SYNTHETIC ION EXCHANGE RESINS, INCORPORATING ASYMMETRIC GROUPS, AS RESOLVING AGENTS.

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BY

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1. INTRODUCTION

The possibility of resolving a racemic compound by means of selective adsorption on an optically active adsorbent has been the subject of numerous papers in the literature. If the adsorption affinities of the d- and l- forms of the compound for the solid adsorbent are different, then it is reasonable to expect selective adsorption to occur when a solution of the racemate is brought into contact with the adsorbent surface. Any selective adsorption will thus result in a total or at least partial resolution of the racemate.

The earliest attempt to show that optical antipodes might be adsorbed to different degrees was made by Willstätter (1) in 1904. Using wool and silk as adsorbents he tried unsuccessfully to demonstrate the selective adsorption of one of the active antipodes of an alkaloid from an aqueous solution of the racemate.

Working with dl-m-α-naphtholazomandelic acid, Porter and Ihrig (2) claimed to have achieved an almost complete resolution by the selective adsorption of the d-form from acetic acid solution on wool, but their results are doubtful and could not be reproduced either by Brode and Adams (3) or by Henderson and Rule (4). Small partial resolutions of dyes on wool have been described by Ingersoll and Adams (5) and by Morgan and Skinner (6).

von Euler and Bucht (7) showed that though α-bromopropionic acid was adsorbed by casein from benzene solutions, there was no preference for either form and therefore concluded that asymmetric sorbtion did not exceed 10% of the whole. In a later investigation by Brode and Brooks (8) both wool and rayon were treated with the active and racemic forms of a bisazo-compound derived by combining 2:2'-diamino-1:1'-dinaphthyl and phenyl-J-acid and in no case was any selective adsorption observed.

An interesting experiment on the selective adsorption of mandelic acid on wool was performed by Martin and Kuhn (9). Since unequal adsorption of the optical isomers will be accompanied by unequal heats of adsorption, then if a temperature gradient is applied across the system an enrichment of the one antipode should occur in the liquid phase and of the other in the solid (adsorbent) phase. The resolving effect was multiplied by using the wool in the form of an endless belt which circulated through an aqueous solution of mandelic acid, one surface of the system being maintained at 100°C whilst the other was kept at room temperature. In some cases a laevorotatory solution was obtained but the results were variable.

Recently, Kogl, Faber and de Boer (10) have prepared two colouring matters by condensing tetra-aminophenarsine with the (+) and (-) forms of camphorquinone. The two isomers showed no significant difference in adsorption on animal tissue, either normal or malignant. Brady and Beaty (11), working on the selective adsorption of optical antipodes by proteins claim that both wool and casein selectively adsorb d-mandelic acid from aqueous solutions of the racemate at room temperature. They also observed that naphthylglycollic acid can be partly resolved when an aqueous-alcoholic solution is brought into contact with wool.

Although a large amount of work has been done on the selective adsorption by proteins, this is by no means the only adsorbent to receive detailed study. Optically active quartz has been used with some degree of success as a resolving agent. Thus Schwab and Rudolf (12) were able to effect a partial resolution of butanol-2 on quartz. Experimenting on racemic metallo-organic complexes such as dl-chlorobis(dimethylglyoxime) diamminocobalt, Tsuchida, Kobayashi and Nakamura (13) found that if a warm saturated solution of the racemate was allowed to cool

over active quartz the residual solution became faintly active, the sign of activity varying with the quartz used. Using the technique of column chromatography, Karagunis and Coumoulos (14) showed the differential adsorption of the d- and l- forms of racemic triethylenediamine chromichloride on powdered active quartz.

The first complete resolution of a racemic substance by means of selective adsorption was achieved by Henderson and Rule (15). Using lactose as the solid adsorbent they were able by means of column chromatography, to completely resolve dl-p-phenylenebisimidocamphor. The lactose was activated by boiling for a short time with chloroform and then dried thoroughly in vacuo to eliminate chloroform traces which diminish the adsorptive power. The racemic compound was adsorbed in the upper part of an upright tube of lactose (solvent, light petroleum containing 12% or 25% benzene) and the adsorbed layer washed with pure solvent until it had expanded the full length of the tube. Material recovered from the upper portion of the tube was found to be d-rotatory while that from the bottom was l-rotatory. The process was then repeated until an optically pure material was obtained.

In a similar manner Prelog and Wieland (16) were able to resolve the Tröger base on a column of chromatographically activated d-lactose hydrate. Activation of the adsorbent was performed by boiling with chloroform, drying, and grinding in a porcelain and then iron ball mill. The solvent used was dry petroleum ether and the technique was similar to that adopted by Henderson and Rule except that the base was completely eluted from the column, the effluent being fractionally collected. The first fractions were strongly d-rotatory, diminishing, and then becoming l-rotatory.

Activated alumina has been used by several workers as a resolving agent. Jamison and Turner (17) found that when

l-menthyl-dl-mandelate was developed with light petroleum on an alumina column, the material contained in the top section yielded upon saponification an acid with $[\alpha] = -18.2^{\circ}$ while the lower section yielded an acid with $[\alpha] = +64^{\circ}$. Similarly Prelog and Geyer (18) obtained a clear separation of two diastereoisomeric N-benzoyl cycloheptano -2:3-pyrrolidines by means of a liquid chromatographic method on a column of alumina.

That the asymmetric nature of the cellulose molecule could be used to effect resolutions of racemic compounds was shown by Kotake, Sakan, Nakamura and Senoh (19). In attempting to resolve various amino acids by means of paper chromatography using l-methyl-(β-phenylisopropyl) amine as solvent, they anticipated that the Rf values of the d- and l- amino acids should be reversed when the d-solvent was used. However, the results for the d-, l-, and dl-solvents were identical and the tendency to resolve was observed even when an inactive solvent such as n-butanol was used. They concluded, therefore, that the resolution was due, at least in part, to the asymmetric character of cellulose.

Curti and Colombo (20) were able to effect a partial resolution of camphorsulphonic and mandelic acids by using specific silica gels as resolving agents. The latter were prepared in the presence of molecules for which specificty was desired and the technique of column chromatography used.

The work so far dealt with involves the preferential adsorption of one enantiomorph of a racemate on an optically active surface. McBath, Hills and Pettit (21) performed a series of experiments designed to study the behaviour of salts in columns containing an excess of base where exchange reactions might be more important than selective adsorption.

In applying column techniques to the resolution of acid esters they combined (±)-2-octyl hydrogen phthalate with brucine

and washed the mixture of salts through a column of brucine mixed with filter aid with light petroleum containing a little acetone. The first fractions contained the brucine salt of (-)-2-octyl hydrogen phthalate and in one experiment 80% of the theoretically possible amount of ester was obtained in a 97% degree of optical purity. Comparable results were obtained with the strychnine salt of (±)-trans-3-methylcyclohexyl hydrogen phthalate in a column of strychnine but the system was not generally applicable and in certain cases e.g. with (±)-2-butyl-hydrogen phthalate on brucine, only partial or slight resolutions were obtained.

In view of the fact that the various salts were only slightly soluble in the solvents used, the authors concluded that differences in solubility rather than exchange reactions in the columns of alkaloid might be the most important factor in achieving a resolution. This conclusion was supported by the fact that the efficiency of the resolution was not affected by altering the degree of fineness of the alkaloid. In the case of the 2-octyl-hydrogen phthalate the alkaloid could be omitted from the columns altogether and a 97% resolution achieved by eluting a mixture of the brucine salts through a column of pure filter aid. This suggests the preferential adsorption of the one diastereoisomer on the filter aid.

These experiments led the authors to suggest the use of a more polar solvent to promote the dissociation of the salts and so facilitate exchange reactions in the columns. They also mention the possibility of resolving racemic bases by passing through an optically active cation exchange resin but did not test this hypothesis by experiment.

Dunnett and Marks (22) appear to be the first workers to actually attempt to resolve racemic substances on ion exchange

resins and were engaged on this work when the suggestions of McBeth et. al. were published. They prepared bakelite-type resins from both the optical isomers of β-(p-hydroxyphenyl)-butyric acid and from N-p-toluene-sulphonyl-l-tyrosine by co-condensing the acids with phenol and formaldehyde. Very little racemisation occured during the condensation process and optically active cation exchange resins resulted. Aqueous and aqueous-alcoholic solutions of racemic amines such as α-phenylethylamine and α-pipecoline were allowed to pass slowly through columns of the resins in the hope that one isomer might be preferentially held by the resin. However, the first samples of amine to break through at the bottom of the column was found in all cases to be inactive. In accounting for this lack of success, Bunnett and Marks assumed that salt formation between the carboxylic acid groups on the resin and the amines was not sufficiently influenced by the steric environment of the former to make any selective adsorption of either isomer possible.

Grubhofer and Schleith (23), working along similar lines were able to resolve mandelic acid on an optically active anion exchanger. The carboxylic groups of the cation exchanger Amberlite XE 64 were converted to the acid chlorides by treatment of the resin with thionyl chloride-pyridine in the usual way. The resulting product contained 20% chlorine and was quite reactive towards phenols, amino acids, proteins, etc. Esterification of this resin with the secondary hydroxyl groups of quinine resulted in a material containing 25% quinine and any unreacted acid chloride groups were rendered neutral by coupling them with β-naphthol. The final resin was thus an optically active anion exchanger. A dilute solution of dl-mandelic acid in chloroform was passed through a 150 c.c. long column of the finely ground resin and the effluent fractionally collected. The first fractions were found to contain optically pure l-

mandelic acid.

Thus despite the discouraging results obtained by Bunnett and Marks, Grubhofer and Schleith became the first workers to achieve a resolution of a racemic compound on an ion exchange resin and so to bear out the predictions of McBeth and his co-workers.

The work to be described in this dissertation is a further investigation into the use of ion exchange resins as specific adsorbents. The work was confined to cation exchangers for two reasons; firstly, they are in general easier to work with than anion exchangers since regeneration and washing is simple and no precautions against carbonate are necessary, while secondly the resolution of a racemic base on a cation exchange resin has not yet been effected.

2. THE AIM OF THIS RESEARCH

As was mentioned in the Introduction, the object of this research is to further investigate the possibility of resolving a racemic base on an optically active cation exchange resin. Any success in this direction would help to establish the reasons for Bunnett and Clark's failure, since the general assumption that selective adsorption of one enantiomorph of a racemic compound on an active exchanger should occur has been verified by Grubhofer and Schleith.

The work can be conveniently divided into two sections: a) the preparation of optically active cation exchange resins, and b) the use of these as resolving agents. It was decided, for reasons which will be explained in a later section of this dissertation, to synthesise resins in which the acidic group was alpha to the asymmetric carbon atom in the matrix. The second section of the work involves the testing of the resins for any selective behaviour towards either of the two antipodes of a dl-base. Column techniques to investigate this possibility were decided upon since any resolving tendency on the part of the resins would thus be amplified.

3. PREPARATION OF THE RESINS

3.1. ION EXCHANGE RESINS - GENERAL CONSIDERATIONS:

The credit for the recognition of the phenomenon of ion exchange is generally attributed to the two English agricultural chemists Thompson and Way (24) who, in 1848, observed that when a soil was treated with either ammonium carbonate or ammonium sulphate, most of the ammonia was removed and lime released. Their discovery aroused a great deal of interest and numerous investigations revealed that similar phenomena were demonstrated by other systems, most work being carried out on various clays and minerals. Amongst the minerals, the zeolites, both natural and synthetic received much study and were used commercially for the softening of water.

The advent of synthetic organic ion exchangers, invented by Adams and Holmes (25) in 1935, greatly stimulated the study of ion exchange and the literature now contains a vast number of papers on this subject.

While the fundamental mechanism of the ion exchange process remains in some doubt, the phenomenon is nevertheless a type of adsorption - the competitive distribution of ionic species between a bulk phase and an interface. Ion exchangers are insoluble substances which bind ions in such a way that the ions may be easily and reversibly replaced by other ions from a solution in contact with the exchanger. Since all ion exchangers are polymeric or macromolecular substances, they are insoluble in common solvents and hence serve to immobilise the ions which they bind.

The discovery of Adams and Holmes of organic exchange resins was based upon the knowledge that in the condensation

of phenol with formaldehyde to form a bakelite-type resin, the phenolic hydroxyl group is not involved and should thus be free to ionise in aqueous solution and therefore be available for ion exchange. The principle of this method has been extended to prepare resins with other acidic groups such as sulphonic ($-SO_3H$), methylene sulphonic ($-CH_2SO_3H$), carboxylic ($-COOH$) and phosphonic ($-PO_3H_2$) in the structure.

If a resin with acid characteristics is denoted as R-H, it will form salts with common bases e.g. R-Na, and exchange reactions of the following type may occur:

$$Na\text{-}R + cation^+ \longrightarrow cation\text{-}R + Na^+$$

or

$$H\text{-}R + cation^+ \longrightarrow cation\text{-}R + H^+$$

The organic anion exchangers rely on primary, secondary, tertiary amine or quarternary ammonium functional groups for the exchange. The exchange mechanism is more complicated than in the case of the cation exchangers and many workers have postulated the complete removal of the acid molecule from the solution. Schwartz (26) et. al., for instance, consider that there is no actual exchange but that the acid molecule is removed by molecular or covalent adsorption on the resin. Thus if a resin containing an amine group is denoted by $R\text{-}NH_2$, then the following reaction will occur with an acid H-X:

$$R\text{-}NH_2 + HX \longrightarrow R\text{-}NH_3^-X^+$$

The exchange equilibrium between a resin and a given ionic species is predominantly influenced by the nature of the polar exchange group and the ion involved but is also dependent on the physical structure of the resin. This is especially true for exchange equilibria involving large organic cations; the position of the equilibrium depends on the ability of the structure to accommodate such ions. The rate of exchange has been shown to

be diffusion controlled, an observation supported by the fact that decrease in particle size greatly increases the rate of exchange. These subjects have been treated in detail elsewhere and will not be considered further here.

