THE USE OF ADSORPTION METHODS FOR THE RESOLUTION
OF RACEMIC COMPOUNDS.

by

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Thesis for the Degree of Doctor of Philosophy.

May 1940. University of Edinburgh.
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INTRODUCTION

The application of chromatographic adsorption methods to the micro-resolution of racemic compounds represents a marked advance in the history of optical activity, being the only new principle of resolution discovered since the time of Pasteur.

The discovery of this method of analysis, which was used primarily for the separation of closely related or otherwise difficultly separable organic compounds, is due to the Russian botanist, Tswett, who first published the details in 1906 (Ber. Dtsch. botan. Ges., 24, 316.) having examined the pigments in green leaves by its aid. Essentially, chromatographic analysis is a method of separating a mixture of compounds contained in solution, by adsorbing them onto a suitable material, in a particular manner, and subjecting them to systematic treatment with solvents. It differs from the use of charcoal in preparative organic chemistry and the use of Fuller's earth in enzyme preparation technique, in that these older methods depend on large differences in the coefficient of adsorption.

Tswett's discovery, however, remained in obscurity
until 1931 when it was revived to deal once more with the separation of the naturally occurring carotenoids, mainly by the schools of Karrer, Kuhn and Winterstein. In 1934, both Winterstein and Karrer showed that the process could be extended to many colourless mixtures by illuminating the chromatogram with ultra-violet light which causes many compounds to glow with a characteristic fluorescence. Karrer called these "ultrachromatograms". (P. Karrer and N. Nielsen. "Trennung von Substanzen gemischen im Chromatogramm und Ultrachromatogramm", Zurich: Rascher and Co., 1934).

The method of analysis employed by Tswett was to have the adsorbent in the form of a column in an upright tube, down which a solution of the coloured material was allowed to flow. The separation is carried out in three stages:

1) **Adsorption.**

The solution (up to 3%), usually in a non-polar solvent, is run slowly into the tube. Assuming that all the solute is adsorbed, the constituents of the mixture are taken up by the adsorbent in order of their adsorbability and a crude series of adsorption bands is obtained, the most strongly adsorbed substance forming the top band and the least adsorbed the lowest.
Adsorption (1)  Development (2)
2) DEVELOPMENT.

At this stage, the bands generally overlap. To complete the separation, a stream of pure solvent, sometimes with the addition of a small amount of a more polar liquid, is allowed to flow down the tube. This tends to detach the coloured particles which move a short distance down the tube before again being adsorbed. The substance with the lower coefficient of adsorption will obviously be easier to detach and so will move down the column faster than one with a higher coefficient. Hence this washing will lead to two changes: the coloured bands will move slowly down the tube and they will, at the same time, tend to separate.

Under favourable conditions, the coloured bands will become completely separated with colourless layers of the adsorbent in between.

The series of coloured bands thus obtained was termed a "chromatogram" by Tswett.

3) ELUTION.

The final isolation of the components may be effected in either of two ways. The tube may be allowed to drain and, after the coloured bands have been separated mechanically on pushing the column out of the tube, each fraction may then be eluted by a highly polar...
solvent; alternatively, washing of the column can be continued and each coloured fraction collected separately as it emerges from the lower end of the tube.

The choice of adsorbent and solvent is of the utmost importance in these adsorption columns. Of the adsorbents, one of the most powerful and most useful is aluminium oxide. Less powerful are calcium carbonate, magnesium oxide, sucrose and lactose. In any specific case, the adsorbent chosen will depend on the nature of the substance to be adsorbed. Thus Winterstein recommends that the chlorophylls of high adsorption coefficient be used with the weakly adsorbing sugars. Xanthophylls are suitably held on calcium carbonate while carotene requires the powerfully adsorbing alumina.

The adsorbent is filled into the tube in the form of a cream or suspension with the chosen solvent and packed down evenly and firmly by allowing the solvent to flow through and at the same time tapping the tube. If the tube is not packed evenly, the bands will develop ragged edges or give "domes". These domes are due to the liquid flowing more rapidly down the walls of the tube than in the interior where the packing may be more dense.

The choice of solvent is of even greater importance than the adsorbent. In general, adsorption takes place best from non-polar liquids of low dielectric constant,
a fact which suggests that the forces involved are of an electrostatic nature. This is in some degree confirmed by the observation that strongly associating molecules such as hydroxy compounds tend to be readily adsorbed whereas the methoxy compound with a much smaller degree of association is much less strongly adsorbed. The best solvent to use, therefore, is light petroleum. This is closely followed by the other non-polar solvents - benzene, carbon tetrachloride and carbon disulphide which have, however, a slightly greater dielectric constant. The more polar solvents - chloroform, alcohol etc. are not nearly so good and are generally only used in small percentages mixed with the benzene or petroleum to allow a reasonable quantity of the mixture undergoing analysis to be dissolved. The best solvent or mixture of solvents in any specific case is only found by experiment. In some cases, the mixture to be separated will not dissolve appreciably in organic solvents, but separation may be effected by using an aqueous solution and a powerfully adsorbing solid such as alumina.

In certain cases, especially with esters on aluminium oxide, hydrolysis may take place in the adsorption tube. Again, actual chemical combination of the compound with the adsorbent, as with certain acids on alumina, may give rise to anomalous results.
### Solvents in Order of their Promotion of Adsorption.

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<th>Dipole Moment</th>
<th>Dielectric Const.</th>
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<tr>
<td><strong>a</strong> Light Petroleum</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>b</strong> (C₆H₆)</td>
<td>0</td>
<td>2.2</td>
</tr>
<tr>
<td><strong>b</strong> (CCl₄)</td>
<td>0</td>
<td>2.3</td>
</tr>
<tr>
<td><strong>b</strong> (CS₂)</td>
<td>0</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>c</strong> CHCl₃</td>
<td>1.05 - 1.2</td>
<td>5.2</td>
</tr>
<tr>
<td><strong>d</strong> Alcohols</td>
<td>1.7</td>
<td>25 - 30</td>
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Arnold (J.A.C.S. 1939, 61, 1611), having made a survey of the data on chromatographic adsorption on a polar medium, concluded that dipole moments had a great influence on the order of adsorption. While acidity or basicity are of little importance, the number of isolated dipoles in the molecule is the dominating factor. Thus picric acid is more strongly adsorbed on alumina than o-nitrophenol although the latter has a larger permanent dipole. Of isomeric molecules containing the same number and kind of functional groups, those with the larger dipole moment are the more strongly adsorbed on polar media. In cases where no permanent dipole exists, those compounds with the highest polarisability should be most...
strongly adsorbed.

