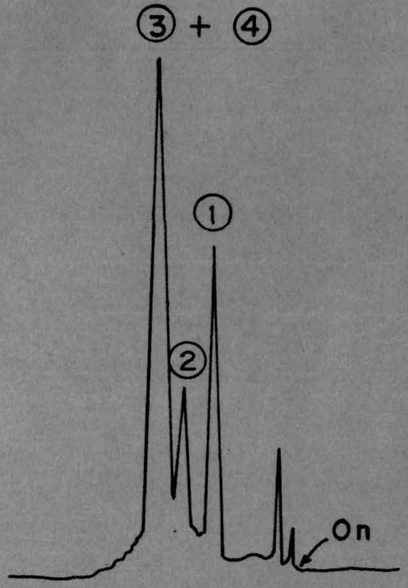
All the three hydrocarbons are low boiling monoterpenic hydrocarbons. But the nature of this stationary phase is such that it resolves all the three compounds even at high column temperature.

Sample: α-Pinene, β-pinene, limonene, methyl heptenone.

Fig. 18 Set of conditions B.

The retention time of the third peak, that corresponds to limonene, is so high that methylene heptenone and limonene emerge together. This may be seen from the large area covered by the third peak. Please compare figure 17 and figure 18.

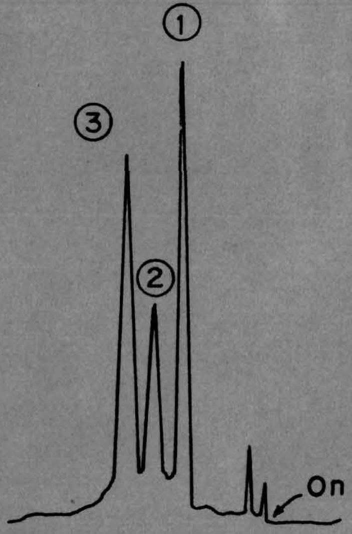
FIG. 18.



Phase: Adipic acid 1, 13-
tridecane diol polyester.

① α- pinene
② β- pinene
③ Limonene.
④ Methyl heptenone

FIG. 17.



Phase: Adipic acid 1, 13-
tridecane diol polyester.

① α- pinene
② β- pinene
③ Limonene

PART III

EVALUATION OF POLYESTERS DERIVED FROM (1) HYDROXY ACIDS (2) DICARBOXYLIC ACIDS AND UNSATURATED GLYCOL

SUMMARY

Polyesters from ω-hydroxy acids have been prepared by self polymerisations and evaluated as substrates for gas-liquid chromatography. Two model compounds (i) ω-hydroxy stearic acid, and (ii) aleuritic acid, which are easily available from indigenous sources, were employed for polyesterification. In the case of aleuritic acid, where cross-linking is possible freely, the usual process of ester formation, was not found to be useful. It gave a rubber like mass, insoluble in common organic solvents. Therefore a novel method of polyester formation on the supporting material in situ, has been described for both the hydroxy acids. Such type of polymers, when subjected to the analyses of the monoterpenic oxygenated compounds, showed acid catalysed isomerisation of the sample vapours. A successfull attempt has been made to retard this process of isomerisation. These polyesters showed very high solubilities for the nonpolar sesquiterpenic hydrocarbons and can be used for the separation of monoterpenic oxygenated compounds and sesquiterpenic hydrocarbons.

Simultaneously, the effect of 'unsaturation in polyester' has also been studied by synthesising two

polyesters, one from adipic acid and 1,4-butane diol and the other from adipic acid and 1,4-butene diol. The effect of 'unsaturation' is found to be opposite to that, which is observed in the case of long chain dicarboxylic acid diethylene glycol polyesters. Unsaturated polyester shows better solubilities for oxygenated compounds than for hydrocarbons.

In our previous investigations, we have described the use of various polyesters as stationary phases in gas liquid chromatography of terpenoids. Initially, polyesters from 1,ω-dicarboxylic acids with diethylene glycol were studied as GLC substrates. It was found that the number of methylene groups in the acid fragment has ~~have~~ a profound influence, giving an arithmetical relationship in the case of retention times of hydrocarbons. When the series of polyesters from various 1,ω-glycols and some fixed acids like adipic acid were used as substrates, the similar effect of spacing of methylene groups in the glycolic fragment was also observed. The effects of molecular weights and temperature gradient have also been studied.

From these observations it was felt that polyesters having properties of both the series may find wide applications. Since the long chain acids or glycols are not always easily accessible, it was thought that some of the hydroxy acids, commercially available from indigenous sources might be very useful for the synthesis of polyesters via self-condensation.