In passing it is worth while mentioning that ion exchange resins by virtue of their large surfaces often exhibit behaviour other than ion exchange. This behaviour includes non-exchange adsorption, occlusions and dissolutions, as well as actual chemical reactions. For example, Cassidy and Cleaver (27) noticed that a number of amino acids were sorbed by a cation exchanger at a pH at which very small amounts of the amino acids could be in the cationic form and concluded that these substances actually dissolved in the resin phase.

3.2. THE SYNTHESIS OF ION EXCHANGE RESINS.

From the foregoing it is clear that the preparation of an ion exchange resin involves the synthesis of a highly crosslinked polymeric network containing ionic groups. There are however, certain basic requirements which must be taken into consideration. The degree of crosslinking must be sufficient to prevent dissolution or excessive swelling of the resin in the solvents with which it is to be treated but must not be so great as to impede the diffusion of ions into the resin network. The resin should also contain a sufficient number of accessible ionic groups, i.e. it should have a reasonable capacity, and should be chemically stable and not undergo any degradation during use.

An ion exchange resin can be synthesised in two ways. The first method consists of polymerising a suitable monomer containing the desired ionic groups while the second method involves the introduction of ionic groups into a preformed

polymeric network. In both cases the polymers may be formed by either polymerisation-condensation or by vinyl polymerisation. For example, a sulphonic acid resin can be obtained by the former method by co-condensing benzaldehyde sulphonic acid with phenol and formaldehyde to give a bakelite-type resin. Alternatively it could be prepared by polymerising styrene (with divinyl benzene as crosslinking agent) and then sulphonating the resulting polymer with concentrated sulphuric acid in the presence of silver sulphate. Either method results in a polymeric network containing sulphonic acid groups as an inherent constituent of the matrix.

These techniques have been adapted for the synthesis of optically active cation exchangers as will be demonstrated in the next section.

3.3 THE SYNTHESIS OF OPTICALLY ACTIVE ION EXCHANGE RESINS

3.3.1. GENERAL CONSIDERATIONS.

In synthesing ion exchange resins suitable for use as resolving agents the desiderata usually associated with exchangers are again applicable. The resin must be sufficiently cross-linked to prevent its dissolving in the solvents to which it will be exposed, and must be chemically stable so as not to undergo any degradation during use. In addition however, it must contain an asymmetric grouping somewhere in the matrix which is also capable of binding molecules of the racemic substance. This active group must also be stable and capable of withstanding racemisation by reagents with which it might come into contact. The application of column techniques to multiply any resolving tendency on the part of the resin presupposes the use of resins which are not subject to any excessive shrinkage or swelling in various solvents.

Two obvious methods of attack are available which will realise the above requirements:

a) A conventional ion exchange resin could be modified by coupling onto it an optically active group which is capable of holding the racemic substance to be resolved.

b) A suitable optically active compound could be synthesised which on polymerisation would yield the required resin.

A modification of the second procedure involves the inclusion of a naturally occuring optically active compound in the condensation recipe for the usual resorcinol-formaldehyde type of resin. A suitable compound of this nature could be either chemically bound into the polymer matrix or inextricably tangled up in it. In either case prolonged washing would not remove the asymmetric molecule.

All of these methods were tried with varying degrees of success, the work being confined to the preparation of cation exchange resins.

3.3.1. MODIFICATION OF A CONVENTIONAL RESIN.

Nalcite HCR, a monofunctional sulphonated copolymer of styrene and divinyl benzene was used as the starting material. The idea was to transform this into an optically active sulphonamide by reaction with an active amino acid according to the scheme:-

$$\text{Polymer-SO}_3\text{H} \longrightarrow \text{Polymer-SO}_2\text{Cl} \longrightarrow \text{Polymer-SO}_2\text{-NH-}\underset{\text{COOH}}{\overset{|}{\text{CH}}}\text{-R}$$

The result would thus be a resin in which the functional carboxylic acid group was alpha to a centre of asymmetry.

The first stage was accomplished by treating the resin with

chlorosulphonic acid which converted the sulphonic acid groups to the sulphonyl chlorides in 100% yield. In order to ascertain whether a sulphonamide could in fact be obtained from an amino acid, attempts were made to react the chlorosulphonylated resin with glycine. The latter was chosen because it is the simplest amino acid and stocks were plentiful. The technique described by Fischer and Bergell (28) for the preparation of the p-toluene sulphonyl derivatives of amino acids was adopted. This involves treating the sulphonyl chloride (in ether) with an alkaline solution of the amino acid but neither this or modifications of it (necessitated by the fact that the resin is insoluble) yielded a nitrogen containing product.

Since the glycine molecule is neutral, increase of the basisity of the latter by esterification of the carboxylic group might cause reaction to take place. The ethyl ester was accordingly prepared by refluxing glycine in absolute alcohol saturated with HCl gas, decomposing the resulting ester hydrochloride with alkali and salting out the product with potassium sulphate. The chlorosulphonylated resin was heated with excess glycine ethyl ester on a water-bath for an hour. A sodium fusion test on the resin showed that nitrogen was present and hence it would appear that the sulphonamide had been formed.

The method was next applied to dl-alanine ethyl ester. The amino acid was prepared according to Kendall and McKenzie (29) and esterified as described for glycine. Once again the chlorosulphonylated resin was heated with excess ester and a semi-micro Kjeldahl analysis performed on the product. This revealed that the resin 4.1%. nitrogen as compared with the theoretical value of 4.9% (assuming no crosslinking of the polystyrene). The ester was hydrolysed by heating with water

on a water-bath for 6 hours yielding the desired product. This resin had a reasonable capacity (± 3 milliequs/gm) but since inactive alanine had been used it was obviously useless as a selective adsorbent, and a method was now sought by which alanine could be resolved in good yield.

The two best methods appeared to be those described by Kipping and Pope (30) and by Pasen and Mullen (31). The first involves the condensation between alanine ethyl ester and dehydroxymethylene camphor. The resulting product is treated with water, the one diastereoisomer extracted with light petroleum and hydrolysed with concentrated hydrochloric acid yielding optically active alanine hydrochloride.

Alanine normally cannot form salts with alkaloids but if the acidity of the molecule is increased by benzoylation then salt formation becomes possible and a resolution can be achieved. This is the basis of the method of resolution outlined by the last-mentioned authors. The strychnine salt of benzoyl-l-(+)-alanine readily separates from the aqueous solution in which it is formed and decomposition with aqueous caustic potash yields benzoyl-l-(+)-alanine from which the l-(+)-alanine can be obtained by hydrolysis with 20% hydrochloric acid solution.

Both these methods were tried and the latter found to be the more satisfactory. Nevertheless, it became obvious that the quantity of optically active alanine required to synthesise a sufficient amount of resin was such as to render the whole proposition completely uneconomic, and hence it was decided to use the naturally occuring active amino acid l-tyrosine.

Once again the ethyl ester of the amino acid was used but since this is a solid, the method of forming a sulphonamide with the chlorosulphonylated resin had to be modified by the introduction

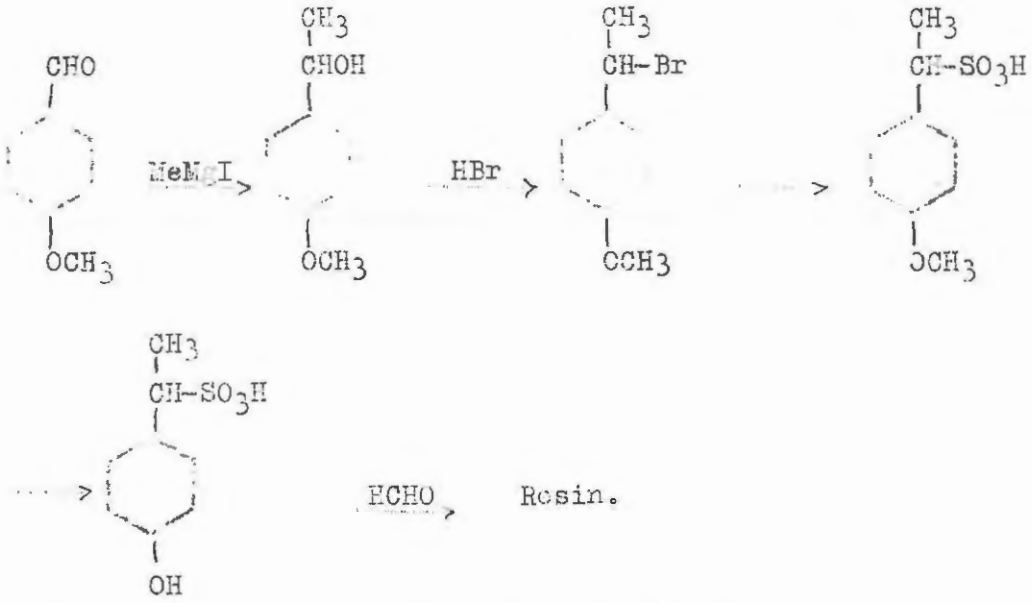
of a solvent. Five different sets of experimental conditions were tried, none of which yielded a product containing more than 0.4% nitrogen. This result was at first sight rather surprising until it was realised that the tyrosine molecule might not, owing to its large size, be able to penetrate the highly crosslinked resin matrix whereas the much smaller glycine and alanine molecules would be able to do so.

The obvious way to test this hypothesis was to make a very much less crosslinked sulphonated polystyrene and use this in the place of the commercial resin. This procedure was accordingly adopted and a 2% crosslinked sulphonated polystyrene resin was prepared according to Popper's (32) instructions. This, after chlorosulphonylation was reacted with l-tyrosine ethyl ester in ethyl acetate in the presence of sodium carbonate when a resin containing 35% tyrosine and which had a capacity of between 3 and 4 meq/gm was obtained. This resin was used in the resolution experiments to be described later.

3.3.3. BY DIRECT SYNTHESIS.

The problem here was to synthesise an optically active acid containing a function allowing it to engage in polymerisation and in which the acidic group was in the alpha position to the asymmetric carbon atom. The monomers selected for preparation were α-p-hydroxy-phenyl-ethyl sulphonic acid and m-phenylene dialanine, both of which should be capable of condensing with formaldehyde to give a resin.

The proposed synthesis in the first case was the following:-



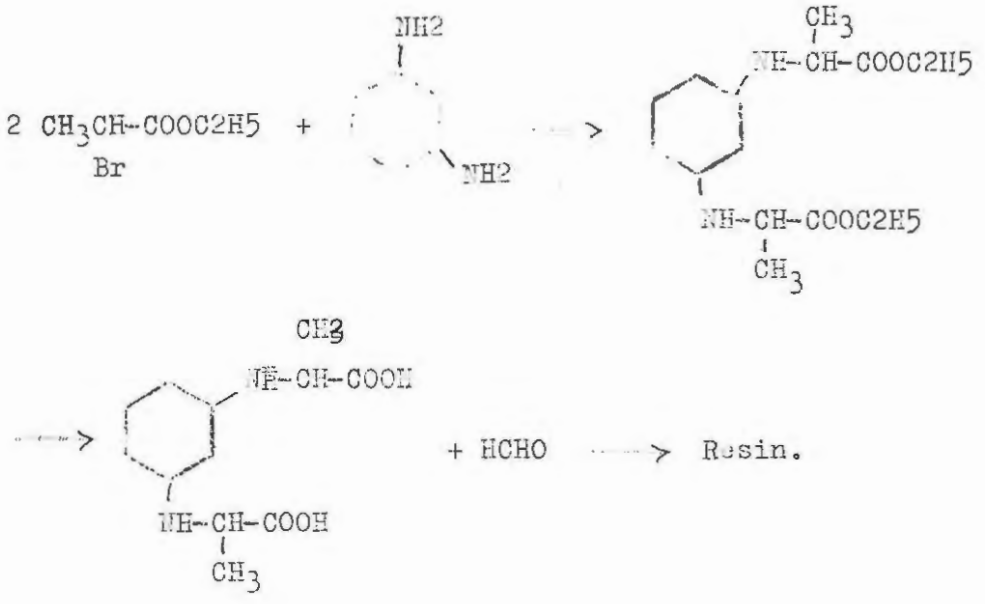
The first two stages have been described by Stedman and Stedman (33) and their method was successfully adopted. However all attempts to obtain a sulphonic acid from the p-methoxy phenyl ethyl bromide resulted in a sulphur-free compound which was probably the corresponding styrene or carbinol, and it became evident that the synthesis was not going to be as straightforward as was hoped. Also, the final product is not described in the literature and a method for its resolution would have to be developed. This line of attack was therefore discontinued.

Gregor, Taifer, Citarel, and Becker (34) describe the preparation of a resin from m-phenylene diglycine and their method, modified by the use of ethyl-α-bromo-propionate was used to prepare m-phenylene dialanine. The synthesis, lengthened by the fact that propionic acid had to be used as

starting material, was the following:-

(i) $CH_3CH_2COOH \longrightarrow CH_3\underset{Br}{CH}.COOH \longrightarrow CH_3\underset{Br}{CH}-COOC_2H_5$

(ii)



Two moles of the bromo-ester, prepared as described by Vogel (35) for the corresponding ethyl bromoacetate, were refluxed with two moles of freshly distilled m-phenylene diamine dissolved in 2-propanol yielding m-phenylene diamine dihydrobromide and the ethyl ester of m-phenylene dialamine. The latter was hydrolysed by refluxing with concentrated hydrochloric acid and on concentrating and chilling the solution, the product crystallised out. Condensation of this with formaldehyde resulted in a gel which on curing gave the desired resin. This resin was not wholly satisfactory since it was slightly soluble in water. It was, however, felt that this disadvantage could be overcome by crosslinking with resorcinol.