Freundlich and Heller (J.A.C.S. 1939, 61, 2228) found that the adsorption of cis-azobenzene on alumina is stronger than that of the trans-form, especially in ligroin, but less so in methyl alcohol. On the other hand, trans-azobenzene is more strongly adsorbed on charcoal, especially in methyl alcohol. These results support the view that the more hydrophilic isomeride (cis) will be more strongly adsorbed by the more hydrophilic adsorbent (alumina), especially in the more hydrophobic medium (ligroin) and vice-versa. Evidence that cis-azobenzene is the more hydrophilic is found in its greater solubility in water and alcohol and lower solubility in ligroin than the trans-form. Previous experimental data on adsorption of maleic and fumaric acids on charcoal in water and of methyl violet on charcoal and silica gel in water and tetralin can also be explained on this view.

As a result of his experiments, Tswett laid down the rule:

"A compound can be considered to be homogeneous if it cannot be separated into two or more zones by adsorption and development under varying conditions."

(BOOK, Warsaw, 1910)
Tswett himself carried out a large number of chromatographic analyses on naturally occurring mixtures of pigments. He worked however, on a micro scale and contented himself with separating the product into its coloured bands and proving these to be homogeneous, without isolating or analysing the individual components. Because of this and also because he published many of his results in the Russian language, his work was neglected for many years.

In more recent times, chromatographic analysis has again been applied to the separation of mixtures which otherwise could only be separated with great difficulty. Highly complex compounds are, of course, adsorbed more readily than simple ones and are also more likely to be coloured. Unsaturation, which is the primary cause of colour, generally leads to a high adsorption coefficient. Thus it is only to be expected that this method of analysis should be used extensively to separate the complicated mixtures obtainable from plant and animal sources, in sterol chemistry and to the separation of products of a carcinogenic nature prepared synthetically.

In connection with the latter example, it has been shown that the three isomeric dibenzanthracenes may be readily separated chromatographically. Of these, the 2:3:6:7 compound is more strongly adsorbed than the
1:2:6:7 and that more strongly than the 1:2:5:6 isomer.

Simpler compounds may also be separated by this process. Thus a mixture of o--, m--, and p-nitranilines, or o--, m-- and p-nitrophenols may be separated by use of calcium carbonate or alumina as adsorbent, the adsorption being p--→m--→o--.

A more unusual case is the separation of two colourless terpene liquids - cineol (b.p. 176°) and dipentene (b.p. 175°), using light petroleum as solvent and aluminium oxide in the column. In this case, ultraviolet light is used to substitute the lack of colour in visible light by a fluorescence colour.

The success of Tswett's chromatographic adsorption analysis in separating closely related and isomeric compounds led chemists to consider whether it might not be employed in separating optical isomers. These, though chemically the same, are not physically homogeneous and, by introducing some asymmetric factor into the adsorbing surface, it should be possible in some degree to effect a resolution. The lack of differentiation in colour or fluorescence between any possible dextro- or laevo- zones is well compensated by a positive or negative activity in polarised light which thus supplies a sensitive test of separation. This method if successful, since it does not depend upon the presence of any
functional group, should be applicable to many compounds which are otherwise incapable of being resolved.

Early attempts to demonstrate asymmetric adsorption were relatively unsuccessful. Willstätter (Ber. 1904, 27, 3758) failed in an attempt to demonstrate asymmetric adsorption of one of the enantiomorphs of a racemic alkaloid using silk and wool as optically active adsorbents.

Porter and Hirst (J.A.C.S. 1919, 41, 1264) claimed to have effected a partial resolution of a racemic dye-stuff by adsorption on wool. However, they gave neither figures nor data and no confirmation of their claim has been forthcoming.

Ingersoll and Adams (J.A.C.S. 1922, 44, 2930) announced the start of a research on the asymmetric adsorption of racemic dye-stuffs on wool. They gave an excellent discussion of the methods they proposed to adopt and mentioned that they were finding a small partial resolution in one case. They carried out their measurements by colourimetric means, only intending to check adsorptions with the polarimeter. Again, no data were given.

Porter and Ihrig (J.A.C.S. 1923, 45, 1990) claimed to have effected an appreciable partial resolution of a racemic dye-stuff, dl-m-azo-β-naphthol mandelic acid. According to their descriptions, after wool had been dyed in a solution of this racemate, the exhausted dye-bath was left strongly laevo-rotatory, owing, they said,
to preferential adsorption of the dextro-isomer. Finally, they claimed that the exhausted dye-bath contained the almost pure laevo-rotatory dye-stuff.

Morgan and Skinner (J.C.S. 1925, 127, 1731) showed that certain "tartaric acid configuration" stereoisomeric azo dye-stuffs showed differential rates of adsorption. They measured the rates of adsorption of the various isomers and enantiomorphs by titration methods. They also mention small but unspecified rotations taken in a polarimeter, after a dying experiment similar to those carried out by Porter and Ihrig.

Adams and Brode (J.A.C.S. 1926, 48, 2193, 2202) failed to confirm any of the findings of Porter and Ihrig and criticised the results of Morgan and Skinner, stating that the differences fell just without the limits of normal error. They did not appear to have repeated Morgan's work, however.

Ryutaro Tsuchida, Kobayashi and Nakamura describe (Bull. Chem. Soc. Japan, Vol. 11, No.1, page 39) some experiments which may have some relation to asymmetric adsorption in the light of later work.

In essential, a warm saturated solution of chlorobisdimethylglyoximoamine-cobalt \([\text{Co dgl}_{2} (\text{NH}_{3}) \text{Cl}]\) was allowed to cool over specimens of powdered dextro or laevo-rotatory quartz. Their residual solutions
after cooling and decanting, generally proved, although not invariably, to be slightly dextro-rotatory from left-handed quartz and slightly laevo-rotatory from right-handed quartz. They explained this fact by assuming that their cobalt complex was of a racemic nature and that asymmetric adsorption had taken place. The work was supported by use of other known racemic metallo-organic complexes. Using the same technique, they report the same type of asymmetric adsorption with a sample of synthetic, racemic adrenaline hydrochloride (J. Chem. Soc. Japan Vol. 56, page 1359). So far as can be calculated from data in the paper, the specific rotations they attained were only of the order of 6° (using the C-line) for the cobalt complex and in the region of 2° (D-line) for the adrenaline, which, when fully resolved, should give a specific rotation of 53°. At no point do they, however, work out the specific rotations.

In all of the above cases, the partial resolutions claimed are very small, the observed rotations being of the order 0.01 to 0.05° with the exception of Porter and Ihrig's work where a single unconfirmed rotation of −0.90° is reported and for this reason, a certain amount of doubt may be attached to them.

Karagunis and Gounoulous (Praktika, 1938, 13, 414; Nature, 1938, 142, 162) reported that by using powdered
optically active quartz, they were able to get a partial resolution of the chromium complex $[\text{Cr} \text{(en)}_3] \text{Cl}_3$.