Previous workers[1] observed that esterified oleic acid, possibly because of its unsaturation, is useful in the separation of monoterpenic hydrocarbons and oxygenated compounds. This suggested that introduction of 'unsaturated centre' in a polyester may be advantageous in the separation of various terpenoids.

The present investigation deals with the results obtained on polyester substrates prepared from self-polymerisation of hydroxy acids and from saturated and unsaturated glycols with saturated dicarboxylic acids.

EXPERIMENTAL

Two varieties of polyesters have been prepared and their solvent capability on GLC columns, studied.

Polyesters from self condensation of hydroxy acids

Polyesters cross-linked with diglycerol or pentaerythritol are fairly stable substrates and are known to give good separation.[2] With a view to prepare a polyester from a hydroxy acid permitting self cross-linking, we selected aleuritic acid $HO\text{-}CH_2\text{-}(CH_2)_5\text{-}CHOHCHOH\text{-}(CH_2)_7\text{-}COOH$. It is available in abundance in India from an indigenous raw material, shellac. It possesses interesting structural features. Besides the terminal primary hydroxy group, it contains two vicinal secondary hydroxyl groups at carbon 9 and 10. Because of the presence of the vicinal hydroxyl groups in the central part of the molecule, it is capable of giving cross-linked polymer.

Along with aleuritic acid, another straight chain monohydroxy acid, ω-hydroxy stearic acid, $HO\text{-}CH_2\text{-}(CH_2)_{15}\text{-}CH_2\text{-}COOH$ has also been employed for self-polymerisation. Unlike aleuritic acid, it is capable of only forming straight chain polymers on self-condensation.

Preparation of polymers:

1. **Aleuritic acid**: This acid when polymerised according to our previous procedure,[1] gave high melting, rubber-like solid mass, which is almost insoluble in chloroform or ethyl acetate at their boiling points or nearly so in β-pinene at 100°.

Due to the insolubility of the polymer in most of the common solvents, it was not found practicable to impregnate it on solid support. Hence, a novel method of preparation of polymer on solid support was employed.

A known weight of aleuritic acid as such, was impregnated on fire-brick support by using ethyl alcohol as a solvent. The solvent was evaporated off on a water-bath and the whole mass dried in an oven at 80° for 2 hr. It was then taken in a 100 ml. flask and heated at about 140-145° for 2 hr., initially as such and then under vacuum (2 mm) for further 2 hr. This material was then used in the column.

A few initial runs on this column revealed some strange retention time data. When retention times of mono-terpenic hydrocarbons were being recorded, it was found that as time elapsed, the retention times of hydrocarbons went on increasing and at the same time, the peaks of the alcoholic and ketonic compounds showed considerable tailing. The base line stability was also poor. These observations indicated that the polymerisation of aleuritic acid was incomplete under the experimental conditions described above.

A number of experiments were therefore designed to trace this phenomenon. Every time a known weight of aleuritic acid was impregnated on fire-brick support, dried and used.

Initially the column was stabilised at 100° or 120° and the retention time data for monoterpenic hydrocarbons and a few medium boiling nonterpenic compounds were recorded. The same column was then stabilised at 163-164° and the retention time data were recorded for monoterpenic oxygenated compounds and sesquiterpenic hydrocarbons. After this, the same column was conditioned at 200° for nearly 3 hr. and again the retention time data were recorded at 100° and 120° as before.

Based on the observations made during the final experiments described above, a final batch of self-polymerisation was prepared as follows.

1. Weight of aleuritic acid 2.425 g. (on the whole column filling).
2. Heated at 200° for6 hr. with constant stirring.
3. Loss during heating 0.381 g.
4. The retention time data were then recorded.

Polymerisation of ω-hydroxy stearic acid

1. Weight of the acid 2.435 g (on the whole column filling)
2. Heated at 200° for 6 hr. with constant stirring.
3. Loss during heating 0.219 g.

RESULTS AND DISCUSSION

In the case of earlier polyesters, derived from 1,ω-dicarboxylic acids and diols, the polyesters are essentially linear with only a bit of cross-linking. But in the case of aleuritic acid polymer, it could be expected that because of the presence of two vicinal 'OH' groups in the central part of the molecule, the resulting polymer would possess cross-linking, representing a graphic structure. This explains the very high melting point of the final polymer. Obviously, the solubilities of hydrocarbons or comparatively non-polar compounds are expected to be high and the results tabulated in Table 1 are in agreement with it.