Before the resin could be used as an agent in the resolution experiments it had first to be prepared in an

optically active form. The simplest way to achieve this is to start with active α-bromopropionic acid but a search of the literature revealed that there is no way of resolving this compound in quantities large enough to be used in synthetic work. Chadwick and Pascu (36), claim that it is possible to resolve α-bromo-propionyl glycylglycine in good yield but it was decided to attempt to resolve the m-phenylene dialanine ethyl ester. This was not achieved and the problem was not investigated further because at this stage optically active resins had been obtained by other means,

3.3.4. FROM NATURALLY OCCURING OPTICALLY ACTIVE ACIDS.

This method involves the preparation of resorcinol-formaldehyde resins, the asymmetric sites in the matrix being due to the inclusion of optically active acids in the condensation recipe. Such acids had to be chosen that would either be chemically held in the resin structure or so firmly tangled up in it that they would not be dislodged by prolonged washing. Four different acids were used, only two of which yielded resins suitable for use as resolving agents. These will now be dealt with separately.

3.3.4a. d-Gluconic acid

The condensation between resorcinol, formaldehyde and gluconic acid resulted in a gel which on curing yielded a hard, brittle, insoluble resin with a capacity of 2.1 milli-equivalents/gm which was capable of 'splitting' neutral salts. That racemisation had not occured during the condensation or curing stages was shown by the fact that a solution of a sample of resin, prepared under identical circumstances but without sufficient formaldehyde to form a highly crosslinked polymer, was strongly dextrorotatory. Prolonged washing of

the resin with both acids and alkalis did not affect the capacity thus showing that the gluconic acid was firmly bound in the matrix.

3.3.4b. l-Tyrosine

According to Brown (37), tyrosine can undergo condensation with formaldehyde to form a polymer with optically active properties. A sample of resin prepared from these materials was found to be unsuitable for use in column experiments since it was soluble in alkali and was subject to great shrinkage and swelling in acids and bases respectively. These drawbacks were overcome by the use of resorcinol as a crosslinking agent and a resin was prepared having a capacity of 3 meq/gm and which was suitable for use as a resolving agent. It is interesting to note that this resin was dextro-rotatory since l-tyrosine reacts with formaldehyde to form a d-rotatory product (37). Different samples of resin prepared by this method were not all equally potent as resolving agents, and the less active preparations were discarded.

3.3.4c. d-Tartaric acid

Some difficulty was experienced in obtaining a proper gel from the condensation of this acid with resorcinol and formaldehyde and when this was eventually achieved, the resulting resin had a negligible exchange capacity after a short washing. The tartaric acid was obviously not firmly lodged in the resin matrix and the resin was discarded.

3.3.4d. d-Camphorsulphonic acid.

A resin was obtained without any difficulty by the co-condensation of camphorsulphonic acid with resorcinol and formaldehyde. The acid was, however, not inextricably bound up in the polymeric network and washing the resin with N hydrochloric acid yielded a sulphur-free product.

4 THE RESOLUTION EXPERIMENTS

4.1. ION EXCHANGERS IN COLUMN OPERATION

Ion exchange resins are commonly employed in two different ways, the static method and the dynamic method. In the former method, known as 'batchwise operation', the exchanger is shaken with a solution of the ions to be exchanged until equilibrium has been reached, the resin filtered off and the solution analysed. This method is not often used since a quantitative exchange can only be achieved in certain cases.

The dynamic method of operation is by far the most common used and is performed by allowing the solution to percolate through a fixed bed of exchanger packed in a column. The flow is generally in a downward direction although in certain cases it is advantageous to force the solution upwards through the resin bed. The solution entering the column is called the influent while the filtrate from the column is known as the effluent.

The principle of the method can be best understood by considering a simple example - a cation exchanger in the hydrogen form being treated with a solution of sodium chloride. When the first part of the influent comes into contact with the top layer of resin a certain number of sodium ions will be adsorbed and an equivalent number of hydrogen ions released. The number of exchanges taking place will depend, inter alia, on the length of time for which the solution is in contact with the resin i.e. on the flow rate, and under ideal conditions will continue until equilibrium is reached. As flow continues this section of influent comes into CONTACT with fresh resin and a further exchange of sodium ions takes place. Simultaneously, a new part of the sodium chloride solution contacts the top layer of the exchanger

which takes up a further amount of sodium ions. This series of events continues, the front of the sodium chloride solution continually contacting fresh resin and being depleted of sodium ions in the process while the resin gradually becomes saturated with sodium ion.

If sufficient solution is introduced into the column, a stage will be reached when the resin can take up no more sodium ions and these will appear in the effluent. This is the so called 'breakthrough point' and thereafter the solution passes unchanged through the resin bed. The total number of exchanging groups in the column, expressed usually as milliequivalents is called the total capacity of the column and in practice is always greater than the breakthrough capacity, defined as the amount of ions which can be taken up under the conditions in question, i.e. the number of milliequivalents which can be retained without any leakage being observed.

If the influent contains two exchangeable ionic species having different adsorption affinities for the resin, then it is clear that the passage of the solution down the column will result in a separation of these ions, the one having the smallest affinity appearing first in the effluent. This method only yields complete separation when there is a large difference in the adsorption affinities of the ions in question and is analogous to the principle of frontal analysis introduced by Tiselius (38).

Separation of ionic species having but slight differences in adsorption affinities is achieved by elution analysis. The two ions to be separated are adsorbed at the top of an exchange column and the adsorbed band eluted with a solution containing a third exchangeable ion. The chief requirement here is that the 'developer' should have an exchange potential lower than

those of the adsorbed ions but sufficiently large to result in some exchange. The elution process results in a series of adsorptions and desorptions of the two ions to be separated; the ion with the smaller potential is desorbed more readily and appears in a band below the other ion. The bands move down the column at different rates and appear in the effluent at different intervals. Thus under appropriate conditions a complete separation can be attained, though in some cases there is a considerable 'tailing' of the separated bands. It should be mentioned that during the whole course of the elution the effluent will contain the third ion originally present in the elutriant.

A modification of the above procedure involves the use of an elutriant containing an exchangeable ion with an exchange potential greater than those of the ions to be separated. This is the principle of the 'displacement development' proposed by Tiselius (39). In this case the components of the mixture are forced down the column ahead of the developer and are not mixed with it as in the previous case. As development proceeds the ions of the mixture displace one another so that they are eventually separated into bands in order of their replacing ability. Once a stationary stage has been reached the bands migrate down the column at constant speed, the band with the lowest adsorption affinity appearing first in the effluent. This method has the advantage that 'tailing' of the separated bands is reduced and also that the effluent is not contaminated with the developer. However, the bands are in such close contact that it is sometimes difficult to cut the fractions properly in the effluent.

4.2. FACTORS INFLUENCING THE SEPARATION

The variables which determine the degree of separation of exchangeable ions on a column can be classified into two groups:

a) Factors influencing the separation for each exchange, i.e. the statical ion exchange equilibrium.

b) Factors influencing the number of exchanges made by ions during their passage down the column, i.e. the column efficiency.

These will now be considered separately.

a) The relative position of the exchange equilibrium for inorganic cations with the same number of charges is largely governed by the size of the ion (ionic radius) which, in conjunction with the charge, influences the electrostatic properties of the ion and largely determines the free energy of adsorption. For ions of roughly the same size and electronic configuration the extent of adsorption is largely determined by the ionic charge. Furthermore the separation of ions independent of valence, is better for resins with a high total ion concentration in the resin phase, i.e. for ion exchangers with a high total capacity per unit volume.

The degree of crosslinking of the resin network is thus important and in analytical work, highly crosslinked resins are usually preferred. It is important, however, not to close the network structure too much by crosslinking since a very dense structure will impede the diffusion of ions into the resin. It is interesting to note that it is possible to effect a separation of high molecular weight and low molecular weight acids by means of an anion exchange resin. By varying the degree of crosslinkage, the size of the pore spaces can be controlled so as to permit a separation of ions by means of the differences in their sizes.

b) The factors of prime importance in influencing the column efficiency are the particle size and the flow rate. Since the separation is improved when the column is operated under such conditions that equilibrium is approached between the solution and the ion exchanger in different parts of the column, a small particle size and slow flow rate will be beneficial. The work of Kotelle and Boyd (40) indicates that the particle size should not exceed 0.1 mm, although in the chromatographic experiments to be described in this dissertation the maximum size was 0.14 mm. Extremely small particles are not desirable since they offer too much resistance against flow and for this reason the resin is freed of 'fines' before use.

A low flow rate (which is a natural consequence of the use of fine particles) is desirable since if the rate is too rapid the ions are carried down the column so fast that they have less time to diffuse through the resin and are thus not able to reach all the exchangeable groups. Also, a high rate of flow will affect the sharpness of the separated bands, resulting in a long trailing edge and overlapping of bands. On the other hand a very slow flow rate makes the experiment very tedious since an inordinately long time is required to collect sufficient effluent.

The length of the column is important and generally a longer column is used in ion exchange chromatography than in ordinary ion exchange work. The use of coupled columns greatly increases the degree of separation as will be shown in a later section (page 29). The length of the column is largely determined by the difference in the exchange potentials of the ions to be separated; a large difference requires a much shorter column than when the difference is very small.

4.3. ATTEMPTS TO RESOLVE RACEMIC BASES ON OPTICALLY ACTIVE CATION EXCHANGE RESINS.

The three resins used in these experiments were the d-gluconic acid resin, the l-tyrosine resin and the l-tyrosine substituted polystyrene resin. An outline of the resolution experiments performed with these resins will be given here, and the exact experimental procedure confined to the Experimental Section of this dissertation. Each resin will be dealt with separately.

4.3.1. EXPERIMENTS WITH THE GLUCONIC ACID RESIN.

4.3.1a. Attempted resolution of alanine.

At this stage of the work stocks of alanine were plentiful and it was decided to attempt a resolution of this amino acid with the resin. A dilute aqueous solution of the alanine was allowed to percolate through a short column of fairly coarsely ground resin (16 - 60 B.S.S. mesh) in the hydrogen form until the effluent gave a positive reaction with ninhydrin. Polarimetric examination of the next few fractions of effluent revealed that no optical activity was present.

A resin of the weakly acid (carboxylic) type would not be expected to quantitatively take up the neutral alanine molecule from an unbuffered solution of the latter but it was hoped that the resin might have had a slightly greater affinity for the one isomer than the other thus allowing a degree of separation to be effected. On the other hand some selective sorbtion of the type noticed by Cleaver (page 11) might have occured. Apparently these hopes had not been realised although a proper chromatographic technique might have produced more positive results.

A.3.1b. Attempted resolution of α-phenylethylamine.

This amine appeared to be well suited for the resolution experiments. It has an ionisation constant of 2.8×10^{-5} and is strong enough to undergo quantitative salt-formation with the resin; it has a reasonably large specific rotation ($[\alpha]_D = +41{\cdot}7^\circ$ in benzene) which would allow a small degree of resolution to be detected, while lastly it is easily prepared pure in large quantities. It has the minor disadvantage that it readily absorbs carbon dioxide from the atmosphere and precautions to prevent this must be taken.

The amine was prepared by the method of Ingersoll (41) and was purified by vacuum distillation. The above author recommended purification by distillation at atmospheric pressure but it was found that a slight amount of decomposition occurred at the boiling point resulting in a pale yellow product. Vacuum distillation yielded a colourless liquid.

Part 1: Preliminary experiments

Before attempting to resolve this amine by chromatographic techniques it seemed advisable to carry out a preliminary experiment to ascertain whether at least a partial resolution could be effected on a short coarse column of resin using ordinary ion exchange methods. In view of the success achieved by Grubhofer and Schleith (page 6) using a non-ionising solvent in their experiments, a dilute solution of amine in benzene was allowed to flow slowly down a short column of resin and the effluent examined for optical activity. The first few fractions were inactive.

It was now apparent that if there was in fact a sufficient difference between the adsorption coefficients of the d- and l-forms of the amine to make separation possible, this could only

be realised by means of ion exchange chromatography.

Using the work of Grubhofer and Schleith as a starting point, a 170 cm. long column was constructed and filled with resin which had been crushed below 60 mesh. The very high flow resistance of this column of resin made washing a very slow process and the column was abandoned in favour of one which was more easily regenerated. This consisted of three segments which could be fitted together by means of ground glass joints to give the required length. Regeneration and washing was speeded up by virtue of the fact that the dismantled sections could be treated separately.

A 1% solution of amine in benzene was allowed to flow slowly down the column of resin (in the hydrogen form) until the breakthrough point of the base was reached and the effluent thereafter examined for optical activity. The first fraction gave a rotation of $+0.05^{\circ}$ while the rest were inactive. This figure, though small, was nevertheless quite detectable on the Hilger polarimeter used and was the mean of 15 readings, none of which differed by more than $\pm 0.01^{\circ}$ from the mean.

This small observed rotation can be attributed to two causes:

a) Either a small degree of resolution had been achieved on the column, or,

b) The resin, which is d-rotatory, was undergoing a certain amount of dissolution in the amine-benzene solution to which it was being exposed.

Thus improved techniques could be expected to increase the former while appropriate pretreatment of the resin prior to use should remove all soluble material and so eliminate the second possibility.

Part 2: Experiments using improved columns.