The first outstanding success of the use of adsorption methods for the resolution of racemic compounds has been gained by Henderson and Rule (J.C.S. 1939, 1568). They examined two compounds — m-azo-$\beta$-napthol mandelic acid and p-phenylene bis-imino camphor. The adsorbents tried were calcium d-tartrate, powdered quartz, emulsin, maltose, glucose, sucrose and lactose. Using commercial d-lactose as adsorbent, the m-azo-$\beta$-napthol mandelic acid yielded indications of small partial resolutions of the order of magnitude of the previous workers. With the camphor derivative, they were again able to get small partial resolutions by using commercial lactose as adsorbent. Then it was discovered that the degree of adsorption was greatly enhanced by first treating the lactose with warm chloroform which removed a small amount of a yellow oil. Using this "activated" commercial lactose, they were able to effect a complete micro-resolution of the p-phenylene bis-imino camphor, though the process had to be carried out in four stages—viz. from the first adsorption tube they obtained a partially resolved product but the quantity was too small to carry on with, so that they made up a synthetic mixture with the same specific rotation and used this in the second tube. This procedure
was repeated for a third and a fourth tube. From the fourth tube, they isolated some of the optically pure product. From this they deduce that if a compound can be partially resolved by adsorption methods, then by further adsorption it may be totally resolved.

From the fourth tube mentioned in the above paragraph these workers isolated their compound in a form which yielded an observed rotation of -1.513° in chloroform (using the mercury green line) which corresponds to a specific rotation of -2000°.

Now that it has been definitely shown that the resolution of a racemic compound is possible by adsorption methods, there still remains the task of finding other cases in which the separation of the optical isomers is practicable in the hope that in the future enough will be learned to lead to the discovery of the mechanism of the process of adsorption and the basic factors governing it. In the ideal case, one optical isomer would remain near the top of the tube while the other would wash down, leading to a rapid, total and quantitative resolution. Unfortunately, before this can be accomplished, a great deal of investigation will have to be carried out.

In the present work, experiments have been carried out with the object of extending our present knowledge of this subject.
EXPERIMENTAL and DISCUSSION.

The aim of this research being to make a further study of the use of adsorption methods for resolving racemic compounds, it was first necessary to select a suitable starting material. Three properties are desirable in such a compound:

A) The compound should be distinctly coloured. Though many colourless substances may be "visible" in ultra-violet light, either due to fluorescence or to quenching of the white colour of the adsorbent, in practice the ultra-violet lamp cannot be used satisfactorily for illumination since the large glass towers holding the adsorbent are necessarily of thick glass through which the ultra-violet rays cannot penetrate. For the coloured racemic compound, yellow is preferable since it allows sodium light or even light of a shorter wavelength to be used when taking rotations in the polarimeter; The shorter the wavelength used, the greater the observed rotation. A disadvantage of pale yellow compounds is that as the band is washed down the adsorption column, its position sometimes becomes difficult to find due to the weakening of the colour caused by the band lengthening.

B) The compound should have a high rotation when fully
resolved.
C) It should be easily adsorbed from non-polar solvents and easily eluted by more polar solvents without undergoing decomposition at any stage, especially during the prolonged adsorption.

In the present investigation, it was decided to use as a basic compound, 1:1'-dinaphthyl-2:2'-diamine from which can be prepared two different types of compound capable of existing in optically active forms.

A) The first type is obtained by condensing the diamine with an α-diketone such as benzil which leads to a structure containing an eight-membered heterocyclic ring.

\[ \text{e.g.} \]

This molecule, as might be expected from the presence of the semi-rigid eight-membered ring, is not planar. Though difficult to show on paper, it can be readily demonstrated by solid models that the bond joining the two naphthalene rings lies at right angles to that linking the central carbon atoms of the benzil residue.
i.e. if one is in the plane of the paper, the other is perpendicular to it. This twisting or warping of the heterocyclic ring can be accommodated in either of two ways, resulting in the formation of two mirror image structures which may be represented as below:

B) The other type is obtained by diazotising the diamine and coupling it with a phenol in alkaline solution. This yields a compound of the form:

Optical activity is conferred on this compound by reason of the restricted rotation about the bond linking the naphthalene rings, owing to the blocking effects exerted by the 3:3'-hydrogen atoms and the two azo groups attached to positions 2:2'.
In the following pages, the method of preparation and purification of each compound will be followed by the results of attempts to resolve it. Where a new compound is made, the analysis figures quoted are those obtained by Mr. W. Brown, Biochemical Department, Edinburgh University, or by Dr. Weiler, Oxford, in both cases micro methods being used.

Concerning solvents mentioned, in all cases where benzene is used, it is the "Analar" product, since the cruder liquid contains traces of various other solvents which might tend to spoil the chromatograms. Also, the light petroleum used had b.p. 60 - 80° unless otherwise stated.

The polarimeter employed in all the following work except that dealing with the condensation compound of dinaphthyl diamine and acenaphthene quinone was a Hilger instrument taking tubes up to 4 dcm. and illuminated by a mercury vapour lamp giving brilliant yellow (5780 Å) and green (5461 Å) lines. In the case of the exception quoted above (a red compound), a Schmidt and Haensch instrument fitted with a wavelength selector (Doppelmonochromator) and deriving its illumination from a small special filament lamp was used. This, however, could only take tubes up to 2 dcm. in length.

In all the work, the zero blank of the instrument
was carefully checked for each set of readings. In finding the rotation of each sample, ten readings were taken, approaching the matching tints alternatively from the positive and negative sides. This set of readings was then averaged. The experimental error is usually taken as ±0.02° though in the majority of cases it is probably less than this.

Preparation of 1:1'-binaphthyl-2:2'-diamine.

This diamine is made by reducing ζ-ζ'-azonaphthalene and causing the hydrazo compound formed to undergo the benzidine transformation. The azonaphthalene can be obtained by the reduction of 2-nitronaphthalene but the latter compound itself is somewhat difficult to prepare so the method of preparation employed was as follows:

ζ-ζ'-Azonaphthalene. (Hantzsch and Schmiedel, Ber., 30, 80).

A suspension of 20 gm. β-naphthylamine in 800 ml. of water containing 60 ml. concentrated hydrochloric acid was diazotised with a solution of 12 gm. sodium nitrite in water. The clear solution was then run into a cooled solution of 50 gm. each of sodium sulphite and sodium hydroxide in 360 ml water with stirring. Frothing occurred. When the reaction had finished, the resultant precipitate was filtered off, washed until alkali free
and allowed to stand till dry. The diazonium sulphite which was formed is only stable in the presence of excess alkali so that on standing it decomposes, mainly changing to the azonaphthalene.

The dry precipitate was then extracted with chloroform and the azonaphthalene obtained by evaporating off the solvent. It was recrystallised from benzene.

Yield: - 11 gm. m.p.: - 204°C.

1:1'-Dinaphthyl-2:2'-diamine. (Kuhn and Goldfinger, Ann., 1929, 470, 183.)