The retention time data were recorded as follows:

Set No.(I) - Monoterpenic hydrocarbons and other low boiling compounds at 120°.

(II)- Sesquiterpenic hydrocarbons and monoterpenic oxygenated compounds at 163-165°.

(III)- Again for monoterpenic hydrocarbons and other low boiling compounds at 120°.

Table 1

Aleuritic acid polymer

Retention time in minutes.

Temperature 120°				Temperature 163-164°		
Compound	Set-I	Set-III	Set-IV	Compound	Set-II	Set-V
α-Pinene	5.91	8.83	9.18	α-Terpineol	5.25	8.00
β-Pinene	9.95	13.96	14.21	Camphor	11.67	17.1
p-Cymene	16.75	25.73	26.83	Borneol	19.0	30.8
1,8-Cineole	17.5	25.46	25.83	Longifolene	21.75	35.0
Cyclohexanone	14.00	18.25	17.00	Humulene	30.75	Borad peak

Then the column was conditioned at 200° for several hours and further readings taken.

Set No.(IV) - Monoterpenic hydrocarbons at 120°.
Set.No.(V) - Sesquiterpenic hydrocarbons and monoterpenic oxygenated compounds at 163-165°.

Comparison of the retention times on set No.III and IV, and II and V shows that there is not much difference in the retention times of the former sets, but a large difference is observed in the latter sets. This can be attributed to the fact that polymeric mass is getting softened at higher temperature (163-165°), thus allowing the sample vapours to dissolve in a better way.

In the case of ω-hydroxy stearic acid polymer the same steps were followed for the determination of retention time data.

In the case of both the columns, mentioned above, some decomposition of tertiary alcohols was observed. In order to retard this acid-catalysed decomposition the following procedure was adopted.

I. <u>Aleuritic acid polymer-column filling</u>

About 3 g. of KOH were dissolved in 300 ml of alcohol and this solution was added to the above mentioned filling. The mixture was stirred from time to time and finally allowed to stand for 15 minutes. The supernatent liquid was decanted off. The remaining column filling was again washed with aqueous alcohol till the pH of the washings was nearly 8. The mass was dried in an oven and again used as column filling to record the retention time data. It was noted that the retention times were very low when compared to the previous values. This indicated that the alcoholic alkali must have extracted out the stationary phase; however, at the same time the decomposition of monoterpenic oxygenated compounds had nearly vanished.

II. ω-Hydroxy stearic acid polymers

This column filling was suspended in 100 ml of alcohol, and with constant stirring, titrated with 0.4 N aqueous KOH solution till it was slightly alkaline. The whole mass was slowly heated on a water bath to dryness and the resulting column filling, heated in an air oven for several hours. This column filling was again filled in the column and the retention time data were recorded. At this stage chromatograms of sesquiterpenic hydrocarbons as well as monoterpenic oxygenated compounds did not show any tailing or decomposition, but the retention time values were less by nearly 20%. This may be due to the fact that some quantity of stationary phase might have been lost during the above mentioned process; or neutralisation of the free carboxylic groups might have changed the general nature of the stationary phase to some extent. Such type of analogus observations have been recorded by the previous workers.[1,3]

The decomposition of monoterpenic oxygenated compounds, seems to be retarded in the present case at least, by neutralisation of the column[4] filling. It may be pointed out at this stage that some of the previous workers have incorporated 1% sodium bicarbonate[4] on the column fillings and have also used potassium or lithium salts of fatty acids as stationary phases in part.[5] It is interesting to note that the nature of some of the popular supporting material was found to be slightly alkaline.[6]

The present ω-hydroxy stearic acid polymer showed very high solubilities for C_{15} hydrocarbons. A mixture of methyl heptenone, linalool, citral, longifolene, longicyclene and humulene was easily resolved as shown in chromatograms complied at the end of this chapter. However, the geometrical isomers of citral are not resolved, though citral can easily be resolved into two geometrical isomers by using succinic acid-diethylene glycol polymer.

Effect of unsaturation

In order to study the effect of unsaturation a polyester was prepared from 1,4-butene diol ($HO-CH_2-CH=CH-CH_2OH$) and adipic acid. For comparative evaluation, another polyester from 1,4-butane diol and adipic acid, was also synthesised. Both the columns were run under identical conditions. Relative retention time data recorded on these two columns are presented in Table No.2 at 120° and Table No.3 at 163°. Retention time data obtained on the two types of hydroxy acid polymers are also compiled in the same table. For comparison, retention time data, compiled from previous series of polyesters are also given in the same table for immediate reference.

