THE SEPARATION OF DIASTEREOISOMERS BY GAS-LIQUID CHROMATOGRAPHY.

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TO MY PARENTS.

A B S T R A C T.

The preparation and the gas chromatographic behaviour of several series of diastereoisomeric compounds is described. The series are the following: (1) Alkyl lactates, (2) Alkyl α-alkanoyloxypropionates, (3) Dialkyl esters of 2,3-butanediol, (4) Di-(haloacetic) esters of 2,3-butanediol and (5) α,β-Diols.

Chromatography was performed at various temperatures and a variety of stationary phases was used. Capillary columns were used for chromatography of series 1 and 2 whereas packed columns were used for series 3 to 5.

It was found that for all of the above series nearly all of the compounds could be separated into diastereoisomers. The separability was found to be a function of the chemical structure of the compounds, of the polarity of the stationary phases and of the temperature at which chromatography was performed.

Under appropriate conditions nearly all of the above compounds could be fully or partially separated into diastereoisomers. The only exception encountered was that of 2,3-di-(trichloroacetoxy) butane which could not be even partially separated into diastereoisomers on any column under any conditions.

The order of elution of the separable diastereoisomers of all the compounds of series 2 to 5 was established. For the α-alkanoyloxypropionates, the diastereoisomers prepared from acid and alcohol both having thesame conventional symbol of configuration were always eluted before the corresponding diastereoisomers of alternative configurations. For the diesters of 2,3-butanediol the meso isomers were always eluted before the corresponding racemic isomers. For the α,β-diols the racemic isomers were always eluted before the corresponding meso isomers.

The order of elution of all the compounds in series 2 to 5 could be explained in terms of appropriate mechanisms of separation.

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SECTION 1 INTRODUCTION.

The original project on which this thesis is based was to investigate the possibility of separating enantiomers by gas-liquid chromatography.

It was decided that the approach most likely to succeed would be to convert the enantiomers into diastereoisomers and to attempt to separate these diastereoisomers by gas-liquid chromatography. It was found that this could be achieved, for certain compounds, with relative ease on capillary columns. The results of this investigation are described in section 5.1.

The compounds described in section 5.1 are relatively complex and their chromatographic behaviour could not be explained in terms of chemical structure. It was therefore decided to study a series of diastereoisomers of simple chemical structure and for this purpose 2,3-butanediol and its diesters were chosen.

The chromatographic behaviour of these compounds was found to be suitable for an investigation of this type. The resolution factors (see section 3) of these diasteroisomeric compounds varied both with their chemical structure and with the polarity (see section 5.6) of the stationary phases used for the separations. It was found possible to rationalize the chromatographic behaviour of these diastereoisomers in terms of mechanisms of separation which predict the order of elution of the diastereoisomers in terms of their chemical structure.

The chromatographic behaviour of certain simple compounds (alkyl halides and ethyl haloacetates) is included in section 5.3.

These compounds were studied to obtain a deeper insight into the behaviour of the haloacetic esters of 2,3-butanediol.

the choice of phase may explain why different authors reported different orders of elution of diastereoisomers.

During the period 1959 to 1962 separations of the following classes of diastereoisomers were also reported: paraffinic hydrocarbons[4], olefinic hydrocarbons[5], alcohols[6,7,8,9], esters[10], dipeptides[11,12] and ketals[13]. Thus it is seen that various classes of diastereoisomers can be separated.

Most of the above references merely refer to the separations of one or two pairs of diastereoisomers, but references 10 and 13 are of more general interest.

Casanova and Corey[13] have shown that it is possible to separate* the enantiomers of racemic camphor by reacting it with one of the enantiomers of racemic-2,3-butanediol, and by separating the resulting pair of diastereoisomers by preparative gas chromatography (i.e. collecting the fractions) and hydrolysing each fraction to obtain the two separated enantiomers of camphor.

These authors reported that while an analytical separation of the two diastereoisomers could be achieved using 1,2,3-tri-(2-cyanoethoxy) propane as stationary phase, five other phases yielded only partial separation of the diastereoisomers. They also reported that the corresponding ketal of norcamphor could not be separated on the 1,2,3-tri-(2-cyanoethoxy) propane column.

* The term resolution is avoided in this sense to prevent confusion with resolution of gas chromatographic peaks (see section 3).

The problems to which these authors have drawn attention, namely the effect of the nature of the stationary phase on the separation of diastereoisomers and the effect of structure of diastereoisomers on their separability, are still only partially understood.

SECTION 2.2 CLASSES OF COMPOUNDS THAT HAVE BEEN MORE FULLY INVESTIGATED.

A. Derivatives of α-Hydroxy Acids.

Gil-Av and Nurok[10] have studied the chromatographic behaviour of a series of sec-alkyl lactates and a series of sec-alkyl α-alkanoyloxypropionates. They found that the lactates could be only partially separated into diastereoisomers on capillary columns coated with either a polar or an apolar phase, whereas each of the members of the series of α-alkanoyloxypropionates could be separated on either column.

They showed that the plot of log retention time against the number of carbon atoms per solute molecule yielded a pair of nearly straight lines and assumed that one line corresponded to diastereoisomers prepared from acid and alcohol both having the same conventional symbol of configuration (i.e. both D or both L) whereas the second line corresponded to diastereoisomers of the alternative configurations. By using optically active starting material for one of the compounds it was shown that the lower line corresponded to diastereoisomers prepared from acid and alcohol both having the same conventional symbol of configuration. Thus they showed that it was possible to assign configuration to a diastereoisomer (and hence in certain cases to an enantiomer) from its gas chromatographic retention

volume. The assignment was checked by Gil-Av and co-workers[14,15,16] by synthesizing eighteen α-alkanoyloxypropionates from optically active starting materials.

These authors have also shown that the relative areas of gas chromatographic peaks are in good agreement with the relative proportions of diastereoisomers as determined by polarimetry. They stated [16] 'that calibration factors are not required for determining the ratio of the diastereoisomeric compounds, except perhaps where very high accuracy is required'.

They reported resolution factors varying from 1.04 to 1.10.

The effect of varying the alkyl group and the α-alkanoyloxy group has been investigated by Gil-Av and co-workers [10,16] and also by Stern and Karger[17]. It was reported that resolution factor was insensitive to the nature of the α-alkanoyloxy group but was sensitive to the nature of the asymmetric alcohol used for esterification.

The separation of the diastereoisomers of 1,1,1-trifluoro-2-propyl O-methylmandelate has been reported[18] but no detail of order of elution has been included in the communication.

B. Derivatives of Amino Acids.

More papers have been published on the separation of diastereoisomers derived from amino acids than on diastereoisomers of any other single class. This is due to the great biological importance of these compounds.

Three approaches to the preparation of diastereoisomers from amino acids have been used. The first is to introduce a second asymmetric

centre by esterifying the amino acid with an asymmetric alcohol. The second is to form a dipeptide by condensing two amino acids. The third is to introduce a second asymmetric centre by acylating the amino group with an acylating agent other than an amino acid.

The first approach has been used by Gil-Av and co-workers[14,15,16,19] who reported the separation of the diastereoisomers of 12 pairs of sec-butyl or sec-octyl N-trifluoroacetyl amino acid esters on capillary columns. Resolution factors varied from 1.01 to 1.10.

Pollock and co-workers[20,21] have used the same approach and have separated the diastereoisomers of 21 pairs of sec-butyl N-trifluoroacetyl amino acid esters on capillary columns. Resolution factors varied from 1.01 to 1.07.

The results of both Gil-Av and Pollock showed that for all amino acids (with the exception of phenylglycine) on all phases, the diastereoisomers formed from acid and alcohol both having the same conventional symbol of configuration (i.e. both D or both L) had lower retentions than the corresponding diastereoisomers of alternative configurations. This is analogous to the behaviour of the α-alkanoyloxypropionates and is to be expected as the difference between the two classes of compounds is due to nitrogen being substituted for oxygen on one of the asymmetric carbon atoms.

Vitt and co-workers[22] have reported the separation of 3 pairs of diastereoisomers of L-menthyl N-trifluoroacetyl amino acid esters on a packed column.

The order of elution of diastereoisomers was the same as that

reported by Gil-Av and Pollock but the resolution factors (calculated from retentions reported in the communication) varied from 1.11 to 1.16. On account of these relatively high resolution factors and of the accessibility of L-menthol it would appear that it is at present the most suitable alcohol for preparing diastereoisomeric esters from amino acids for subsequent chromatographic separation by this first approach.

The second approach is due to Weygand and co-workers[11,12,23] who have investigated the separation of the diastereoisomers of 35 N-trifluoroacetyl dipeptides on capillary and (in a few cases) packed columns at temperatures ranging from 180° to 220°.

Resolution factors (calculated from retentions reported in the paper) varied from 'too small to measure' (i.e. only a shoulder obtained) to 1.25 for the proline-valine dipeptide.

In reporting separations of such amino acids as N-trifluoroacetyl dipeptide methyl esters care must be taken to indicate which amino group is attached to the trifluoroacetyl group and which amino acid is attached to the carboxyl group of the second amino acid. By inspection of the retentions reported by Weygand it may be concluded that the order of elution of diastereoisomers is not affected by which amino acid of the dipeptide is methylated, but that in some cases the resolution between chromatographic peaks may be markedly affected. Thus for the dipeptide alanine-methionine, resolution* (under identical chromatographic conditions) changed from 51% to 10% according to which amino acid was methylated.

* These authors used a different definition of resolution to that used in this thesis. By their definition, 100% resolution corresponds to two completely separated peaks.

The order of elution was such that diastereoisomers formed from two amino acids both having the same conventional symbol of configuration were eluted before the corresponding diastereoisomers of alternative configurations. The only pair of diastereoisomers having the opposite order of elution were those of the alanine-alanine dipeptide.

The authors investigated the degree of racemization in synthesizing the valine-valine dipeptide by eight different methods and found that it varied from 'no racemization' to 80% racemization depending on the method used.

Halpern and Westley [24,25] have separated the diastereoisomers of six dipeptides on a packed column at temperatures ranging from 176° to 220°. They reported that the dipeptides formed from amino acids of the same conventional symbol of configuration were eluted after the corresponding /dipeptides of alternative configurations. This is opposite to the order of elution reported by Weygand even though two of the dipeptides (proline-valine and proline-leucine) were chromatographed by both groups of workers.

The third approach to the formation of diastereoisomers from amino acids is to treat the amino group with an asymmetric acylating agent other than a second amino group. This method has been used by Halpern and Westley[26] and by Landowne and Lande[27]. Both pairs of authors claim the method to be superior to the dipeptide method for certain amino acids.

The former authors have separated the diastereoisomers of 15 N-α-chloroacyl amino acid methyl esters on packed columns at temperatures between 140° and 214°. They report that in all cases the

diastereoisomers formed from the amino acid and the acylating reagent of the same configuration had higher retentions than the corresponding diastereoisomers of alternative configurations. The acylating reagents were prepared from amino acids by a completely stereospecific conversion of the amino group to a chloro group. They investigated both a series of α-chloroisovaleryl-amino acid methyl esters (α-chloroacyl group derived from valine) and a series of N-chloroacyl-valine methyl esters in which the chloroacyl group was derived from various amino acids. For each series resolution factors varied from about 1.06 to 1.25.

The approach of Landowne and Lande[27] was to use either α-chloropropionic acid or α-bromopropionic acid as acylating agent. They reported that the chloro derivatives had larger resolution factors than the bromo derivatives whereas the electron capture detector was more sensitive to the bromo derivatives than the chloro derivatives. No retentions or resolution factors were reported.

C. Diastereoisomeric Alcohols.

Gault and Felkin[7,28] have investigated the separation between diastereoisomers of a series of alcohols of the general formula $RCH(M_e)CHOHR'$. The diastereoisomers were either of the threo or erythro configuration. (These configurations are defined in reference 28). R corresponds to any of various hydrocarbon groups that are either saturated or unsaturated and R' corresponds to various saturated hydrocarbon groups.

Chromatography was performed with packed columns using phases that could form hydrogen bonds with alcohols. Resolution factors varied from about 1.0 (i.e. 'no resolution') to 1.49 and temperatures

at which chromatography was performed were in the range 70° to 178°.

These authors reported that the diastereoisomers fall into two classes. The first class consisted of those diastereoisomers where an intramolecular hydrogen bond could exist between the hydroxyl and an unsaturated group. For a pair of these diastereoisomers it was that isomer which had the smaller population of molecules in the hydrogen bonded form that had the higher retention. This is the erythro isomer.

The second class of compounds consists of those that cannot form intramolecular hydrogen bonds either because R is saturated or because the position of unsaturation was considered to be too distant from the hydroxyl for intramolecular hydrogen bonding. For this class of compounds the diastereoisomers of threo configuration had the larger retentions. The authors considered that this is due to the threo isomers existing in conformations such that the hydroxyl is more exposed to the stationary phase.

Diastereoisomers of the first class were easier to separate than those of the second class.

From the above examples it is seen that several classes of diastereoisomers may be separated and that for certain classes the order of elution may be predicted for most members of the class.

SECTION 3. PRINCIPLES OF GAS CHROMATOGRAPHY.

DEFINITION AND DESCRIPTION OF GAS CHROMATOGRAPHY.

Gas-liquid chromatography is a method of separation in which the components to be separated are introduced into a column where they are distributed between a mobile gas phase and a stationary liquid phase, the two phases being in intimate contact. The mobile gas phase is called the carrier gas.

Components are eluted by the carrier gas from the column in an order that is dependent on the gas-liquid partition coefficient of each component. The volume of gas required for elution of a component is called the retention volume of that component.

The retention volume is dependent on column geometry. This difficulty may be overcome by relating the retention volume of a component to that of a standard substance chromatographed under the same conditions. This is done by calculating the ratio of the adjusted* retention volume (see ahead) of the component to the adjusted retention volume of the standard substance. The ratio is called the relative retention volume of the component.

The degree of separation between diastereoisomers is conveniently expressed as a relative retention with the diastereoisomer of lower retention volume as standard. To avoid any ambiguity in this thesis the relative retention between diastereoisomers is called resolution factor.

The adjusted retention volume of a component is defined as its retention volume minus the retention volume of a substance that

* This nomenclature is according to reference 29.

does not interact with the stationary phase. In work using a thermal conductivity detector air is chosen for the latter substance.

Retention volume is dependent on temperature. Isothermal gas chromatography involves the operation of a gas chromatograph at constant temperature as in the work described in this thesis.

A conventional gas chromatograph is so arranged that there is a device at the end of the column whereby components are detected as they are eluted from the column. The device is called a detector, and in the work described in this thesis a thermal conductivity detector (katherometer) was used for this purpose, unless otherwise stated.

THERMODYNAMIC EQUATIONS AND SOLUTE-SOLVENT INTERACTION FORCES.

A pair of diastereoisomers may in principle be separated by gas-liquid chromatography if a phase is available for which they have different free energies of solution at any temperature at which they are chemically stable and sufficiently volatile for analysis. If the difference in free energy of solution is small, the separation may be very difficult to achieve in practice.

The free energy of solution, ΔG_s, is related to the adjusted retention volume (V_R') by eqn (1).

$$\Delta G_s = -RT\ln(kV_R') \quad \ldots\ldots\ldots\ldots (1)$$

k is a constant that is dependent on chromatographic conditions, and is the same for any solute chromatographed under the same conditions.

Now $$\Delta G_s = \Delta H_s - T\Delta S_s \quad \ldots\ldots\ldots\ldots (2)$$

where ΔH_s is the heat of solution and ΔS_s is the entropy of solution.

Considering two substances 1 and 2

$$\Delta(\Delta G_s) = (\Delta G_s)_1 - (\Delta G_s)_2 = (\Delta H_s)_1 - (\Delta H_s)_2 + T\left[(\Delta S_s)_2 - (\Delta S_s)_1\right] \quad . \quad . \quad . \quad (3)$$

It is however reasonable to assume that entropy of solution is the same, or very nearly so, for each of a pair of diastereoisomers. Any differences in entropy of solution most probably arise from differences in entropy of mixing for those diastereoisomers that exist in a number of conformations.

Separation of diastereoisomers by gas-liquid chromatography is thus normally due to differences in heats of solution of the isomers.

The heat of solution of each diastereoisomer is a function of the various cohesion forces that operate between solute and solvent. These are:

(a) Dispersion Forces.

Due to vibrations associated with the zero point energy of any molecule, rapidly varying transient dipoles occur. Dipoles that are in phase with these transient dipoles are induced in neighbouring molecules and attractive forces result. These forces operate between all types of molecules. The interaction energy is given by equation (4).

$$E = -\frac{3}{2}\,\alpha_A \alpha_B \times \frac{I_A I_B}{r^6 (I_A + I_B)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Where α_A and α_B are the polarizabilities of the solute and solvent molecules, I_A and I_B are their ionization potentials and r is the distance between a pair of solute and solvent molecules.

The polarizability of a molecule may be calculated from the Clausius-Mossotti equation:

$$\alpha = \frac{3}{4\pi N} \times \frac{n^2-1}{n^2+2} \times \frac{M}{\rho} \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

The quantity $\frac{n^2-1}{n^2+2} \times \frac{M}{\rho}$ is called the molar refraction of a compound. N is Avogadro's number, M is the molar mass of the solute molecules, n is the refractive index of the liquid solute and ρ is its density. Ideally refractive index at infinite wavelength should be used but n_D may be used as a good approximation.

Equation (4) was originally derived for two small molecules, but Littlewood[30] has discussed how it may be used to estimate dispersion forces operating in gas-liquid chromatography.

For estimating dispersion forces operating for a particular solvent, equation (4) may be written $E = -k(\frac{n^2-1}{n^2+2})\frac{M}{\rho}$ ..... (6) Where k is a constant and n, M and ρ refer to the solute molecules. Implicit in equation (6) is the assumption that I_A and r^6 are the same for each compound considered. For a pair of diastereoisomers this should be true, or very nearly so. It should also be approximately correct for cases where ionization potential varies (as in a series of haloacetates) in that the variation in $\frac{I_A I_B}{I_A+I_B}$ is less than that in I_A.

Dipole-Dipole Interactions: The energy of interaction of a solute dipole and a solvent dipole is given by the expression

$$E = \frac{2\mu_1\mu_2}{3kTr^6} - \frac{2\mu_2^4}{3kTr^6} \quad \ldots\ldots\ldots\ldots\ldots (7)$$

Where μ_1 and μ_2 are the permanent dipoles of solute and solvent respectively, r is the distance between dipoles, k is the Boltzman constant and T is the absolute temperature. The term in μ_2^4 is due

to the fact that a fraction of the solvent dipoles are paired with other solvent dipoles. The magnitude of this fraction is dependent on solvent type. For solvents with many polar functional groups per molecule the fraction approaches unity, whereas for solvents with only one polar group per molecule the fraction is smaller.

Induction Forces.

A permanent dipole in either the solute or the solvent can induce a dipole in the other, and will then interact with it. The mean energy of this interaction is $E = \frac{1}{r^6}(\alpha_1 \mu_2^2 + \alpha_2 \mu_1^2)$ . . . . (8).

Hydrogen Bonding. A hydrogen bond is of the form X-H-X where X may be nitrogen, oxygen or fluorine. Carbon can also participate in a hydrogen bond but only when a strongly electronegative group is attached to the carbon atom[40]. The hydrogen bond is generally accepted to be electrostatic or ionic in nature and varies in bond energy from 2 to 8 k cal per mole. It is not necessarily colinear.

Complex Formation.

This is not a physical cohesion force, but an example of chemical bonding.

Solvents incorporating metal ions are capable of forming complexes with certain solutes. Thus olefins are selectively retarded on phases containing silver nitrate while amines and amino acid esters are selectively retarded on phases of various transition metal salts of fatty acids. Separations may be spectacular (e.g. the often quoted separation of γ-picoline and 2,6-lutidine[31]) and it is surprising that the separation of diastereoisomeric amino acid esters has not been

attempted using these phases.

The above cohesion forces operate in any solute-solvent system even though in many systems any of the interactions may make a vanishingly small contribution or in the cases of hydrogen bonding and complex formation, no contribution at all.

Phenomena related to free energy of solution are not the only factors influencing separations by gas-liquid chromatography.

The degree of separation between two substances is best discussed in terms of resolution which has been defined in many ways. A convenient expression is due to Golay[32].

$$R = N^{\frac{1}{2}}(1-\frac{x_1}{x_2}) \qquad \ldots\ldots\ldots\ldots\ldots (9).$$

Where N is the column efficiency and is a measure of the degree of peak broadening which is caused by various physical effects. x_1 and x_2 are the retention volumes of the two substances being considered ($x_2 > x_1$).

From equation (9) it is seen that as $\frac{x_1}{x_2}$ approaches unity the resolution becomes relatively more sensitive to minor variations in this ratio. In general diastereoisomers have a ratio of retention volumes that is close to unity.

The quotient $\frac{x_1}{x_2}$ is called separation factor and is used by some authors to report separations between pairs of diastereoisomers. It is however dependent on column geometry and thus separation factor will not be the same as the resolution factor for diastereoisomers of low retention volume. The term separation factor is not used in this thesis.

SECTION 4.1. SYNTHESIS OF DIASTEREOISOMERS.

PREPARATION OF ALKYL LACTATES AND α-ALKANOYLOXYPROPIONATES.

Alkyl Lactates. Esterification was carried out by refluxing in benzene a solution which contained 20% by weight of reactants. These consisted of 1 mol. proportion of lactic acid (40% aqueous solution) per four mol. proportions of the appropriate alcohol. Two percent, by weight of solution, of p-toluenesulphonic acid was used as catalyst. After all the water formed had been removed by azeotropic distillation (2 to 3 hours), the reaction mixture was cooled, neutralized with 30% aqueous sodium carbonate solution, washed with water, dried over anhydrous sodium sulphate and distilled in vacuo.

The boiling points of the lactates are listed in table 1.

The infrared spectra of the lactates have normal ester carbonyl absorptions (1740 to 1750 cm^{-1}) and hydroxyl absorptions (3460 - 3480 cm^{-1}).

The microanalyses of these compounds are not satisfactory. This is attributed to the presence of dilactide which is formed under the reaction conditions and which could be detected as a high boiling fraction. These lactates were identified by converting them to α-acetoxypropionates which gave satisfactory microanalyses. The percentage yields of the lactates varied between 50% and 80%.

The α-Alkanoyloxypropionates. 3 gm of each of the lactates was treated with four times the theoretical quantity of the appropriate acyl chloride under anhydrous conditions, and in each case the mixture was heated to gentle reflux for four hours. In each case the product

TABLE 1.

BOILING POINTS OF ALKYL LACTATES.

ALCOHOL FROM WHICH DERIVED	BOILING POINT
2-n-BUTANOL	75-76°/19 m m.[a]
2-n-PENTANOL	99.5°/27 m m[b]
2-n-HEXANOL	110-113°/23 m m
2-n-HEPTANOL	123°/23 m m.
2-n-OCTANOL	152-155°/25 m m.
MENTHOL	156-161°/19 m m.
BORNEOL	168-174°/21 m m.

a. Wood, Such and Scarf[51] reported b.p. 65-67°/12 m m

b. Bailey and Hass[37] reported b.p. 75-78°/10 m m.

was cooled, neutralized with 30% aqueous sodium carbonate solution, washed with water, dried over anhydrous sodium sulphate and distilled *in vacuo*.

It was later found that a satisfactory yield of the α-acetoxypropionates could be obtained without isolating the intermediate lactate. After the alkyl lactate had been formed, the solvent was removed and the acyl chloride added.

It was also found that the α-acetoxypropionates could be obtained in high purity by fractional distillation of the untreated acylation mixture. The infrared spectra of these compounds have normal ester carbonyl absorptions (1752 - 1755 cm^{-1}) and have no absorption in the 3400 cm^{-1} region of the spectrum. The microanalyses and boiling points of these compounds are listed in tables 2 and 3. Their percentage yields varied between 55% and 88%.

The fact that the plot of log retention on squalane vs molar mass is a straight line for these compounds substantiates the fact that they form an homologous series.

THE PREPARATION OF 2,3-BUTANEDIOL AND THE IDENTIFICATION OF ITS ISOMERS.

Commercial 2,3-butanediol, purified by distillation, was used for the syntheses of the diesters described in the next section. Its identity was proved by comparing it to a sample of 2,3-butanediol prepared by reducing diacetyl with sodium borohydride by the method of Chaikin and Brown[33]. Both the synthetic and the commercial 2,3-butanediol have an identical infrared spectrum. They each have the same boiling point and their diacetates have identical retention volumes on a 1.6 metre, 20% CYB column at 150°.

Meso-2,3-Butanediol. Wilson and Lucas[34] have published a plot of melting point vs isomer composition for a mixture of the isomers of

TABLE 2.

MICROANALYSES AND BOILING POINTS OF ALKYL α-ACETOXYPROPIONATES PREPARED FROM RACEMIC MATERIAL.

ALCOHOL FROM WHICH DERIVED	MOLECULAR FORMULA	MICROANALYSIS FOUND		MICROANALYSIS CALCULATED		BOILING POINT
		C	H	C	H	
2-n-BUTANOL	$C_9H_{16}O_4$	57.5	8.6	57.4	8.6	102-103°/23 m m [a]
2-n-PENTANOL	$C_{10}H_{18}O_4$	60.2	8.8	59.4	9.0	120°/30 m m [b]
2-n-HEXANOL	$C_{11}H_{20}O_4$	61.0	9.2	61.1	9.3	129-131°/23 m m
2-n-HEPTANOL	$C_{12}H_{22}O_4$	62.6	9.5	62.6	9.6	139-141°/23 m m
2-n-OCTANOL	$C_{13}H_{24}O_4$	63.9	9.9	63.9	9.9	152-155°/25 m m
MENTHOL	$C_{15}H_{26}O_4$	66.6	9.5	66.7	9.6	170-176°/18 m m
BORNEOL	$C_{15}H_{24}O_4$	66.8	8.9	67.1	9.0	170-174°/23 m m

a. Bailey and Hass[37] report b.p. 90-92°/18 m m

b. Bailey and Hass[37] report b.p. 110-111°/25 m m.