To a suspension of 10 gm. finely ground azonaphthalene in 90 ml. of alcohol was added 40 gm. stannous chloride. Dry hydrogen chloride gas was then passed into the mixture with cooling. The azo compound goes into solution and, after about an hour, the double salt of stannous chloride and diamine begins to crystallise out. The temperature was then raised to 50° and kept there for nearly 12 hours.

The precipitate was filtered off and, after washing with a little alcohol, it was dissolved in the minimum amount of dilute hydrochloric acid and the bulk of the tin precipitated by means of hydrogen sulphide. The filtrate was then made alkaline and extracted with ether. After washing and drying the ether solution, the solvent was evaporated off leaving the white dinaphthyl diamine
1:1'-Dinaphthyl-2:2'-diamine

Diazotise and treat with Na₂S₂O₃

Reduction and benzidine transformation

Condense with benzal

Condense with phenanthroline

Condense with dinitro benzal

Condense with phenanthroline quinone

NH₂  NH₂

NO₂  NO₂
which may be purified by dissolving it in acid, filtering and precipitating with alkali, or by dissolving in pyridine and precipitating with water.

Yield: - 5 gm.  

The first derivative of this diamine to be made was the condensation compound with benzil which was prepared as below.

Condensation of Dinaphthyl Diamine with Benzil.
(Kuhn and Goldfinger, loc. cit.)

A mixture of 2 gm. dinaphthyl diamine and 1.8 gm. benzil was heated in a pyrex tube on an oil-bath. At 150°, the mixture melts to a clear orange-red liquid which at about 200° begins to froth and give off steam. After being kept at 200° for three quarters of an hour, the product was cooled and crystallised from glacial acetic acid. It can also be crystallised from acetic anhydride or benzene-ligroin.

The purified product consisted of bright yellow prisms which melted at 276° but almost immediately formed crystals of a different type which melted again at 288-289°. Repeated crystallisation produced no further change. On one occasion, crystallisation from acetic anhydride gave crystals which melted at 288° but a further crystallisation from acetic acid brought the m.p.
back to 276°. This shows that it is not some type of decomposition that is happening at the melting point. The compound is readily soluble in pyridine and chloroform less soluble in benzene and ether and nearly insoluble in alcohol.

Adsorption of the Condensation Compound of Dinaphthyl Diamine with Benzil.

In this and following experiments, it was decided to rely mainly on lactose as adsorbent. This was shown by Henderson and Rule (loc. cit.) to be the best of the available carbohydrates. It has the advantages of being practically insoluble in benzene and light petroleum and of being procurable in large quantities in the fine state necessary for adsorption without being so fine as to choke completely the flow of solvent.

A comparison was carried out in small "pilot" tubes (30 by 1 cm.) between "B.P. Lactosum" which had been thrice extracted with chloroform and dried in vacuo and "Analar" lactose similarly dried. The solvents examined were light petroleum and 12½% benzene in light petroleum. In both cases the "Analar" product proved to be the better adsorbent, though the adsorption of this benzil derivative proved to be exceedingly weak. Of the solvents, light petroleum led to the better adsorption.
A solution of this compound is pale yellow and when it is run onto the lactose it is very difficult to follow the movement of the coloured layer. Under ultra-violet illumination, the white of the lactose is quenched by the solution though, as was found later, this method of following the progress of the band in the large glass tube or tower is not practicable due to the thickness of the glass.

Having completed the preliminary tests, preparations were made for running a larger tube in which the attempt to resolve the compound would be made. The following description is applicable to all of the adsorption towers used and so will not be repeated unless any change was made.

The tower used was a tube of soft glass five feet long by two inches internal diameter. This was clamped vertically and the lower end closed by a rubber stopper through which passed a glass tube on which was a tap. On top of the rubber bung was placed a layer of cotton wool and then a sheet of filter paper, to prevent solid lactose washing out through the tap.

The "Analar" lactose to be used was first dried for a day in a warm vacuum chest. It was then stirred to a cream with light petroleum and poured into the tower. The tower was only filled about six inches at a time so
that by tapping the glass sides, air bubbles could be got rid of and the lactose made to settle. When the tower was nearly full, excess solvent was allowed to drain away though care must be taken at this and other stages that the solvent level never drains below that of the upper level of the lactose since this would allow the formation of air bubbles in the lactose column which would decrease its efficiency as well as hinder the flow of solvent. The drainage of excess liquid completed the packing down of the adsorbent and more of the suspension could be added till the desired level (four or six inches from the top) was reached. A disc of filter paper was placed on top of the lactose to prevent disturbance of the upper layers on entry of the solution or wash liquid. A column of the size described here holds approximately two and a quarter kilos of lactose.

A nearly saturated solution of the benzil condensation compound in light petroleum was prepared. By allowing a volume of this to evaporate and weighing the solid, its concentration was found to be 0.13 gm./litre. A litre of this solution was allowed to run down the column. The method of regulating the inflow of solution is to have it contained in a large separating funnel (2 litre) fitted with a stopper. The funnel is suspended above the tube so that the end of the stem terminates about an inch above
the level of the lactose. The tap is left open. Air bubbles up into the funnel and liquid flows out till the level of the liquid in the tower rises past the end of the stem and prevents the ingress of air. Flow then ceases till the tower has drained far enough to allow more air into the funnel.

After the litre of solution had been run onto the lactose, a faint yellow colour could be seen to reach nearly half-way down the tower. Washing was then begun with pure light petroleum. The speed of flow with the tap at the foot of the tower full open was approximately 460 ml./hour. After a litre of wash liquid had been used, the yellow colour was no longer distinguishable. A note having been kept of the position of the head of the band at various times, it was, however, easy to estimate its new position. When about 1200 ml. of solvent had been run into the tower, a yellow solution started to drip from the lower end. This was collected in portions as follows:

<table>
<thead>
<tr>
<th>No. of Fractions</th>
<th>Volume of each Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20 ml.</td>
</tr>
<tr>
<td>B</td>
<td>100 ml.</td>
</tr>
<tr>
<td>C</td>
<td>300 ml.</td>
</tr>
<tr>
<td>D</td>
<td>100 ml.</td>
</tr>
<tr>
<td>E</td>
<td>20 ml.</td>
</tr>
<tr>
<td>F</td>
<td>100 ml.</td>
</tr>
<tr>
<td>G</td>
<td>250 ml.</td>
</tr>
</tbody>
</table>
H This was obtained by extracting the solid lactose with warm chloroform.

Solutions F and G were very dilute.

The portions in section A were each evaporated to dryness, dissolved in 10 ml. of chloroform and examined for optical activity in a 4 dcm. capillary polarimeter tube, using the mercury green line. No activity could be detected in them. The first five were then mixed, evaporated down to 10 ml. and re-examined in the polarimeter. This now showed slight activity, the observed rotation being $-0.07^\circ$. A mixture of the other five portions in section A showed no activity.

The active fraction was evaporated to dryness and washed with hot distilled water to remove traces of lactose. It was then transferred to a weighed basin and the basin reweighed, using a semi-micro balance which was accurate to $0.02$ mg.