Examination of the relative retention time data reveals some striking differences in the case of the stationary phases discussed above. Aromatic compounds have higher relative retention values on unsaturated polyester, compared to those obtained on saturated polyester (p-cymene). Similarly, oxygenated compounds also show higher relative retention values on unsaturated polyester. The relative retention time of cyclohexanone is 2.37 on the unsaturated polyester, while on the saturated polyester, its relative retention value is 1.73 only. In the case

Table 2

Relative retention times with respect to limonene. Temp. 120°

Compound	1,4-Butane diol adipic acid polyester	1,4-Butene diol adipic acid polyester	ω-Hydroxy stearic acid polymer	Aleuritic acid polymer	Pentadecane 1,15-dicarboxylic acid-diethylene glycol polyester	Apiezon
α-Pinene	0.4009	0.4140	0.4574	0.4355	0.4184	0.5052
β-Pinene	0.6790	0.6719	0.6915	0.6755	0.640	0.5825
^{3}-Carene	0.8091	0.7812	0.7939	0.8654	0.7703	0.8404
Ocimene	1.161	1.219	-	-	-	-
p-Cymene	1.416	1.50	1.151	1.196	1.144	0.9330
Limonene	9.22=1.0	6.4=1.0	9.4=1.0	12.6=1.0	12.5=1.0	19.4=1.0
Tricyclene	0.4122	0.4031	0.4468	0.3761	0.3960	0.4890
Camphene	0.5424	0.5671	0.5958	0.5859	0.5208	0.6081
1:8-Cineole	1.237	1.479	1.191	1.1630	1.1280	1.021
Cyclohexanone	1.728	2.369	0.9318	0.9506	0.8508	0.3299
Benzene	0.2332	0.2547	0.1229	0.1109	0.1212	0.0773
Toluene	0.4122	0.3751	0.2666	-	-	-
Methyl alcohol	0.1356	0.1719	0.046	-	-	-
Ethyl alcohol	0.1660	0.2266	0.065	-	-	-

Table 3

Relative retention times (with respect to camphor) at 163-164°

Compound	Succinic acid diethylene glycol polyester.	Azelaic acid diethylene glycol polyester.	Brassylic acid diethylene glycol polyester.	Adipic acid 1,4-butene diol polyester.	Adipic acid 1,4-butane diol polyester.	ω-Hydroxy stearic acid polymer*	Aleuritic acid polymer*
α-Terpineol	1.435	1.644	1.608	1.577	1.729	1.547	1.696
Linalool	0.6296	0.7568	0.7476	0.6893	0.7762	0.6373	0.7365
Borneol	-	-	-	1.496	1.646	1.418	1.798
Isoborneol	-	-	-	1.234	1.399	1.2019	1.560
Menthol	-	-	-	1.190	1.454	1.235	-
Camphor	5.40=1.0	9.25=1.0	10.3=1.0	11.1=1.0	12.15=1.0	5.75=1.0	3.95=1.0
Carvone	-	-	-	2.072	2.173	-	-
Dihydrocarvone	-	-	-	1.365	1.473	-	-
Longifolene	0.6685	1.329	1.971	1.0	1.338	2.28	1.63
Caryophyllene	-	-	-	-	1.427	2.423	-
Humulene	1.019	1.909	2.617	1.432	1.904	2.869	2.15
Longicyclene	-	-	-	0.7523	1.050	1.826	-
Cyclopentadecane	-	-	-	1.712	2.301	5.217	-

* Relative retention time data were recorded on neutralised column filling.

of the remaining terpenic hydrocarbons, there is no significant change in the relative retention values. 1,8-Cineole presents an interesting example. It has an oxygen atom in the form of 'ether linkage'. Its relative retention time (limonene = 1) on saturated polyester happens to be 1.24 only, but the same figure rises to 1.48, when recorded on unsaturated polyester.

When we take into consideration the relative retention time data recorded at 163-165°, we find the same differences in the two cases. For example a mixture of camphor and longifolene can be easily resolved on the corresponding saturated polyester. But the same pair cannot be separated easily on the unsaturated polyester. The ω-hydroxy stearic acid polymer column seems to be the best for the separation of camphor and longifolene. Here relative retention value for this pair happens to be 2.28. At the same time it is interesting to note the relative retention time value for this pair on succinic acid diethylene glycol polymer. This value happens to be 0.669, which indicates that the order of emergence of these two compounds is reversed due to the change in the nature of the polyester. It must be noted that, on succinic acid diethylene glycol column, camphor and humulene would overlap. But this pair can be easily separated on ω-hydroxy stearic acid polymer.