Claesson (42) and Hagdahl (43) have shown that improved chromatographic separations can be achieved using a series of columns of decreasing diameter arranged one after the other. Between each pair of columns there is a small chamber in which turbulent mixing can occur. Since a front with a high concentration moves down the column more rapidly than does a front with a low concentration then if a solution corresponding to an imperfect front is mixed and spread over the whole area of a subsequent column, the more concentrated solution will overtake the mixed, more dilute solution and a sharpening of the front will be achieved. Turbulent mixing between the columns is very necessary since an imperfect front can, by means of "streamlined flow", move down several successive columns without any appreciable sharpening taking place.

For the next series of resolution experiments a modified form of Hagdahl's apparatus was used, consisting of two columns of unequal size mounted one above the other. The lower, smaller column acted as a "front-sharpener" and connection between the columns was effected by means of ground glass adaptors. The liquid level above each resin bed could be adjusted and the set-up was arranged in such a manner as to prevent accidental drainage of the columns. The apparatus has been described in detail in the Experimental Section.

The resin (the particles of which were crushed below 100 mesh) was, before introduction into the columns, subjected to a Soxtlet extraction with acetone and then benzene until the extracts were colourless. After drying in an oven it was cycled repeatedly with N sodium hydroxide and hydrochloric acid solutions in order to 'condition' it and remove any further impurities. The resin in the sodium form was slurried

into the columns, converted to the acid form and washed until the washings were neutral. This treatment of the resin should have removed any soluble impurities which might result in the effluent from a resolution experiment exhibiting optical activity.

Breakthrough analyses:

Run 1.

The water in the columns was displaced by washing with absolute alcohol and the columns then washed with benzene. A 1% solution of α-phenylethylamine in benzene was then allowed to percolate through the resin beds at a rate not exceeding 15 ml/hour until the breakthrough point of the amine was reached. The effluent was thereafter fractionally collected and examined for optical activity. The first three fractions, each of 4 ml were diluted to 10 ml (since a micro-polarimeter tube was not available) and found to have rotations of $+0.22^{\circ}$, $+0.05^{\circ}$ and 0.00° respectively. These figures indicate that a certain amount of selective adsorption was occuring resulting in the appearance of optical activity in the effluent. However, it is apparent that the activity falls away rapidly with the passage of further influent.

Since it is possible that the two isomers influence the adsorption of each other, the more strongly adsorbed displacing the less adsorbed isomer, then there should be a nett amount of l-amine bound on the resin. The resin was accordingly washed with alcohol, water and then with N hydrochloric acid solution to strip off the amine. The free base was isolated from the hydrochloride by decomposition of the latter with alkali and subsequently steam-distilling out the amine. A benzene solution of the latter showed no optical activity.

Run 2.

It seemed advisable at this stage to repeat the above procedure to ensure that the pretreatment of the resin had been effective in removing any soluble material which might be the cause of the observed rotations. Accordingly another run was carried out under the same conditions as before and the first fraction of 4 ml containing amino again diluted to 10 ml and the rotation measured. In this case the value was $+0.12^{\circ}$ This fraction was then subjected to a washing with N sodium hydroxide solution which would remove any traces of resin which might have been present in the effluent. The washed benzene solution was again checked for optical activity and the value obtained was found to be unchanged. This indicated that some degree of resolution of the amino had been obtained and it now remained to find out what this value was. Before doing so, the column was eluted with 0.3 N hydrochloric acid in another attempt to obtain the l-isomer. This was up in unsuccessful

Run 3.

The above procedure was again repeated on the regenerated column. The rotations of the first few fractions containing amino were measured and the amount of amino present in each fraction determined by bubbling dry HCl gas through a known volume of the solution, evaporating to dryness and weighing the resulting hydrochloride. The concentration in gms/ml was thus obtained and the specific rotations calculated. The results were as follows:

Fraction	1	2	3
$[\alpha]_D^{\prime}$	+7.04	+6.47	+5.96

Small rotations +0.01° were observed for the 4th and 5th fractions but as this value is within the error of the instrument the specific rotations for these fractions were not calculated. The value of +7.04° for the first fraction indicated that a 17% excess of the d-form was present.

These experiments demonstrate that by using the technique of frontal analysis it is possible to partially resolve dl-α -phenylethylamine, though it is apparent that the resolving power of the resin is decreased when the latter is used more than once.

Elution analysis

Frontal analysis, as has already been pointed out (page 22) only yields good separations when there is a large difference between the adsorption affinities of the two compounds to be separated, and in cases where only a small difference exists it is preferable to use elution analysis techniques.

Run 1.

The column in the H^+ form was treated with a solution of 2 gm of amine in a small volume of alcohol and the column then washed with water. This procedure resulted in the formation of a band of adsorbed amine at the top of the column. The band was eluted with 0.3% sodium hydroxide solution, i.e. subjected to displacement development, but unfortunately the amount of swelling undergone by the resin during this process was sufficient to cause flow to cease.

Run 2.

The column was regenerated and a new band of adsorbed amine formed by treatment of the resin with a solution of 2 gm of amine in water. In this case the elutriant was a 0.8% hydro-

chloric acid solution, the idea being that the one isomer would be more rapidly desorbed than the other and hence the amine hydrochloride breaking out first at the bottom of the column would be the one with the lowest adsorption affinity. Consider the equation:

$$\text{Resin} - COO^{-}{}^{+}H_3N\cdot\underset{\displaystyle CH_3}{\underset{|}{CH}}\cdot Ph + HCl \longrightarrow \text{Resin} - COOH + Ph\cdot\underset{\displaystyle CH_3}{\underset{|}{CH}}\cdot NH_3{}^{+}Cl^{-}$$

This represents the elution of amine from the column and it was hoped that the reaction would be more rapid for the least adsorbed isomer. The pH of the effluent was measured at frequent intervals during the course of the elution since any sharp change would indicate the presence of amine hydrochloride. However, the pH decreased steadily during the course of the elution until it reached the value of the influent and at no stage was there a sharp jump.

The above procedure was repeated, but modified by using 0.1% HCl solution as elutriant and fractionally collecting the effluent. The pH of each fraction was measured but once again no sharp change could be observed.

Run 3.

Selective desorption appeared to be completely ineffective and work was once more reverted to displacement development. On this occasion, piperidine was chosen as the developing agent because this is not as strong a base as sodium hydroxide and does not cause the resin to undergo much swelling. The adsorbed band of phenylethylamine was again formed and eluted with a 1% aqueous solution of piperidine. The first fractions of effluent containing amine were a light brown in colour and appeared to be optically inactive. The results of the polarimetric examination were not wholly conclusive since the colour

of the fractions made accurate measurements difficult to obtain. The cause of the colour was probably due to a slight amount of oxidation of the amine during the elution procedure.

Run 4.

In view of the fact that a certain degree of success was experienced in the frontal analysis experiments using a non-ionising solvent (benzene) it was decided to perform some elution analysis experiments using this solvent. The water in the regenerated columns was displaced with alcohol and the latter with benzene. A solution of 1.5 gm. of amine in a little benzene was then run through the column to form an adsorbed band of material and the latter developed with a 1% benzene solution of ethylamine. Ethylamine is the stronger base, the dissociation constant, being 5.6×10^{-4} as compared with that of 2.8×10^{-5} for phenylethylamine and hence this is also an example of displacement development and not elution analysis. Ethylamine has the disadvantage as an eluting agent in that it is rather volatile (b.pt. 19°C) and if room temperature rises above this value the benzene solution of the amine becomes more and more dilute. However, during these experiments, room temperature was less than 15°C and the very slight loss of amine from the developing solution was not troublesome.

The effluent was fractionally collected and the first samples containing amine examined in the polarimeter. The first six fractions had the following rotations:

Fraction	1	2	3	4	5	6
α°	$+0.65^\circ$	$+0.60^\circ$	$+0.60^\circ$	$+0.60^\circ$	$+0.46^\circ$	$+0.42^\circ$

Further observations were not possible due to the pale yellow colour of the effluent. The first fraction was found to have

a specific rotation of $[\alpha]_D = +12{\cdot}2^{\circ}$ i.e. a 29% excess of the d-form. Displacement was continued to obtain the l-rotatory material but was not very successful, only one fraction with a measurable rotation of $-0{\cdot}07^{\circ}$ being obtained. This was probably due to a certain amount of tailing of the resolved band.

Run 5

It was now decided to use a stronger developer in the form of a 1% benzene solution of piperidine. The above procedure was accordingly repeated but the effluent was strongly coloured and polarimetric measurements were difficult to obtain with any degree of accuracy. These measurements did however, indicate that little or no resolution had occured since no activity could be detected in any of the fractions.

4.3.1c. Attempted resolution of methyl amarine

A 3gm sample was adsorbed at the top of the column from an alcoholic solution of the base and eluted with 1% alcoholic solution of acetic acid. The situation here was similar to that in Run 2 of the elution experiments involving phenyl-ethylamine (page 32), i.e. it was hoped that selective desorption of the one isomer might occur. The effluent was allowed to drip into dilute ammonium hydroxide solution, the presence of a turbidity indicating that the base was present.

This experiment did not achieve any resolution of the methyl amarine; it did, however, yield a very pure sample of the latter. When prepared, methyl amarine is usually contaminated with methyl lophine and is very difficult to obtain in the pure state. The above elution with alcoholic acetic acid stripped the methyl lophine from the resin while the methyl amarine, being the stronger base was retained on the

column. It was then subsequently recovered by elution with a 3% alcoholic HCl solution.

4.3.1d. Attempted resolution of dl-β-phenylisopropylamine

This amine, like phenylethylamine is also well suited for the resolution experiments since it is easy to obtain in large quantities and has a reasonably large specific rotation of $+ 35.6^{\circ}$ (no solvent). It was obtained from the makers in the form of the sulphate and the free base was isolated by decomposition with alkali followed by ether extraction of the aqueous solution.

A 1% solution of the amine in benzene was percolated through the column and the effluent, after the break-through point had been reached, examined for optical activity. All the fractions were inactive indicating that no resolution had occured. A possible reason for this result will be discussed later.

4.3.2. EXPERIMENTS WITH THE TYROSINE RESIN

The physical characteristics of this resin were very similar to those of the gluconic acid resin. The resin was crushed and sieved, the particles passing 100 mesh freed of fines by floatation and then treated to remove any soluble material. It was then packed into a pair of columns identical in all respects to those used for the gluconic resin.

4.3.2a. Attempted resolution of dl-α-phenylethylamine

In view of the fact that this amine had been partially resolved by means of breakthrough analysis techniques on the gluconic acid resin it was decided to repeat the procedure using this new resin. A 1% solution of the amine in benzene was again used.

It was anticipated from a knowledge of the capacity of the resin that the break-through point for the amine should occur after ±1000 ml of effluent had been collected. However, the base actually appeared in the effluent after only 200 ml had been collected; apparently the capacity of the resin with respect to the amine in benzene is only 1/5th of the value for the aqueous-alcoholic solution used in the capacity determinations (page 54).

The first fraction had a rotation of $+0{\cdot}17^{\circ}$ and the specific rotation, after determining the concentration of amine in the solution, calculated to be $[\alpha]_D = 8{\cdot}06^{\circ}$ i.e. a 19% excess of the d-form. Thus this resin is also capable of effecting a partial resolution of the dl-amine and the experiment was repeated to follow the course of the resolution more closely. The results were as follows:

Fraction	1	2	3	4
$[\alpha]^{\circ}$	+9·5	+6·04	+3·51	+3·16

Elution or displacement procedures were not feasible on this resin using benzene as solvent because the very low capacity of the resin for the amine in benzene made it impossible to load the column with sufficient amine to make the analysis possible. However, an attempt to develop an adsorbed band of amine with aqueous piperidine was made but unsuccessful because exposure of the resin to this reagent caused it to swell sufficiently to stop the flow.

4.3.2b. Attempted resolution of dl-β-phenyl-isopropylamine

A 1% solution of the amine in benzene was passed down the column until the break-through point was reached. This occured after some 200 ml had been collected and the solution was there-

after examined for optical activity. None of the fractions had any rotation indicating that negligible resolution had occured.

4.3.2c. Attempted resolution of dl-β-naphthol phenylaminomethane

This amine was chosen in order to see whether the introduction of the bulky naphthol group into the asymmetric system would result in the attainment of a greater degree of resolution of the amine than in the case of the phenylethylamine. The hydrochloride of the base was prepared as described by Betti (44) and the free amine isolated according to Betti's original method (45). The above author gives the melting point of the dl-amine as 125°, but although several samples were isolated from the hydrochloride on different occasions no sample had a melting point above 120°C (the constant figure obtained after three recrystallisations from ether).

The method of estimating the concentration of amine in the effluent from the column by precipitation and weighing as the hydrochloride is tedious and inelegant and a new method for this determination was sought. The very dilute (ca. 1%) solutions used made the measurement of the refractive index of the solution useless as a means of determining the concentration since the difference between the reading for the pure solvent and that of the solution was so small (ca. 0.0003), as to be negligible.

It was felt that measurement of the optical density would be a promising method and this possibility was further investigated. A Beckman Model DU spectrophotolometer was used. A plot of optical density versus concentration of amine in A.R. benzene at 365 mμ gave a straight line passing through the origin. (The wavelength of 365 mμ was chosen after a plot of wavelength versus percentage absorption from 350 mμ (100% absorption) to 600 mμ (100% transmission) on a solution of the

amine in benzene had been obtained. This method seemed ideally suited for the concentration determination since once the instrument had been standardised over the concentration range in question, the optical density of samples of effluent could be measured and the concentration obtained from the standardisation graph. This method presupposes that the effluent will not be contaminated with any other substance which is strongly absorbing at the wavelength used.