TABLE 3.

MICROANALYSES AND BOILING POINTS OF ALKYL α-ALKANOYLOXYPROPIONATES PREPARED FROM RACEMIC MATERIAL.

$RCOOCH(CH_3)COOCH(CH_3)C_2H_5$

NATURE OF R	MOLECULAR FORMULA	MICROANALYSIS FOUND		MICROANALYSIS CALCULATED		BOILING POINT
		C	H	C	H	
C_2H_5	$C_{10}H_{12}O_4$	60.0	8.9	59.4	9.0	110-112°/21 m m
n-C_3H_7	$C_{11}H_{20}O_4$	61.2	9.2	61.1	9.3	125-126°/24 m m
n-C_4H_9	$C_{12}H_{22}O_4$	62.5	9.6	62.6	9.6	136-137°/26 m m
n-C_5H_{11}	$C_{13}H_{24}O_4$	63.8	10.0	63.9	9.9	154°/25 m m

2,3-butanediol. The isomer composition of a mixture containing more than about seventy percent of meso diol (i.e. having a melting point greater than 7.6° which is the melting point of the racemic diol) may be unambiguously determined from the plot. From this plot and from gas chromatography it was determined that a commercial sample of pure 2,3-butanediol (m.p. 27-29°) consisted of about 95% of the meso isomer and about 5% of the racemic isomer.

Racemic-2,3-Butanediol. This was prepared by the method of Bottari and Macchia[35] from a mixture of racemic- and meso-2,3-butanediol. The method involves refluxing 2,3-butanediol monosodium alkoxides in toluene for 18 hours, decanting the bulk of the toluene from the solid alkoxides, neutralizing the alkoxides with dilute aqueous acid, extracting the aqueous solution with ether, drying the ether extract with sodium sulphate and then distilling the extract.

PREPARATION OF DIESTERS OF 2,3-BUTANEDIOL.

2,3-Diacetoxybutane and 2,3-di-(trichloroacetoxy)butane were prepared in both the racemic and the meso form by separately esterifying both racemic- and meso- 2,3-butanediol using methods indicated below. The other diesters were prepared by esterifying 2,3-butanediol which consisted of 75% meso diol and 25% racemic diol. As the yields were greater than 50% of theoretical and as the stereochemistry of the asymmetric carbon is not affected by esterification, the relative areas of the gas chromatographic peaks could be used to indicate which peak corresponded to which isomer. The detector response is the same for both series of diastereoisomers (See section 4.2).

The Dialkyl Esters. These were prepared by a method analagous to that used by Wilson and Lucas[34] for the preparation of 2,3-diacetoxybutane. A weighed amount of 2,3-butanediol (4 to 10 gm depending on the molar mass of the diester being prepared) was mixed with the appropriate acid anhydride. The proportion of reactants was 1 mol. proportion of 2,3-butanediol per 3 mol. proportions of anhydride. A drop of concentrated sulphuric acid was added as catalyst. After an induction period of about fifteen seconds the mixture became hot and in the case of 2,3-diacetoxybutane started to reflux spontaneously. The mixture was cooled to about 80° to prevent decomposition. It was allowed to stand for twenty-four hours and was then heated at 100° for thirty minutes. On distillation the required diesters were obtained in about 75% yield.

The above method was used with the anhydride of each of the following acids: acetic, propionic, butyric and hexanoic.

The infrared spectra of these compounds have normal ester carbonyl absorptions (1747 to 1748 cm^{-1}) and have no absorption in the 3400 cm^{-1} region of the spectrum. The microanalyses and boiling points of these compounds are listed in table 4. The diacetate and dipentanoate of 2,3-butanediol were purified by preparative scale gas chromatography before microanalysis.

The fact that the plot of log retention on squalane vs molar mass is a straight line for these compounds substantiates the fact that they form an homologous series.

TABLE 4.

MICROANALYSES AND BOILING POINTS OF DIESTERS OF 2,3-BUTANEDIOL. (A MIXTURE OF BOTH ISOMERS OF 2,3-BUTANEDIOL WAS USED FOR PREPARING THE BELOW COMPOUNDS).

ACID FROM WHICH DERIVED	MOLECULAR FORMULA	MICROANALYSIS FOUND			MICROANALYSIS CALCULATED			BOILING POINT
		C	H	HALOGEN	C	H	HALOGEN	
ACETIC	$C_8H_{14}O_4$	54.9	8.0		55.2	8.1		115-123°/16 m m [a]
PROPIONIC	$C_{10}H_{18}O_4$	59.4	9.0		59.4	9.0		112-113°/19 m m
BUTYRIC	$C_{12}H_{22}O_4$	62.8	10.1		62.6	9.61		133-137°/20 m m
VALERIC	$C_{14}H_{26}O_4$	65.1	10.0		65.1	10.1		96-97°/0.5 m m
HEXOIC (AN)	$C_{16}H_{30}O_4$	67.2	10.3		67.15	10.58		113.5°/0.4 m m
FLUOROACETIC	$C_8H_{12}F_2O_4$	45.4	5.8	18.0	45.71	5.76	18.08	---
CHLOROACETIC	$C_8H_{12}Cl_2O_4$	39.7	5.0	28.9	39.55	4.97	29.2	107-108°/0.4 m m
DICHLOROACETIC	$C_8H_{10}Cl_4O_4$	31.2	3.3	45.8	30.8	3.23	45.45	100-104°/0.2 m m
TRICHLOROACETIC	$C_8H_8Cl_6O_4$	25.4	2.0	55.9	25.2	2.1	55.9	126°/.6 m m
BROMOACETIC	$C_8H_{12}Br_2O_4$	29.1	3.3	47.8	28.9	3.6	48.1	175-181°/1.2 m m

a. Wilson and Lucas[34] report b.p. 66-70°/5.5 m m

Di-(haloacetic) esters. Esterification was carried out in refluxing toluene solution which contained 20% by weight of reactants. These consisted of 1 mol. proportion of 2,3-butanediol per mol. proportions of the appropriate acid. Two percent by weight of solution, of p-toluenesulphonic acid was used as catalyst. After all the water formed had been removed by azeotropic distillation the reaction mixture was cooled, neutralized with saturated aqueous sodium bicarbonate solution, washed with water, dried over anhydrous sodium sulphate and distilled to give the required diesters in about 75% yield.

The above method was used with each of the following acids: chloroacetic, dichloroacetic and bromoacetic. It was also used with pentanoic acid but the yield was only 10% in this case.

For trichloroacetic acid the same method was used but no catalyst was necessary. In the case of dichloroacetic acid a catalyst was necessary. In the case of fluoroacetic acid the required diester precipitated out on cooling the reaction mixture. The purity of this product was satisfactory as determined by infrared spectrometry, gas chromatography and microanalysis (see table 4). Recrystallization from petroleum ether (80-100^{o} fraction) yielded only the meso isomer.

The infrared spectra of the haloacetic derivatives have normal ester carbonyl absorptions (in the range 1762 to 1772 cm^{-1}) and have no absorption in the 3400 cm^{-1} region of the spectrum. The microanalyses and boiling points of these compounds are listed in table 4.

CYCLOPENTANE DERIVATIVES.

Cis-cyclopentane-1,2-diol.

Cis-cyclopentane-1,2-diol was prepared by the method of Owen and Smith[36] by the hydroxylation of cyclopentene with neutral aqueous potassium permanganate at -40°. The diol has the following physical constants: b.p. 74-78°/1.8 mm (lit. 88-92°/2mm), N_D^{17} = 1.4772 (lit. N_D^{21} = 1.4770). Found: C = 58.7%, H = 9.7%. $C_5H_{10}O_2$ requires C = 58.7%, H = 9.9%.

Trans-cyclopentane-1,2-diol.

Trans-cyclopentane-1,2-diol was prepared by the method of Owen and Smith[36] by the hydroxylation of cyclopentene with performic acid. The diol has a boiling point: 90-100°/2.5 mm, 131-135°/18 mm (lit. 93°/2 mm, 136°/22 mm). The diol did not, however, have a satisfactory microanalysis and gas chromatography showed it to be impure. The corresponding di-p-nitrobenzoate was prepared from p-nitrobenzoyl chloride. It had m.p. = 138-141° (lit. 143°).

Cis-2,3-diacetoxycyclopentane and Trans-2,3-diacetoxycyclopentane.

These two compounds were prepared from the corresponding cyclopentanediols and acetic anhydride by the same method as used for preparation of the dialkyl esters of 2,3-butanediol (see page 24). The cis-diacetate has boiling point 122-124°/19 mm. The trans-diacetate has boiling point 118°/20 mm. The cis-diacetate has C = 58.2%, H = 7.4%. The trans-diacetate has C = 58.2%, H = 7.6%. $C_9H_{14}O_4$ requires C = 58.1%, H = 7.6%.

Alanine and Valine derivatives.

The sec-butyl esters of N-acetoxyalanine and N-acetoxyvaline were purchased from the Yeda laboratories of the Weizmann Institute of Science.

Bicycloheptanols.

Borneol, isoborneol and norborneol were obtained as a gift from Mr. J. H. Leftin of the Weizmann Institute of Science.

Ethyl Haloacetates.

Commercial samples of these compounds were used. In cases where the compounds were acid to litmus, they were neutralized with sodium bicarbonate, dried over sodium sulphate and then distilled.

SECTION 4.2. GAS CHROMATOGRAPHY.

EXPERIMENTAL TECHNIQUES USED.

All the chromatography described in this thesis, with the exception of that described in section 5.1. was performed using packed columns in a Beckman G.C. 2A gas chromatograph equipped with a katherometer detector. A 1 mv Texas 'Servoriter' pen recorder was used in conjunction with the G.C.2A. The chart speed was set at $\frac{3}{4}$ inch a minute. The columns were constructed of copper tubing with an internal diameter of a quarter inch. Helium was used as carrier gas. Except where otherwise stated the packing consisted of a 20% loading of stationary phase on 80 to 100 mesh chromosorb P (grade HMDS). The chromosorb was supplied by Applied Science Laboratories. This grade of chromosorb was sufficiently inactive for use with all phases except squalane where its use resulted in poorly resolved tailing ester peaks. Satisfactory peaks were obtained when squalane was coated on chromosorb P that had been acid washed, neutralized and then silanized with hexamethyldisilazane and chlorotrimethylsilane. The chromosorb was coated by the slurrying technique.

The relatively high percentage loading was chosen with the purpose of minimizing the effect of adsorption of solute on the chromosorb and on the surface of the liquid phase. It was originally hoped that separation might be correlated with the molecular parameters of the stationary phase. Better peak resolution (see section 3) might have been obtained for the same retention time by using longer columns with about a tenth of the liquid loading. The resolution factors obtained on such columns however would not be dependent solely on the nature of the stationary phase.

The high liquid loading resulted in long retention times for

some of the compounds of high molar mass. Accordingly, these compounds were chromatographed on 25 cm columns, whereas the compounds of low molar mass were chromatographed on 1 metre columns. Even with the 25 cm columns retention times were as high as sixty minutes for some of the compounds of high molar mass.

The gas chromatograph was allowed a warm-up period of about an hour and a half before chromatography was started. Samples were injected into the chromatograph using a Hamilton 10 microlitre syringe. Sample sizes varied between 0.2 microlitre and 1.5 microlitre depending on the retention time of the compounds. No variation in retention time with variation in sample size of diastereoisomers was detected.

Each compound was chromatographed two to four times, (depending on its retention time) on each phase. In a few cases where compounds had retentions higher than sixty minutes, compounds were chromatographed once only. Error in the determination of relative retention volume for compounds of short retention time, is most probably due to error in chart measurements. This may result in appreciable error in the calculation of resolution factor, but the error is minimized by chromatographing the compound several times.

Error in the determination of relative retention volume for compounds of longer retention time is most probably due to variations in carrier gas flow rate. Slight variations in flow rate are to be expected with the G.C.2A as it does not have a thermostatted gas flow valve. However, variations in flow rate result in very small error in the calculation of resolution factor.

Capillary Columns.

A Perkin Elmer model 154 gas chromatograph provided with appropriate attachments and a hydrogen flame ionization detector were used for work involving capillary columns. The coated columns were purchased from the Perkin Elmer Corporation. They were 150 ft. long and of 0.01 inch internal diameter.

Samples of 0.5 and 1.0 microlitre were injected, with a split ratio of about 1500 to 1.

Detector Response.

Both the katherometer and the flame ionization detector have the same response to each member of a pair of diastereoisomers. This was shown by chromatographing a pair of diastereoisomers as a 50:50 mixture. The chromatographic peaks were of equal area. The isomeric purity of each of the diastereoisomers was first checked by chromatography and was found to be greater than 95%.

The katherometer was checked with the diastereoisomers of 2,3-diacetoxybutane. The flame ionization detector was checked with the diastereoisomers of sec-octyl α-acetoxypropionate.

5.1. LACTIC ACID DERIVATIVES.

INTRODUCTION.

In 1941 Bailey and Hass[37] reported a method of partial separation of enantiomers. Racemic acids were converted into diastereoisomeric esters by reaction with optically active alcohols, and these diastereoisomers were then partially separated by distillation.

A complete separation corresponds to a method of resolving optically active compounds if no racemization occurs during the preparation, the separation or the saponification of the diastereoisomers.

The authors estimated that a distillation column with an efficiency of between 60 and 300 theoretical plates would separate the diastereoisomers completely. Efficiencies far greater than this may be realized by gas chromatography.*

Gas Chromatography.

Two of the compounds that Bailey and Hass had studied, sec-butyl lactate (I) and sec-butyl α-propionoxypropionate (II), were prepared from racemic material.

Compound I could not be separated into diastereoisomers either on a 6 metre silicone fluid 703 column or on a 5 metre octoil column. The separation of II into its constituent isomers was attempted on the former column and was unsuccessful. The chromatography was performed at 130°. As the retention of I was about 2 hours on either column it was impractical to work at a lower temperature.

* The separation of two components by gas chromatography is however governed not only by the efficiency as measured by the number of theoretical plates. See section 3.

The separation was then attempted on a 150 ft., 0.01 inch I.D. capillary column coated with polypropylene glycol (PPG) of molar mass 550. On this column the diastereoisomers of I were partially separated into two overlapping peaks which could not be further separated either by varying the temperature from 40° to 150° or by varying the pressure from 10 to 20 p.s.i.g.

The diastereoisomers of II were completely separated on the PPG column at 120° using a pressure of 20 p.s.i.g.

Diastereoisomers Studied.

The following compounds were synthesized to study the influence of structure on the separability of diastereoisomers.

Series A: lactates of secondary alcohols, $CH_3CHOHCOOCH(CH_3)R$

R = C_2H_5, n-C_3H_7, n-C_4H_9, n-C_5H_{11} and n-C_6H_{13}.

Series B: α-acetoxypropionates of secondary alcohols, $CH_3CH(OOCCH_3)COOCH(CH_3)R$. R as for series A.

Series C: α-alkanoyloxypropionates of secondary butanol, $RCOOCH(CH_3)COOCH(CH_3)C_2H_5$.

R = CH_3, C_2H_5, n-C_3H_7, n-C_4H_9 and n-C_5H_{11}.

Series D: N-acetyl sec-butyl esters of alanine and valine.

Series E: α-acetoxypropionates of menthol and borneol.

Results:

Two capillary columns were used. One was coated with polypropylene glycol (PPG) of molar mass 550 and the second was coated with squalane (SQ). Each column was 150 ft. long and was of 0.01 inch I.D.

Series A: Attempts to separate diastereoisomers of series A

completely were not successful on either phase.

Series B and C: Each compound of series B and C gave two distinct peaks on both PPG and SQ. Separation without overlap was achieved for all compounds on PPG at 120°, P = 20 p.s.i.g. which corresponds to a flow rate of 1.5 ml min^{-1}. The retentions of these compounds relative to n-decyl acetate are listed in tables 5 and 6.

The peaks of each pair had equal areas for compounds synthesized from racemic starting material, each peak corresponding to a racemic mixture of one of the diastereoisomers. However, in the case of D-sec-octyl L-α-acetoxypropionate of relatively high optical purity, the second peak had an area about twenty times larger than the first. The order of elution of the isomers was the same on both phases. For L-sec-octyl L-α-acetoxypropionate the first peak was many times larger than the second. A mixture of either of these two diastereoisomers with the sec-octyl α-acetoxypropionate prepared from racemic material results in two peaks of unequal area depending on the ratio of the mixture.

Figure 1 is a plot of the log of relative retention for compounds of series B and C at 120° on SQ against the total number of carbon atoms in the compound (i.e. against the molar mass of the compounds). For each series the values fall on two nearly straight lines, as would be found in two different homologous series. The plot on PPG is of a similar shape. It is reasonable to assume that each line corresponds to diastereoisomers having identical or the related mirror image configurations. Thus the order of appearance of the diastereoisomers is dependent on their configurations.

TABLE 5.

α-ACETOXYPROPIONATES OF 2-n-ALKANOLS.

RELATIVE RETENTIONS (1-n-DECYL ACETATE = 1.00) AND RESOLUTION FACTORS.

ALCOHOL FROM WHICH DERIVED	SQ[a] T = 120°			PPG[a] T = 125°		
	LL OR DD RELATIVE RETENTION	LD OR DL RELATIVE RETENTION	RESOLUTION FACTOR	LL OR DD RELATIVE RETENTION	LD OR DL RELATIVE RETENTION	RESOLUTION FACTOR
2-n-BUTANOL	0.095	0.099	1.04	0.245	0.254	1.04
2-n-PENTANOL	0.174	0.183	1.05	0.363	0.379	1.04
2-n-HEXANOL	0.335	0.352	1.05	0.60	0.63	1.05
2-n-HEPTANOL	0.664	0.720	1.08	1.00	1.04	1.04
2-n-OCTANOL	1.36	1.50	1.10	1.69	1.79	1.06

a. See list of abbreviations on page 121.

TABLE 6.

α-ALKANOYLOXYPROPIONATES OF 2-n-BUTANOL

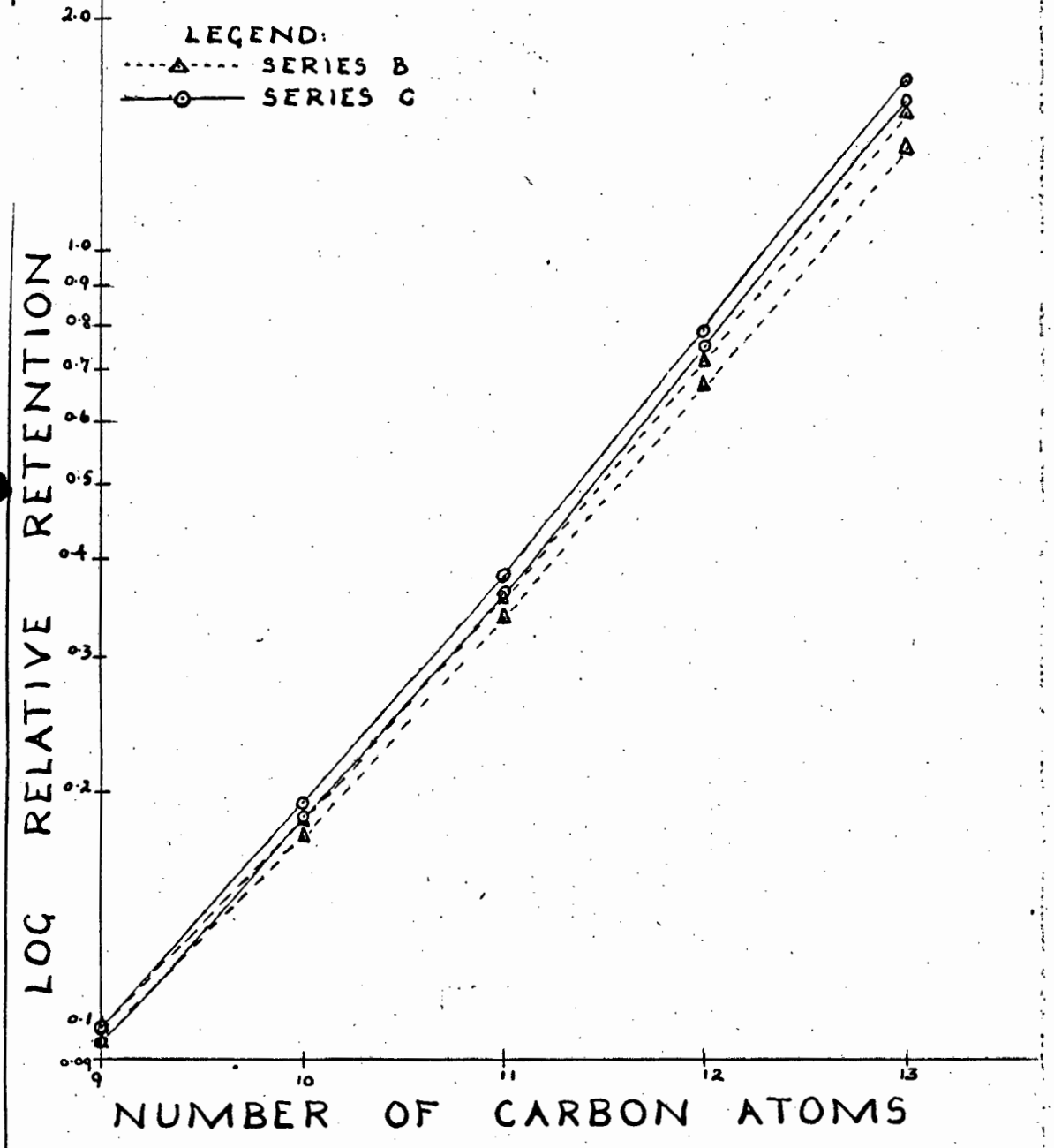
$RCOOCH(CH_3)COOCH(CH_3)C_2H_5$

RELATIVE RETENTIONS (1-n-DECYL ACETATE = 1.00) AND RESOLUTION FACTORS

NATURE OF R (see above)	SQ[a] T = 120°			PPG[a] T = 120°		
	LL OR DD RELATIVE RETENTION	LD OR DL RELATIVE RETENTION	RESOLUTION FACTOR	LL OR DD RELATIVE RETENTION	LD OR DL RELATIVE RETENTION	RESOLUTION FACTOR
CH_3	0.095	0.099	1.04	0.227	0.236	1.04
C_2H_5	0.185	0.193	1.04	0.342	0.356	1.04
$n\text{-}C_3H_7$	0.358	0.377	1.05	0.588	0.614	1.04
$n\text{-}C_4H_9$	0.743	0.782	1.05	1.010	1.050	1.05
$n\text{-}C_5H_{11}$	1.55	1.65	1.06	1.642	1.725	1.05

a. For list of abbreviations see page 121.

FIG: 1



α-ALKANOYLOXYPROPIONATES OF SEC-ALCOHOLS. PLOT OF LOG RELATIVE RETENTION ON SQUALANE AT 120° vs NUMBER OF CARBON ATOMS PER SOLUTE MOLECULE.

From the retention time for the main peak of the D-sec-octyl L- α-acetoxypropionate it can be concluded that the upper curves for series B and C in figure 1 correspond to compounds derived from either L-lactic acid and D-sec-alcohol or D-lactic acid and L-sec-alcohol, whereas the lower curves for series B and C in figure 1 correspond to compounds of the alternative configurations. (Gil-Av and co-workers[15,16] have confirmed this by synthesizing other members of series B and C from optically active material).

These results establish that it is possible to assign the configuration of an asymmetric compound by gas chromatography. The sepcial advantages of this method are:

a) Very small quantities (10^{-8} gm or less depending on detection unit) of material may be used.

b) Impure material may be used. It should in principle be possible to assign configuration to diastereoisomers that are present in reaction mixtures in concentrations of 1% or less. This should be of very great value (i) in biochemistry in dealing with small quantities of impure but valuable material as in the case of a protein hydrolysate where it is desired to know the configuration of constituent amino acids, and (ii) in reaction mechanism for determining the ratio of concentrations of a pair of diastereoisomers in a reaction mixture. Any attempt to purify a mixture of diastereoisomers may effect the ratio of their concentrations.

c) It is in principle possible to determine a very small amount of diastereoisomeric impurity in a compound. Most commercial gas chromatographic detectors may be attenuated over a range of several

thousand. Thus it should be possible to detect isomeric impurities in the order of 1% or less, provided peak tailing is kept to a minimum. Detection of such small impurities by conventional methods can be very difficult.

d) If it can be shown to be the general behaviour of diastereoisomers that in a given series the isomers of a certain configuration are always eluted first under specified gas chromatographic conditions, it will be possible to assign configuration to each member of an unknown pair of diastereoisomers from their retention volumes. If only one member of a pair is available it is frequently possible to obtain the second member by isomerisation.

e) It is possible to predict the retention of an unknown member of a homologous series of diastereoisomers by interpolation or to a limited extent by extrapolation of the log retention vs molar mass plot.

The resolution factor (R.F.) for series B changes from 1.04 to 1.06 on PPG and from 1.04 to 1.10 on SQ. For series C, R.F. changes from 1.04 to 1.05 on PPG and from 1.04 to 1.06 on SQ. All results are at 120°. Thus the increase in R.F. with increasing molar mass is much greater in series B than in series C. The increase in alkyl chain length increases the separability of the diastereoisomers while increases in the acyl chain length have little effect.