Therefore it seems that for over all separation of monoterpenic oxygenated compounds and C-15 hydrocarbons, ω-hydroxy stearic acid polymer column would give a better performance.

It is interesting to observe the separation of the individual components of the monoterpenic oxygenated compounds. Separation of borneol and camphor, may serve as a typical example. The relative retention time of borneol with respect to camphor, on aleuritic acid polymer, happens to be 1.80. This is the maximum value amongst all recorded in Table 3. This in turn may indicate that in the case of aleuritic acid polymer, some of the 'OH' groups are remaining 'free'. These free groups in turn may have some strong interactions with alcohols.

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Description of the chromatograms

Numerous chromatograms had to be recorded during the investigations, described in this part. So only those chromatograms are chosen, which represent the important points of the present investigations. The experimental conditions are described along with each figure.

Fig. 1a Sample: Camphor.

Phase: Aleuritic acid polymer column filling (before alkali washing).

Temperature: 163-164°;

Flow rate: 4 lit./hr., hydrogen.

When camphor was analysed on this stationary phase, it recorded a peak as shown in Fig. 1a. The sharp front and the enormous tailing may be noted (retention time: 17.1 minutes).

Fig. 1b Sample: Camphor.

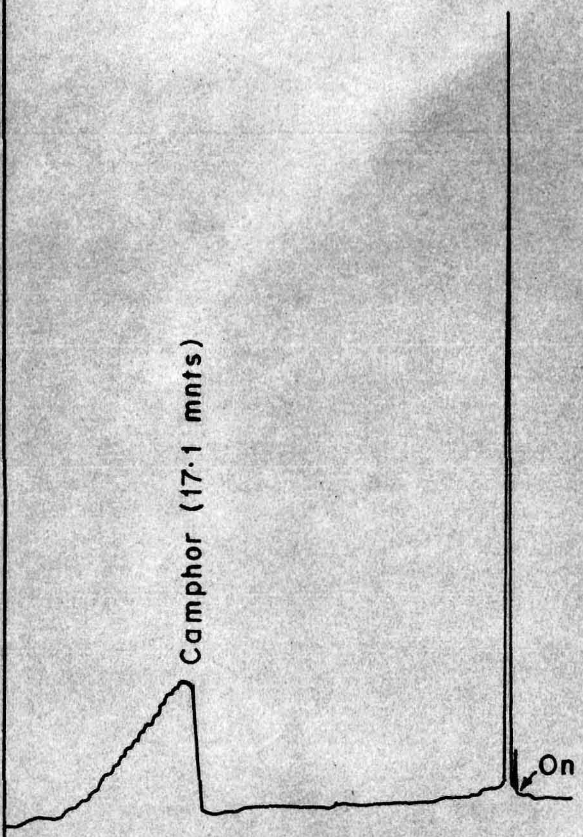
Phase: Aleuritic acid polymer column filling (after washing with alkali).

Temperature: 163-164°

Flow rate: 4 lit./ hr., hydrogen.

When camphor was analysed on this column filling, a sharp peak was obtained as shown in Fig. 1b. Tailing was

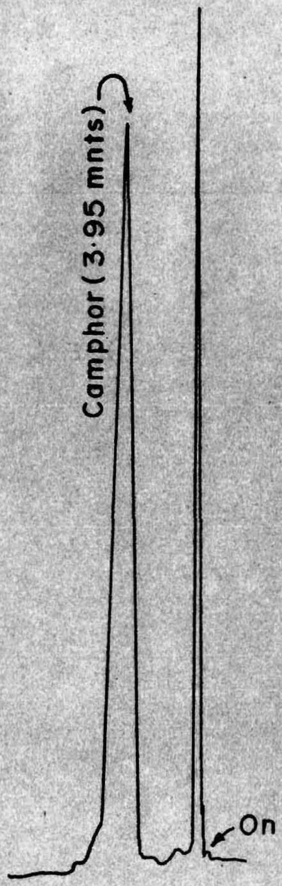
FIG. 1a.