A 1% solution of the amine in benzene was passed down the column at a rate of 1 drop/10 sec. The amine, which is a very weak base was not, as anticipated, quantitatively bound by the resin. The first 15 fractions containing amine all possessed considerable optical activity, the 5th having the highest rotation of $+5{\cdot}42^{\circ}$. All the fractions were, however, a pale yellow in colour due to a slight amount of oxidation of the amine and the spectrophotolometric method of determining the concentration could not be used. Accurate polarimetric measurements were also difficult to obtain due to the colour of the fractions. The first eight fractions were combined and the solvent removed under reduced pressure leaving a brownish oil. Addition of a small amount of petroleum ether caused the latter to crystallise and the product purified by three recrystallisations from ether. The resulting white crystals melted at 138°C and had $[\alpha]_D = +45{\cdot}3^{\circ}$ in benzene. According to Betti (45) the pure d-form has a melting point of 137°C and $[\alpha]_D = +58{\cdot}92^{\circ}$ in benzene. On the basis of the latter figure the partially resolved material consists of 89% of the d- and 11% of the l-forms.

4.3.3. RESOLUTIONS USING THE TYROSINE SUBSTITUTED POLYSTYRENE RESIN.

Breakthrough techniques involving 1% benzene solutions of dl-α-phenylethylamine, dl-β-phenylisopropylamine and dl-β-naphtholphenylaminomethane did not result in any detectable degree of resolution of any of the three amines. In view of the considerable amount of time devoted to the preparation of this resin, these results are singularly disappointing. A possible reason for the inability of the resin to exhibit any selective adsorption is that there might have been unsubstituted free sulphonic acid groups present in the matrix which result in a disruption of the resolved front.

7. DISCUSSION OF RESULTS

Ion exchange resins whose molecules carry asymmetric cationic groups have been synthesised and experiments have indicated that it is possible to use these to effect at least a partial resolution of a racemic base.

A normal cation exchanger would not be expected to exhibit any selective behaviour towards either of the two antipodes of a dl-base because the factors which normally affect the exchange affinity of an ion for the resin, viz., ionic radius, charge, valency, etc., are the same for each isomer. However, if the environment of the functional groups of the exchanger is such that the steric 'fit' of one isomer is better than that of the other, then it is anticipated that a difference in exchange potentials will result which will allow a separation to be effected. In other words, if salt formation (using this term to include van der Waal's and other weak binding forces) is influenced by steric factors then it is possible that selective adsorption will result.

It is thus apparent that if the functional acidic groups on the exchanger are directly connected to an asymmetric carbon atom, the surrounding groups will exert a greater influence on salt formation than if the acidic groups were separated from the center of asymmetry and were thus able to project well clear of the asymmetric system. For this reason emphasis was laid on the synthesis of resins in which the carboxylic group was alpha to the asymmetric carbon atom.

Bunnett and Marks (page 8) felt that their failure to achieve any resolution using the active resins which they prepared was due to salt formation not being sufficiently influenced by the environment of the carboxylic group. Though

this is likely to be so for their β-(p-hydroxyphenyl)-butyric acid resin where the -COOH group is both to the center of asymmetry, the present author feels that a partial resolution at least should have been effected on the N-p-toluene-sulphonyl-l-tyrosine resin and considers that their lack of success was due to the fact that ionising solvents were used. The work described in this dissertation and that of Grubhofer and Schleith (page 8) indicates that the use of non-ionising solvents to promote covalent or molecular adsorption of the substance in question is essential if a resolution is to be effected.

It is interesting to consider the steric configuration of the three amines, dl-β-naphthol phenylaminomethane, dl-α-phenyl ethylamine and dl-β-phenylisopropylamine where in each case the functional amine group is connected to a centre of asymmetry. In the first compound the asymmetric carbon atom is connected to the bulky naphthol and phenyl groups; in the α-phenylethylamine the steric environment is less pronounced while in the last compound the bulky phenyl group is separated from the centre of asymmetry by a methylene linkage. This gradation is reflected in the degrees to which the three compounds were resolved on the tyrosine resin. In the case of the β-naphthol phenylaminomethane there was a sufficient difference between the exchange potentials of the two isomers to make an almost complete separation possible using the relatively ineffective frontal analysis technique, whereas with β-phenylisopropylamine the difference between the exchange potentials was so slight that little if any selective adsorption was possible and frontal analysis did not effect any separation.

In the light of these results it is tentatively suggested that if a resin could be synthesised in which the functional group was linked to a centre of asymmetry surrounded by a very

bulky steric environment, it could be used to resolve a large number of racemic compounds even though they themselves were not sterically very bulky.

EXPERIMENTAL SECTION.

6. PREPARATION OF THE RESINS.

6.1. MODIFICATION OF A CONVENTIONAL RESIN BY SUBSTITUTION WITH VARIOUS AMINO ACIDS

6.1.1. USING NALCITE HCR AS STARTING MATERIAL (Page 13)

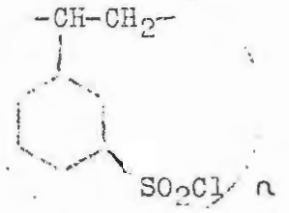
6.1.1a. Attempts to chlorosulphonylate Nalcite HCR

(i) 18 gm of resin in the bead form supplied by the makers were dried in an oven overnight at 50°. 20 gm (i.e. excess) chlorosulphonic acid was added in small portions to an ice-cold suspension of the resin in chloroform, the vigorous reaction accompanying each addition being allowed to subside before the next addition was made. When all the acid had been added the reaction mixture was allowed to stand with occasional shaking for two hours at room temperature to complete the reaction The excess chlorosulphonic acid was decomposed by pouring the mixture onto crushed ice and the resin filtered off.

In order to ascertain whether or not the chlorosulphonylation had been successful, a small sample of the resin was boiled with 6N sodium hydroxide solution, the solution acidified with nitric acid and silver nitrate solution added. No silver halide precipitate was obtained indicating that the sulphonyl chloride had not been formed.

(ii) The chlorosulphonylation was now repeated in the absence of any solvent. Small portions of resin were dropped into ice-cold chlorosulphonic acid, the latter again being present in excess. After isolation of the resin as before, a test for chloride ion was positive and the percentage chlorine was determined as follows:- A small (±0.5 gm) dry, weighed sample of resin was refluxed with 25 ml of 30% sodium hydroxide solution for 30 minutes, the resin quantitatively filtered off

with hardened paper, and the filtrate and washings neutralised with dilute HNO_3 solution using phenolphthalein indicator. The chloride was then titrated with standard approx. 0·1 N silver nitrate by Mohr's method. Assuming the basic structure of the chlorosulphonylated resin to be



i.e. neglecting crosslinking, the theoretical value for the percentage chloride is 17·5%. The value obtained was 8·0%.

(iii) It would appear that moisture was still present in the resin and resulting in the decomposition of the chlorosulphonic acid. Accordingly, a further portion of resin was dried in vacuo over P_2O_5 for 24 hours and treated with chlorosulphonic acid as described in attempt (i). This time however, the mixture was refluxed for 2 hours after all the acid had been added in order to complete the reaction. The chlorosulphonylated product obtained in this experiment contained the required amount of chlorine.

6.1.1b. Reaction of the chlorosulphonylated resin with glycine.

(i) Two grams of resin were shaken with excess glycine in the form of a saturated solution in water. After several hours the resin was filtered off, washed, and tested for chloride ion. This was found to be present and so apparently reaction had failed.

(ii) The previous experiment was repeated using 1 gm of resin and 2 gms glycine dissolved in 30 ml N sodium hydroxide solution. A test for nitrogen on the resin was negative. Reaction had failed.

(iii) 1 gm of resin was boiled under reflux with a solution of 2 gms of glycine in 30 ml of 60% pyridine solution for one hour. A nitrogen test on the resin was again negative indicating that once again reaction had failed.

6.1.1c. Reaction of the chlorosulphonylated resin with glycine ethyl ester.

The ester was prepared by bubbling dry HCl gas through a boiling suspension of glycine in absolute alcohol until solution was complete. On cooling, the ethyl ester hydrochloride separated and was filtered off. The free ester was obtained by the decomposition of an ice-cold solution of the hydrochloride with 33% sodium hydroxide solution, salting out with potassium carbonate, and extraction from the alkaline solution with ether.

One gram of resin was treated with 3 gms of ester and allowed to stand on a boiling water-bath for one hour. During this time white crystals of glycine ester hydrochloride separated and the resin, after being washed with alcohol and water gave a positive test for nitrogen. This time reaction had apparently occurred and it now remained to be seen whether alanine could be reacted in a similar manner.

6.1.1d. Reaction of the chlorosulphonylated resin with alanine ethyl ester.

The alanine was prepared as follows:-
To a cold ($\pm 5^{\circ}C$) solution of 13·1 gm (0·3 moles) of acetaldehyde in 10 ml ether was added 18·0 gm (0·34 moles) of ammonium chloride dissolved in 55 ml of water, followed by the slow addition of an ice-cold solution of 15·0 gm (0·31 moles) of sodium cyanide in 40 ml water. The reaction flask was then corked and the mixture shaken for 4 hours at room temperature.

Hydrolysis of the product was effected by distilling the reaction mixture with 60 ml conc. HCl solution until salt formation prevented further heating. The residue was evaporated to dryness on a steam bath, extracted with 80 ml 95% ethyl alcohol and the extract, after filtration, evaporated to dryness in vacuo. All but the last traces of NaCl and NH_4Cl were removed by dissolving the residue in 50 ml of 95% ethyl alcohol containing 2% HCl and adding 20 ml of ether. The solution after filtration was evaporated to dryness under reduced pressure yielding the alanine hydrochloride.

The free alanine was isolated from the salt by boiling a solution of the latter in 150 ml water successively with 22 gms yellow lead oxide, 10 gms of lead oxide and 2 gms freshly precipitated lead hydroxide and filtering off the resulting lead chloride each time. The last traces of chloride were removed by boiling the solution with the calculated amount of silver oxide (as determined by a Volhard titration) and filtering off the silver chloride. Excess lead was precipitated with H_2S, the solution evaporated to 40 ml and 60 ml of 95% ethyl alcohol added. On cooling, the alanine crystallised out and a second crop was obtained by evaporating the residue to low bulk and adding an excess of alcohol.

Yield: 14 grs. (53% theoretical).

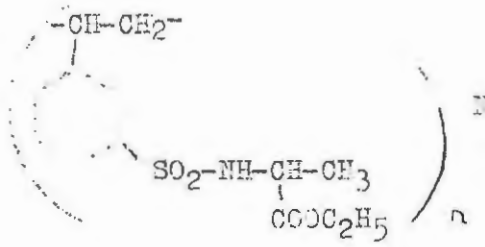
A large amount of alanine was prepared more simply by adding 3 litres of conc. aqueous ammonia solution to 100 gms of cold α-bromopropionic acid. The solution, after standing for 4 days was concentrated to 200 ml and the alanine precipitated with methyl alcohol.

Yield: 35 gms. (60% theoretical).

The ethyl ester of alanine was prepared by bubbling dry

HCl gas into a boiling suspension of 5 gms of alanine in 15 ml absolute alcohol until solution was complete and then concentrating the solution under reduced pressure at a temperature not exceeding 35°C. The free ester was then isolated as described for glycine.

One gram of the resin was heated on a water bath with 2·5 gms alanine ethyl ester for one hour, the resin then filtered off and thoroughly washed with alcohol and water. A nitrogen test was again positive and hence a semi-micro Kjeldahl analysis was performed on the resin in order to obtain the percentage nitrogen.

Calculated for  N = 4·95%

Found: N = 4·06%

6.1.1e. Reaction of the chlorosulphonylated resin with l-tyrosine ethyl ester.

The ester was prepared by bubbling dry HCl gas into a suspension of the amino acid until solution was complete. Refluxing for several hours completed the esterification and the solution was concentrated under reduced pressure at 35°C, causing the ester hydrochloride to precipitate. This was decomposed with potassium carbonate and the free ester extracted with ethyl acetate.

(i) 4 gms of ester were dissolved in 20 ml of absolute alcohol and added to 2 gms of dried resin. The mixture was stirred and boiled under reflux for 2 hours. The resin after being filtered off, was washed and tested for nitrogen. The

test was negative.

(ii) 9.7 gm of l-tyrosine methyl ester hydrochloride (prepared as for the ethyl ester hydrochloride) was covered with a solution 1.2 gm of sodium carbonate in 6 ml of water and 36 ml chloroform added. The mixture was thoroughly shaken 5 gm resin added, and the mixture magnetically stirred for one hour. During this period CO_2 was evolved. A further 1·2 gm sodium carbonate in 20 ml water was then added and the stirring continued for a further two hours. A nitrogen test on the resin was positive but on heating the latter on a water-bath with 30 ml 2N NaOH solution to hydrolyse the ester, a nitrogen free product was obtained.

(iii) The above procedure was repeated but the stirring continued overnight. Once again a nitrogen-free product was obtained.

(iv) 1·6 gm of resin were heated with 3·8 gm of tyrosine ethyl ester on an oil-bath at 120°C for an hour. A nitrogen test on the resin was negative.

(v) 2 gm resin were added to a boiling solution of 4 gm ester in 12 ml of benzene. The mixture was then boiled under reflux with stirring for 7 hours. During this period a white material crystallised out on the walls of the flask. This presumably was the ester hydrochloride, indicating that reaction was taking place. The resin, after being isolated and washed, was heated on a water-bath with 30 ml 2N NaOH solution for half an hour, the solution acidified and the resin filtered off, washed and dried. A semi-micro Kjeldahl analysis performed on the resin indicated that 0·4% nitrogen was present.