Weight of compound = 6.91 mg.

This fraction was then taken up in 6.9 ml. of chloroform which was just sufficient to fill the 4 dcm. polarimeter tube.

Observed rotation now = $-0.054^\circ$.

From these figures, the specific rotation can be calculated.

$$[\alpha]_{546} = -13.5^\circ$$ in chloroform.
Fraction $F$, after concentration, was also examined. It showed a rotation of $+0.03^\circ$. Fraction $G$ was too dark even after filtration for polarimetric measurement but was purified by running it down a column of alumina in benzene. No activity could, however, be detected due, probably, to the dilution of the solution. Fraction $H$ was also too dark and was not further examined.

$F$ was evaporated to dryness, washed with water and weighed.

Weight of $F = 2.95$ mg.

This was dissolved in 7 ml. of chloroform and its rotation observed.

Observed rotation now = $+0.061^\circ$

<table>
<thead>
<tr>
<th>Fraction</th>
<th>$\alpha$(observed)</th>
<th>Wt. of compd.</th>
<th>$[\alpha]_{546}^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>$-0.054^\circ$</td>
<td>6.91 mg.</td>
<td>$-13.5^\circ$</td>
</tr>
<tr>
<td>$F$</td>
<td>$+0.061^\circ$</td>
<td>2.95 mg.</td>
<td>$+36.2^\circ$</td>
</tr>
</tbody>
</table>

Fraction $A$ had m.p. 260-265° (Pure compound m.p. 276°) Recrystallisation from acetic acid brought the m.p. up to 265-272°. This lowering of the melting point is probably due to the presence of excess of the laevo-rotatory isomer.
The partial resolution recorded above, though small, is greater than the experimental error involved in the optical measurements and these figures might possibly be improved by altering the conditions. One improvement would be to use more lactose or less of the compound since, in order to obtain an appreciable separation, the band, before washing, should not reach further down the tube than one third or one quarter of its length.

In this case of the condensation compound of dinaphthyl diamine and benzil, no further adsorptions were tried since the compound is so weakly adsorbing that no very great separation was probable. This lack of adsorption is somewhat surprising since the compound is coloured (showing unsaturation) and has a fairly complex ring system. On the other hand, the molecule, since it contains no side-chain groupings of high polarity, will only have a small dipole moment, approximating in fact to a hydrocarbon. Thus there will be a tendency for it to remain in the solvent (hydrophobic) rather than be adsorbed on the hydrophilic lactose.

It was decided to condense the dinaphthyl diamine with a more complex diketone in the hope of improving the adsorbability of the condensation compound. The diketone selected was acenaphthene quinone.
Condensation of Dinaphthyl Diamine with Acenaphthene Quinone.

A mixture of 1.5 gm. diamine and 0.9 gm. acenaphthene quinone was kept at 240–250° for an hour. On cooling, the product was dissolved in benzene, filtered and precipitated with light petroleum (b.p. 40–60°). Extraction with alcohol separated the product into two fractions—a brown residue and a red solution.

The brown residue was further extracted with ligroin. The brown material left was crystallised from acetic acid yielding 0.025 gm. of a product which did not melt below 385°.

The analysis figures obtained for this compound were: %C = 85.0, %H = 4.2, %N = 4.8.

This corresponds to formula C_{44} H_{24}N_{2}O_{2} which requires: %C = 86.2, %H = 4.0, %N = 4.6.

The brown compound has therefore the structure below.

The red fraction which crystallised from alcohol melted at 235–245°. It crystallised from ligroin and then melted at 245–270°. It was finally dissolved in benzene.
and run down a column of alumina. This left a brown band at the top (along with several others) but the red part ran through readily and was collected. Concentration and precipitation with light petroleum yielded a bright red crystalline compound which melted at 379-380°.

Yield: 0.16 gm.

On analysis, the percentage of nitrogen was found to be 6.49 which agrees well with the expected figure of 6.51%. This was therefore taken as the desired compound (for structure see page 21 A).

Adsorption of the Condensation Compound of Dinaphthyl Diamine with Acenaphthene Quinone.

Experiments were carried out to find the best solvent for the adsorption of this compound on lactose. The solvents tried were light petroleum, 12.5% benzene in light petroleum, 2.5% benzene in light petroleum, ligroin, benzene, carbon tetrachloride, carbon disulphide and dichlorethane. In all these pilot tubes it was easily seen that the compound adsorbed exceedingly weakly, even less strongly than the benzil derivative. Because the compound is readily soluble in benzene, it was decided to use benzene in a large tube experiment since this would allow the compound to be run down over a short distance of the lactose column, leaving a long distance
to be traversed during the washing. It was decided, however, to use light petroleum for the washing in the hope that, the compound being less soluble in it, the band would have a greater tendency to spread.

The glass tower (5 feet by 2 inches) was filled with a paste of lactose in benzene. Onto this was run 0.064 gm. of the acenaphthene quinone derivative dissolved in 15 ml. of benzene. After draining in, the top four inches of the lactose column was deep red in colour. Washing was then carried out with light petroleum.

The red band moved rapidly down the tube leaving a faint pinkness on the lactose and at the same time spreading slightly. After six and a half hours, during which two litres of solvent run through the column, the red band reached the lower end of the tube, having spread to twice its original length in the process. After the tube had drained, the column of lactose was allowed to slide carefully out of the tube (using the tap as regulator) and was cut up into sections. The band itself was cut into six portions (C to H reading downwards) while the bulk of the lactose which contained only absorbed compound was cut into two parts (A and B).

The large fractions A and B were extracted with chloroform in large copper Soxhlets while the small portions C to H were warmed with chloroform on the
steam-bath and filtered. The solutions obtained were evaporated down to a suitable concentration for use in the polarimeter, the volume of A and B being 80 to 90 ml. each while C to H could be reduced to 30 or 40 ml. without becoming too dark in the polarimeter. These solutions were then examined for optical activity using the Schmidt and Haensch instrument. The shortest wavelength usable was the C-line ($\lambda = 6563 \text{ Å}$).

After washing fractions A, B, C, E, G and H each four times with water to remove possible traces of lactose, only one fraction, H, was found to show measurable activity, the observed rotation being $+0.096^\circ$ (length of tube = 2 dcm.). Owing to accident it was not possible to complete this experiment, but the conditions were in any case unsatisfactory on account of the extremely low degree of adsorption and the necessity of measuring rotations with light in the red region, thus further reducing the values of the observed rotations.

The next diketone to be condensed with dinaphthyl diamine was phenanthraquinone. This should yield a compound midway in some of its properties between the two previous ones.
Condensation of Dinaphthyl Diamine with Phenanthraquinone.