Temp. 163-164°

Phase:
Aleuritic acid polymer column filling

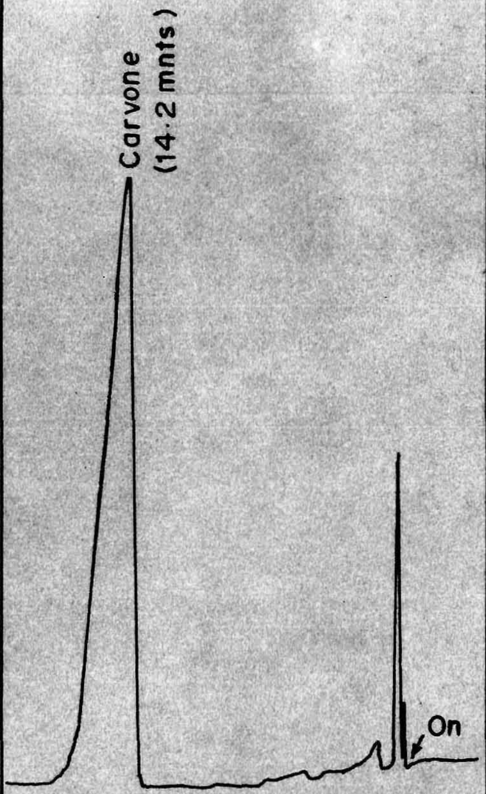
FIG. 1b.



Temp. 163-164°

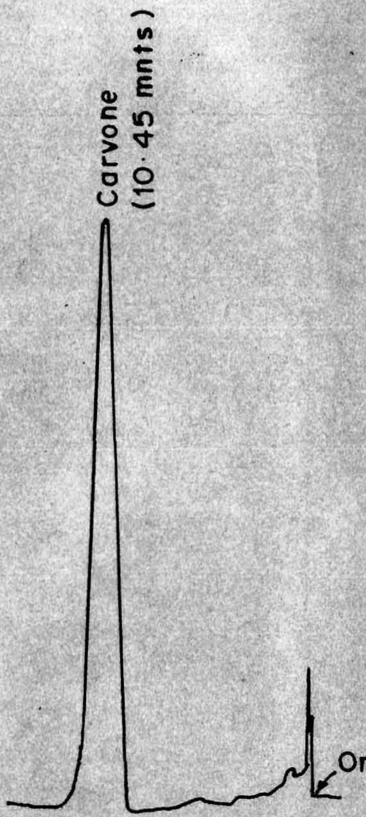
Phase:
Aleuritic acid polymer column filling
(After washing with alkali)

FIG. 2a.



Phase:
ω -hydroxy stearic acid polymer column filling.

FIG. 2b.



Phase:
ω -hydroxy stearic acid polymer column filling (Neutralised)

greatly reduced. But the retention time was found to be 3.95 minutes only.

<u>Fig. 2a</u> Sample: Carvone.

Phase: ω-Hydroxy stearic acid polymer column filling.

Temperature: 163-164°

Flow rate: 4 lit/hr., hydrogen.

It can be seen from this figure, that tailing does exist even after prolonged column conditioning.

<u>Fig. 2b</u> Sample: Carvone.

Phase: ω-Hydroxy stearic acid polymer column filling (Neutralised).

Temperature: 163-164°.

Flow rate: 4 lit./hr., hydrogen.

The same sample, carvone, was analysed. It may be seen that tailing is reduced.

Note the loss in retention time.

<u>Fig. 3a</u> Sample: Linalool.

Phase: ω-Hydroxystearic acid polymer column filling.

Temperature: 163-164°

Flow rate: 4 lit./hr., hydrogen.

It may be seen from this figure, that linalool, a tertiary alcohol, is decomposed.

<u>Fig. 3b</u> Sample: Linalool.

Phase: ω-Hydroxystearic acid polymer column filling (Neutralised).

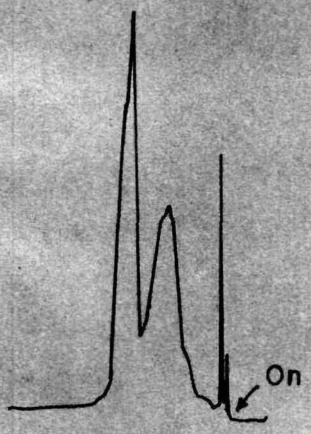
Temperature: 163-164°

Flow rate: 4 lit./hr., hydrogen.

In this case, linalool does not decompose.

FIG. 3a.