6.1.2. Using a 2% crosslinked sulphonated polystyrene resin as starting material (Page 16)

6.1.2a. Preparation of a sulphonated polystyrene resin.

Three ml of freshly distilled divinyl benzene (b.pt. 41 - 5°C/2mm) were dissolved in 75 ml freshly distilled styrene (b.pt. 42 - 3°C/18mm) containing 0·75 gm benzoyl peroxide. This solution was then thoroughly shaken with 400 ml of a 3% aqueous solution of 'Promulsin' until dispersion was complete. Polymerisation was effected by heating the emulsion in a water-bath at 80 - 85°C for 18 hours. The Promulsin was hydrolysed by the addition of M H_2SO_4 solution and heating for 2 hours in a water-bath at 60 - 80°C. Any colloidal resin was decanted off and the remainder filtered and dried at 50°C. Sieving the dry resin removed the coarser particles, the portion passing 100 mesh being used in the next stage.

11 gm of resin, 200 gm concentrated sulphuric acid and 2 gm silver sulphate were heated on a water-bath for 11 hours. After cooling, the suspension was poured into a large volume of cold water, colloidal particles decanted and the remainder filtered and washed with distilled water until the washings were neutral to methyl orange. A sample of the resin was dried in a vacuum over P_2O_5 at 56°C and used in the capacity determination outlined below.

The total capacity was determined by leaching a weighed amount of the dry resin with a 5% calcium chloride solution and titrating the liberated hydrochloric acid with standard alkali. The capacity was found to be 4·7 millieqvs./gm, indicating the presence of one sulphonic acid group per benzene nucleus.

6.1.2b. Chlorosulphonylation of the resin.

A modification of the previously described method (page 45) was used. 15 gm of dry resin was suspended in the minimum amount of carbon tetrachloride in a two necked flask fitted with a dropping funnel and condenser, the latter carrying a calcium chloride guard tube. The suspension, which was maintained by magnetic stirring was cooled in ice and then 20 gm chlorosulphonic acid added dropwise through the funnel. A certain amount of swelling of the resin took place during this process, necessitating the addition of small amounts of CCl_4 at regular intervals. When the addition of the acid was complete, the mixture was boiled under reflux for two hours. The excess acid was removed in the usual way and the resin filtered off and washed with water and alcohol.

6.1.2c. Reaction of the chlorosulphonylated resin with l-tyrosine ethyl ester.

(i) 2 gm of resin were added to a solution of 4 gm of ester in 10 ml of hot benzene. The mixture was magnetically stirred and refluxed for 17 hours during which time a large amount of white material crystallised out on the walls of the flask. The resin, after being filtered off and washed with hot ethyl alcohol was treated with 30 ml of 2N NaOH solution and heated on a water-bath for 30 minutes. A Kjeldahl analysis on the resin gave 0·7% nitrogen, i.e. 5% tyrosine.

(ii) Two gm of resin were added to a solution of 2 gm ester dissolved in the minimum amount of ethyl acetate and the mixture magnetically stirred. A solution obtained by dissolving one gram of sodium carbonate in 6 ml of water was then added slowly over a period of half an hour and the mixture stirred for a further 2 hours. The resin, after hydrolysis with 2N NaOH solution as before, was found to contain 2% nitrogen, i.e.

26% tyrosine. The capacity, determined as described on page 54 was found to be 3.4 meq/gm of dry resin.

Another batch of resin prepared on a larger scale was found to contain 35% tyrosine. The method of preparation was the same as before except that a more concentrated solution of sodium carbonate was used and added over a period of three hours. This resin was used in the resolution experiments.

A note on the semi-micro Kjeldahl analyses.

Since micro-digestion flasks and a digestion oven fitted with micro-burners was not available, a semi-micro method was adopted. The micro-distillation apparatus of Parnas and Wagner (46) was used and the method of Niederl and Niederl (47) followed.

The digestion was performed in flasks of 50 ml capacity on a digestion stand constructed of asbestos and retort stands, full-sized burners being used. Samples of 20 - 50 mg were taken and an ordinary analytical balance used for the weighings. The digestion catalyst was a mixture of copper and potassium sulphates and 4 ml of concentrated sulphuric acid were used in the digestion process. The latter required a slightly longer time to reach completion than in the true micro method and 25 ml of 40% sodium hydroxide solution were required for the liberation of the ammonia. The distillation was continued for six minutes, the ammonia trapped in 10 ml of a saturated boric acid solution and directly titrated with standard 0.05 N HCl solution using the mixed indicator of methyl red and methylene blue, recommended by Yuen and Pollard (48).

6.2. BY DIRECT SYNTHESIS (Page 16)

6.2.1. PREPARATION OF A RESIN FROM M-PHENYLENE DIALANINE

6.2.1a. Preparation of ethyl-α-bromopropionate.

110 gm of α-bromopropionic acid, prepared as described by Vogel (34), 46 ml of absolute alcohol and 2.5 ml of concentrated sulphuric acid were boiled under reflux for 5 hours. The ester was washed twice with water, once with saturated sodium bicarbonate, again with water and then dried over anhydrous magnesium sulphate and finally distilled under reduced pressure. (B.pt. 70-72°C/26mm)

6.2.1b. Preparation of the ethyl ester of m-phenylene dialanine.

The procedure adopted was the same as that used by Gregor et. al. (33) except that ethyl-α-bromopropionate was reacted with the m-phenylene diamine. 36.6 gm. of the bromo-ester (0.2 moles) and 10.8 gm. (0.1 mole) of re-distilled m-phenylene diamine in 25 ml of 2-propanol were boiled under reflux for 6 hours. The solution was then allowed to cool and the m-phenylene-diamine hydrobromide filtered off. The precipitate was washed with 2-propanol and the united washings and filtrate concentrated and refrigerated causing the product to crystallise out. This was filtered off, washed well with water and then sparingly with 2-propanol. The yield was 7 gm of a product melting at 131°C.

6.2.1c. Preparation of mephenylene dialanine

This was prepared by refluxing 4.6 gm. of the above with 50 ml of conc. HCl solution for 4 hours. The solution was then concentrated under reduced pressure and chilled, causing the product to precipitate. This was filtered off, suspended in ice-cold 2-propanol, refiltered and washed with ether. The yield was 3 gm of a product melting at 172-174°C.

6.2.16. Preparation of a resin from the above product.

Five grams of m-phenylene diamine were dissolved in 3.3 ml of dilute sodium hydroxide, the solution warmed and 1.3 gm of 40% formalin added. The resulting gel was cured at 110°C for 24 hours when a hard brittle resin resulted.

6.3. FROM NATURALLY OCCURRING OPTICALLY ACTIVE ACIDS

6.3.1. FROM GLUCONIC ACID (Page 19)

39.2 gm (0.2 moles) of gluconic acid and 22 gm (0.2 moles) of resorcinol were dissolved in the minimum amount of hot water and the solution made alkaline by the addition of 6N sodium hydroxide solution. Ten ml of 40% formalin were then added, the solution heated to 80°C and vigorously stirred until the smell of formaldehyde had completely disappeared. After cooling to 30 - 40°C a further 30 ml of formalin was added, thus making a total of just over 0.4 moles, and the solution stirred until the whole mass gelled. The gel was broken up and cured by heating in an oven at 100°C for 24 hours.

The hard brittle resin was ground in a mortar and then soaked in successive amounts of 10% sodium hydroxide solution until no more colour was discharged. It was then allowed to soak in N HCl solution for several hours to convert it to the acid form. A small sample was removed, washed until free of chloride ion and the total capacity determined as outlined below

Approximately 1 gm of air-dried resin was accurately weighed out and shaken with 100 ml of 0.1 N sodium hydroxide solution overnight. The resin was then filtered through a filter funnel contained in the mouth of a 250 ml volumetric flask and washed until the flask was filled up to the mark. A 100 ml aliquot of this solution was withdrawn and titrated

with standard 0.1 N HCl solution. The capacity was found to be 2 meq/gm of air-dried resin.

This resin was found to be capable of holding ammonia quantitatively and was also able to 'split' neutral salts, thus showing that the carbonyl groups had been unaffected by the condensation and curing processes. It now remained to be shown that racemisation had not occurred during these processes and that an optically active resin had in fact been formed.

A polarimetric examination of a solution of the resin would establish whether optical activity was present or not but since the resin was insoluble in all solvents, the preparation of a solution was not possible. Accordingly, another sample of resin was prepared under the same conditions as before but with the exclusion from the condensation recipe of sufficient formaldehyde to form a highly cross-linked polymer. The recipe used was: 9.3 gm gluconic acid, 5.5 gm resorcinol, and 3 ml of 40% formalin.

As was to be expected, a syrup, rather than a gel, was obtained and this was cured for 16 hours at 100°C, the resulting resin ground in a mortar and dissolved in water with the aid of a Soxtlet apparatus. The solution of resin was dark red in colour with the result that a polarimetric examination was impossible. However, the addition of dilute HCl to the solution caused it to turn a light yellow in colour and a small amount of brownish material to precipitate. The latter was filtered off and the resulting clear solution placed in the polarimeter.

The solution was found to be dextro-rotatory and thus this experiment, though only qualitative, showed that the resin was optically active. The original insoluble resin was used in the resolution experiments described in the next section.

6.3.2. FROM L-TYROSINE. (L-P-HYDROXY-PHENYLALANINE.) (Page 20)

A resin from l-tyrosine and formaldehyde was prepared by using a slightly modified version of Brown's (36) procedure. 3.6 gm of tyrosine was dissolved in 10 ml of 10% sodium hydroxide solution and one ml of 40% formalin added. The solution was then boiled until the smell of formaldehyde had disappeared and then a further 2 ml of formalin added. The resulting thick syrup was cured at 100°C for 2½ hours, when a transparent orange-brown resin was obtained. This, after crushing, was placed in a column and treated with N hydrochloric acid to convert it to the acid form. During this process the resin shrank to one half of its original volume and when N sodium hydroxide solution was introduced into the column, the resulting swelling of the resin caused the flow to cease altogether.

This difficulty was overcome by cross-linking the resin with resorcinol and the final method of preparation was the following:- 7.27 gm (0.04 moles) of tyrosine were dissolved in 40 ml of water containing 3.2 gm (0.08 moles) of sodium hydroxide. Six ml of 40% formalin were then added and the solution kept at the boiling point for ten minutes. After cooling slightly, 4.4 gm (0.04 moles) of resorcinol dissolved in a little water was added and the solution boiled until the smell of formaldehyde had disappeared. The solution was then cooled to 60°C and a further 6 ml of formalin added. After stirring for a short while the solution gelled. The gel was broken up and cured in an oven at 100°C for 16 hours.

A sample of the solution withdrawn just prior to the addition of the resorcinol was submitted to a polarimetric examination and found to be strongly dextrorotatory. This is in accordance with the work of Brown who showed that l-tyrosine combines with formaldehyde to give a d-rotatory product.

The cured resin was much more resistant to shrinkage and swelling than the previously prepared uncrosslinked sample and appeared to be suitable for column work. It was crushed in a mortar, soaked in successive amounts of 10% sodium hydroxide solution until no more colour was discharged and then converted to the hydrogen form by treatment with N hydrochloric acid. A sample of the resin was then washed until the washings were free of chloride ion, air dried, and the total capacity determined as described on page 54. This was found to be 2.6 meq/gm of air-dried resin.

Since α-phenylethylamine was extensively used in the resolution experiments the capacity of the resin with respect to this base was also determined. The procedure was exactly the same as before except that the sodium hydroxide solution was replaced by a 0.1 N solution of the amine in 50% alcohol and bromophenol blue used as the indicator in the back-titration. The total capacity with respect to α-phenylethylamine was found to be 2.3 meq/gm of air-dried resin. The percentage moisture in the air-dried sample of resin was determined by drying it over P_2O_5 in vacuo, this was found to be 21% and the total capacity of the resin with respect to the amine calculated to be 3 meq/gm of dry resin. This resin was used in the resolution experiments.

6.3.3. TWO D-TARTARIC ACID (Page 20)

0.1 moles of tartaric acid and 0.1 moles of resorcinol were dissolved in the minimum amount of water, the solution made alkaline with NaOH, and the solution heated to 70°C. A 40% formalin solution was then added slowly with stirring. An immediate exothermic reaction took place and after 0.05

moles had been added the solution came to the boil, a white dough-like mass separating out in the process. This was broken up and cured at 110°C for 24 hours. An amorphous brown product resulted, which, though insoluble in all solvents, was very brittle and crumbled easily and hence was discarded.

After some experimentation it was found that a proper gel could be obtained by using the same techniques as for the gluconic acid resin provided that the temperature was kept as low as possible. The first mole of formaldehyde was added to the solution of tartaric acid and resorcinol at a temperature of 40°, the solution stirred until the smell of formaldehyde had disappeared and the solution then allowed to cool to room temperature. The second mole of formaldehyde was then added when the solution gelled on standing with occasional stirring. The gel was broken up and cured at 100° for 16 hours. The resulting dark transparent resin was found to have a negligible exchange capacity and consequently was quite useless for ion exchange purposes.

6.3.4. FROM D-CAMPHORSULPHONIC ACID (Page 20)

0.1 moles of camphorsulphonic acid and 0.1 moles of resorcinol were dissolved in the minimum amount of water and the solution made alkaline by the addition of 6N sodium hydroxide solution. 0.1 moles of formaldehyde in the form of 40% formalin was then added and the solution boiled until the smell of formaldehyde had completely disappeared and then cooled to 40°C. On the addition, with stirring, of a further 0.1 moles of formaldehyde, the solution gelled. This was cured at 100°C for 16 hours, when a dark red hard resin was obtained. At this point a sodium fusion test for sulphur on the resin was positive. The resin was crushed, washed with

N HCl solution and water and the test for sulphur repeated. This time a negative result was obtained indicating that the camphorsulphonic acid had been washed out of the resin matrix. The resin was hence discarded.