It is interesting to note that the resolution factor is higher on SQ than on PPG (i.e. on the less polar of the two columns). This is most probably due to the resolution factor being not dependent only on the nature of the stationary phase but also on the nature of the support (i.e. the walls of the stainless steel capillary tubing). In the case of the more polar phase (PPG) solute adsorption on the walls

of the capillary column should have a very much smaller effect than in the case of SQ. In packed column gas chromatography a trace of polyethylene glycol is occasionally coated on the solid support to deactivate it.

Thus the higher resolution factor on SQ is attributed to the nature of the support rather than to the nature of the phase.

Chromatography of Series D.

The N-acetyl sec-butyl esters of alanine and valine could not be separated into diastereoisomers on either PPG or SQ under varying conditions. Reproducibility of the chromatograms was very poor and compound decomposition in the column or in the injection block was suspected. Subsequent to the completion of the above work several authors have successfully separated the N-trifluoroacetyl derivatives of several amino acids (see section 2).

The Chromatography of Series E and of Some Members of Series C on a Packed Column.

To investigate the possibility of separating diastereoisomeric α-acetoxypropionates using a packed column, a 25 ft. column loaded with 2.5% 1,2,3,4-tetrakis-(2-cyanoethoxy)butane (CYB) was prepared. The excellent separating power of this phase had been encountered in the work on 2,3-disubstituted butanes.

Both sec-octyl and menthyl α-acetoxypropionates (see table 7) were separated on this column, the latter having the high resolution factor of 1.1. It was thought that this might possibly be associated with the greater rigidity of the menthyl group. However since the

TABLE 7.

ALKYL α-ALKANOYLOXYPROPIONATES

RELATIVE RETENTIONS (ETHYL BENZOATE = 1.00) ON

1,2,3,4-TETRAKIS-(2-CYANOETHOXY)BUTANE AT 150°.

COMPOUND	FIRST ISOMER ELUTED	SECOND ISOMER ELUTED	RESOLUTION FACTOR
2-n-BUTYL α-ACETOXYPROPIONATE	0.60	a	--
2-n-OCTYL α-ACETOXYPROPIONATE	1.78	1.93	1.08
MENTHYL α-ACETOXYPROPIONATE	3.62	4.07	1.12
BORNYL α-ACETOXYPROPIONATE	52.7	54.5	1.03

a. No resolution achieved under the experimental conditions.

resolution factor of the corresponding bornyl compound is very low, it is unlikely that rigidity alone has any connection with separability. It is interesting to note the behaviour of sec-butyl α-acetoxypropionate. The resolution factor for this compound on CYB at 150° is most probably of the same magnitude as found in the capillary work at 120° (R.F. = 1.04) because the resolution factor of sec-octyl α-acetoxypropionate is of about the same magnitude in the capillary work (R.F. = 1.06,1.10) as on CYB (1.08). Thus a R.F. of 1.04 would be expected for sec-butyl α-acetoxypropionate. In spite of this however the isomers of sec-butyl α-acetoxypropionate were not separated under the experimental conditions on CYB whereas the isomers of bornyl α-acetoxypropionate (R.F. = 1.03) were sufficiently separated for their resolution factor to be measured. Thus for the α-acetoxypropionates on the 2.5% CYB column resolution appears to increase with increasing retention volume. For this reason it would be expected that the isomers of sec-butyl α-acetoxypropionate should be separable on the same column at lower temperature due to larger retention volume.

The significance of separating these compounds on a packed column is that using the same phase at a higher loading (possibly at a higher temperature and somewhat longer column length) it should be possible to separate these compounds on a preparative scale.

Recently (1966) Stern and Karger[17] have also separated similar compounds on packed columns.

SECTION 5.2. THE DIALKYL ESTERS OF 2,3-BUTANEDIOL.

5.2.a. GENERAL TRENDS.

Table 8 lists the relative retentions (Ethyl benzoate = 1) and table 9 the resolution factors of five dialkyl esters of 2,3-butanediol chromatographed at 150°.

The diastereoisomers were chromatographed on the following phases: squalane (SQ), dinonyl phthalate (DNP), polyethylene glycol 6000 (PEG), ethylene glycol succinate (EGS) and 1,2,3,4-tetrakis-(2-cyanoethoxy)butane (CYB).

The racemic member of any of the pairs of diastereoisomers listed in table 8, always has a higher retention that that of the meso isomer on all the phases listed above. It is suggested that this behaviour is quite general for these diastereoisomers since the above phases span the polarity range and that as is shown in section 5.6. the magnitude of resolution factor is to a first approximation dependent on phase polarity.

Figures 2 and 3 are plots of log retention vs n, the total number of carbon atoms (i.e. molar mass) for these compounds, on SQ and CYB (n = 8 corresponds to the diacetate and n = 16 corresponds to the dihexanoate of 2,3-butanediol). The shapes of the plots on other phases are intermediate between those on SQ and CYB. Each plot consists of two curves corresponding to the meso and to the racemic series of isomers. The relative retention of the racemic isomer is always higher than of the respective meso isomer. Where only one member of a pair of diastereoisomers is available its configuration

TABLE 8.

RELATIVE RETENTIONS (ETHYL BENZOATE = 1.00)[a] OF DIALKYL ESTERS OF 2,3-BUTANEDIOL.

ACID FROM WHICH DERIVED	SQ[b] 150°		DNP[b] 150°		PEG[b] 150°		CYB[b] 150°		EGS[b] 100°		EGS[b] 125°		EGS[b] 150°		EGS[b] 175°	
	meso	racemic	meso	racemic	meso	racemic	meso	racemic	meso	racemic	meso	racemic	meso	racemic	meso	racemic
ACETIC	.32	.34	.42	.48	.41	.48	.55	.73	.52	.70	.51	.66	1.00	1.24	.51	.62
PROPIONIC	.92	.97	1.03	1.16	.70	.80	.74	.95	.79	1.06	.71	.90	1.34	1.63	.66	.79
BUTYRIC	2.54	2.71	2.65	3.01	1.29	1.48	1.15	1.43	1.53	2.06	1.26	1.55	2.14	2.59	1.01	1.20
VALERIC	7.27	7.84	7.40	8.44	2.75	3.12	1.95	2.40	3.40	4.75	2.48	3.19	3.82	4.59	1.69	1.99
HEXANOIC	c	c	20.80	23.86	5.95	6.80	3.41	4.19	7.93	11.00	5.01	6.26	7.13	8.51	2.88	3.41

a. Retentions on EGS at 150° are relative to meso-2,3-diacetoxybutane = 1.00.

b. See list of abbreviations on page 121.

c. Retentions not determined.

TABLE 9.

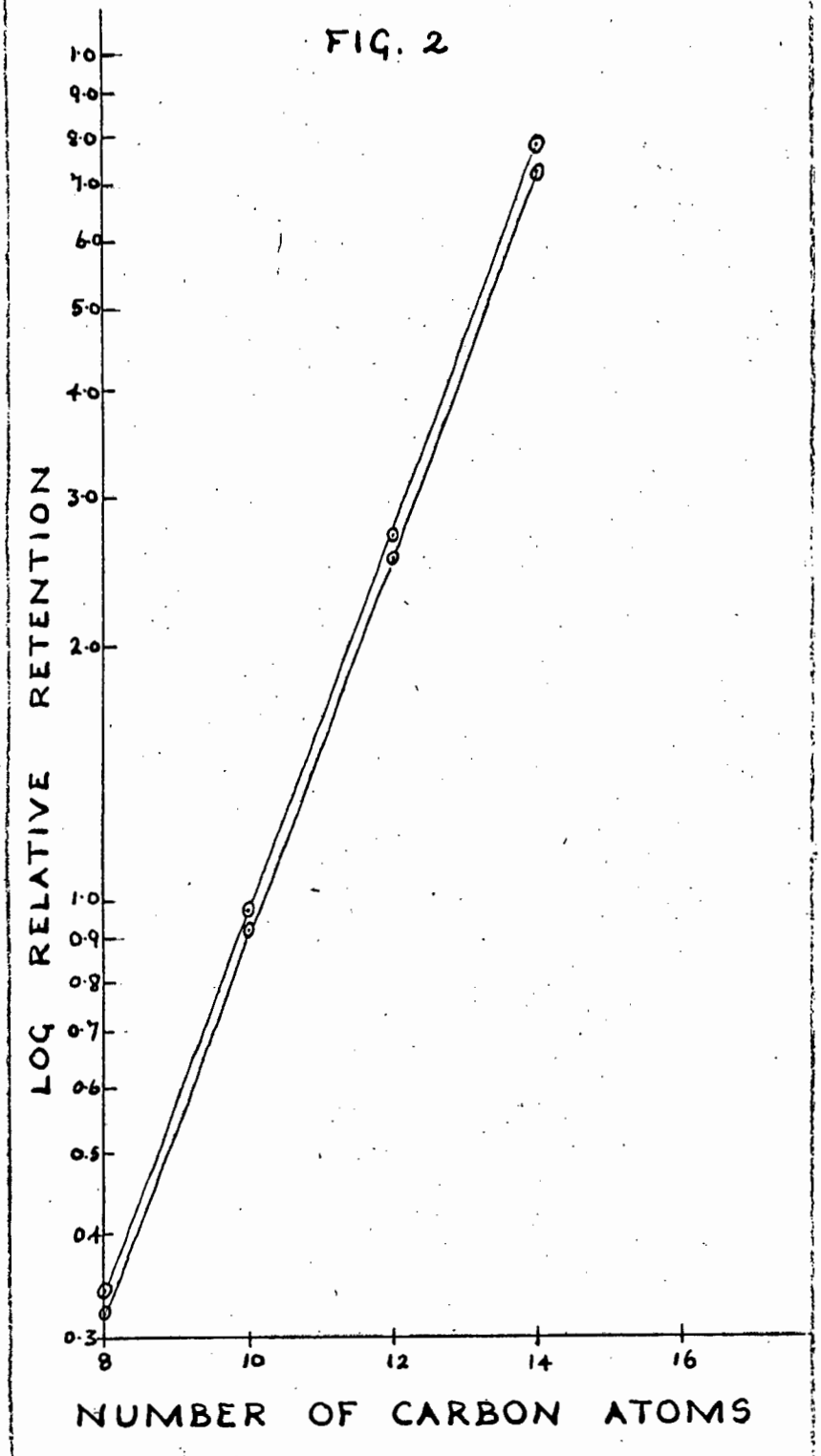
RESOLUTION FACTORS OF DIALKYL ESTERS OF 2,3-BUTANEDIOL.

ACID FROM WHICH DERIVED.	SQ[a] 150°	DNP[a] 150°	PEG[a] 150°	CYB[a] 150°	EGS[a] 100°	EGS[a] 125°	EGS[a] 150°	EGS[a] 170°
ACETIC	1.06	1.15	1.17	1.31	1.33	1.29	1.24	1.23
PROPIONIC	1.05	1.12	1.14	1.28	1.36	1.27	1.22	1.20
BUTYRIC	1.06	1.13	1.14	1.24	1.33	1.24	1.21	1.19
VALERIC	1.08	1.14	1.14	1.23	1.39	1.30	1.20	1.18
HEXANOIC	b	1.15	1.14	1.23	1.40	1.25	1.20	1.19

a. See list of abbreviations on page 121.

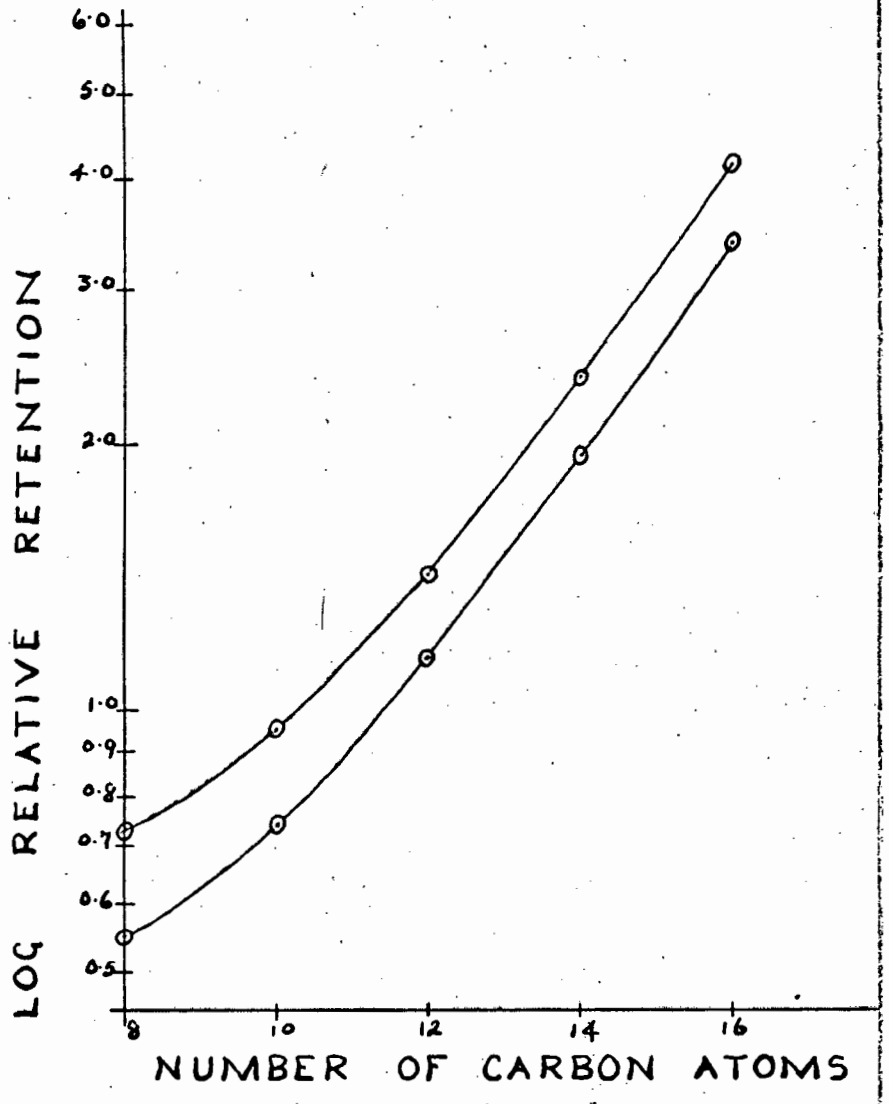
b. Retentions not determined.

FIG. 2



DIALKYL ESTERS OF 2,3-BUTANEDIOL. PLOT OF LOG RELATIVE RETENTION ON SQUALANE AT 150° vs. NUMBER OF CARBON ATOMS PER SOLUTE MOLECULE.

FIG: 3



DIALKYL ESTERS OF 2,3-BUTANEDIOL. PLOT OF LOG RELATIVE RETENTION ON CYB AT 150°. vs. NUMBER OF CARBON ATOMS PER SOLUTE MOLECULE.

can therefore be deduced from a plot of this nature if the retentions of other members of the homologous series have been determined.

The plot for SQ consists of two closely spaced parallel straight lines. As the polarity of the phases is increased the plots exhibit the following regular changes:

(i) The separation between the lines increases, i.e. the isomers are better separated.

(ii) The lines become curved, the curvature being greatest in the region where n decreases from 12 to 8.

(iii) Resolution factor of the first member of the series increases relative to the factors of higher members of the series.

(iv) The slope of the plots, measured by the increment between the fourth and fifth member of the series, decreases as polarity is increased. Even for CYB, the most polar phase, the plot is nearly a straight line in this region.

The curvature of the plot noted in point (ii) can be explained by postulating that the polar ester group becomes progressively more sterically shielded from interacting with the stationary phase as n increases. The contribution of the polar group to the overall retention would be greatest on the more polar phases, and hence one would expect that the shielding would become fully effective only at higher values of n. On the other hand, for SQ, where the contribution of the polar group is less important, the maximum shielding is reached for the first member of the series. There is no increase in shielding for the other members of the series and the

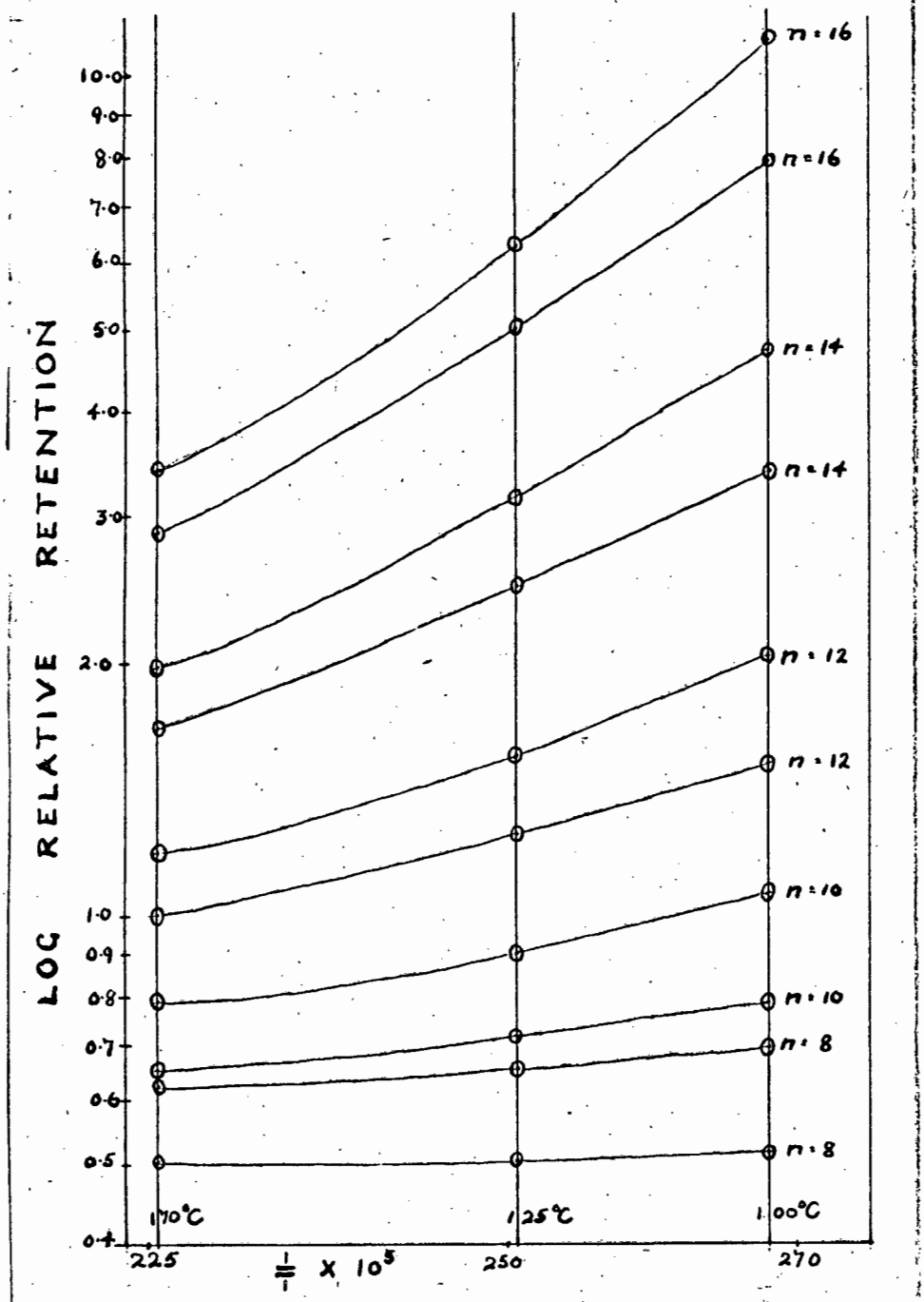
plot consists of two parallel straight lines. For more polar phases such as EGS and CYB linearity is found only for the higher members of the series, and the increment between the first and second member of the series is the lowest increment due to the fact that the increase in retention due to the methylene group is offset by the decrease in retention due to the screening of the ester group. Thus the plot for the early members of the series is curved. It is significant that the diastereoisomers that fall on this curved section of the plot have the highest resolution factors. It is reasonable to assume that their separation is in some way connected with the interaction of the polar ester group with the stationary phase.

Effect of Temperature on the Separation of Diastereoisomers.

The series of diastereoisomers were chromatographed on EGS at temperatures of 100°, 125°, 150° and 175°. The general shape of the plot of log retention vs molar mass is not temperature dependent. At the higher temperatures the lines are closer together and the plots extend over a smaller retention range than at lower temperatures.

Fig. 4 is a plot of log retention vs inverse temperature for these compounds. It consists of a family of slightly curved lines, the curvature of which increases with increasing homologue number. The curvature may be due to error in temperature measurement or due to variations in the heat of solution of these compounds. Generally the heat of solution of a compound is considered to be constant only over a smaller range of temperatures than considered here. It is tempting to explain the variation in heat of solution in the following manner. Each diastereoisomer exists in a number of conformations and each of these conformations may have a different heat of solution. There is

FIG: 4



DIALKYL ESTERS OF 2,3-BUTANEDIOL. PLOT OF LOG RELATIVE RETENTION ON EGS VS INVERSE TEMPERATURE.

an equilibrium* between the conformations, and the position of this equilibrium is temperature dependent. Thus the overall heat of solution of a diastereoisomer may change with changing temperature. This will lead to a non-linear plot of log retention vs inverse temperature.

When log retention of these compounds on one phase is plotted against log retention on a second phase two parallel curves are obtained, each corresponding to either the meso or the racemic series of isomers. The closer together the phases are in polarity (see section 5.6.), the closer together are the two lines. Thus figure 5a, the plot for CYB and EGS - two phases of high polarity - shows two closely spaced lines whereas figure 5b the plot for CYB and SQ (the most polar and the least polar phases studied) shows two lines more widely spaced.

5.2.b. MECHANISM OF SEPARATION.

The separation of a pair of diastereoisomers on a particular phase is dependent on each member of the pair having a different free energy of solution in that phase. In general differences in free energy of solution may arise from a variety of factors and it may be difficult to predict which of a pair of diastereoisomers will have the higher

* This position of equilibrium will not be the same in the gas and the liquid phase. This discussion is simplified by ignoring this fact. For a more sophisticated treatment of heat of solution see appendix .

FIG: 5a.

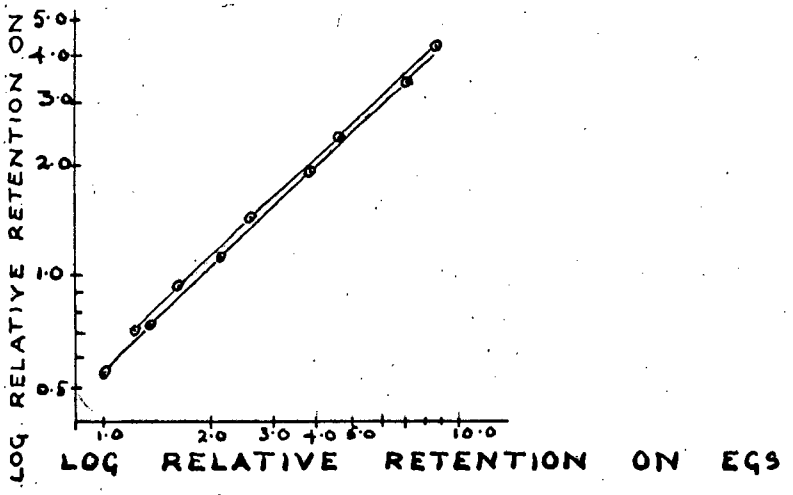
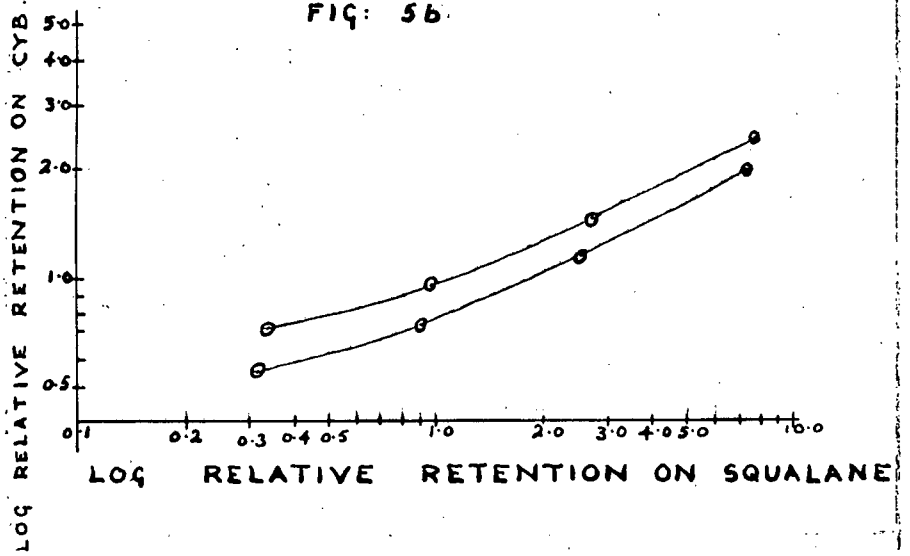


FIG: 5b.



DIALKYL ESTERS OF 2,3-BUTANEDIOL. PLOTS OF LOG RELATIVE RETENTION ON ONE PHASE vs LOG RELATIVE RETENTION ON A SECOND PHASE.

retention volume. There are, however, certain classes of diastereoisomers for which it has been possible to correlate resolution factor with structure. The diesters of 2,3-butanediol constitute one of these classes of diastereoisomers. The correlations are interpreted in terms of specific 'mechanisms' of separation.

Separation Mechanism 1.

This mechanism is applicable to diastereoisomers that contain two adjacent polar functional groups that repel each other.