A mixture of 1.2 gm. diamine, 0.75 gm. phenanthraquinone and 0.5 gm. naphthalene was heated at 200-220° for one hour, the naphthalene being added to act as a solvent in the hope of preventing some of the decomposition that usually attends these fusions. On cooling, the dark mass was dissolved in benzene and precipitated with light petroleum. The precipitate was extracted with ligroin and on cooling, the filtrate deposited yellow-brown crystals which melted 344-356°. These were dissolved in benzene and run down a short column of alumina. The yellow solution which came through first was evaporated down and precipitated with light petroleum.

m.p. 361-365°.

The range in m.p. is apparently due to the compound subliming, leaving a trace of impurity which becomes concentrated in the small amount of condensation product still unsublimed.

Analysis figures:

% C = 89.83   % H = 4.88   % N = 6.21

This corresponds to C_{34}H_{28}N_{2} which requires

% C = 89.43   % H = 4.42   % N = 6.14

This yellow compound is therefore the desired product (for structure see page 21A).
Adsorption of Condensation Product from Dinaphthyl Diamine and Phenanthraquinone.

The yellow compound proved to be readily soluble in benzene but practically insoluble in light petroleum. Tests were made in pilot tubes as for the two previous compounds and as in these cases the degree of adsorption was found to be negligible. One large scale experiment was, however, carried out using benzene as solvent, since the coloured band (probably containing very little adsorbed material) spread well with this medium.

A solution of 0.05 gm. of the compound in 15 ml. of benzene was run onto the lactose and washed down with benzene. No adsorption was apparent. When the yellow solution emerged from the lower end of the tube, it was collected in fractions of 25 mls.

After careful washing with water and drying over calcium chloride, the fractions were concentrated to 10 mls. and examined. In no case, either from the first or the last runnings, were any definite rotations obtained. The failure to observe any sign of resolution is in agreement with the fact that no appreciable adsorption occurred.

The previous three adsorption experiments having shown that the unsubstituted polycyclic compounds were
so weakly adsorbing that no appreciable degree of resolution could be expected, it was decided to attempt to increase the degree of adsorption by introducing one or more polar groupings into the molecule. One of the strongest of these is the nitro group and it was decided to substitute this in the benzil residue of the dinaphthyl diamine condensation product since, of the three products already tried, this yielded the most promising results.

The preparation of 4-nitrobenzil is described by Hausmann (Ber. 1890, 23, 531). Benzoin was therefore nitrated with fuming nitric acid and the oil obtained oxidised to the nitrobenzil with concentrated nitric acid. Repeated crystallisation of the product obtained did not improve the melting-point (132-142°) nor did running it down a column of alumina. This preparation was therefore abandoned.

An attempt to make 4:4'-dinitrobenzil from p-nitrobenzaldehyde by means of potassium cyanide failed. The product consisted of a resin and nitrobenzoic acid. Finally the 3:3'-dinitrobenzil was prepared.

Preparation of 3:3'-Dinitrobenzil.

(Klinger and Martinoff. Ann., 389, 234.)

To 300 gm. of water-free nitric acid (Sp. G. 1.52) cooled to - 10° was added 30 gm. powdered benzil, the temperature being kept below 2° during the addition.
After standing for several hours at 0°, the mixture was poured onto ice and the yellow precipitate washed and dried.

Crystallisation from alcohol and acetic acid brought the melting-point up to 108-128°.

Further purification was effected by the method of Boon and Nisbet (J.C.S., 1929, 1901).

The crude 3:3'-dinitrobenzil (25 gm.) was dissolved in boiling alcohol (400 ml.) and 18 gm. of pure benzidine added. After boiling for a few minutes, the orange-brown addition compound was filtered off from the hot solution and decomposed by acetic acid containing a little hydrochloric acid.

Addition compound: Yield- 18 gm. m.p. 163°

Dinitrobenzil Yield- 10 gm. m.p. 129-131°

Condensation of Dinaphthyl Diamine with Dinitrobenzil.

To try to prevent loss due to decomposition during the fusion, this condensation was carried out in a high-boiling solvent.

A mixture of 1·5 gm. diamine and 1·5 gm. dinitrobenzil was dissolved in 40 ml. hot tetralin and refluxed for three hours. After a few minutes, the yellow solution turned brown and then black. On cooling, the product was precipitated from the tetralin by adding light petroleum
and purified by running a solution of it in benzene down a column of alumina.

The pure yellow product thus obtained, on being heated, frothed at 170° and turned liquid, but solidified again at 240° and finally melted about 350°. This suggests that the condensation had only been half completed.

The product (0.5 gm.) was therefore heated at 250° for five minutes and crystallised from acetic acid and acetic anhydride. This compound shows to a less extent the change in crystal form near the melting-point observed with the benzil derivative.

Yield: 0.25 gm. m.p. 332-334°.

Analysis figures:
%C = 74.37  %H = 3.86  %N = 10.53

This corresponds to the expected compound which requires:
%C = 74.42  %H = 3.67  %N = 10.22

This yellow product is therefore the desired compound (for structure see page 21A).

Adsorption of the Condensation Product from Dinaphthyl Diamine and Dinitrobenzil.

This compound is soluble in benzene but practically insoluble in light petroleum. Pilot tubes were run using benzene and various percentages of benzene in light petroleum as solvent. Of these, the best medium was found
to be a 50-50 mixture of the two. The adsorption was, however, very disappointing because, though stronger than in any of the previous three cases, it was still very weak. It was therefore decided not to carry out any experiments with it on a large scale, but endeavour to find a compound which adsorbed strongly enough to have a reasonable chance of being resolved.

Tetrazo Derivatives of 1:1'-Dinaphthyl-2:2'-Diamine.

The four previous compounds being of a similar configuration and having shown no great degree of adsorbability, it was decided to examine a different type of structure. It might have been possible to make these eight-membered ring compounds more strongly adsorptive if hydroxyl groups could be introduced, but since there were neither symmetrical hydroxybenzils nor hydroxy-dinaphthyl-diamines mentioned in the literature, it was not considered worth while investigating their preparation.

In the paper by Kuhn and Goldfinger (loc. cit.), mention is made, however, of a compound obtained by diazotising 1:1'-dinaphthyl-2:2'-diamine and coupling with resorcinol in alkaline solution. The tetrazo compound
thus formed is stated to be dark red in colour and only slightly soluble in the common solvents, except in pyridine in which it is very soluble. It is recorded as decomposing about 300° after crystallisation from alcohol and yielded a good analysis figure for nitrogen corresponding to the structure shown.

The active form, prepared by diazotising the d-diamine and coupling with resorcinol, was found to have a specific rotation of 3230° in pyridine using the D-line, optical isomerism being due to restricted rotation about the bond joining the two naphthyl nuclei.

For the present work, a quantity of this compound was prepared as follows:

A solution of 1.5 gm. dinaphthyl diamine in 20 ml. of moderately concentrated hydrochloric acid was diazotised in the usual way by adding a solution of 0.75 gm. sodium nitrite in water. The slight excess of nitrite was destroyed in the clear yellow solution thus obtained by adding urea. The acid diazo solution was then added to
an alkaline solution of 1.2 gm. resorcinol, the mixture remaining alkaline. A dark red dye was formed which was precipitated on acidification. This was filtered off and well washed with water.