6.4. PREPARATION OF THE AMINES USED IN THE RESOLUTION EXPERIMENTS.

6.4.1. PREPARATION OF α-PHENYLETHYLAMINE (Page 27)

This amine was prepared according to the following reactions

$$Ph.CO.CH_3 + 2HCOONH_4 \longrightarrow Ph.CH.(NH.CHO)CH_3 + 2H_2O + NH_3 + CO_2$$

$$Ph.CH.(NH.CHO)CH_3 + H_2O + HCl \longrightarrow Ph.CH.(NH_3Cl)CH_3 + HCOOH$$

$$Ph.CH(NH_3Cl)CH_3 + NaOH \longrightarrow Ph.CH(NH_2)CH_3 + NaCl + H_2O$$

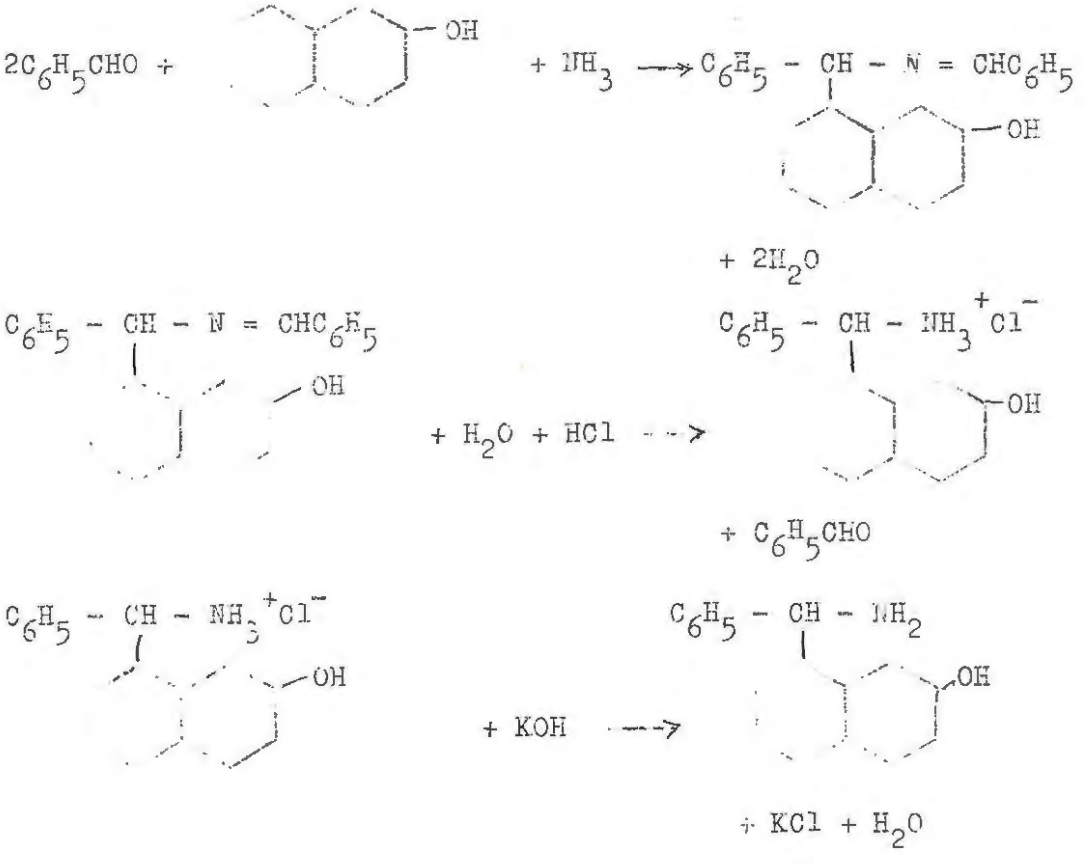
250 gm (4 moles) of ammonium formate and 150 gm (1.25 moles) of acetophenone were placed in a 500 ml Claisen flask. The flask was fitted with a cork carrying a thermometer extending nearly to the bottom and the side arm connected to a condenser fitted for distillation. The mixture was heated over a flame until the temperature reached 185°C, and the acetophenone which collected in the distillate during this process removed and returned to the flask. The heating was then continued for a further three hours at 180 - 185°C, allowed to cool, extracted with 200 ml water and the crude α-phenylethyl-formamide drawn off into the original flask.

Hydrolysis of the crude formamide was effected by boiling with 150 ml of concentrated HCl solution and the cool solution then extracted with several portions of benzene to remove excess

acetophenone. The aqueous acid solution was then treated with a solution of 125 gm NaOH in 250 ml of water and the solution steam distilled until the distillate was only faintly alkaline. The amine in the distillate was extracted with benzene and the extracts after drying over powdered sodium hydroxide, distilled under reduced pressure. The yield was 82 gm (55% theoretical based on acetophenone) of a product boiling at 87°C/22 mm.

6.4.2. PREPARATION OF DL-β-NAPHTHOLPHENYLAMINOMETHANE (Page 38)

The amine was prepared according to the following scheme:-



A cold solution of 144 gm (1 mole) of β-naphthol in 200 ml of 95% ethyl alcohol was placed in a one litre round bottomed

flask, 212 gm (2 moles) of freshly distilled benzaldehyde added, followed by the addition of 200 ml of 95% ethyl alcohol which had been saturated with ammonia at room temperature. The flask was stoppered and allowed to stand for 2 hours, the excess ammonia then allowed to escape and the flask allowed to stand for at least another two days. The condensation product was filtered off and washed with a little alcohol.

It was hydrolysed by steam distilling with 3 or 4 times its volume of concentrated HCl until no more benzaldehyde appeared in the distillate. The hydrochloride which separated out during this process, was filtered off. The amine was stored as the hydrochloride and only isolated when required. This was achieved as follows. 20 gm of the salt was stirred to a smooth paste with 30 ml of water and 5 gm of crushed ice. An ice-cold solution of 20% sodium hydroxide was then carefully added to give an almost clear solution. The solution was extracted several times with ether, the extracts dried over anhydrous sodium sulphate and the free amine obtained by concentrating and cooling the ethereal solution. A sample of the amine was recrystallised three times from ether and found to have a melting point of 119°C.

6.4.3. PREPARATION OF DL-β-PHENYLISOPROPYLAMINE (Page 36)

The amine was obtained from the makers in the form of the sulphate. The salt was decomposed with dilute sodium hydroxide solution and the free base extracted with ether.

7. THE RESOLUTION EXPERIMENTS

7.1. USING THE GLUCONIC ACID RESIN (Page 26)

7.1.1. ATTEMPTED RESOLUTION OF DL-ALANINE

A sample of the resin was crushed in a mortar, sieved, and the portion between 16 and 60 mesh washed free of 'fines' by flotation of the latter in water. The resin was then cycled in N NaOH and N HCl solutions for several hours and was finally converted to the acid form by treatment with 1. HCl solution. A slurry of resin was transferred into the column which, in this case, consisted of a burette containing a plug of glass wool situated above the stopcock to support the resin bed. The column was then washed with distilled water until the washings were free of chloride ion.

A solution of 8.4 gm of alanine in 1 litre of water was allowed to run slowly down the column at a rate not exceeding 6 ml/minute, the effluent being periodically tested for the presence of amino acid with ninhydrin solution. Alanine broke through after 70 ml of effluent had passed and the latter was thereafter collected in 50 ml portions until 250 ml had been collected. Each fraction was submitted to a polarimetric examination but no rotation was observed in any of the fractions.

7.1.2. ATTEMPTED RESOLUTION OF DL-α-PHENYLETHYLAMINE

7.1.2a. Preliminary experiments

The above sample of resin was regenerated and washed free of acid and then dried at 100°C for three hours. It was made into a slurry with benzene and transferred into the column. A solution of 10.34 gm of dl-α-phenylethylamine in 1 litre of benzene was poured down the column at a rate not exceeding 1 ml/

minute. The resin darkened considerably in contact with the benzene and the progress of the amine down the column could not be followed visually. The amine was therefore detected at the bottom of the column by shaking one drop of effluent with 5 ml of an aqueous solution of phenolphthalein. Amine broke through after 120 ml of effluent had been collected and the next 25 ml were examined in the polarimeter. No rotation was observed.

It was decided to use a longer fine-grained column in an effort to achieve a resolution of the amine. A large sample of resin was prepared, crushed in a mill, sieved, and particles passing 60 mesh freed of fines by flotation. The resin was then transferred into a column 170 cm long with a diameter of 1½ cm, and treated with N HCl solution to convert it to the acid form. However, the very slow rate of flow made washing extremely tedious and so a new column was designed.

This column consisted of three segments, each 50 cm long and 1½ cm in diameter. The segments could be joined together by means of ground-glass joints and each was fitted with a burette type tap at the bottom. The column of resin in each section was supported on a glass-wool plug. The use of this column greatly speeded up the regeneration and washing processes since it could be dismantled and each section treated separately.

The resin was converted to the H^+ form with HCl, washed free of acid and then washed with acetone to remove the water. (Since air is more soluble in acetone than in water, the acetone was first 'degassed' by subjecting it to reduced pressure for a short while. If this is not done the air released on contact between the acetone and the water in the column forms bubbles in the resin bed thus causing channeling and in some cases a complete cessation of flow.) The column was finally washed with benzene since it was intended to perform the experiment in benzene solution.

The segments were then connected together and a 1% solution of amino in benzene poured down the column at a rate not exceeding 0.5 ml/minute. Amino broke through after 673 ml of effluent had been collected and the next 25 ml were submitted to a polarimetric examination. A rotation of $+0.05^{\circ}$ was observed.

7.1.2b. Using improved columns

In order to prevent 'streamlined flow' down the columns a modified version of the columns designed by Haddahl (page 34) was used.

The columns described below were used in all the resolution experiments from this point onwards and hence their construction will be dealt with in some detail. Two columns were used; a large one containing the bulk of the resin and a smaller 'front straightening' column connected directly beneath it. A photograph of the set-up appears on page 78. The dimensions of the columns were as follows:

Large column: 40 cm long, $\frac{3}{4}$" internal diameter;
Depth of resin bed 31 cms.

Small column: 15 cm long, $\frac{1}{2}$" internal diameter,
Depth of resin bed 12 cms.

Stopcocks and inlets carrying liquid were made of thick-walled capillary tubing of 1-2 mm. bore. The inlet to each column consisted of a small adaptor which fitted by means of a ground glass joint into the top of the column. Each adaptor contained a central liquid inlet and an outer air outlet to which was connected a short piece of Tygon tubing carrying a pinchcock. The height of liquid above the resin bed could thus easily be controlled. The influent was contained in a 1 litre reservoir connected to the top of the large column by means of a long piece of Tygon tubing in the form of a "U" such that the lowest point of the "U" was below the outlet of the small column.

This arrangement ensured that the columns could not accidently drain. Since all the resolution experiments were performed in benzene solutions, all connections had to be made of Tygon and not rubber tubing.

It was observed that after several months use the Tygon darkened and became extremely hard and brittle. The makers state that this is due to the extraction of the plasticiser by the solutions in contact with the tubing. Since most conventional stopcock greases dissolve in benzene, the stopcocks and ground glass joints were greased with a compound made from glycerine and starch, which was insoluble in benzene but soluble in water. This grease proved to be very effective.

A sample of resin, prepared as described, crushed below 100 mesh and washed free of fines was extracted in a Soxhlet extractor with acetone and benzene until no more colour was discharged. It was then dried in an oven, cycled in N sodium hydroxide and N hydrochloric acid solutions and finally soaked in the former to convert it to the sodium form. After a thorough washing with water it was introduced into the columns as a slurry in water and allowed to settle. (The glass wool plugs supporting the resin beds had previously been carefully levelled off.) A small 'disc' of cotton wool was allowed to rest on the surface of each bed to prevent incoming liquid from disturbing the level surface of the resin.

The resin was converted to the hydrogen form by treatment with N HCl solution, and washed free of chloride ion. Washing with 'deaerated' ethyl alcohol removed the water and the columns were then finally washed with redistilled thiophene-free benzene. At no stage during these operations was the level of liquid in the columns allowed to fall below the resin surface. The columns were now ready for use in the resolution experiments.

7.1.2b (i) Breakthrough techniques

Run 1.

A 1% w/v solution of dl-α-phenylethylamine was placed in the reservoir and the latter fitted with a soda-lime guard tube. This precaution was necessary since the solution gradually absorbed carbon dioxide from the atmosphere during the experiment and caused the amine to precipitate as the carbonate.

The amine solution was then passed down the column at a rate not exceeding 15 ml/hour, flow being stopped at night. The effluent was periodically tested for amine with phenolphthalein as before. After 850 ml of effluent had been collected, amine broke through and the next 4 ml of effluent was diluted to 10 ml and examined in the polarimeter. A rotation of $+0.22^{\circ}$ was observed. A further two fractions of 4 ml were collected, diluted to 10 ml and found to have rotations of $+0.05^{\circ}$ and 0.00° respectively. (A note on the polarimeter tubes: Since micro-polarimeter tubes were not available the normal size tube was modified to enable the rotations of the small fractions to be measured. This was effected by placing two concentric glass tubes in the polarimeter tube, the inner tube having a diameter of 3.5 mm. Both surfaces of the inner tube were silvered to prevent reflected and refracted light from obscuring the field of view. In later experiments the two tubes were replaced by a thick-walled capillary tube which fitted snugly into the polarimeter tube. Both of these modifications resulted in a reduction of tube volume from 21 ml to 8 ml.)