The repulsion between two adjacent polar groups is greatest when the group dipole moments are adjacent and parallel i.e. when the groups are cis to each other. In solution there is a solvent stabilization of this dipole-dipole repulsion. This results in a lowering of the potential* energy of the system and results in a more negative free energy of solution.

Infrared and Raman spectroscopy studies (see ref: 38 p.41) show that for 1,2-dibromoethane and 1,2-dichloroethane the gauche$^{\emptyset}$ conformation is more favoured in the liquid than in the gas phase by about 1 k cal mole^{-1} (different authors give different values). In solution it is more favoured in solvents of high dielectric than in

* It should in principle be possible to calculate this lowering of potential energy, for when a simple molecule of dipole moment μ is transferred from a vacuum into a medium of dielectric constant ϵ it loses potential energy by an amount of

$$E = \frac{(\epsilon - 1)\mu^2}{(2\epsilon+1)a^3}$$

where a is the molecular radius (see ref: 38 page 42).

$\emptyset$ Gauche here refers to a staggered conformation with the polar groups cis to each other.

those of low dielectric. This has been shown[38] by observing the change of intensity ratio of two lines in the Raman spectrum with change of solvent. One line (ν_1) is assigned to the anti conformation and the second (ν_2) to the gauche conformation. The intensity ratio $\frac{I(\nu_1)}{I(\nu_2)}$ decreases (i.e. the gauche conformer becomes more favoured) from 5 to 1.2 in 1,2-dichloroethane and from 15 to 7 in 1,2-dibromoethane when the dielectric constant of the solvent is changed from 2 (n-hexane) to 33 (methanol).

The gas chromatography of cis- and trans-dichloroethylene and cis- and trans-dibromoethylene may also be explained in terms of solvent stabilization of cis dipoles. When these compounds are chromatographed on various polar phases the cis isomer has the higher retention.[41,42]

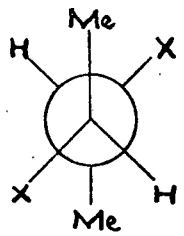
The order of elution of 'rigid' or 'near rigid' diastereoisomers may also be explained in terms of this effect. Thus cis-1,2-diacetoxycyclopentane has a higher retention than the trans isomer on a diethylene glycol succinate column at 145°. The resolution factor is 1.14.

For an understanding of the solute-solvent interactions in acyclic diastereoisomers it is necessary to consider all the conformations in which these molecules can exist.

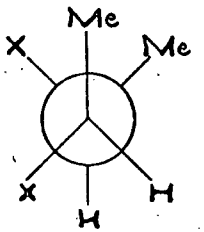
Consider a 2,3-disubstituted butane, the nature of the substituents being that they are polar and repel each other. The repulsion between the two polar groups is assumed to be greater than that between the two methyl groups. Figure 6 represents all the

FIG. 6

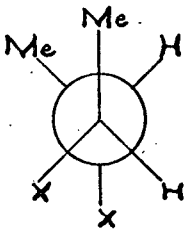
MESO ISOMER



A

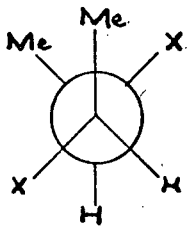


B

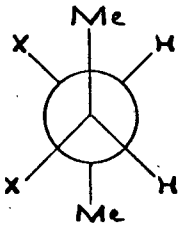


C

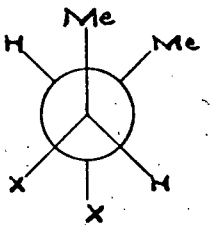
RACEMIC ISOMER.
(ONE ENANTIOMER SHOWN.)



D



E



F

POSSIBLE CONFORMATIONS OF A 2,3— DISUBSTITUTED BUTANE.

staggered conformations that the meso and racemic compounds are capable of assuming. Conformers A and D are expected to be the preferred conformers in the meso and the racemic series respectively due to their polar groups being anti to each other. There is, however, more steric interaction in D than in A due to an extra methyl-methyl interaction in the latter conformer. There is also more steric interaction in B and C than in F due to an extra polar group - methyl interaction in the latter conformer. The overall effect of these steric interactions is that the racemic isomer is expected to have the greater population of molecules in conformations such that the polar groups are cis to each other. The net result is that conformer A (with polar groups anti) is more favoured in the meso series than conformer D in the racemic series.

The work of Bothner-By and Naar-Colin[43] confirms this conclusion. These authors have estimated the population of the conformations of 2,3-dichlorobutane and 2,3-dibromobutane by n.m.r. spectroscopy. They find that about 66% of the molecules of racemic 2,3-dichlorobutane are in conformations with the chlorine atoms cis to each other, whereas only 48% of the molecules of the meso isomer are in such conformations. For 2,3-dibromobutane the corresponding percentages are: racemic isomer 40%; meso isomer 30%.

Racemic isomers of 2,3-disubstituted butanes would thus be expected to have higher retention volumes than meso isomers on all phases that are capable of stabilizing dipole-dipole interactions.

The racemic isomer does have the higher retention in the

case of 2,3-dichlorobutane[3], 2,3-dibromobutane[2] and for all the compounds listed in tables 8 and 15.

It would be expected that the greater the solvent stabilization of the adjacent polar groups, the greater would be the difference in free energy of solution between the racemic and the meso isomer. It is reasonable to assume that the greater the dielectric constant (which is a measure of polarity) of a phase, the greater will be the stabilization of adjacent polar groups in a solute molecule. It is seen that the resolution factors of nearly all the diastereoisomers listed in tables 8 and 15 increase regularly with increasing phase polarity.

These ideas are developed more fully in section 5.3.d. in which the separation of diastereoisomers derived from 2,3-butanediol and the haloacetic acids is discussed.

It should be noted that when the polar groups of a pair of diastereoisomers are not adjacent to each other the order of elution may be different to that of the compounds described above. Thus Pritchard and Vollmer[50] have reported that in the case of 2,4-diacetoxypentane, 2,4-dichloropentane and 2,4-dibromopentane the meso isomers have higher retentions than the racemic isomers. This may be due to either a different distribution of conformer populations or due to the operation of a different mechanism of separation.

SECTION 5.3. THE HALOALKANES AND HALOESTERS.

The gas chromatography of the haloesters of 2,3-butanediol exhibits several features that are not found in that of the dialkyl esters discussed in section 5.2. It was thus decided to investigate the behaviour of various haloalkanes and ethyl haloacetates in order to obtain a deeper insight into the effect of introducing halogen atoms into the esters of 2,3-butanediol. The results of this investigation are presented below.

5.3.a. THE HALOALKANES.

Gas Chromatography of Halomethanes.

Table 10 lists the relative retention volumes (methyl chloride = 1) of the chloromethanes and bromomethanes at 80^{o} on squalane (SQ), dibutyl phthalate (DBP) and polyethylene glycol 400 (PEG4).

Figure 7 is the plot of log relative retention vs the number of chlorine atoms (which is proportional to the molar mass) of the four chloromethanes on SQ, DBP and PEG4. It is seen that for SQ the plot is nearly linear whereas for DBP and PEG4 the plots are parabaloid.

Figure 8 is the corresponding plot for the four bromomethanes. For SQ the plot is nearly linear. For this series of compounds the plot on DBP is only slightly more curved than on SQ.

The near-linear plot for both sets of compounds on SQ is to be expected as on this phase retention is due to dispersion forces only (see ref: 30) and these increase nearly linearily as

TABLE 10.

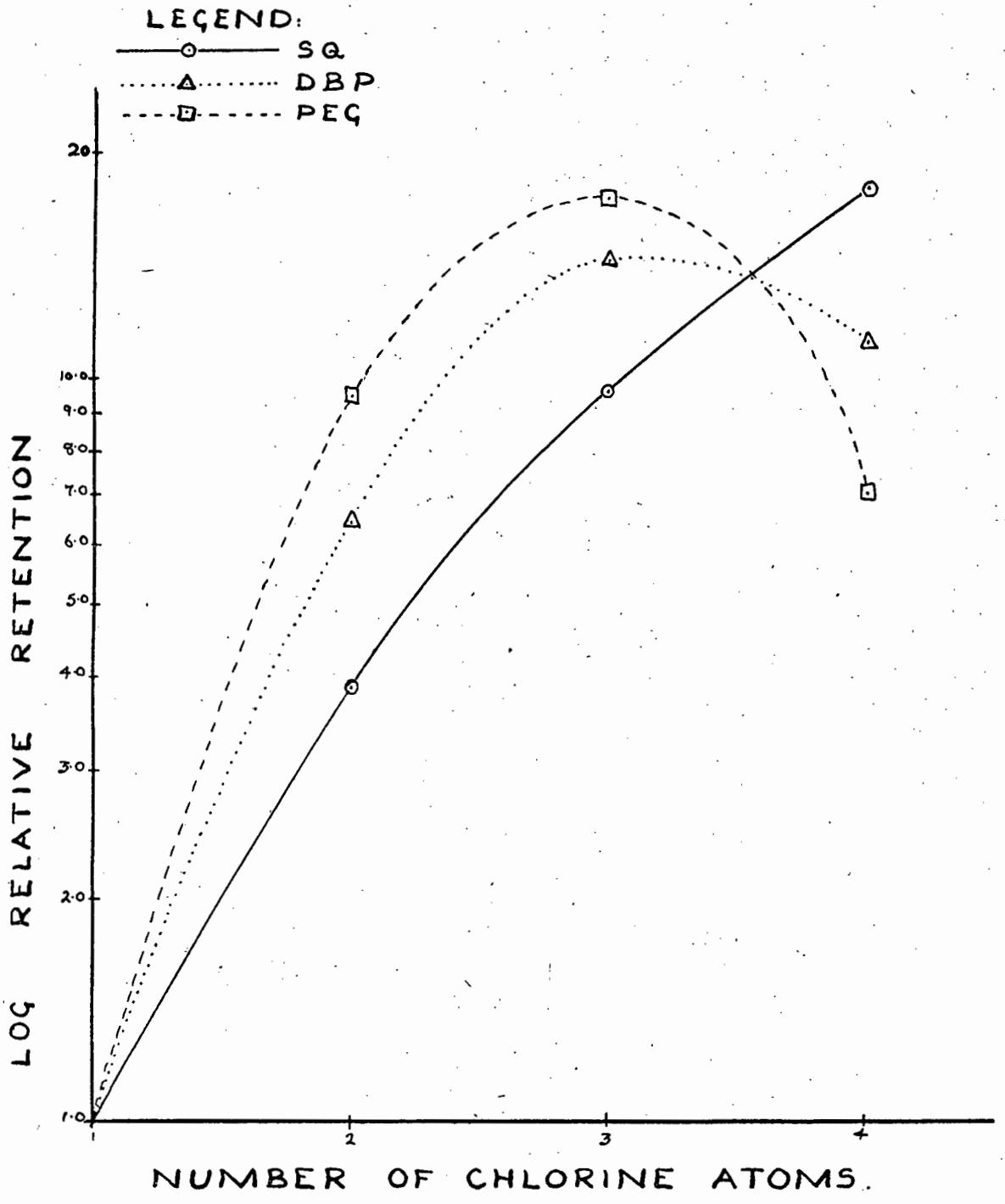
RELATIVE RETENTIONS (CH_3Cl = 1.00) OF HALOMETHANES AT 80°

COMPOUND	SQ[a]	DBP[a]	PEG4[a]
CH_3Cl	1.00	1.00	1.00
CH_2Cl_2	3.88	6.42	9.45
$CHCl_3$	9.70	14.5	17.5
CCl_4	18.1	11.2	7.08
CH_3Br	2.19	2.27	1.69
CH_2Br_2	20.4	31.5	43.8
$CHBr_3$	97.4	165	245
CBr_4	376	494	b

a. For list of abbreviations see page 121.

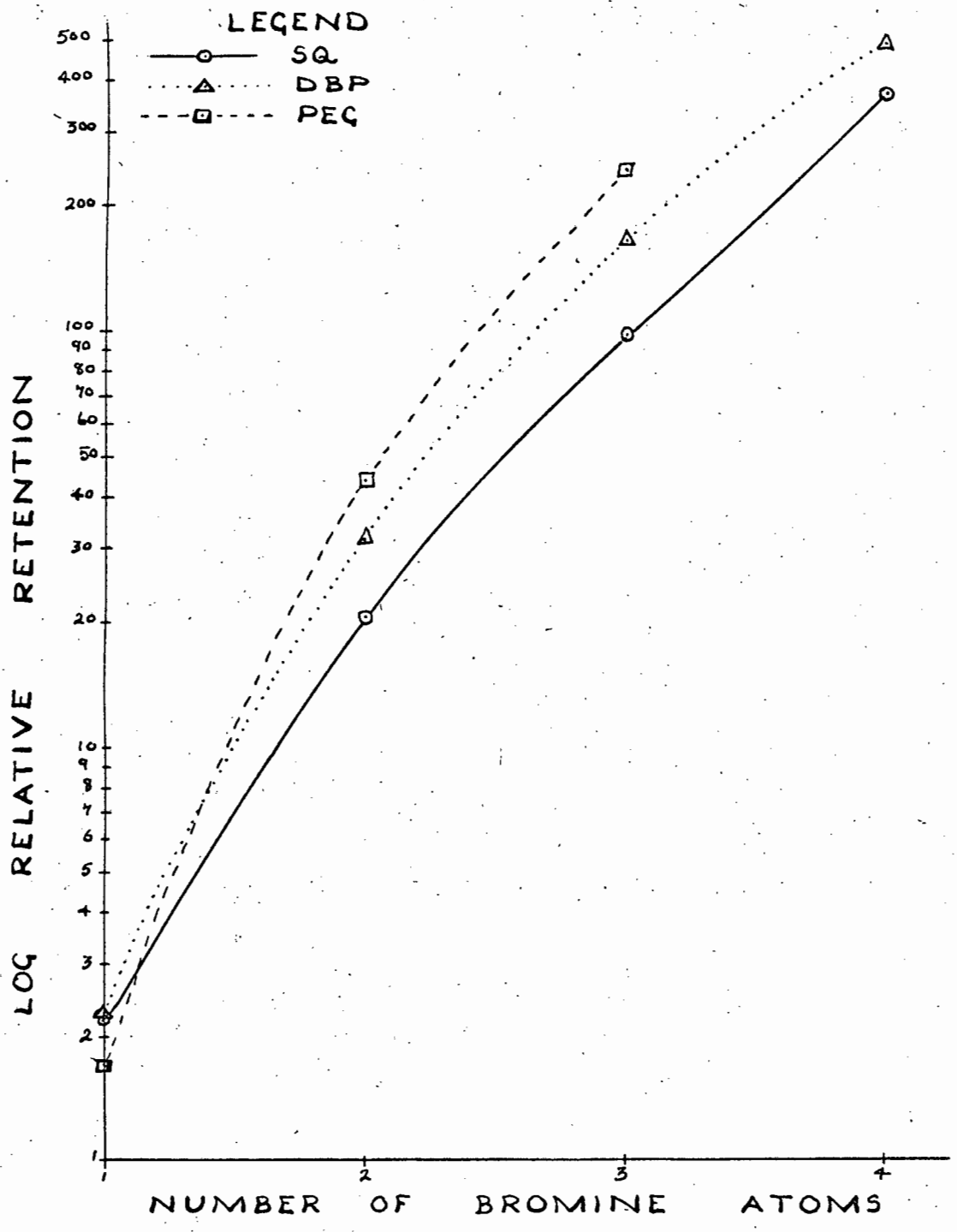
b. Retention not determined.

FIG: 7



CHLOROMETHANES. PLOT OF LOG RELATIVE RETENTION ON THREE DIFFERENT PHASES AT 80° vs NUMBER OF CHLORINE ATOMS PER SOLUTE MOLECULE.

FIG. 8



BROMOMETHANES. PLOT OF LOG RELATIVE RETENTION ON THREE DIFFERENT PHASES AT 80° vs NUMBER OF BROMINE ATOMS PER SOLUTE MOLECULE.

the series is ascended. The deviation from linearity may be due to variations in ionization potential with increasing halogen substitution (see equation (4) section 3).

The behaviour of the chloromethanes on DBP and PEG4 may be due to non-linear variations in either the dipolar interaction or the hydrogen bonding capacity of these compounds with increasing molar mass.

The dipole moments of each of the four chromomethanes are nearly the same as the moments of the corresponding bromomethanes. Thus if the chromatographic behaviour of these compounds on PEG4 and DBP is governed by dipolar interactions both sets of compounds should have similar plots of log retention vs molar mass. This is not the case.

If the deviation from linearity in these plots is related to induced dipole interactions, carbon tetrachloride would be expected to have the highest retention of the chloromethane series. This is also not the case.

It is however reasonable to assume that the variations from linearity in these plots is due to the non-linear variation of hydrogen bonding* capacity of these compounds with increasing molar mass.

* Pollard and Hardy[44] have shown that the order of elution of chloromethanes is dependent on which phase is used for chromatography and have postulated that this behaviour is due to the active hydrogens of methylene chloride and chloroform. They have however not investigated hydrogen bonding in detail as is done here.

Carbon tetrachloride has no hydrogens and the hydrogens of methyl chloride are activated only by a single chlorine atom. Chloroform however has three chlorine atoms activating a single hydrogen and methylene chloride has two chlorine atoms activating two hydrogens. If a straight line is drawn between the points for methyl chloride and carbon tetrachloride in the plots for DBP and PEG4 it is seen that the deviation from linearity is nearly the same for both methylene chloride and chloroform.

The order of hydrogen bonding is expected to be the same in the analogous bromine compounds but the degree of hydrogen bonding is far less, due to bromine being less electronegative than chlorine.

Heats of Mixing of Chloromethanes.

Heats of mixing were determined to obtain a quantitative measure of the interaction energies between the chloromethanes and the stationary liquids.

The stationary liquids studied were squalane and oxydipropionitrile (ODPN). These were selected because they represent one of the least polar liquids (SQ) and one of the most polar liquids (ODPN) available. The shape of the log retention vs molar mass plot for ODPN is very similar to that of PEG4.

The heats of mixing were determined by calorimetry. They are listed in table 11 as heats of mixing per mole of chloromethane.

The heats of mixing of ODPN with methylene chloride and chloroform were determined for 25, 50 and 75 mole % of chloromethane. Carbon tetrachloride is immiscible with ODPN at these concentrations.

TABLE 11.

HEATS OF MIXING OF CHLOROMETHANES AND STATIONARY LIQUIDS

(EXPRESSED AS CAL MOLE^{-1} OF CHLOROMETHANE)

	ODPN[a]			SQ[a]
	MOLE % HALOMETHANE			
CHLOROMETHANE	25	50	75	50
CH_2Cl_2	259	156	87	-905
$CHCl_3$	478	368	249	-401
CCl_4	b	b	b	-250

a. For list of abbreviations see page 121.

b. ODPN and CCI_4 are not completely miscible at these concentrations.

Figures 9a and 9b are the plots of heat of mixing per mole of chloromethane vs mole percent of chloromethane. Figure 9a is for ODPN and chloroform and figure 9b is for ODPN and methylene chloride. For both systems the heat of mixing increases with decreasing mole fraction of chloromethane. This is to be expected as the lower the concentration of chloromethane the greater is the number of ODPN molecules that can be associated with each chloromethane molecule. The heat of mixing of ODPN and chloroform extrapolated to zero concentration of chloroform is about 600 cal $mole^{-1}$ whereas for ODPN and methylene chloride it is about 380 cal $mole^{-1}$. The difference in heats of mixing between the chloroform and methylene chloride systems is of the same magnitude (about 120 cal $mole^{-1}$) at 25 and 50 mole % chloromethane.

Thus it is seen that the order of increasing interaction of halomethane with ODPN is carbon tetrachloride < methylene chloride < chloroform. This order can be explained in terms of hydrogen bonding. It is difficult to explain the order in terms of dipolar interactions or in terms of dispersion forces.

The heats of mixing of methylene chloride, chloroform and carbon tetrachloride with SQ at 50 mole percent chloromethane are listed in table 11. It is seen that the order of increasing interaction (i.e. decreasing negative heat of mixing) is not the same as the order of increasing interaction with ODPN. The order of increasing interaction with SQ is methylene chloride < chloroform < carbon tetrachloride. This is due to dispersion forces,

FIG : 9a

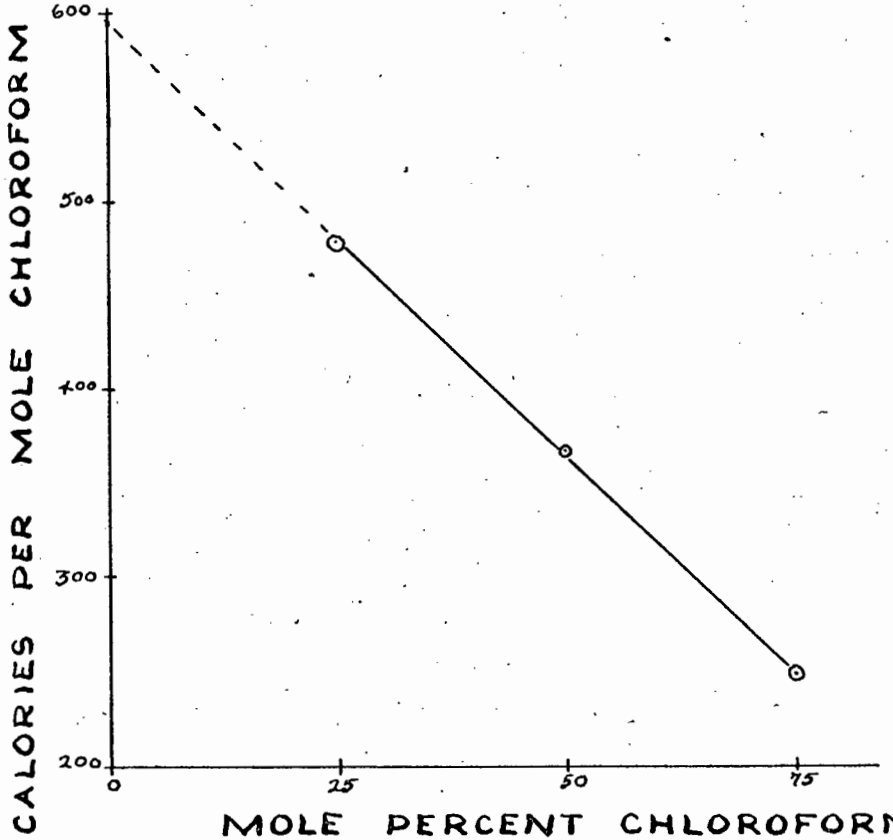
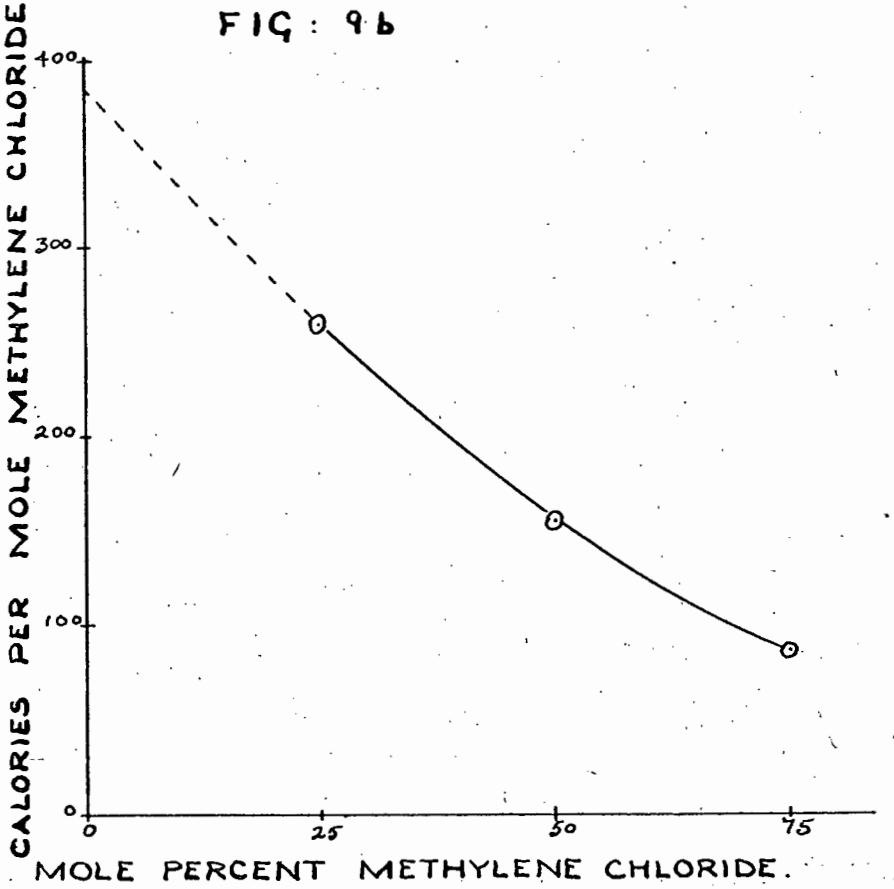


FIG : 9b



PLOTS OF HEAT OF SOLUTION OF CHLOROMETHANE AND ODPN vs MOLE PERCENT HALOMETHANE.

which are expected to be the only significant interaction forces in the system, increasing in the same order. This is illustrated in figure 10 which is the plot of heat of mixing of chloromethane with SQ vs molar refraction of chloromethane. It is seen that there is a regular increase of heat of mixing with increasing molar refraction. For these compounds molar refraction increases with increasing chlorine content due to chlorine being a more polarizable atom than hydrogen. The relationship between molar refraction and dispersion forces is discussed in Section 3.

Gas Chromatography of Chloroethanes.