Attempts to crystallise this compound from alcohol were unsuccessful since practically none separated on cooling the saturated solution. It was found, however, to crystallise from nitrobenzene or chlorobenzene yielding a product which decomposed over a long range (250-320°). Further crystallisation effected no change in the decomposition temperature. This dye is only sparingly soluble in benzene, a cold saturated solution containing 0.0124 gm. per 100 ml., giving a solution with a strong red colour.

First Adsorption of the Tetrazo Compound.

Experiments with pilot tubes showed that, from a benzene solution, lactose strongly adsorbs this dye and, on washing, the band spreads slowly down the tube till the whole column is red in colour.

Accordingly, a large tower having been filled with a paste of lactose in benzene, a solution of 0.05 gm. of the compound in 400 ml. of benzene was run onto it. The dye was adsorbed on the uppermost six inches of the column and, as was expected, washing spread the band down
the column. It was soon apparent, however, that the compound was not homogeneous since the top of the band was dark red while the lower part was orange-red. A yellow forerunner was also observed. This lack of homogeneity was borne out by the results obtained when, after the washing had been completed and the column cut into sections and extracted, the solutions were examined in the polarimeter. Quite definite rotations were observed but the only negative one came from the centre of the band.

In one respect, the results obtained by running this tube were interesting since they suggested that, after further purification, the compound should be capable of being partially resolved by adsorption methods.

The further purification of the dye proved to be somewhat difficult. Since it has no melting-point, it is not possible to check the degree of purification by the usual method. Also, if the pure compound sought should prove to correspond to the yellow-orange band, then the impurity present is probably of a lower solubility and will be very difficult to eliminate. It was realised that the best method of purification was to use an adsorption column. Alumina adsorbs the dye very strongly and washing with an organic solvent does not move the band. A quantity, however, was dissolved in pyridine and run onto alumina
Second Adsorption of the Tetrazo Compound.
where it was adsorbed as a narrow dark red band. This was washed with pyridine and alcohol and then the band was taken out of the tube and eluted with ammonia, leaving a strongly adsorbed fraction still on the oxide. The product thus obtained, though still decomposing over a long range (270-330°), was much lighter in colour and more homogeneous.

**Second Adsorption of the Tetrazo Compound.**

It was decided to use the material obtained as above for a resolution experiment in another large tower. To improve the magnitude of the observed rotations, a larger glass tube was used this time. This was five feet long by three and a quarter inches diameter and holds nearly six kilos of lactose.

Onto this column was run 455 ml. of an almost saturated solution of the azo dye in benzene which yielded an orange-red band on the top six inches of the lactose. Washing was then carried out over 42 hours with pure benzene of which 13 litres were required. As in the first adsorption, there was a faint yellow forerunner. This time, however, the band that washed down the tube was orange coloured and it gradually left a number of red bands as in sketch.

1 and 2 are fairly strong red bands.

3 is nearly white. 4 is faintly red.

5 to 11 is a continuous orange-yellow band.
The tube was drained and the column of lactose cut up in portions corresponding with the numbering shown. Each portion was extracted with cold alcohol which is the most efficient solvent for eluting this compound and the filtered solutions evaporated to dryness. Each was then left on the steam-bath for half an hour with dilute hydrochloric acid to hydrolyse any trace of lactoside which might possibly have been formed and then washed thoroughly with hot distilled water and dried. The fractions were dissolved in alcohol, filtered and examined in the polarimeter for optical activity. The solutions as thus obtained varied in colour from bright red to yellow so that to obtain comparative results, the rotations were all observed in a 2 dcm. tube using light of wavelength 6085 Å which is the shortest wavelength to which all the fractions were transparent.

Fractions 6 to 5 had no activity.

6   7   8   9   10   11
-0.086 -0.080 -0.028 +0.066 +0.234 +0.074

Thus it is seen that the main band (6 to 11) has been partially resolved. To check against any traces of lactose, fractions 6, 7, 10 and 11 were evaporated to dryness, taken up in approximately 40 ml. of benzene and shaken for nearly an hour with distilled water. This was thought necessary because the product submitted to the first
washing was obtained in semi-solid form on the side of the basin and it was doubtful if the water could penetrate and dissolve out all the lactose from it. The benzene solutions were evaporated to dryness and the remaining products taken up in the minimum volume of alcohol necessary to fill the polarimeter tube (9 to 10 ml.).

Using the same polarimeter tube and wavelength as before, the results now obtained were:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Rotation</th>
<th>Concentration</th>
<th>Specific Rotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-0.115°</td>
<td>6.21 mg./9.5 ml.</td>
<td>-88°</td>
</tr>
<tr>
<td>7</td>
<td>-0.056°</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>+0.194°</td>
<td>9.82 mg./9.0 ml.</td>
<td>+89°</td>
</tr>
<tr>
<td>11</td>
<td>+0.059°</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

From the appearance of the tube before elution, it was seen that the original compound was still impure. The observed rotations themselves are quite definite and they should be capable of improvement if the compound can be obtained pure.

**Purification of the Tetrazo Compound.**

To effect the required purification, it was finally decided to retain chromatographic methods but instead of the too strongly adsorbing alumina, to employ a weaker
adsorbent. A number of likely adsorbents were tested, e.g. gypsum, kieselguhr, etc. The best of these proved to be calcium carbonate and the "Analar" product had to be used since the precipitated powder, though more strongly adsorbing, did not permit a reasonable flow of solvent, whereas the "Analar" calcium carbonate, being granular, allows a comparatively rapid flow. Of the pure and mixed solvents tested, benzene was found to be most satisfactory. The first purification was carried out as follows.

A tube was packed with a column (8 by 35 cm.) of calcium carbonate (3½ pounds) in benzene and onto this was run slowly one litre of a benzene solution of the crude dye (0.12 gm.). This yielded a strong red band on the top 7½ cm. of the column. Washing was then carried out with benzene (3½ litres). This process separated the band into three portions.

(a) A pale yellow forerunner which, on evaporation, yielded a dark sticky product.

(b) A "clean" orange compound which washed out slowly as a yellow solution in the benzene.

(c) A dark red compound which remained fixed on the carbonate but which could be eluted by alcohol.

From knowledge gained in the lactose adsorptions, it was considered that the orange substance (b) was the
desired compound. The yield obtained from this chromatogram was 0.014 gm. which decomposed at 360-365° though this decomposition point depends on the speed with which the temperature is raised; if the temperature is raised more slowly, the decomposition point is lower and over a longer range.