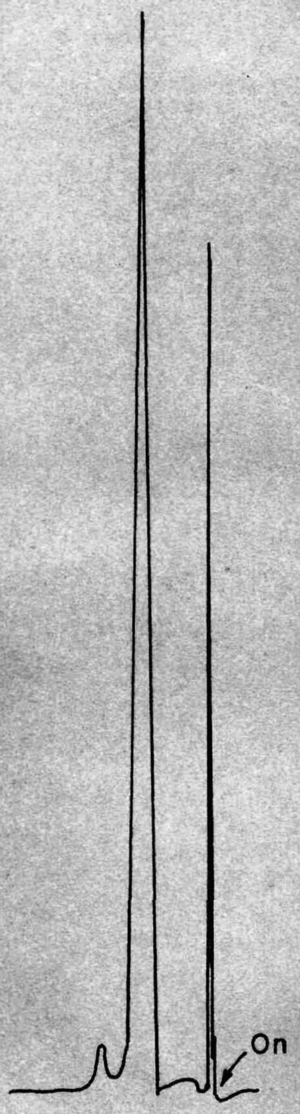
Linalool (decomposed)



Phase: ω-hydroxy stearic acid polymer column filling.

FIG. 3b.

Linalool



Phase: ω-hydroxy stearic acid polymer column filling (**Neutralised**).

Fig. 4

Sample: A mixture of methylheptenone, linalool citral, longicyclene, longifolene and humulene.

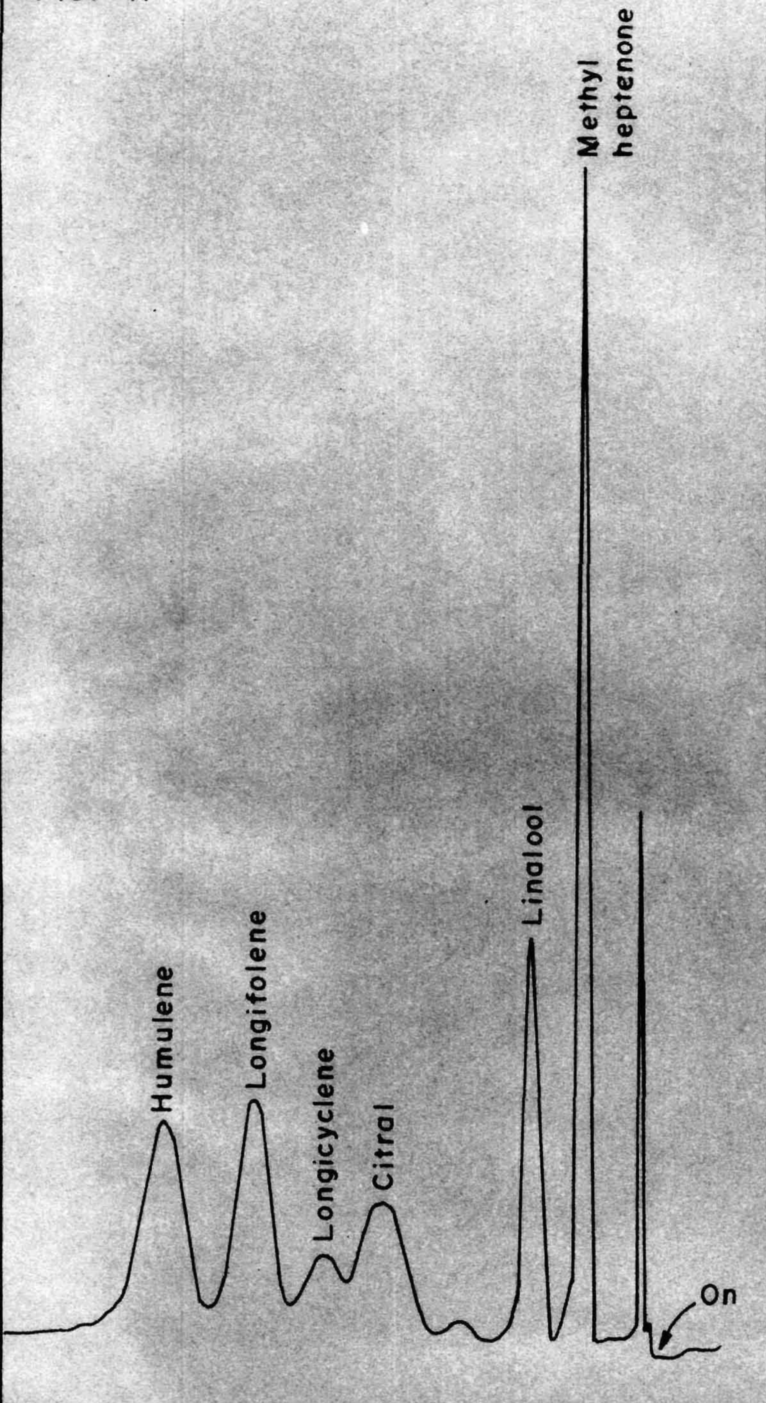
Phase: ω-Hydroxystearic acid polymer column filling.