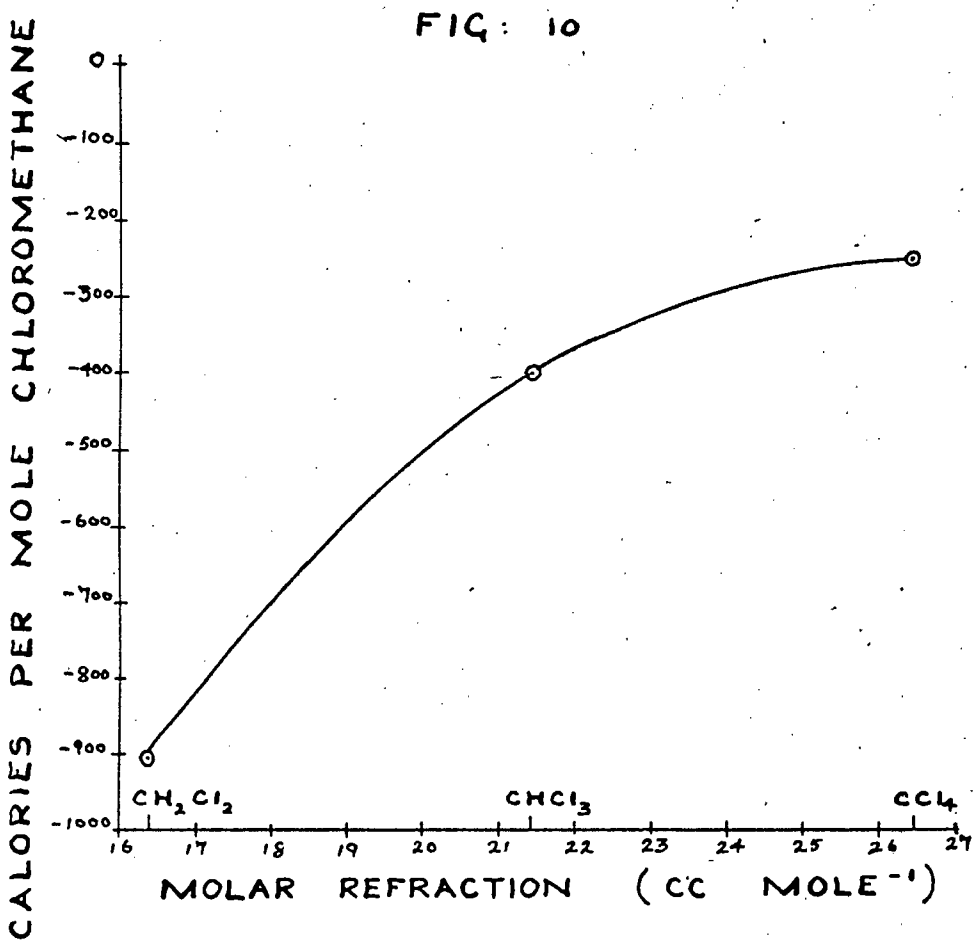
Table 12 lists the relative retention times (diethyl ether = 1) of the following chloroethanes at 100°; chloroethane; 1,2-dichloroethane; 1,1,2-trichloroethane; 1,1,2,2-tetrachloroethane; pentachloroethane, hexachloroethane. The stationary phases used were squalane (SQ), dinonyl phthalate (DNP) and oxydypropionitrile (ODPN). These represent a non polar (SQ), a moderately polar (DNP) and a very polar (ODPN) phase.

Figure 11 is the plot of log relative retention vs number of chlorine atoms (which is proportional to the molar mass) for the above chloroethanes.

For SQ the plot is slightly curved, showing that for this series of compounds retention increases nearly linearily with increasing molar mass.

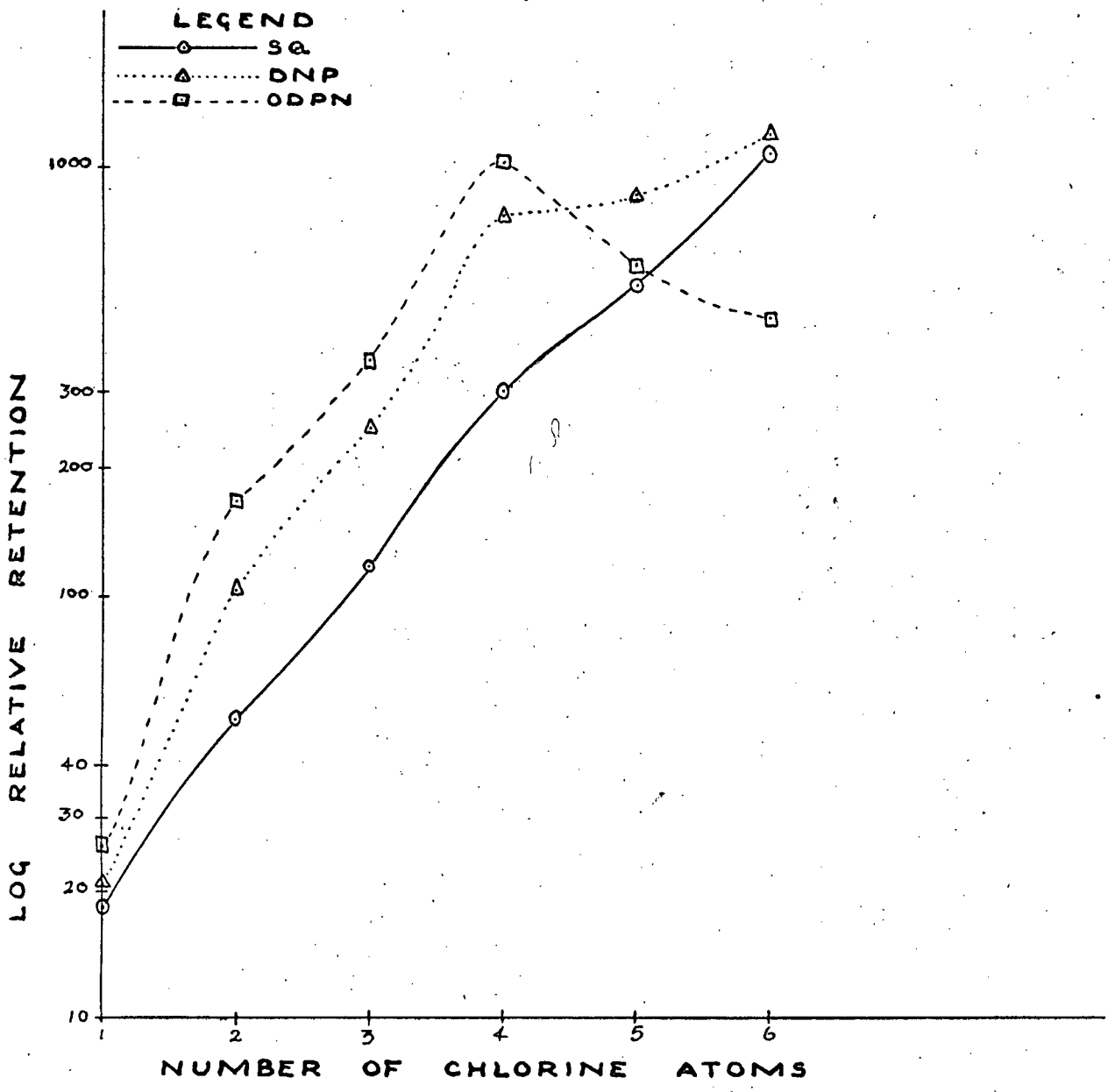
For DNP and ODPN the plots exhibit a greater deviation from linearity, the deviation being greater for ODPN than for DNP. By

FIG: 10



PLOT OF HEAT OF SOLUTION OF CHLOROMETHANE AND SQ vs MOLAR REFRACTION OF CHLOROMETHANE.

FIG: 11



CHLOROETHANES. PLOT OF LOG RELATIVE RETENTION ON THREE DIFFERENT PHASES AT 100° vs NUMBER OF CHLORINE ATOMS PER SOLUTE MOLECULE.

analogy to the chloromethanes it is postulated that the deviation from linearity is dependent on the number of active hydrogens and on the degree of their activity.

From figure 11 it is seen that on DNP and ODPN, tetrachloroethane exhibits the greatest positive deviation from linearity. By analogy to the chloromethanes, pentachloroethane might be expected to exhibit the greatest positive deviation from linearity, due to the single hydrogen on it being the most active in the series. However, due to the activating effect of the adjacent halomethyl group, tetrachloroethane exhibits the greatest degree of hydrogen bonding due to its having two hydrogens that are nearly as active as the single hydrogen of pentachloroethane. The fact that the two hydrogens are on different atoms - as opposed to methylene chloride where they are on the same atom - may also be relevant in connection with hydrogen bonding.

There is a regular increase in hydrogen bonding from chloroethane to tetrachloroethane.

Conclusions for the Haloalkanes.

From the above section it is seen that in halogenated chloroalkanes the free energy of solution (which is proportional to log retention volume) increases nearly linearily with the number of halogen atoms when these compounds are chromatographed on SQ.

When these compounds are chromatographed on polar phases there is a non-linear relationship between free energy of solution and number of halogen atoms. The more polar a phase, the greater is

this deviation from linearity.

On any given polar phase the deviation is dependent on the number of active hydrogens and on the degree of their activity. Thus methylene chloride and chloroform have nearly the same deviation from linearity due to the single hydrogen of chloroform being more active than the two hydrogens of methylene chloride. However 1,1,2,2-tetrachloroethane has a greater deviation from linearity than pentachloroethane due to its two hydrogens being nearly as active as the single hydrogen of pentachloroethane.

Difference in Behaviour between Bromoalkanes and Chloroalkanes.

From the behaviour of the bromomethanes it is seen that bromoalkanes exhibit the same trends as the chloroalkanes, but to a lesser extent due to the bromine being a less electronegative atom than chlorine.

5.3.b. THE ETHYL HALOACETATES.

These compounds were studied because of their close structural similarity to the haloesters of 2,3-butanediol. In fact the latter class of diesters may be considered as dimers of the corresponding ethyl haloacetates, linked through the α-carbon atoms of the ethyl group.

The compounds were chromatographed on the following phases: squalane (SQ), dinonyl phthalate (DNP), polyethylene glycol 6000 (PEG), sorbitol (SL), 1,2,3,4-tetrakis-(2-cyanoethoxy) butane (CYB) at 150°. The results are listed in table 13. Figures 12, 13 and 14 are plots of log retention of the ethyl haloacetate on SQ, CYB and SL vs molar mass.

TABLE 13.

RELATIVE RETENTIONS (ETHYL BENZOATE = 1.00) OF ETHYL ACETATE AND ETHYL HALOACETATES.

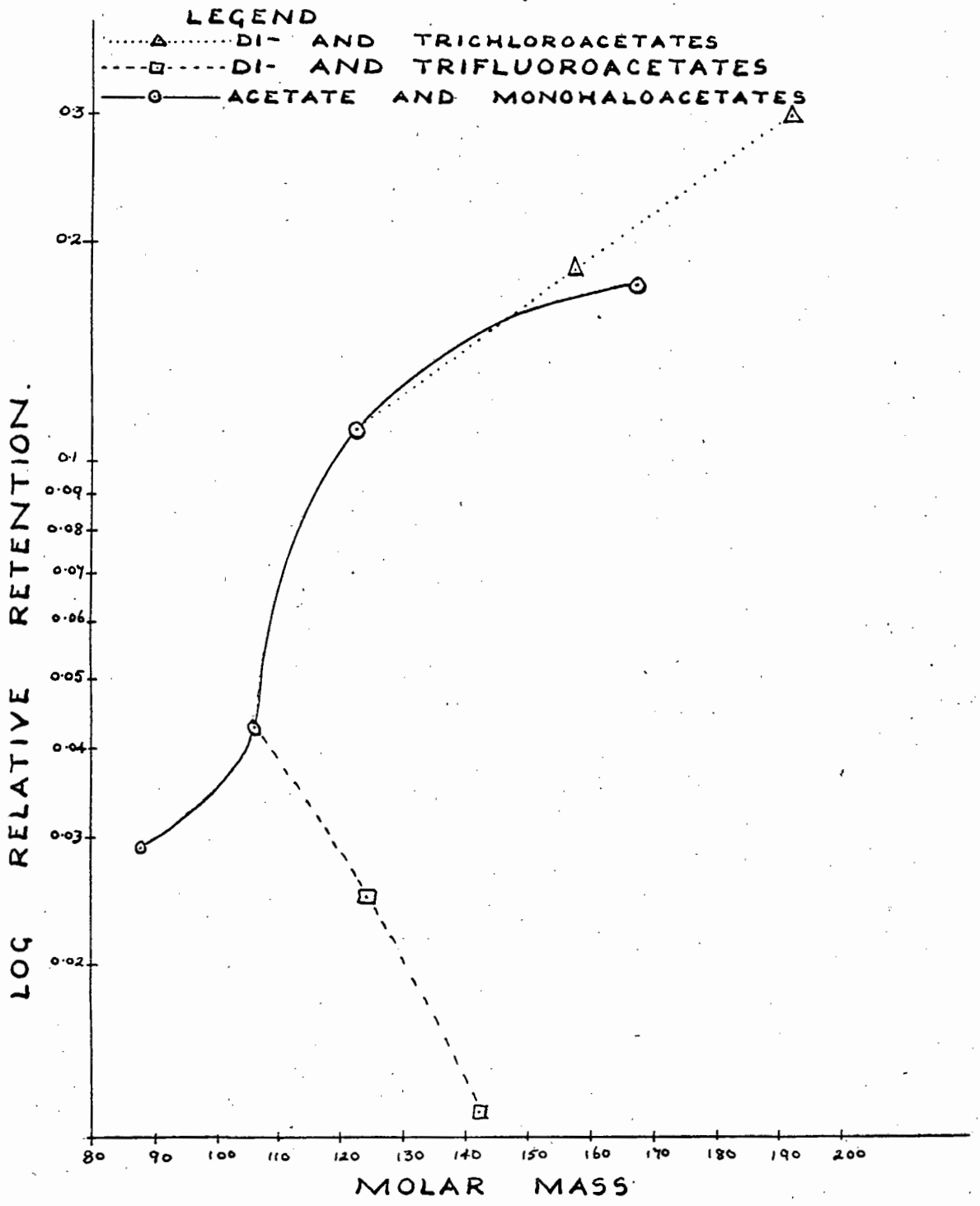
ACID FROM WHICH DERIVED.	SQ[a]	DNP[a]	PEG[a]	CYB[a]	SL[a]
ACETIC	.029	.029	.031	.040	0.17
FLUOROACETIC	.043	.056	b	.140	4.73
DIFLUOROACETIC	.025	.033	b	.066	c
TRIFLUOROACETIC	.012	.011	b	c	c
CHLOROACETIC	.111	.128	.183	.270	7.40
DICHLOROACETIC	.184	.205	.275	.307	5.35
TRICHLOROACETIC	.299	.274	.268	.239	3.96
BROMOACETIC	.175	.204	.300	.398	11.0

a. See list of abbreviations on page 121.

b. Retention not determined.

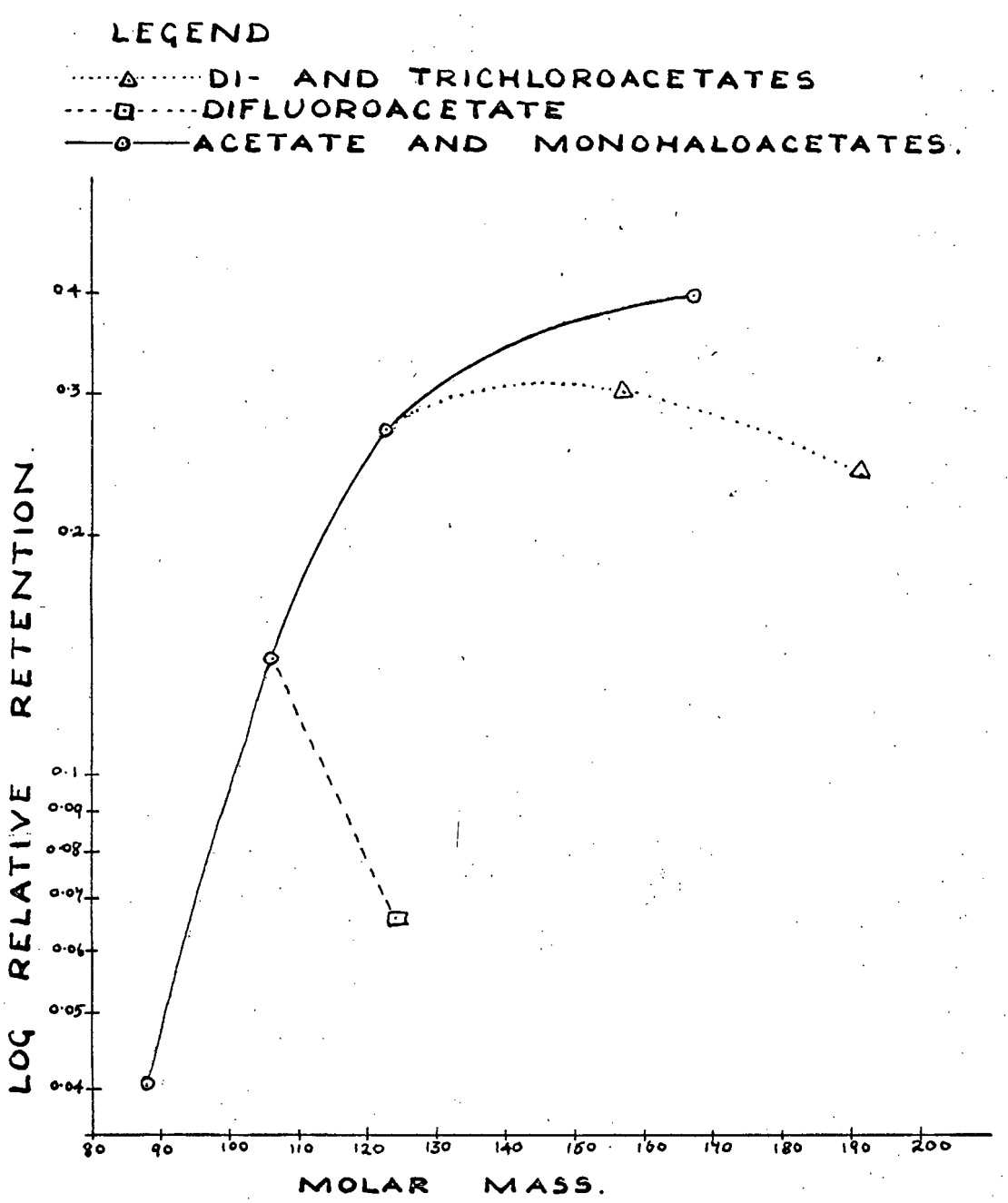
c. Retention too small to measure under the experimental conditions.

FIG: 12



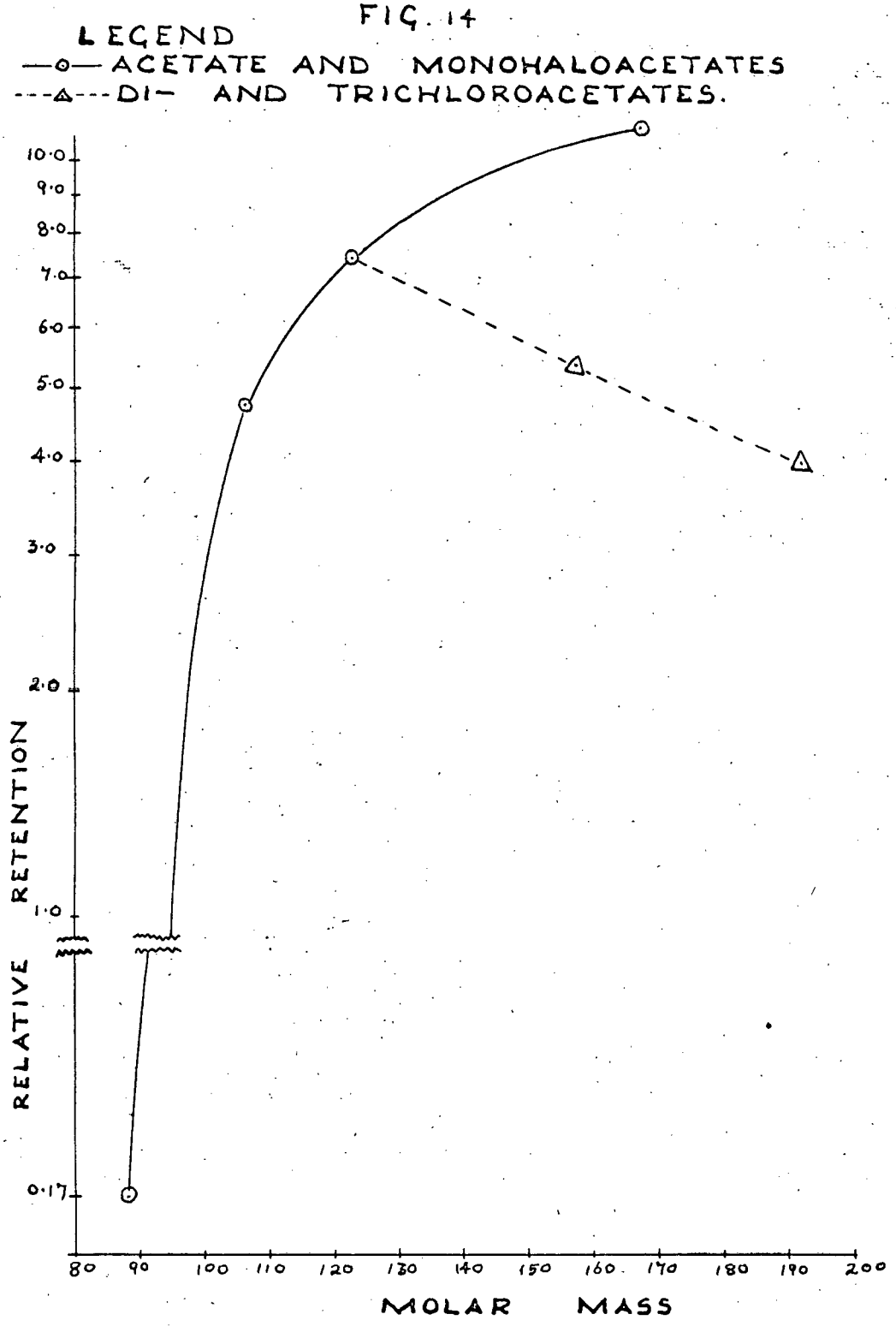
ETHYL HALOACETATES. PLOT OF LOG RELATIVE RETENTION ON SQ AT 150° vs MOLAR MASS.

FIG: 13



ETHYL HALOACETATES. PLOT OF LOG RELATIVE RETENTION ON CYB AT 150° vs MOLAR MASS.

FIG. 14



ETHYL HALOACETATES, PLOT OF LOG RELATIVE RETENTION ON SL AT 150° vs MOLAR MASS.

The plots are best discussed under the following headings.

1. The Ethyl Monohaloacetates.

On all phases the plot for the three monohaloacetates is a curve of nearly the same shape. The curve can be extrapolated to include the point for ethyl acetate but in the case of SQ and DNP the plot becomes sigmoidal.

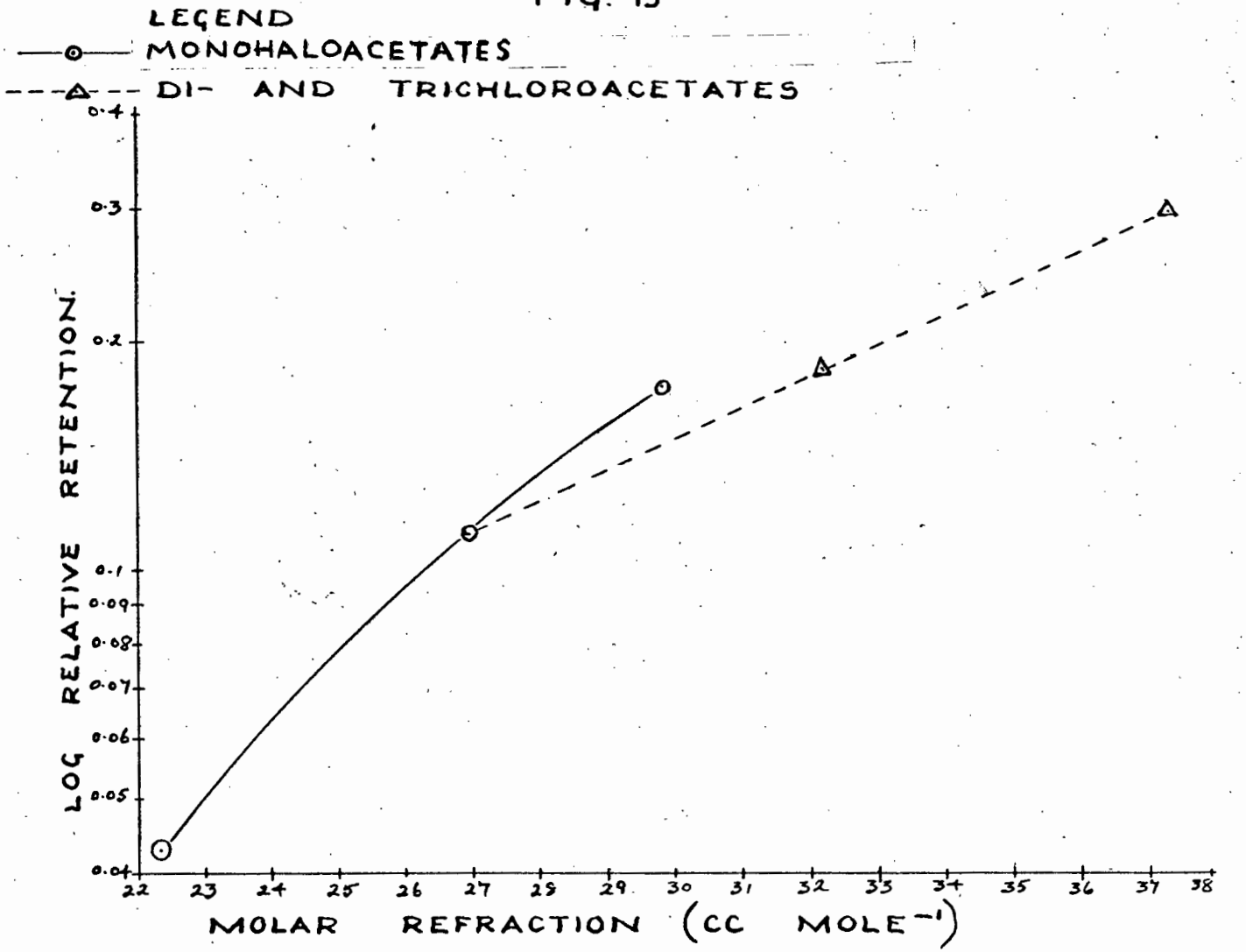
The spread of retentions of the monohaloacetates decreases slightly with increasing phase polarity. This can be rationalized in the following way.