An attempt was then made to elute the l-rotatory isomer from the column. The latter was washed with benzene, alcohol water, and finally with N HCl to strip off the amine. The acid washing was continued until the pH of the influent was equal to the pH of the effluent and the combined washings made alkaline

with NaOH. The solution was steam distilled, the distillate extracted with benzene and the extracts dried with powdered NaOH. The benzene solution, after concentration under reduced pressure to a volume of 25 ml was polarimetrically examined. No rotation was observed.

Run 2.

The regenerated resin from the previous run was washed free of acid and then washed with alcohol and benzene as before. A solution of 16 gm amine in 2 litres of benzene was percolated through the resin at a rate of 15 ml/hour. After 1050 ml had been collected the amine broke through and the effluent was thereafter collected in 4 ml fractions. The first fraction after dilution to 10 ml was found to have a rotation of $+0.12^{\circ}$. In order to exclude any possibilty of this rotation being due to a small amount of dissolved resin, the fraction was washed by shaking with N sodium hydroxide solution and the rotation again measured. This was found to be the same as before, indicating that a small amount of resolution had been effected.

Another attempt was made to wash off the l-amine on the column. The resin was washed with alcohol and water and then eluted with 0.3 N HCl at the rate of 1 ml/minute. The effluent was collected in 10 ml fractions, the pH of each fraction being measured. Flow was stopped when the pH of influent = pH of effluent. The last six fractions were examined polarimetrically but none possessed any rotation.

Run 3.

The procedure described above was again repeated, the flow rate being 1 drop/10 seconds. Amine broke through after 1055 ml had passed and 4 ml fractions were thereafter collected. Each fraction was diluted to 10 ml and the rotations measured. The

amount of amine present in each fraction was determined by bubbling dry HCl gas through a known volume of fraction, evaporating to dryness in vacuo and weighing the resulting hydrochloride The specific rotation of the amine in each fraction could then be calculated. The results for the first three fractions were the following:-

Fraction	1	2	3
Conc. gm/ml	0.0032	0.0023	0.0027
α°	+0.09	+0.06	+0.06
$[\alpha]_D$	+7.04	+6.47	+5.56

The 4th and 5th fractions each had a rotation of $+0.01^{\circ}$ but as this figure is too low to be significant the specific rotations are not shown. An attempt to obtain a laevo-rotatory sample of amine was again unsuccessful.

7.1.2b (ii) Elution techniques

Run 1.

The resin was converted to the hydrogen form by treatment with N HCl and washed free of acid. Two gm of amine dissolved in a little 'degassed' alcohol was poured down the column and the latter then washed with water. The adsorbed band of amine was eluted with 0.3% sodium hydroxide but the resin underwent a large amount of swelling and flow ceased. The run was abandoned.

Run 2.

Two gm amine dissolved in water was introduced into the regenerated washed column and the adsorbed band eluted with 0.8% HCl solution. The effluent dripped into a small container in which the electrodes of a Beckman pH meter were immersed and the overflow allowed to siphon continuously into the collecting vessel. In this manner the pH of the effluent could be measured at any desired moment. The flow rate was adjusted to 1 drop/10

sec and the pH recorded at regular intervals. At no stage in the experiment was there a sudden change in pH (corresponding to a sharp front of amine hydrochloride in the effluent) and the run was abandoned.

The procedure was repeated using a 0.1% HCl solution as elutriant and the effluent collected in 10 ml fractions. The pH of each fraction was measured but once again no sharp change was observed.

Run 3.

The above procedure was again repeated using a 1% aqueous piperidine solution as elutriant. Since the experiment was performed in aqueous solution and no acid was present, the adsorbed amine caused a distinct darkening of the resin and the progress of the amine down the column could be followed. During the elution the resin underwent a certain amount of swelling but this was not sufficient to completely stop the flow. When the dark band had nearly reached the bottom of the column the effluent was fractionally collected. The first fractions containing amine were light brown in colour and appeared to possess no optical activity although the colour made accurate measurements difficult to obtain.

Run 4.

The regenerated resin (liquid phase being benzene) was treated with a solution of 1.5 gm amine in a little benzene and the adsorbed band developed by elution with a 1% solution of ethylamine in benzene at the usual speed of 1 drop/10 sec. The effluent was collected in 10 ml fractions and when amine appeared at the bottom of the column the fractions were examined in the polarimeter. The rotation of the first six fractions

was the following:-

Fraction	1	2	3	4	5	6
α°	+0.65	+0.60	+0.60	+0.60	+0.46	+0.42

The fractions were light yellow in colour.

The amount of amine in the first fraction was determined by precipitation and weighing as the hydrochloride as previously described (page 89), after first subjecting the solution to reduced pressure (12 mm Hg) for a few minutes to remove any ethylamine which might be present. The specific rotation of the amine present in this fraction was calculated as $[\alpha]_D = +12.2^{\circ}$. Elution was continued to obtain a sample of the l-rotatory amine but only one fraction with a negative rotation (of -0.07°) was obtained.

Run 5.

The above procedure was repeated using a 1% solution of piperidine in benzene as the developing agent. After approximately 200 ml of effluent had been collected the latter became highly coloured although at this stage no amine had broken through. This colour persisted and was still present when amine was finally detected in the effluent. Polarimetery was impossible and the run was stopped. The first highly coloured fraction (which contained no amine) was diluted with benzene until a polarimetric examination was possible but no activity was observed. Apparently the presence of the piperidine was causing a certain amount of 'colour-throwing' from the resin but no gluconic acid was being extracted from the matrix.

7.1.3. ATTEMPTED RESOLUTION OF DL-METHYL AMARINE

Three gm of a sample of methyl amarine dissolved in 100 ml of absolute alcohol was run slowly through the regenerated

column which had previously been washed with alcohol. The adsorbed band was eluted with 1% alcoholic solution of glacial acetic acid at a rate of 20 ml/hour. The presence of methyl emarine in the effluent was detected by allowing the latter to drip into dilute ammonium hydroxide solution. The methyl emarine is insoluble and appears as a turbidity in the alkaline solution. The effluent was thereafter fractionally collected.

Eight fractions of 10 ml each were collected, a further 15 of 20 ml each and finally 10 fractions of 30 ml each, the latter being obtained by eluting the column with 5% alcoholic acetic acid. The material in the fractions was precipitated by the addition of 6N NH_4OH. The material in fractions 13 and 14 was filtered off and the melting point taken; this was found to be 145-148°C, indicating that the substance was methyl lophine.

To ensure that the column had been completely stripped of adsorbed material, it was washed with a 3% alcoholic solution of HCl and the effluent collected in three fractions each approximately 50 ml in volume. Ammonia solution was added, causing a considerable amount of material to precipitate - this proved to be the methyl emarine. The methyl emarine in the last fraction was purified by dissolution in N HCl and precipitation with ammonia. 0.2 gm of the material in 25 ml alcohol exhibited no optical activity. Apparently no resolution had occurred, which under the circumstances is not surprising. The procedure can be used to purify methyl emarine when contaminated with methyl lophine.

7.1.4. ATTEMPTED RESOLUTION OF DL-β-PHENYLISOPROPYLAMINE

A 1% solution of the amine in benzene was passed through the column exactly as was described for the phenylethylamine. The presence of amine in the effluent was again detected with phenolphthalein. No optical activity was detected in the first

few amino-containing fractions to break through.

7.2. USING THE TYROSINE RESIN (Page 36)

7.2.1. ATTEMPTED RESOLUTION OF DL-α-PHENYLETHYLAMINE

The resin, after preparation as described on page 27 was crushed in a mill, sieved, and the particles below 100 mesh freed of 'fines' by flotation. Any iron present in the resin due to rust flakes from the mill was removed by heating the resin on a steambath with 5N HCl for several hours. Finally the resin was cycled with N NaOH and N HCl solutions until no more colour was thrown. It was then slurried into the columns in the hydrogen form and washed free of chloride ion. The water in the column was displaced with degassed alcohol and benzene as has already been described

Frontal analysis

Run 1

A 1% solution of the amine in benzene was run through the column at a rate not exceeding 1 drop/10 sec. and the effluent tested at regular intervals with phenolphthalein. Amine broke through after 200 ml had passed and the next 25 ml fraction was examined polarimetrically. A rotation of $+0.17^{\circ}$ was observed and hence the concentration of amine present was determined by precipitation and weighing as the hydrochloride. From these results $[\alpha]_D = +8.06^{\circ}$, i.e. a 19% excess of the d-form was present.

Run 2.

The procedure was repeated and the effluent, after the breakthrough point, collected in 4 ml fractions. Each fraction was diluted to 10 ml, the rotation measured and the concen-

tration of amine measured as before. The results are shown in the following table:-

Fraction	1	2	3	4
Conc.g/ml	0.00159	0.0025	0.00428	0.00476
α°	+0.06	+0.06	+0.06	+0.06
$[\alpha]_D$	+9.5	+6.00	+3.51	+3.16

The other readings were too low to be significant.

Elution analysis

The regenerated column (liquid phase:water) was treated with 2 gm of amine in a little water and the adsorbed band eluted with a 1% aqueous piperidine solution. Unfortunately the resin underwent considerable swelling and the flow ceased.

7.2.2. ATTEMPTED RESOLUTION OF DL-β-PHENYLISOPROPYLAMINE

A 1% solution of the amine in benzene was passed down the column at a rate of 4 ml/hour. The amine in the effluent was detected with phenolphthalein, the breakthrough point occurring after 200 ml had been collected. No rotation was observed in the first fractions containing the base.

7.2.3. ATTEMPTED RESOLUTION OF DL-β-NAPHTHOLPHENYLAMINOMETHANE

A 1% benzene solution of the amine was passed down the column at a rate of 1 drop/10 sec and the effluent collected in 10 ml fractions. The breakthrough point occurred after some 50 ml had been collected and the rotations of the next 15 fractions are shown in the following table.

Fraction	1	2	3	4	5	6	7	8
α°	+2.76	+5.34	+4.60	+4.66	+5.42	App. +4.8	App. +5.0	App. +5.0
Fraction	9	10	11	12	13	14	15	
α°	+3.6	+3.2	+2.2	+1.4	+1.1	+1.0	+0.6	

The fractions were a pale yellow colour and polarimetry on some of the fractions was difficult. The field of view was no longer visible after the 15th fraction and further readings could not be obtained.

The first eight fractions were combined and evaporated down in vacuo. A brownish oil remained which would not crystallise. However, the addition of 60-80° petroleum ether caused a whitish material to separate which was filtered off and recrystallised three times from ether. It was found to have $[\alpha]_D$ = +45.3° (in benzene) and had a m.pt. of 138°C.

7.3. USING THE TYROSINE SUBSTITUTED POLYSTYRENE RESIN (Page 40)

The preparation of the resin yielded particles of the desired size and no further crushing was necessary. The column of resin underwent considerable shrinkage when the water was displaced with alcohol and benzene and the resin had to be removed from the column prior to regeneration since the swelling on reverting back to an aqueous phase caused blockage of the column.

1% solutions of the amines α-phenylethylamine, β-phenyl-isopropylamine and β-naphtholphenylaminomethane were each run through the column as previously described and in no case was there any detectable amount of rotation in the fractions at the breakthrough point.

7.3. NOTE ON THE COLLECTION OF FRACTIONS

In the initial resolution experiments the fractional collection of effluent was performed manually. The long duration of some of the runs (often lasting several days) made this a very tedious process and a method for the automatic collection of fractions was sought. Phillips (49) has described a convenient fraction collector and a modified form of his apparatus was constructed.

This consisted of two circular aluminium discs drilled just within their peripheries with holes just slightly larger than the tubes to be used for the collection of the fractions. The holes were situated as close together as drilling would permit. The discs were then fixed a few inches apart on a central shaft in such a manner that each hole in one disc was directly opposite a corresponding hole in the other disc. This assembly was then mounted, by means of suitable bearings at each end of the shaft, in a container of water so that the discs were parallel to the surface of the water. A series of rimless test-tubes were introduced into the holes and the water level in the container adjusted until the tubes floated freely in their constraining holes. By means of a weight and a system of pulleys, torque was applied to the shaft and the motion of the assembly of tubes prevented by a knife-edge resting against the leading tube.

The column was mounted directly above the leading tube and the effluent allowed to drip into it. As the tube filled, it sank down into the water, eventually slipped under the knife-edge and the torque applied to the shaft caused the next tube to move into place. By adjusting the position of the knife-edge fractions of any desired size could (theoretically) be collected. If the torque was too great the friction of the knife-edge against the tube prevented its descent.

This apparatus was not very satisfactory for several reasons. It is very difficult to obtain a series of tubes all of which are exactly the same size, with the result that the size of the fractions collected was not constant. A more serious objection to the appartus, and the one which led to its eventual abandonment, was that the slight friction between tube, discs and knife-edge was sometimes sufficient to prevent the former from sinking under the knife-edge. The tube would then overflow and the run would have to be abandoned and the whole experiment repeated. Careful smoothing of the holes in the discs and adjustment of the weight responsible for the torque did, to a certain extent, minimize the trouble but the apparatus was never completely trustworthy.

Eventually an automatic fraction collector was obtained from the Shandon Scientific Company and found to be very satisfactory. The chromatographic set-up incorporating this instrument can be seen in the photograph on the next page.

THE ION EXCHANGE COLUMNS AND AUTOMATIC FRACTION COLLECTOR.

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