Temperature: 180°

Flow rate: 2.66 lit./hr., hydrogen.

It may be seen from this figure, that all the components are well separated. Note that the geometrical isomers of citral are not separated. But citral can be easily resolved into two geometrical isomers by using succinic acid-diethylene glycol polyester as a stationary phase, as may be seen from Fig. 5.

FIG. 4.

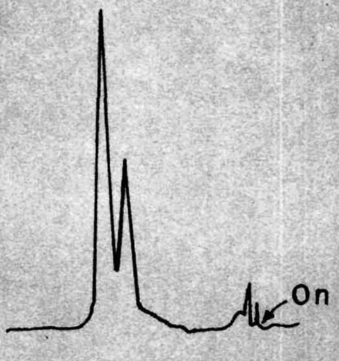


Phase: ω-hydroxy stearic acid polymer (<u>Neutralised</u>)

Temp. 180°.

FIG. 5.

Citral.



Phase: Succinic acid - diethylene glycol polyester.

Temp. 179 - 180°.

Fig. 6

Sample: Longicyclene and longifolene.

Phase: ω-Hydroxystearic acid polymer column filling
(Neutralised).

Temperature: 180°

Flow rate: 2.66 lit./hr., hydrogen.

The separation of these two compounds, is somewhat incomplete. The relative retention time of longifolene, with respect to longicyclene, was found to be 1.18 only.

Fig. 7

Sample: Longicyclene and longifolene.

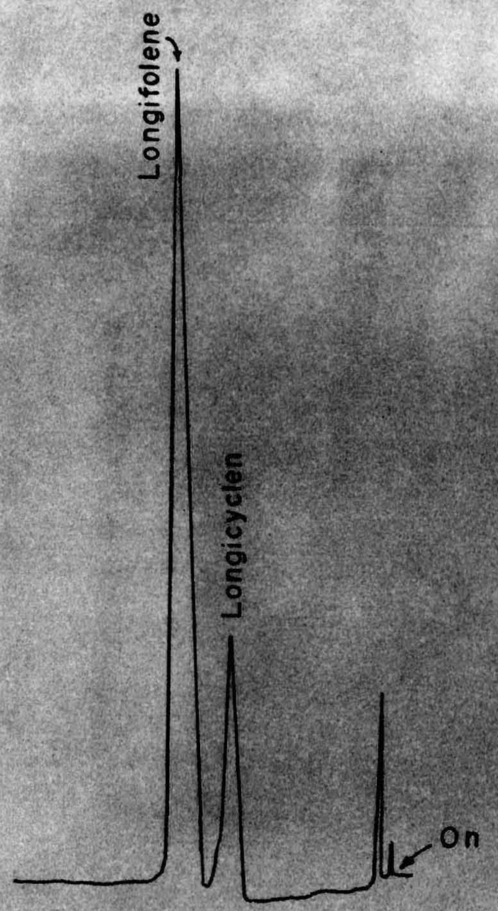
Phase: 1,4-Butene diol adipic acid polyester.

Temperature: 163-164°

Flow rate: 4 lit./hr., hydrogen.

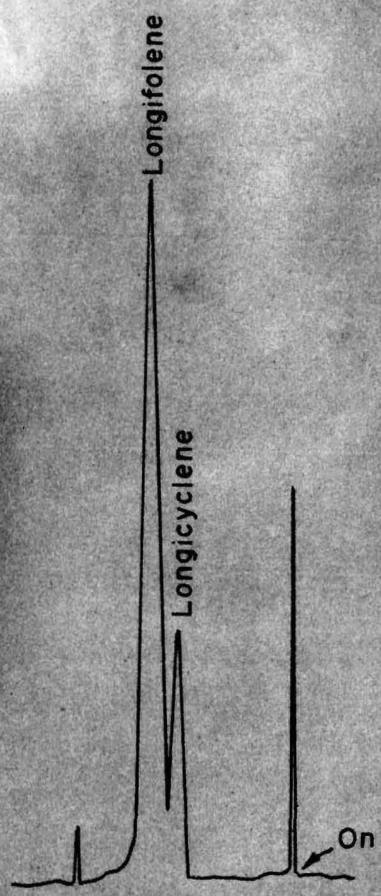
The separation of the two compounds is almost complete. The relative retention time of longifolene, with respect to longicyclene, was found to be 1.32.

FIG. 7



Phase: Adipic acid-1,4-butene diol polyester

FIG. 6



Phase: ω-hydroxy stearic acid polymer column filling (Neutralised)