On apolar phases retention is determined entirely or to a large extent by dispersion forces. This is illustrated by figure 15 which is a plot of log relative retention of the ethyl haloacetates on SQ vs their molar refraction (see section 3 for the relationship between molar refraction and dispersion forces). The plot for the monohaloacetates is nearly linear, indicating an almost linear increase in dispersion forces as the halogen series is ascended. The deviation from linearity is possibly due to differences in ionization potential.

The molar refractions of the ethyl haloacetates is listed in table 14. For the monohaloacetates, molar refraction increases regularly as the halogen series is ascended.

On polar phases retention is determined mainly by dipolar forces and by hydrogen bonding and to a lesser extent by dispersion forces. It is postulated that the variation in these polar forces with increasing molar mass is less than the variation in dispersion

FIG. 15



ETHYL HALOACETATES. PLOT OF LOG RELATIVE RETENTION ON SQ AT 150° vs MOLAR REFRACTION.

TABLE 14.

MOLAR REFRACTIONS OF ETHYL ACETATE AND ETHYL HALOACETATES

ACID FROM WHICH DERIVED	MOLAR REFRACTION $MOLE^{-1}$ CM^3
ACETIC	22.14
FLUOROACETIC	22.32
TRIFLUOROACETIC	22.86
CHLOROACETIC	26.92
DICHLOROACETIC	32.18
TRICHLOROACETIC	37.26
BROMOACETIC	29.80

forces with increasing molar mass.

It is interesting to note that the difference between acetate and fluoroacetate increases with increasing phase polarity. Thus on the more polar phases a large proportion of total intermolecular interaction must be due to the highly polar C - F bond.

(II) The Ethyl Chloroacetates and Fluoroacetates.

For the three chloroacetates the plot of log retention vs molar mass is linear with a positive slope on squalane and is linear with a negative slope on sorbitol. On squalane the only solute-solvent interaction is due to dispersion forces and these are seen to increase linearily with molar mass and with molar refraction for these compounds (figures 12 and 15). Sorbitol is a phase that is insensitive to dispersion forces but that is very sensitive to hydrogen bonding. It is to be expected that the hydrogen bonding capacity of the chloroacetates decreases with increasing chlorine substitution. It is however interesting that this decrease is linear. Thus it appears that sorbitol does not distinguish between the activity of active hydrogens but that for the ethyl chloroacetates the retention on sorbitol is a measure of the number of active hydrogens present. (It should be noted that the hydrogens of the acetate group of ethyl mono/chloroacetate should be approximately as active as those of methylene chloride due to the activating effect of the ester group).

For the other phases investigated the plot of log retention vs molar mass is not linear for the chloroacetates. This may be due to the fact that solute-solvent interaction is composed of various

interaction forces on these phases.

The results for the three fluoroacetates are difficult to understand in that the retention volume decreased with increasing fluorine substitution on both polar and apolar phases. The decrease in retention on apolar phases may be explained in either of the following two ways.

(a) By postulating that the heat of solution decreases with increasing fluorine substitution. On squalane the heat of solution is due to solute-solvent dispersion interaction. The molar refraction gives an indication (see ref: 30) of relative dispersion interaction which should be fairly accurate for closely related compounds. The molar refractions of ethyl fluoroacetate and ethyl trifluoroacetate are 22.3 and 22.8 cc mole^{-1} respectively whereas the corresponding chloroacetates have molar refractions of 26.9 and 37.3 cc mole^{-1} (see table 14). This explains why the slope of the log retention vs molar mass plot should be small for the fluoroacetates as compared with the chloroacetates but not why it should be negative. However there may be error in assuming that the ionization potential (see eqn. 4) is the same for all of the three fluoroacetates.

(b) By postulating that the entropy of solution increases with increasing fluorine substitution and that the heat of solution remains nearly constant as indicated by the molar refractions of these compounds. At 150^{o} an increase of 2.5 entropy units accounts for the decrease in retention on squalane assuming that there is no decrease in heat of solution on squalane. If there is a decrease in the heat of solution the increase in entropy of solution will be

smaller than 2.5 e.u.

No values are available for entropies of solution for these compounds, but the above value of 2.5 e.u. appears reasonable. By inspection of fig 2 ref: 45 it is seen that carbon tetrachloride has an entropy of solution on squalane that is about 2 e.u. greater than that of methylene chloride.

It is thus possible that for the ethyl haloacetates entropy of solution also increases with increasing number of halogen atoms.

It is postulated that for the chloroacetates the effect of increasing heat of solution with increasing number of chlorine atoms is large enough to result in an increasing free energy of solution even if there is also an increasing entropy of solution.

5.3.c. HALOACETATES OF 2,3-BUTANEDIOL.

The retention of these compounds is to a large degree determined by the same factors as determine the retention of the ethyl haloacetates. As noted earlier the haloacetates of 2,3-butanediol may be considered dimers of the corresponding ethyl haloacetates. In fact the plot of either the meso or the racemic series of diesters on any phase is nearly parallel to that of the corresponding ethyl haloacetates on the same phase at the same temperature (compare figures 12 and 16, figures 13 and 18).

Table 15 lists the relative retentions (ethyl benzoate = 1) and table 16 the resolution factors of these compounds. The same phases and temperature were used as for the chromatography of the dialkyl esters of 2,3-butanediol described in section 5.2.

Figures 16, 17 and 18 are the plots of log retention vs molar

TABLE 15.

RELATIVE RETENTIONS (ETHYL BENZOATE = 1.00)[a] OF DIESTERS DERIVED FROM 2,3-BUTANEDIOL AND HALOACETIC ACIDS.

ACID FROM WHICH DERIVED	SQ[b] 150°		DNP[b] 150°		PEG[b] 150°		CYB[b] 150°		EGS[b] 100°		EGS[b] 125°		EGS[b] 150°		EGS[b] 170°	
	meso	racemic	meso	racemic	meso	racemic	meso	racemic	meso	racemic	meso	racemic	meso	racemic	meso	racemic
FLUOROACETIC	.49	.52	1.44	1.74	2.48	2.97	5.90	7.83	.70	1.04	4.95	6.70	7.00	9.04	3.03	3.80
CHLOROACETIC	3.0	3.2	7.21	8.20	11.31	13.57	17.01	21.71	c	c	19.6	25.8	24.0	30.2	10.08	12.41
DICHLOROACETIC	7.65	d	16.74	18.22	19.34	21.90	21.66	24.19	c	c	27.8	30.0	36.3	40.9	14.05	15.72
TRICHLOROACETIC	16.9	d	22.75	d	17.15	d	12.11	d	c	c	c	c	23.5	d	c	c
BROMOACETIC	7.52	7.92	15.54	17.53	26.8	32.9	33.15	43.27	c	c	45.8	61.3	50.5	63.4	19.98	24.80

a. Retentions on EGS at 150° are relative to meso-2,3-diacetoxybutane = 1.00.

b. See list of abbreviations on page 121.

c. Retention not determined.

d. No resolution obtained under the experimental conditions.

TABLE 16.

RESOLUTION FACTORS OF DIESTERS DERIVED FROM 2,3-BUTANEDIOL and HALOACETIC ACIDS.

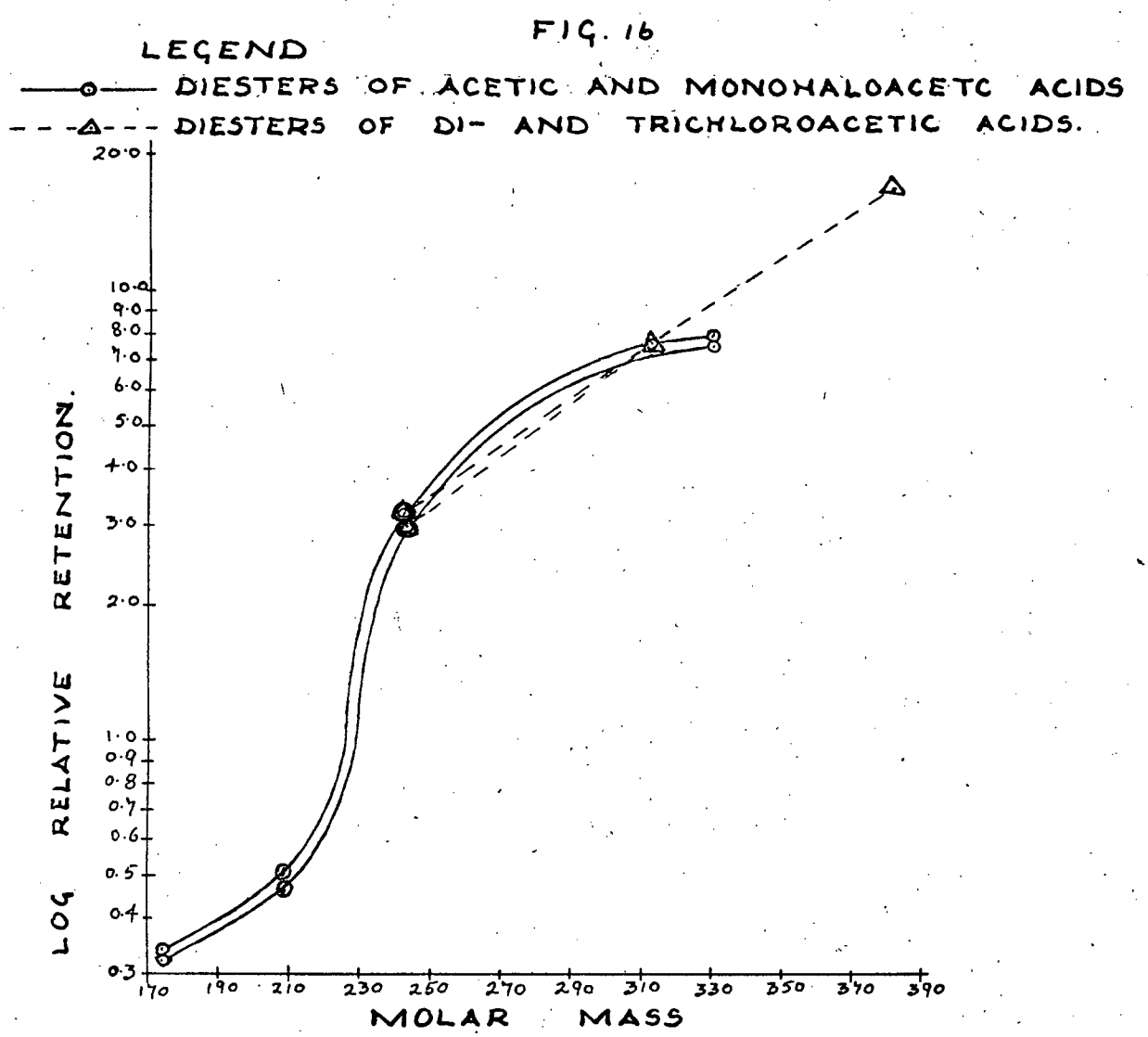
ACID FROM WHICH DERIVED,	SQ[a] 150°	DNP[a] 150°	PEG[a] 150°	CYB[a] 150°	EGS[a] 100°	EGS[a] 125°	EGS[a] 150°	EGS[a] 170°
FLUOROACETIC	1.07	1.20	1.21	1.33	1.48	1.35	1.30	1.26
CHLOROACETIC	1.06	1.15	1.20	1.28	b	1.32	1.26	1.23
DICHLOROACETIC	c	1.09	1.13	1.12	b	b	1.14	1.12
TRICHLOROACETIC	c	c	c	c	b	b	c	b
BROMOACETIC	1.05	1.13	1.23	1.28	b	1.33	1.25	1.24

a. See list of abbreviations on page 121.

b. Retention not determined.

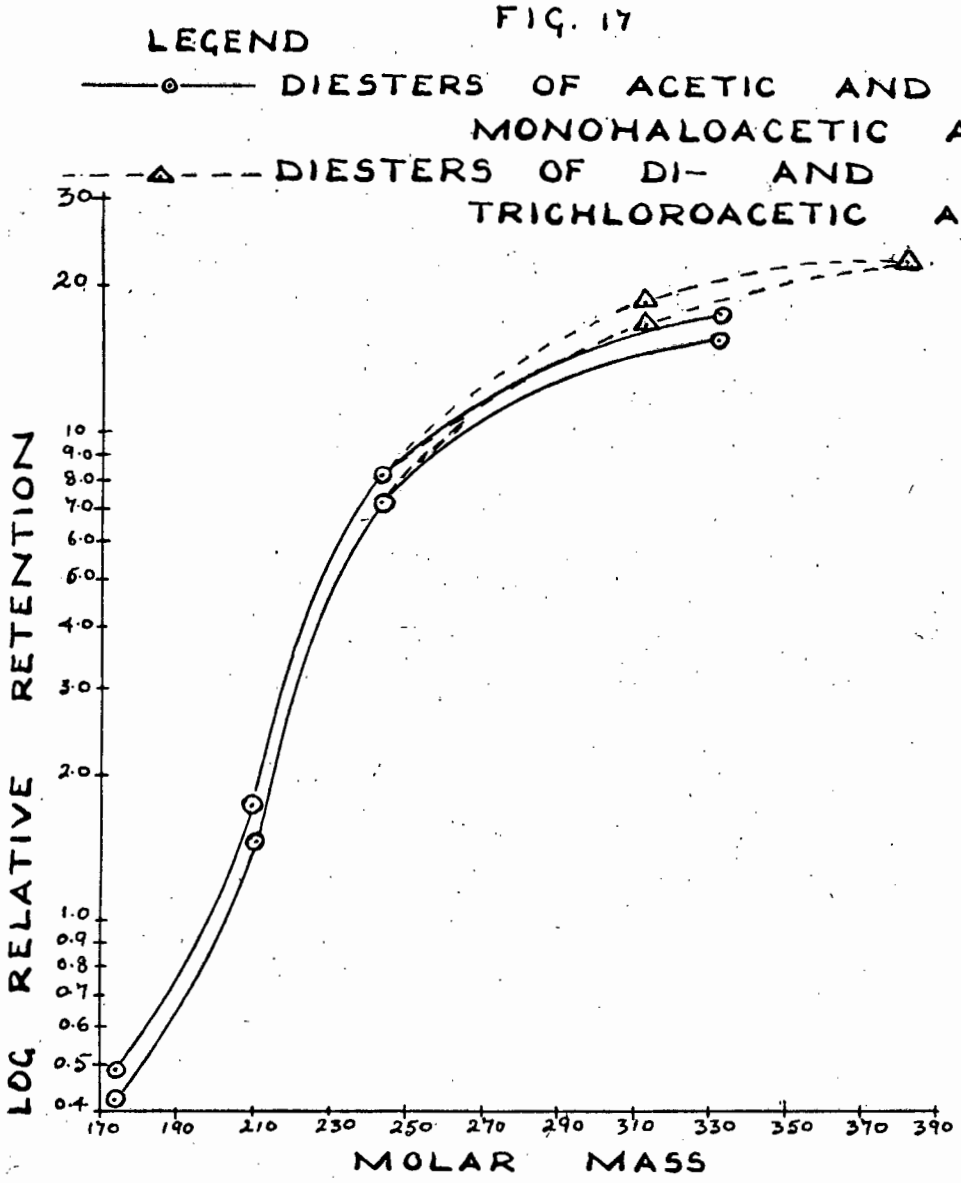
c. No resolution achieved under the experimental conditions.

FIG. 16



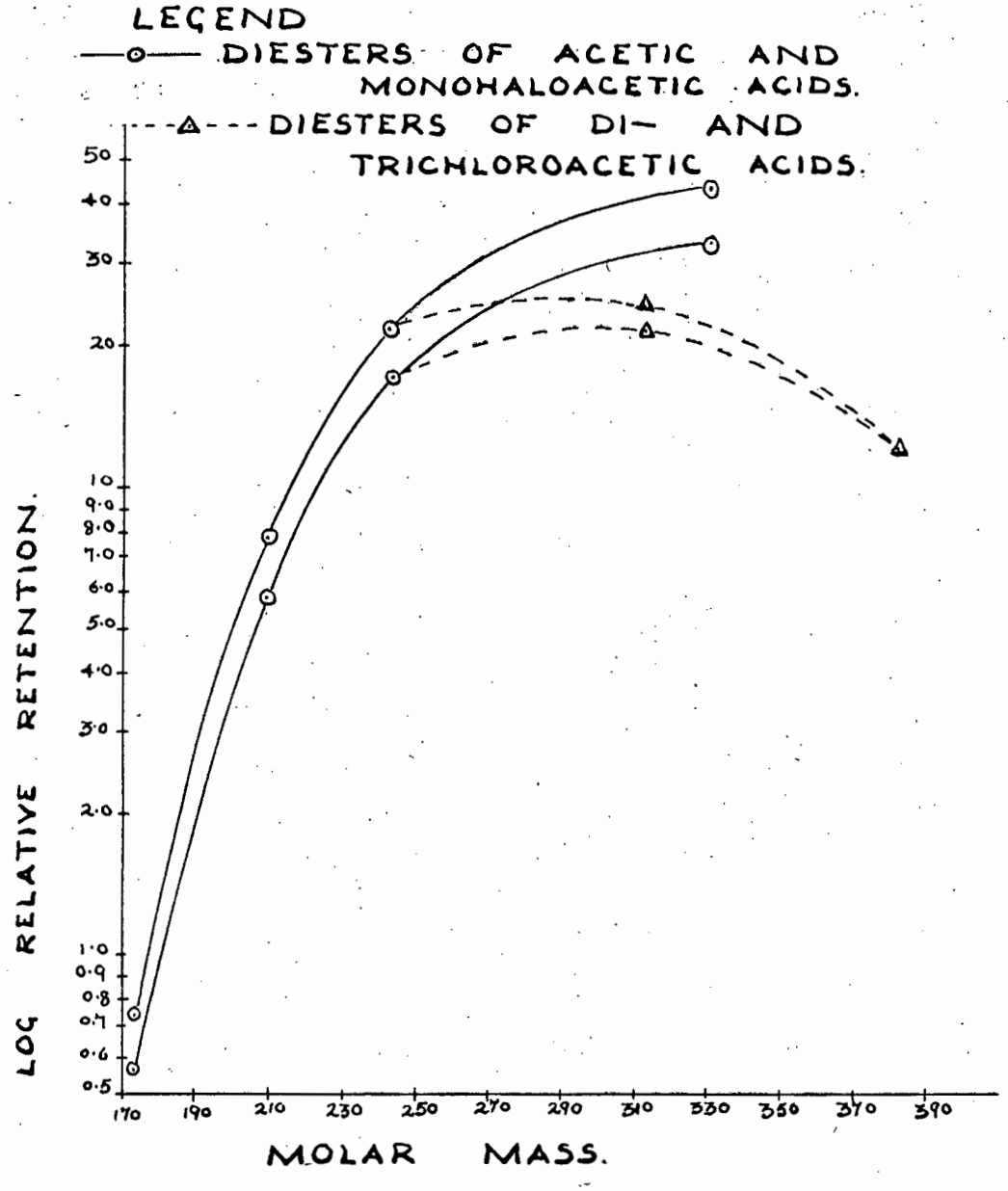
DIESTERS OF 2,3-BUTANEDIOL.
PLOT OF LOG RELATIVE RETENTION ON SQ AT 150° vs MOLAR MASS.

FIG. 17



DIESTERS OF 2,3-BUTANEDIOL. PLOT OF LOG RELATIVE RETENTION ON DNP AT 150° vs MOLAR MASS.

FIG. 18



DIESTERS OF 2,3-BUTANEDIOL, PLOT OF LOG RELATIVE RETENTION ON CYB AT 150° VS MOLAR MASS.

mass for these compounds on SQ, DNP and CYB. The points for 2,3-diacetoxybutane are also included in the plots. The plots for PEG and EGS are similar in shape to that on CYB.

The compounds were also chromatographed on EGS at 125° and 170°. The plots at these temperatures are of the same shape as at 150°.

The plots exhibit the following features:

(i) The acetate and monohaloacetates form one series whereas the three chloroacetates form a separate series.

(ii) For each series there are two separate lines. The upper line corresponds to racemic isomers and the lower line corresponds to meso isomers.

(iii) The lines are close together for the phases of low polarity and are more widely spaced for the more polar phases.

(iv) The lines for the monohaloacetates become more widely spaced with increasing molar mass for the more polar phases whereas the lines for the three chloroacetates converge on all phases.

(v) The monohaloacetates have retentions that increase with increasing molar mass. For the three chloroacetates there is a decrease in the retention of the trichloroacetate relative to the monochloroacetate as the polarity of the phase is increased.

These trends are partially explained in section 5.2.b. and in the next section which is a discussion of the variation in resolution factor of the haloacetic esters of 2,3-butanediol.

5.3.d. FURTHER ASPECTS OF SEPARATION MECHANISM 1.

As in the case of the dialkyl esters of 2,3-butanediol the chromatographic behaviour of the haloacetic esters can be correlated with structure in terms of 'mechanisms' of separation. Mechanism 1 (see section 5.2.b.) accounts for the order of elution of these compounds. There are however certain differences between the chromatographic behaviour of the dialkyl esters which were discussed in section 5.2.a, and that of the haloacetic esters. Thus on any phase there is a large variation in resolution factor between the various haloacetic esters whereas the variation in resolution factor for the dialkyl esters is small. The variation in resolution factor of the haloacetic esters may be due to either (or both) a 'steric effect' or an 'interaction effect'. These are discussed below.

'Steric effect'.

The variation may be due to different compounds having different mole fractions of conformers with adjacent polar groups due to different distributions of conformer population.

A good example of where this effect may be operating is in the case of the three diesters of 2,3-butanediol formed from each of the three chloroacetic acids. On any phase the separation of 2,3-di-(dichloroacetoxy) butane is lower than that of 2,3-di-(monochloroacetoxy) butane. No separation of the isomers of 2,3-di-(trichloroacetoxy) butane was achieved on any of the phases listed in table 15.

This behaviour is explained by postulating that as the halomethyl groups become more bulky the repulsion between them increases

with the result that conformers A and D (see fig. 6) become more favoured. These conformers would be expected to have similar free energies of solution. Any differences would be related to steric effects as discussed in mechanism 3.

It is, however, interesting to note that the resolution factors of the monohaloacetoxy derivatives are always of the same magnitude as that of 2,3-diacetoxybutane. This may be explained by postulating that the halomethyl groups of these compounds have favoured conformations with the halogens so orientated that they do not interact with each other. Thus the distribution of conformer populations would be expected to be nearly the same for the different monohaloacetoxy derivatives.

The slightly greater resolution factor of the fluoro derivative on all phases studied is most probably due to the highly polar carbon-fluorine bond inducing dipoles in the stationary phase. These induced dipoles will increase the effective polarity of the stationary phase and will result in a higher resolution factor for this derivative. It is significant that for the compounds listed in table 15, the difference in resolution factor between the fluoro derivative and the next best separated derivative is greatest on dinonyl phthalate which is the most polarizable of the phases studied.

Interaction Effect.

It has been noted earlier in this section that the degree to which a solvent can stabilize a dipole-dipole repulsion is dependent on solvent polarity. It is also logical to assume that the stabilization of dipole-dipole repulsion is dependent on how strongly the functional groups concerned interact with the stationary phase. It would thus be

expected that in a hypothetical series of diastereoisomers in which each pair of diastereoisomers had the same populations of the various conformers and in which the functional groups were of the same dipole moment, it would be those diastereoisomers whose functional groups interacted most strongly with the stationary phase, that would be best separated.

The order of interaction of functional groups on an apolar phase is chloroacetoxy < dichloroacetoxy < trichloroacetoxy. This is due to dispersion forces increasing in that order. On polar phases there is always a decrease in retention between dichloroacetoxy and tricholoroacetoxy while the increase between chloroacetoxy and dichloroacetoxy is small or negative (as in the case of ethyl chloroacetates on sorbitol). However even on polar phases the contribution of dispersion forces increases with increasing molar mass. Thus the decrease in polar forces must be greater than that indicated by the decrease in the retention. Therefore it may be concluded that on any stationary phase, polar interaction of the trichloroacetoxy group is less than that of the dichloroacetoxy group. On sorbitol the polar interaction of the dichloroacetoxy group is less than that of the monochloroacetoxy group. This is most probably true for all polar phases but can be proved unequivocally only if the magnitudes of the dispersion forces acting for these two groups is known for the phases considered.

An attempt to estimate the relative magnitude of polar interaction for various functional groups on a particular phase, is described below.

The relative retentions of simple monofunctional compounds containing the functional groups are divided by the relative retentions

of the same compounds on squalane or any other paraffinic (apolar) phase. The values so obtained are expressed relative to the value of a compound chosen as a standard. It should be noted that these relative values are independent of the nature of the original standard used in determining the relative retentions of the compounds on each phase. If for each phase, the same experimental conditions are used for determining the retention of each compound, retention times instead of relative retentions may be used for calculating these relative values.

It is proposed to call the relative value for each compound the polar interaction constant (P.I.C.) of the functional group in the compound. P.I.C. for any group apart from the standard is dependent on the nature of the stationary phase. It is most probably also dependent on temperature and on the nature of the monofunctional compound.

Table 17 lists the polar interaction constants for the acetoxy and the various haloacetoxy groups on DNP, PEG and CYB. The constants are relative to trichloroacetoxy = 1.00.

It is assumed that P.I.C. of a functional group is a measure of its polar interaction with the stationary phase. It is interesting to note that the fluoroacetoxy group has the highest P.I.C. on any phase and 2,3-di-(fluoroacetoxy) butane the highest resolution factor on any phase. The trichloroacetoxy group has the lowest P.I.C. on any phase and 2,3-di-(trichloroacetoxy) butane the lowest resolution factor on any phase.

Figure 19 is a plot of P.I.C. of the acetoxy, chloroacetoxy and bromoacetoxy groups vs resolution factor of corresponding diastereoisomers on three different phases. It is seen that there is a

TABLE 17.

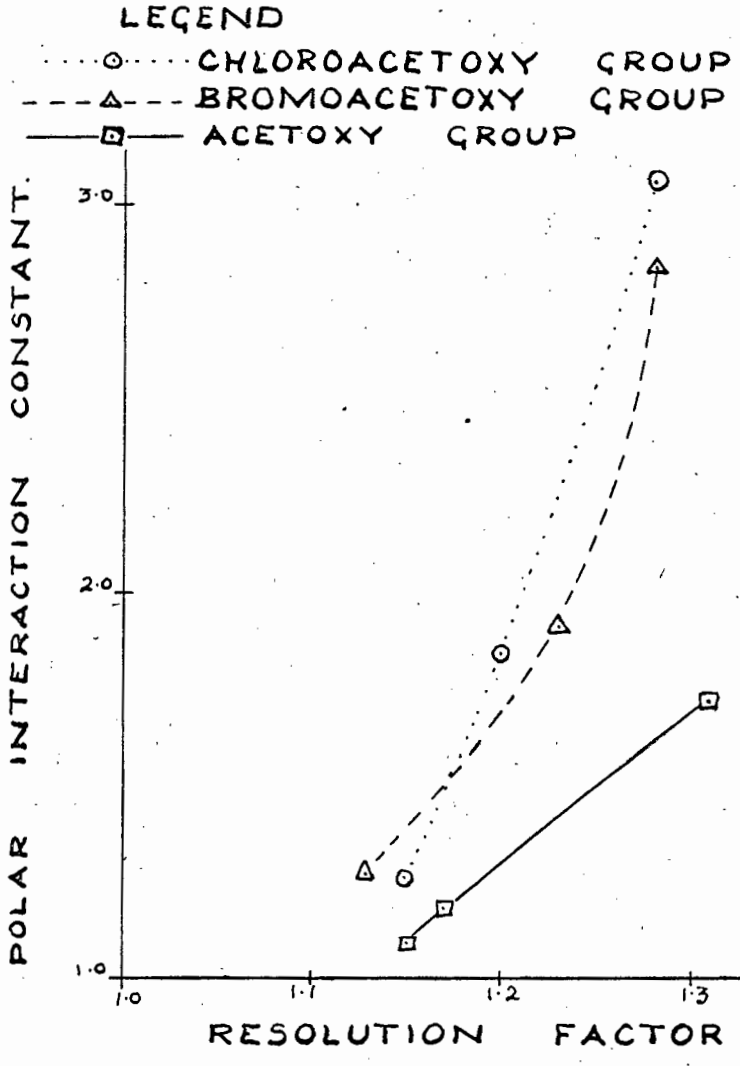
POLAR INTERACTION CONSTANTS OF FUNCTIONAL GROUPS AT 150° DETERMINED FOR ETHYL HALOACETATES AND EXPRESSED RELATIVE TO TRICHLOROACETOXY GROUP = 1.00.

FUNCTIONAL GROUP	DNP[b]	PEG[b]	CYB[b]
ACETOXY	1.09	1.18	1.72
FLUOROACETOXY	1.42	a	4.08
CHLOROACETOXY	1.26	1.84	3.06
DICHLOROACETOXY	1.22	1.67	2.09
TRICHLOROACETOXY	1.00	1.00	1.00
BROMOACETOXY	1.27	1.91	2.84

a. Retention of Ethyl fluoroacetate on PEG not determined.

b. For list of abbreviations see page 121.

FIG. 19



DIESTERS OF 2,3-BUTANEDIOL.
PLOT OF POLAR INTERACTION CONSTANT OF FUNCTIONAL GROUPS vs RESOLUTION FACTOR ON DNP, PEG AND CYB AT 150°.

regular increase in resolution factor with increasing P.I.C. This increase is linear for the acetoxy and chloroacetoxy groups. This demonstrates the close relationship between P.I.C. and resolution factor and supports the argument that resolution factor is dependent on the degree of polar interaction of a functional group with the stationary phase.

Plots of this type should be of importance for predicting the resolution factor of a pair of diastereoisomers on a phase for which the P.I.C. of the functional groups is known.

Figures 20a and 20b are plots of P.I.C. of various functional groups vs resolution factor for the corresponding 2,3-disubstituted butanes on one phase. Figure 20a is for DNP and figure 20b is for PEG. A resolution factor of 1.02 was arbitrarily chosen for 2,3-di-(trichloroacetoxy) butane as the true value most probably lies between 1.00 and 1.04.

Figures 20a and 20b illustrate that on DNP and PEG there is a semi-quantitative relationship between P.I.C. and resolution factor. The plot for CYB (not shown) is not as regular as the latter two plots.

On any of the three phases (DNP, PEG and CYB) the point for 2,3-diacetoxybutane lies off the curve for the haloacetoxy derivatives. The distance that the point lies off the curve increases with increasing phase polarity. This fact may be explained by postulating that while resolution factor is proportional to P.I.C., the constant of proportionality is dependent on the nature of the functional group. Thus it is postulated that the various haloacetoxy groups all have similar constants of proportionality that are however different from that of the acetoxy group.

It should be noted that a dependence of separation of diastereoisomers on a structural constant has been observed by Hagens[52].

FIG. 20a

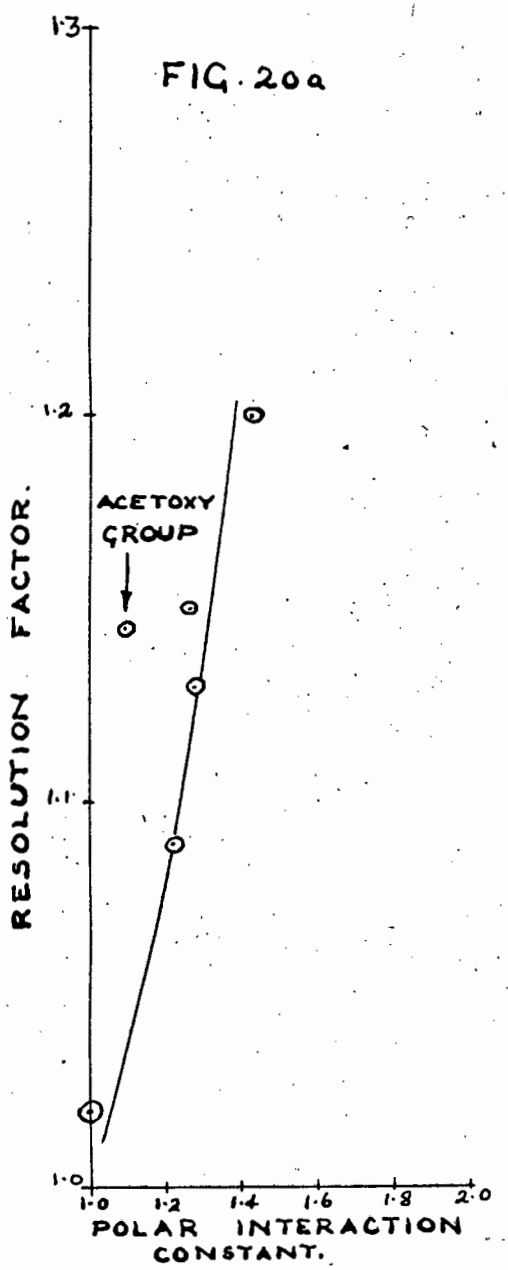
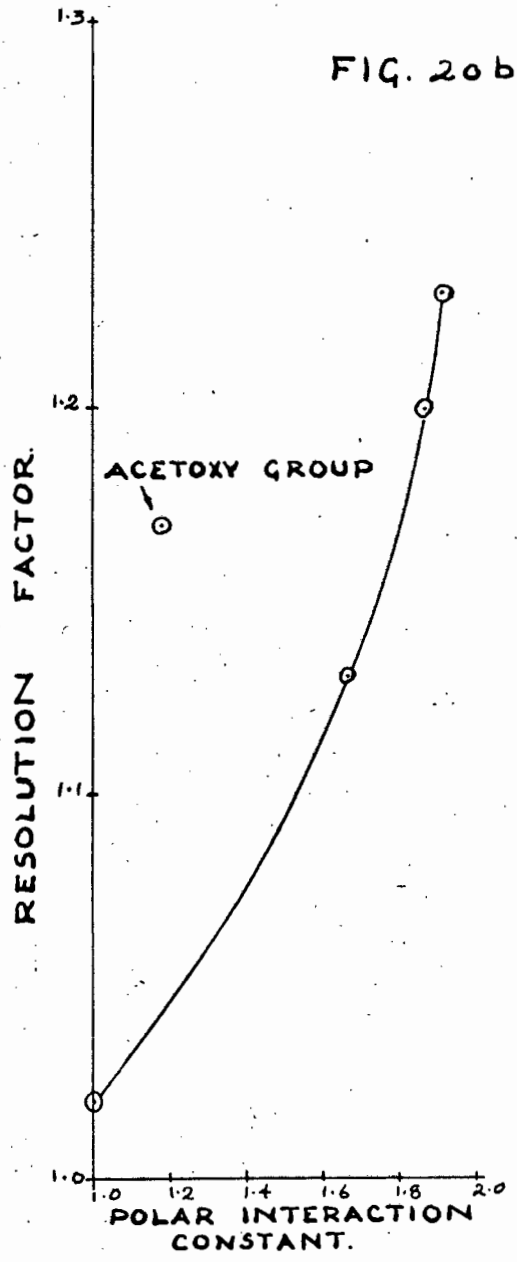


FIG. 20b



DIESTERS OF 2,3-BUTANEDIOL. PLOTS OF RESOLUTION FACTOR vs POLAR INTERACTION CONSTANT OF FUNCTIONAL GROUPS ON DNP(20a) AND PEG(20b).

The effective polar interaction is implicit in Hagens's 'group retention factor' whereas in the present work it is explicitly expressed via the P.I.C.

While the derivation of P.I.C. is not similar to that of 'group retention factor' it is similar to that of the interaction constant, $r_{(b)}$, derived by Littlewood[45]. The latter constant involves the retentions of both an alkane and a polar solute on two different phases.

SECTION 5.4 α,β-DIOLS.

5.4.a. ORDER OF ELUTION.

The diastereoisomers of 2,3-butanediol are difficult to separate on apolar phases but may be separated with ease on sorbitol. At 150° the resolution factor is 1.23, with the meso isomer having the higher retention i.e. having the opposite order of elution to that of the diastereoisomers discussed earlier.

The diastereoisomers of 1,2-cyclopentanediol have the very high resolution factor of 2.65 on sorbitol at 145°, with the trans isomer having the higher retention volume i.e. having the opposite order of elution to that of 1,2-diacetoxycyclopentane.

The following mechanism of separation is postulated for these compounds.

5.4.b. SEPARATION MECHANISM 2.

This mechanism is applicable to diastereoisomers that contain two functional groups that attract each other. α,β-Diols fall into this class of compounds. There is experimental evidence that this mechanism is applicable also to compounds where there is intramolecular hydrogen bonding between hydroxyl and a double bond[7,28] (see section 2) or hydroxyl and an ether group[46].

In the case of 2,3-butanediol there is a very strong attractive interaction between the hydroxyl groups due to intramolecular hydrogen bonding. Thus conformers B, C, E and F (see figure 6 page 55) are more favoured than A or D. By a similar argument to that used earlier (see page 56) it can be shown that conformers B and C have higher free energies than conformers E and F. The overall effect is that racemic-2,3-butanediol

has the greater population of conformers with adjacent hydroxyl groups. This results in a greater degree of intramolecular hydrogen bonding in racemic-2,3-butanediol than in meso-2,3-butanediol.

In the case of cyclopentanediol, only the cis isomer is capable of intramolecular hydrogen bonding. The infrared spectrum[47] of the cis isomer in dilute carbon tetrachloride solution has absorptions due to intra- as well as intermolecularly hydrogen bonded hydroxyl, whereas the trans isomer has only an absorption due to intermolecularly hydrogen bonded hydroxyl.

Thus it is that diol isomer which exhibits the lesser degree of intramolecular hydrogen bonding that has the larger retention on sorbitol, since the stabilization of the molecule, due to intramolecular hydrogen bonding, results in a more negative value of its free energy, and hence a less negative value for the corresponding free energy of solution (assuming that the free energies of both isomers are very nearly the same in hydroxylic solvents - a reasonable assumption).

The isomers of 1,2-cyclopentanediol separate better than those of 2,3-butanediol because (a) the stereochemistry of cis-1,2-cyclopentanediol is more favourable for intramolecular hydrogen bonding than is the stereochemistry of racemic-2,3-butanediol and (b) there is no intramolecular hydrogen bonding in trans cyclopentanediol whereas there is some degree of intramolecular hydrogen bonding in meso-2,3-butanediol. Thus there is a greater difference in the degree of intramolecular hydrogen bonding in the case of the isomers of 1,2-cyclopentanediol than in the isomers of 2,3-butanediol.

A semiquantitative approach to the calculation of resolution factor for the isomers of 2,3-butanediol is considered in the Appendix.

SECTION 5.5 SEPARATION MECHANISM 3.

The separation of certain bifunctional diastereoisomers has been correlated with certain structural features in mechanisms 1 and 2. There are however certain diastereoisomers that have fairly high resolution factors but which have one functional group only. Thus the resolution factor for the pair of diastereoisomers borneol/isoborneol is 1.40 on sorbitol at 150°. The separation of diastereoisomers of this class is explained by separation mechanism 3 which is discussed below.

The contribution of any functional group to the total retention of a molecule is dependent on the group's steric environment. A functional group that is screened by any other part of the molecule will interact with the stationary phase to a lesser extent than an unscreened group.

A pair of diastereoisomers will contain the same functional groups, but these groups may not be in the same steric environment in each member of the pair. If this is so, identical functional groups may make different contributions to the total retention of different diastereoisomers. This effect will be dependent both on the degree of steric hindrance and on the strength of the interaction of the functional group with the stationary phase.

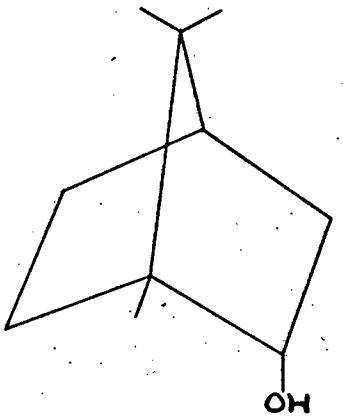
In general this effect will be small, may be cancelled out when all groups are considered and will in any event be difficult to predict. There are, however, some stationary phases that to a first approximation interact only with certain functional groups. Sorbitol falls into this class in that it interacts strongly with hydroxyls and very weakly with alkyl groups.

Figure 21 lists three bicycloheptanols and their relative retentions (ethanol = 1.00) on sorbitol. It is seen that for the isomeric borneol and isoborneol, the isomer that has the less sterically hindered hydroxyl has the higher retention volume. Norborneol which has a less hindered hydroxyl than either of the borneol isomers, has the highest retention of the three compounds.

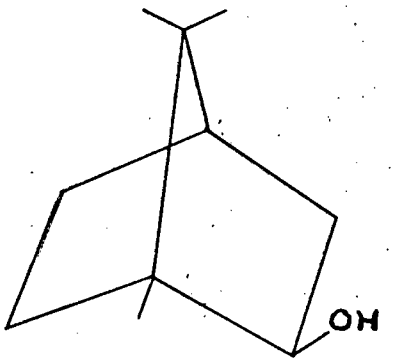
Norborneol is not isomeric with borneol and isoborneol due to the lack of three methyl groups (see fig. 21). On sorbitol, however, the contribution of these methyl groups to retention is very small.

A similar effect should operate also in the case of diastereoisomers that contain more than one functional group. It should operate also where each diastereoisomer exists in a number of conformations as in the case of the diesters of 2,3-butanediol. However this mechanism of separation most probably does not play an important role in the separation of the latter class of diastereoisomers.

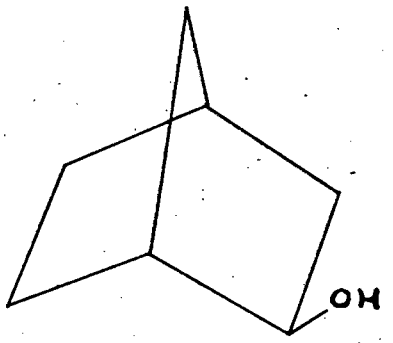
FIG. 21



BORNEOL
R = 3·5



ISOBORNEOL
R = 2·5



EXO-NORBORNEOL
R = 5·0

BICYCLOHEPTANOLS AND THEIR RETENTIONS RELATIVE TO ETHYL ALCOHOL = 1·00

SECTION 5.6. PHASE CLASSIFICATION AND SELECTION.

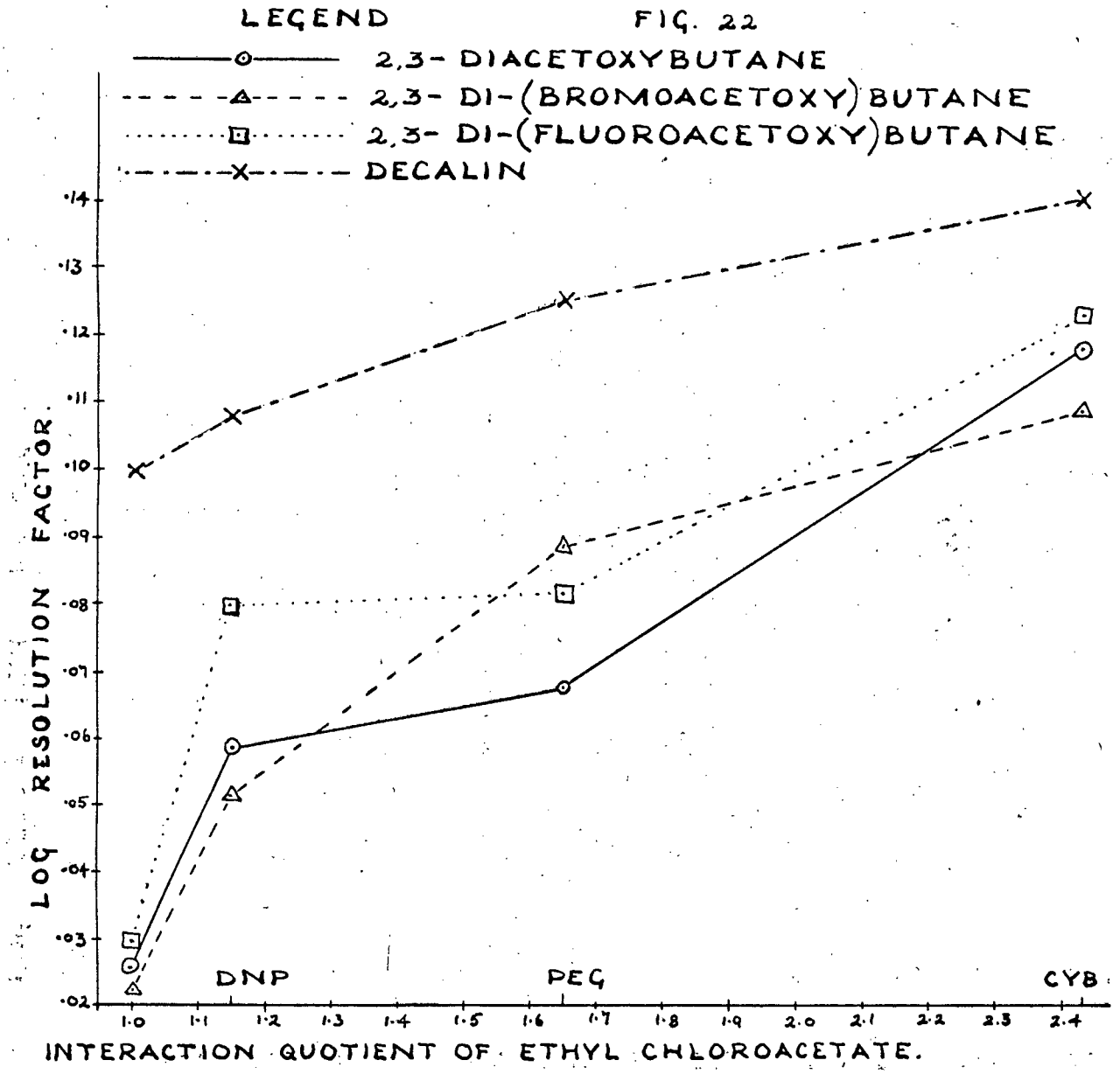
Ideally the choice of phase for the separation of a particular pair of diastereoisomers should be made on purely theoretical grounds in terms of solute-solvent interactions. Unfortunately these interactions are poorly understood for most solute-solvent systems encountered in gas chromatography.

An alternative approach is to classify phases on an empirical or semi-empirical basis according to their suitability for separating diastereoisomers. In the methods described below the parameter is termed polarity in accordance with general gas chromatographic practice.

1) Classification by a Method Based on Group Interaction.

It has been postulated that diastereoisomers separate due to various polar solute-solvent interactions (see section 5.3.d.). An estimate of the degree of polar interaction for a particular phase can be obtained by dividing the relative retention volume of a polar solute on that phase by the relative retention of the same solute on squalane. It is proposed to call this quotient the interaction quotient. It does not quantitatively allow for dispersion forces as the dispersion forces decrease with increasing polarity.

In figure 22, interaction quotient of ethyl chloroacetate is used for assigning polarity to various phases. The log of resolution factor for various pairs of diastereoisomers is plotted against the interaction quotient of the various phases on which the diastereoisomers have been separated. Such a plot of retention (or relative retention as in fig. 22) vs polarity is called a retention diagram after the practice of Littlewood (see ref: 30).



PLOT OF LOG RESOLUTION FACTORS OF SOME DIASTEREOISOMERS AT 150° vs INTERACTION QUOTIENT OF ETHYL CHLOROACETATE.

To illustrate the general nature of the retention diagram decalin is included. The resolution factors for decalin at 150° are 1.26 on SQ, 1.29 on DNP, 1.33 on PEG and 1.38 on CYB.

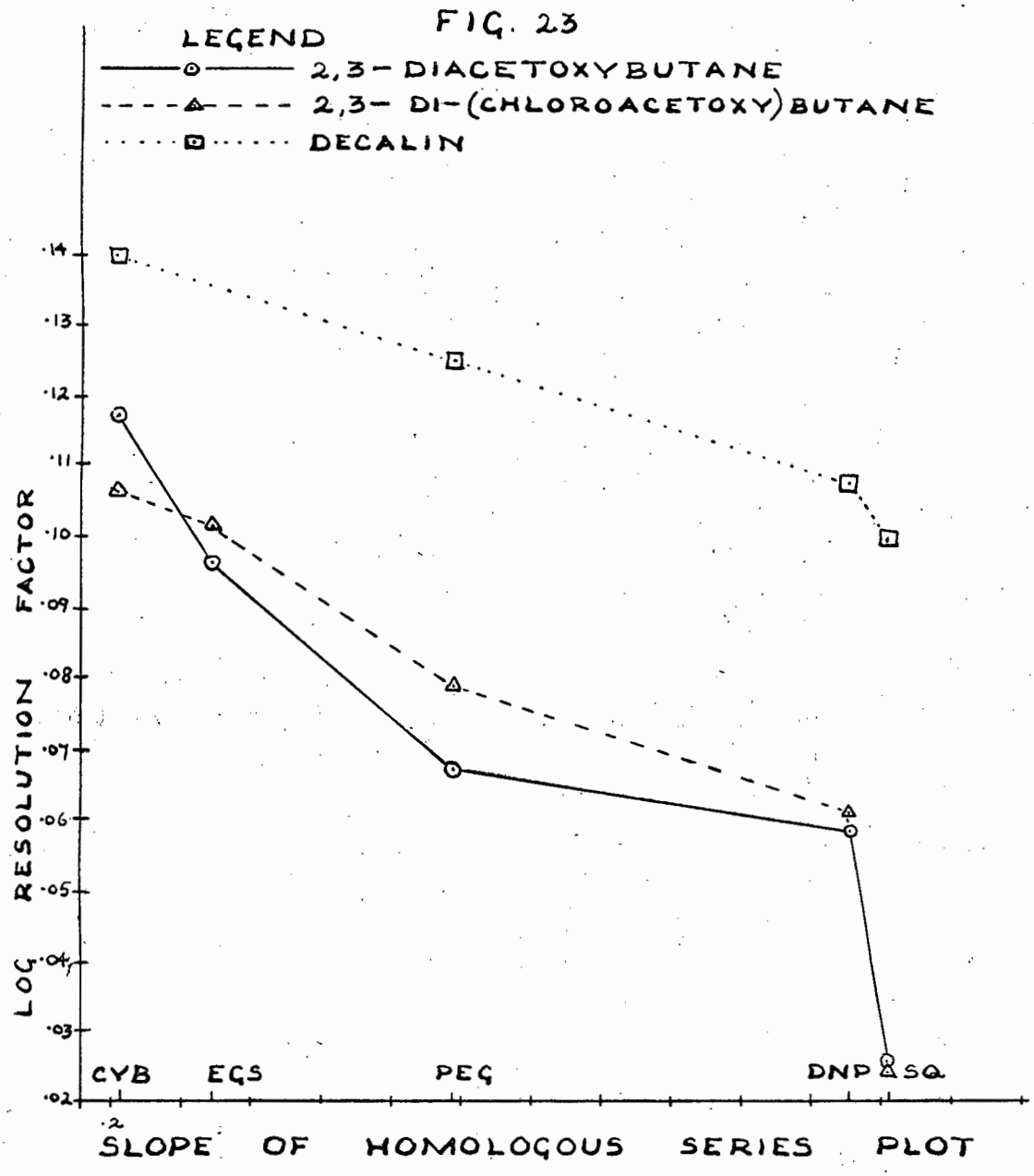
It is seen that log resolution factor increases regularly with increasing phase polarity even for the apolar decalin. The rate of increase of resolution factor with increasing polarity is however less for decalin than for any other compound [with the exception of 2,3-di-(dichloroacetoxy) butane] investigated.

Thus it is possible to estimate the suitability of a phase for separating diastereoisomers by determining the interaction quotient for simple compounds such as ethyl chloroacetate which is commercially available.

2) Classification by Means of the Slope of the Plot of Retention vs Molar Mass for a Homologous Series.

Littlewood[30] has shown that for polar phases the slope of the plot of the log of retention of a homologous series of compounds vs carbon number (i.e. vs molar mass) is a good measure of solvent polarity. Fig. 23 is the plot of log resolution factor of a few pairs of diastereoisomers vs the slope of the homologous series plot for the dialkyl esters of racemic-2,3-butanediol. It is seen that with the exception of the points for DNP, the resolution factors of the polar diastereoisomers vary regularly with polarity. The irregular behaviour of DNP may be due to the fact that this method of phase classification is essentially a measure of the affinity of a particular phase for a methylene group. A methylene group is incapable of causing an induced dipole in another molecule whereas an ester group will cause an induced

FIG. 23



PLOT OF LOG RESOLUTION FACTOR OF SOME DIASTEREOISOMERS AT 150° VS SLOPE OF HOMOLOGOUS SERIES PLOT.

dipole in a polarizable molecule such as DNP, i.e. it will increase the polarity of DNP. The ratio of the resolution factor of 2,3-di-(fluoroacetoxy)butane to that of 2,3-di-(bromoacetoxy)butane is greatest on DNP for an analogous reason. Bromine is less effective than fluorine in inducing polarity.

Thus phase classification by the slope of the homologue plot is unsatisfactory when considering the chromatography of polar diastereoisomers on polarizable phases. The method however appears to be satisfactory for all phases when considering the chromatography of apolar diastereoisomers such as decalin.

3) Classification by Means of the Log Resolution Factor of a Pair of Diastereoisomers.

This is the most straightforward of all the methods. Fig. 24 is a plot of log resolution factor of some haloacetoxy derivatives vs log resolution factor of 2,3-diacetoxybutane. It is seen that there is a regular trend and that the lines may reasonably be extrapolated to the origin. A linear relationship would not be expected for all pairs because of the different nature of the forces causing separation.

In the case of very closely related compounds there may be a linear relationship between log resolution factors. This is seen in figure 25 which is a plot of log resolution factor of the divaleric ester of butanediol vs log resolution factor of 2,3-diacetoxybutane.

4) Classification by Means of a Modified Rohrschneider Plot.

Phases are classified by this method by assigning SQ and CYB polarities of 0 and 100 respectively. 2,3-Diacetoxybutane is used as a standard, and the log of its resolution factor is plotted against

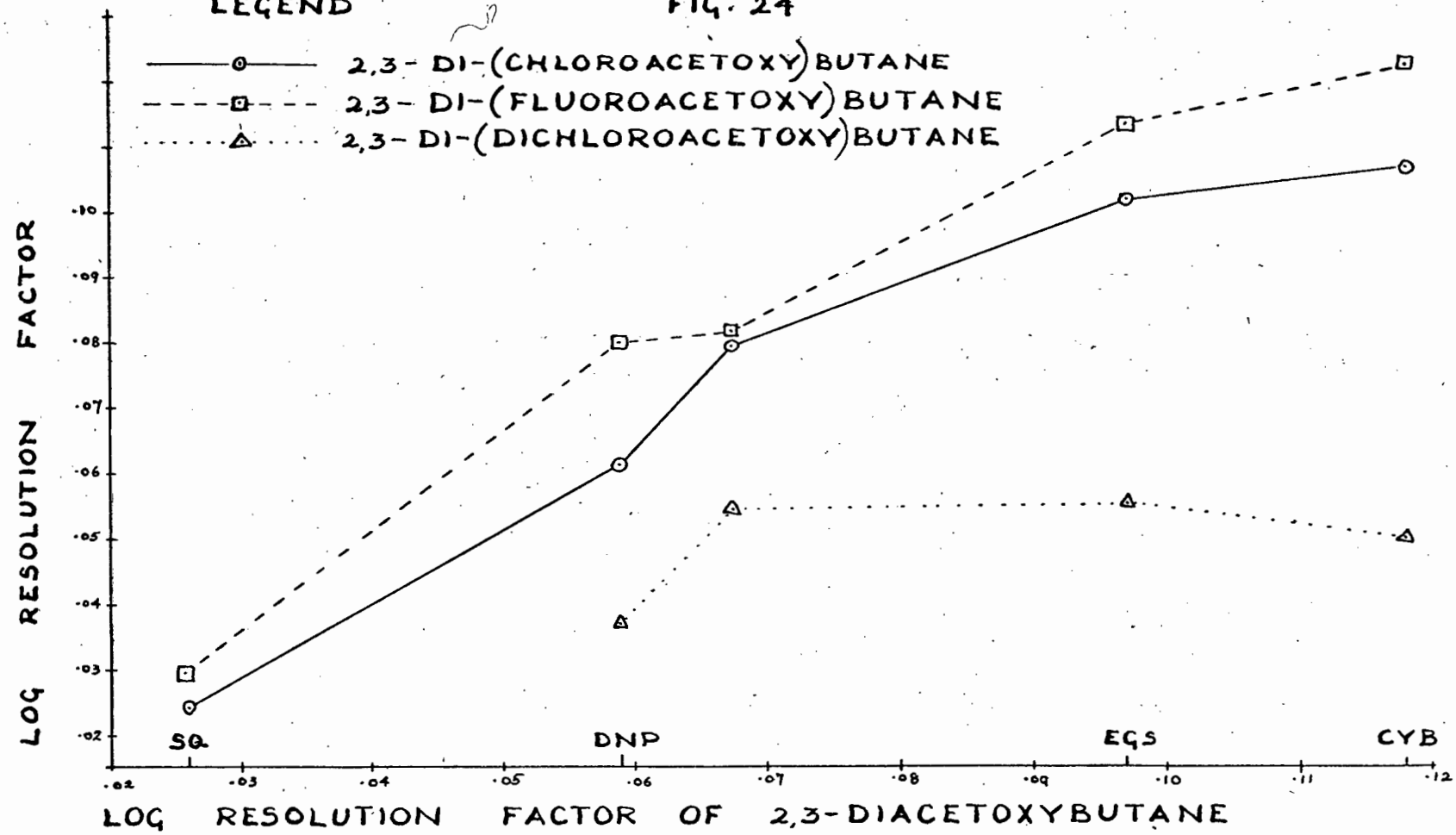
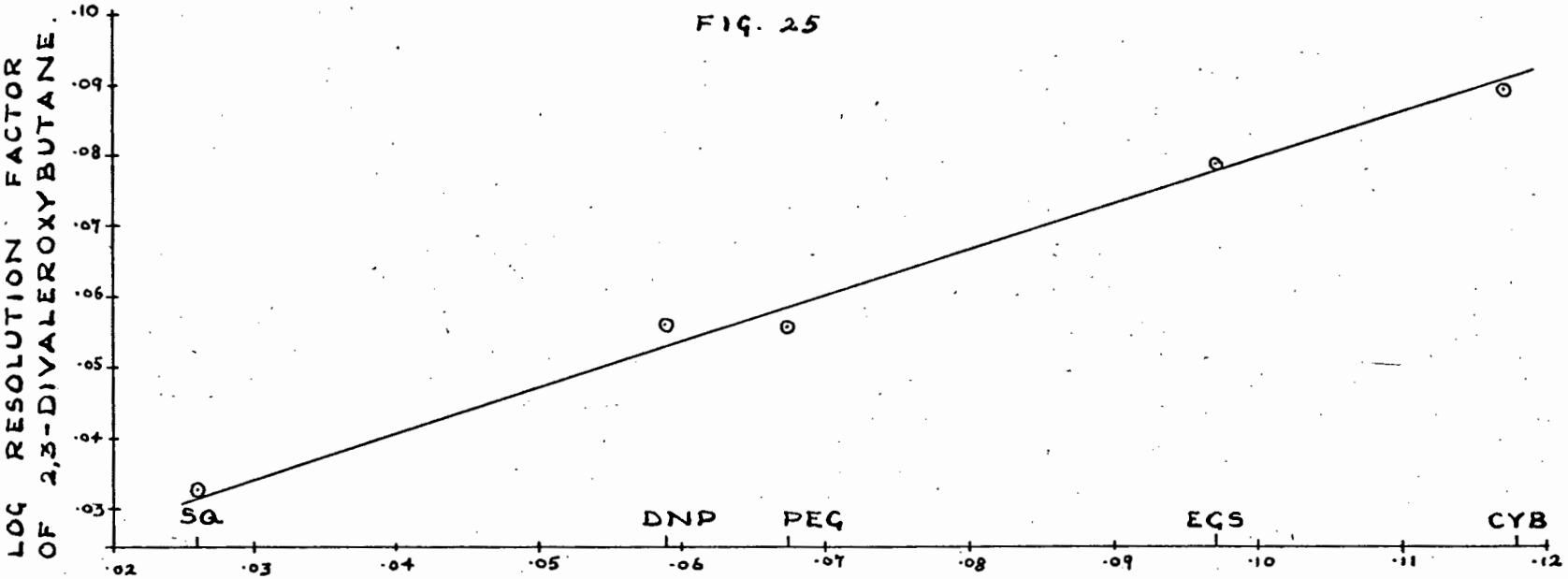


FIG. 24

PLOT OF LOG RESOLUTION FACTOR OF SOME DIASTEREOISOMERS AT 150° vs LOG RESOLUTION FACTOR OF 2,3-DIACETOXYBUTANE.

FIG. 25



PLOT OF LOG RESOLUTION FACTOR OF 2,3-DIVALEROXYBUTANE AT 150° vs LOG RESOLUTION FACTOR OF 2,3-DIACETOXYBUTANE.

polarity for SQ and CYB. The other phases are then assigned polarities such that the points for all the phases lie on the straight line joining the points for the two reference phases.

Figure 26 is a plot of log resolution factor of a few diastereoisomers vs polarity assigned by this method. The reference line for 2,3-diacetoxybutane is included in the plot.

Conclusions.

The above examples show that the various methods of phase classification are of some value in that there are regular trends between phase polarity and resolution factor for all diastereoisomers investigated.

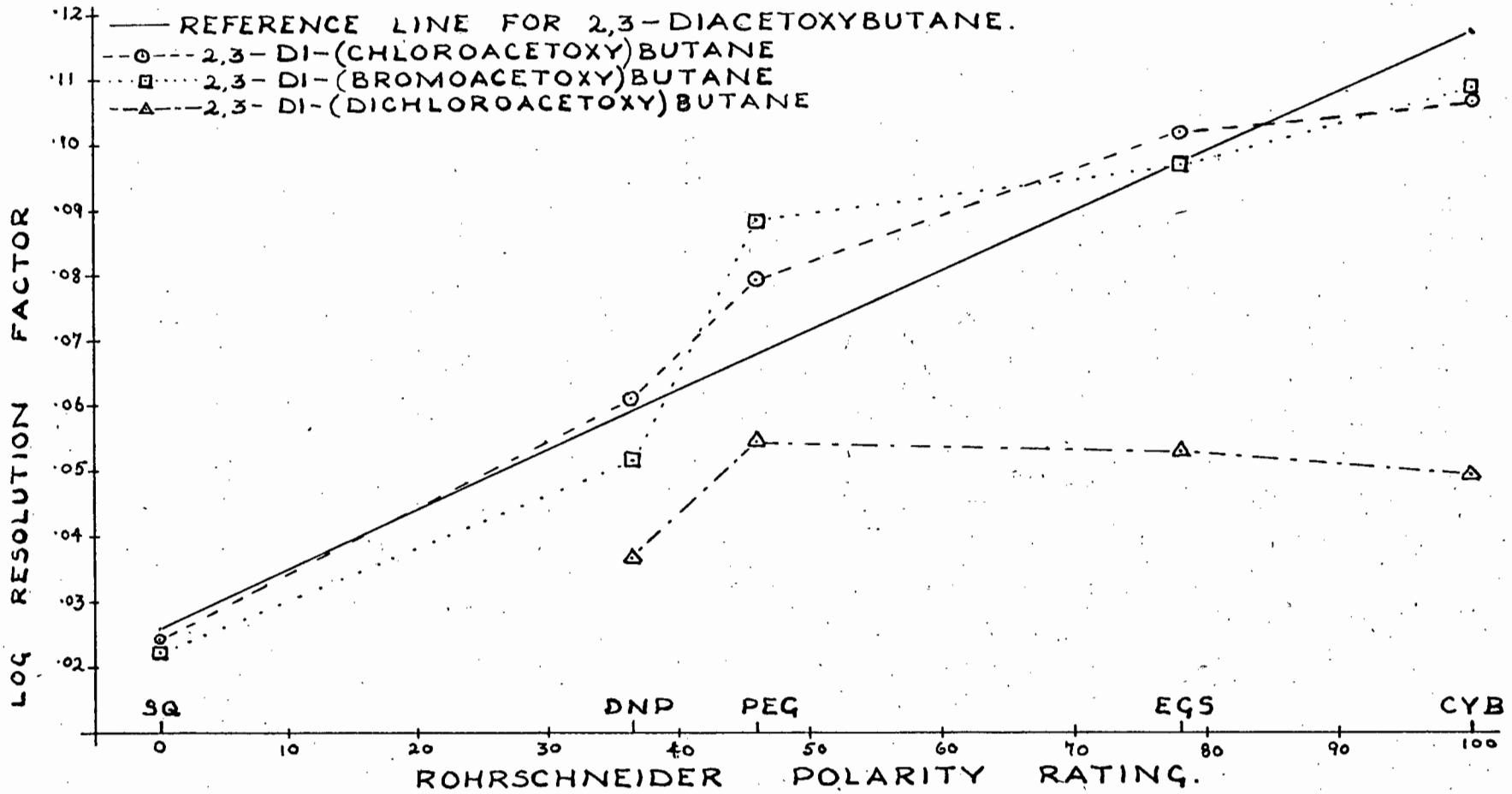
A fair indication of the best phase for separating a pair of diastereoisomers may be obtained from the retention diagram of a chemically related compound. Thus the retention diagram of any two dialkyl esters of 2,3-butanediol should be the same (see fig. 25).

Care must be exercised in deciding which are 'chemically related' compounds. Thus while the resolution factors of all the monohaloacetoxy derivatives increase with increasing phase polarity, the resolution factor of 2,3-di-(dichloroacetoxy)butane decreases with increasing phase polarity. There is no detectible separation between the diastereoisomers of 2,3-di-(trichloroacetoxy)butane.

Even though diastereoisomers of the same general class may have very different retention diagrams, there are also diastereoisomers of completely different chemical structure that have similar retention diagrams. Thus decalin and the dialkyl esters of 2,3-butanediol have similar retention diagrams.

For cases where no / retention diagrams are available polar interaction

FIG. 26



PLOT OF LOG RESOLUTION FACTOR OF SOME DIASTEREOISOMERS AT 150° vs ROHRSCHNEIDER POLARITY RATING.

constants (see section 5.3.d.) may prove of value. These have been investigated only for the haloacetoxy groups. On any particular phase diastereoisomers with functional groups of high polar interaction constant have higher resolution factors than diastereoisomers with functional groups of low polar interaction constants.

A further use of polar interaction constants is that they should determine which is the best phase for separating a pair of diastereoisomers.

From a general knowledge of group interaction it is often possible to predict the phase on which a particular group will have a high polar interaction constant. Thus the hydroxyl group is expected to have a high polar interaction constant on a polyol. This is borne out by the fact that sorbitol separates 2,3-butanediol better than any of the other phases investigated even though it is a poor phase for separating any of the diastereoisomers listed in table 8.

In general phases of high polarity are the best phases for separating diastereoisomers. Not only are the resolution factors generally higher on these phases but the retention times tend to be lower with the result that longer columns with a larger number of theoretical plates can conveniently be used. Both these factors - higher resolution factor and larger number of theoretical plates - result in higher resolution.

C O N C L U S I O N S.

The conclusions that may be drawn from the work described in this thesis are discussed under the following subheadings.

ALKYL LACTATES.

Some of these compounds can be partially separated into diastereoisomers on capillary columns.

ALKYL α-ALKANOYLOXYPROPIONATES.

All of these compounds can be separated into diastereoisomers on capillary columns and in some cases can be separated into diastereoisomers on packed columns. The order of elution is such that the diastereoisomers prepared from acid and alcohol both having the same conventional symbol of configuration (i.e. both D or both L) are eluted before the corresponding diastereoisomers of alternative configurations.

DIALKYL AND DI-(HALOACETIC) ESTERS OF 2,3-BUTANEDIOL.

All of these compounds [with the exception of 2,3-di-(trichloroacetoxy) butane] can be separated into diastereoisomers on packed columns. The order of elution is such that for any compound the *meso* isomer is always eluted first.

The resolution factors of nearly all of these compounds are of approximately the same magnitude on any given phase. The resolution factor of 2,3-di-(dichloroacetoxy) butane is however always lower than that of any other compound on any phase and the diastereoisomers of 2,3-di-(trichloroacetoxy) butane could not be even partially separated on any phase.

The resolution factors of nearly all of these compounds increase

with increasing phase polarity.

α,β-DIOLS.

For these compounds it is the isomer that has the greater degree of intramolecular hydrogen bonding that is eluted first. In the case of 2,3-butanediol this corresponds to the racemic isomer and in the case of 1,2-cyclopentanediol this corresponds to the cis isomer.

A P P E N D I X

THE SEMIQUANTITATIVE CALCULATION OF RESOLUTION FACTOR.

INTRODUCTION.

The semiquantitative calculation of resolution factor of the diastereoisomers of 2,3-butanediol at 150^{o} on sorbitol is described below.

Eliel and co-authors (ref: 48 page 23) have demonstrated that it is possible to calculate the equilibrium constant between a pair of diastereoisomers by assuming that differences in free energy are due to differences in overall group interaction and differences in entropy of mixing. Their method is extended in this section.

The free energies of each of a pair of diastereoisomers may be calculated in both the gas phase and the liquid phase relative to a standard state. The standard state may be chosen[49] as a hypothetical conformer of one of the diastereoisomers such that the overall group interaction of this conformer is zero.

From the relative free energies it is possible to calculate the free energy of solution for each diastereoisomer and hence the resolution factor for the pair of diastereoisomers.

Only groups that are adjacent to each other were considered in calculating the overall group interaction of a conformer. The following values were assumed in calculating enthalpy changes (due to group interactions) referred to a hypothetical standard state in which no group interactions were encountered.

methyl-methyl	0.8 k cal mole^{-1} (gas or liquid phase)
methyl-hydroxyl	0.35 k cal mole^{-1} (gas or liquid phase)
hydroxyl-hydroxyl	0.35 k cal mole^{-1} (liquid phase)
hydroxyl-hydroxyl	-0.8 k cal mole^{-1} (gas phase)

These values (taken from ref: 48) were obtained for different

diols. It is however assumed that gauche group interactions are to a first approximation independent of the rest of the molecule.

Interactions between hydrogens and any other groups are assumed to be negligible. The methyl-methyl and methyl-hydroxyl values are assumed to vary by less than 0.1 k cal $mole^{-1}$ on solution. The methyl-methyl value is that calculated for the gauche interaction of the methyl groups of butane; the methyl-hydroxyl and hydroxyl-hydroxyl* (liquid) values are for the interaction of two equatorial groups in cyclohexane derivatives in aqueous solution, and the hydroxyl-hydroxyl (gas) value is for the interaction of two equatorial hydroxyls of a cyclohexane derivative in tetrachloroethylene solution.

The assumption that the hydroxyl-hydroxyl interaction has (a) the same value in the gas phase as in tetrachloroethylene solution and (b) has the same value in sorbitol solution as in aqueous solution, is at best poor. It is however reasonable to assume that the direction of error is the same in both cases. The variation in interaction energy is dependent on hydrogen bonding. The greater the degree of intermolecular hydrogen bonding, the greater is the repulsive interaction between the two hydroxyls. There is more intermolecular hydrogen bonding in tetrachloroethylene solution than in the gas phase, and also more intermolecular hydrogen bonding in aqueous solution than in sorbitol solution.

* It may be considered surprising that there is an appreciable hydroxyl-hydroxyl interaction in aqueous solution. This interaction may be steric in origin due to the hydroxyls being 'bulky groups' because of solvation by water molecules.

The final assumption is that the entropy difference between the conformers is small enough to ignore. Thus the enthalpy difference between them is taken as the free energy difference.

The Calculation

Consider one mole of any diastereoisomer that has three staggered conformations A, B and C. The conformers are in equilibrium and the ratio of the mole fractions of any two is $e^{\frac{-\Delta G}{RT}}$, where ΔG is the free energy difference between them, R is the gas constant and T is the absolute temperature. The mole fractions may then be calculated from the ratios between conformers and the relationship $N_A + N_B + N_C = 1$, where N_A, N_B and N_C are the mole fractions.

The enthalpy of the diastereoisomer relative to the previously mentioned standard state is $\Delta H = \sum_i N_i H_i$

$$= N_A\Delta H_A + N_B\Delta H_B + N_C\Delta H_C.$$

The entropy of mixing of one mole of the diastereoisomer is

$$\Delta S = -2.3R\ (N_A \log N_A + N_B \log N_B + N_C \log N_C).$$

If the diastereoisomer is a racemic pair there is an additional entropy term of Rln2. This constant term is however ignored in this work as it disappears when the free energy of solution is calculated.

The free energy, due to group interactions, of the diastereoisomers is $\Delta G = \Delta H - T\Delta S$.

Using the above method the following relative values of thermodynamic constants were calculated for the isomers of 2,3-butanediol.

meso isomer (gas phase): $\Delta H = 0.44$ k cal mole^{-1}, $\Delta S = 2.1$ cal mole^{-1}deg^{-1}, $\Delta G = -0.45$ k cal mole^{-1}

meso isomer (liquid phase): $\Delta H = 1.05$ k cal mole^{-1}, $\Delta S = 2.0$ cal mole^{-1}deg^{-1}, $\Delta G = 0.21$ k cal mole^{-1}

racemic isomer (gas phase): $\Delta H = 0.06$ k cal mole^{-1}, $\Delta S = 1.8$ cal mole^{-1}deg^{-1}, $\Delta G = -0.70$ k cal mole^{-1}

racemic isomer (liquid phase): $\Delta H = 1.19$ k cal mole^{-1}, $\Delta S = 2.2$ cal mole^{-1}deg^{-1}, $\Delta G = 0.26$ k cal mole^{-1}

For the meso isomer the free energy of solution, $\Delta(\Delta G)$, is $.21-(-.45) = 0.66$ k cal mole^{-1}, and for the racemic isomer it is $.26-(-.70) = 0.96$ k cal mole^{-1}

Now $\Delta(\Delta G) = -RT\ln(V_g)$

$$\therefore \quad \log\frac{(V_g)_m}{(V_g)_r} = -\frac{\Delta(\Delta G)_m - \Delta(\Delta G)_r}{2.3\,RT}$$

where $(V_g)_m$ and $(V_g)_r$ are the retention volumes of the meso and the racemic isomers and $\Delta(\Delta G)_m$ and $\Delta(\Delta G)_r$ are the free energies of solution of the meso and the racemic isomers.

By substitution of the calculated values of free energy of solution.

$$\frac{(V_g)_m}{(V_g)_r} = 1.43$$

The experimental value is 1.23. Considering the approximations that have been made the agreement is good and may be partly fortuitous.

The above example involves only two functional groups, but in principle the method can be extended to any number of functional groups provided the appropriate group interactions are known. The method can

also be extended to compounds having more than two centres of asymmetry if all conformations are considered.

ABBREVIATED NAMES OF STATIONARY PHASES.

CYB	1,2,3,4-Tetrakis-(2-cyanoethoxy) butane.
DBP	Dibutyl Phthalate
DNP	Dinonyl Phthalate.
EGS	Ethylene Glycol Succinate polymer.
ODPN	Oxydipropionitrile.
PEG	Polyethylene Glycol 6000.
PEG4	Polyethylene Glycol 400.
PPG	Polypropylene Glycol 550.
SL	Sorbitol.
SQ	Squalane.

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