Analysis figures found were:

\[ %C = 72.83 \quad %H = 4.50 \quad %N = 10.88 \]

Figures calculated from the structure shown on page 39

\[ %C = 72.96 \quad %H = 4.21 \quad %N = 10.65 \]

To obtain a sufficient quantity of this pure compound, other adsorption tubes were run as below.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>4½ lb.</td>
<td>3 litres.</td>
<td>0.045 gm.</td>
<td>5 days.</td>
</tr>
<tr>
<td>6 lb.</td>
<td>8 litres.</td>
<td>0.12 gm.</td>
<td>8 days.</td>
</tr>
<tr>
<td>5½ lb.</td>
<td>7 litres</td>
<td>0.11 gm.</td>
<td>8 days.</td>
</tr>
</tbody>
</table>

It was suspected that the dark red compound which remained adsorbed on the carbonate might be an isomer since there are three possible geometric isomers due to the arrangements of the two azo groups, viz. cis-cis, trans-trans and cis-trans. Accordingly, a portion of this compound eluted from the carbonate with alcohol
was crystallised from chlorobenzene and sent for analysis. The percentage of nitrogen found was 10.66. The percentage required if an isomer was 10.65.

The small amount of yellow forerunner was not obtained in solid form and no further examination of it was carried out, nor of the above supposed isomeric products.

**Third Adsorption of the Tetrazo Compound.**

Having now obtained the resorcinol azo compound in a pure state, it was decided to use it in another large tower. Since it was already known to yield satisfactory results from benzene solution, the effect of a mixture of light petroleum and benzene was tried in this tube. Light petroleum generally improves the degree of adsorption and also, being less viscous than benzene, allows a more rapid flow of solvent down the column.

The compound, however, proved to be nearly insoluble in a mixture of 25% light petroleum in benzene. By reducing the percentage of petroleum to five, it was found possible to dissolve 0.075 gm. of the dye in a little over five litres of the solvent. The solution was run onto a column of nearly six kilos of lactose in the same solvent mixture contained in a tube five feet long by three and a quarter inches diameter where it was strongly adsorbed on the top fifth of the column. Washing
was then carried out with the same solvent mixture.

To minimise any tendency to diffusion of the partly resolved band, the washing in this case was carried out continuously, day and night; it also effected a great saving of time. This was accomplished by using a five and two two-litre separating funnels, each with its stem dipping into the solvent lying above the lactose suspension and fitted as described previously. This method gives a reservoir volume of nine litres without the introduction of any rubber joints which are readily attacked by benzene.

After seven days' washing, during which approximately fifty litres of wash liquid was run through, the yellow band had reached the lower end of the tube, having spread at the same time to one third of the length of the column. This time there were no red bands, only a yellow one which was deepest in colour near the bottom and faded off to a very pale yellow or cream colour one third of the way up the tube.

After draining, the column of lactose was allowed to slide out of the tube and was cut up as follows:

The top two-thirds which was of an even cream colour was divided into three portions (1 to 3 reading down).

The lower third, on which the compound was adsorbed, was divided into nine portions (4 to 12 reading downwards),
the lower fractions being cut slightly smaller since the colour was more intense there.

Each fraction was eluted with alcohol, evaporated to dryness, well washed with hot distilled water and, after drying, dissolved in 10 ml. of alcohol. The filtered solutions were then examined in the polarimeter. It was found possible, though very difficult, to use a 4 dcm. capillary tube and the mercury yellow line, the most difficult solutions to get light through coming from the upper end of the adsorption column. These solutions were red, and the amount of redness progressively diminished so that the solutions from the lower end of the tube were clear yellow.

Solutions 1 to 3 showed no signs of optical activity. The other solutions, 4 to 12, varied progressively from \(-0.076^\circ\) to \(+0.096^\circ\).

Solutions 4 and 12 were then evaporated to dryness and made up into nearly saturated solutions in benzene. These solutions (approx. 300 ml.) were run onto columns of calcium carbonate. On washing with benzene, yellow solutions were obtained, leaving a red band on each adsorbent resembling that eliminated during the original purification. There was no yellow forerunner. The yellow solution in each case was evaporated to dryness and the purified compound, after weighing on a semi-micro balance,
was redissolved in 7.5 ml. of alcohol and its rotation observed as before.

Rotation of 4 = \(-0.042^\circ\). \hspace{1cm} \text{Weight} = 1.33 \text{ mg.}

Rotation of 12 = \(+0.064^\circ\). \hspace{1cm} \text{Weight} = 0.70 \text{ mg.}

(Solution 12 being very dilute, its rotation could also be observed using the mercury green line. In this case, the observed rotation was \(+0.106^\circ\).)

In each of these two cases, purification has resulted in a lower observed rotation. In an effort to trace the loss in rotation, the red band on the carbonate was eluted with alcohol from each tube and examined for activity. None was found. The lower part of the adsorption column had been discarded, being white, and so could not be examined. It is highly probable, considering the later use of this purification method, that too much calcium carbonate had been used in these trial columns, with the result that nearly half of the active dye remained deeply adsorbed and lost since it could not be extracted by elution with benzene. In all later purifications by chromatographic methods, therefore, the very minimum of adsorbent was employed.

Solutions 5, 6, 10 and 11 were treated as above. Each of these purifications resulted in an increase in the observed rotation. This is due to the fact that after the removal of a deep red fraction, the purified compound
can be used dissolved in the minimum volume of alcohol necessary to fill the polarimeter tube, whereas previously the solution required to be diluted to enable the yellow mercury line to pass through. In none of these cases was the mercury green line observed.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>( \alpha_{5780} ) of crude dye</th>
<th>( \alpha_{5780} ) of pure dye</th>
<th>Weight of pure dye</th>
<th>Specific Rotation ([\alpha]_{5780})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1.33 mg.</td>
<td>-60.7°</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2.87 mg.</td>
<td>-60.1°</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1.87 mg.</td>
<td>-45.2°</td>
</tr>
<tr>
<td>4</td>
<td>-0.076°</td>
<td>-0.042°</td>
<td>1.33 mg.</td>
<td>-60.7°</td>
</tr>
<tr>
<td>5</td>
<td>-0.072°</td>
<td>-0.092°</td>
<td>2.87 mg.</td>
<td>-60.1°</td>
</tr>
<tr>
<td>6</td>
<td>-0.036°</td>
<td>-0.045°</td>
<td>1.87 mg.</td>
<td>-45.2°</td>
</tr>
<tr>
<td>7</td>
<td>-0.046°</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-0.022°</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>+0.031°</td>
<td>+0.043°</td>
<td>1.67 mg.</td>
<td>+48.2°</td>
</tr>
<tr>
<td>11</td>
<td>+0.073°</td>
<td>+0.176°</td>
<td>2.16 mg.</td>
<td>+152.8°</td>
</tr>
<tr>
<td>12</td>
<td>+0.096°</td>
<td>+0.064°</td>
<td>0.70 mg.</td>
<td>+172.0°</td>
</tr>
</tbody>
</table>

The decomposition temperature of one of the purified fractions was taken. It was found to be 275-310°. It may be that this fall in the decomposition temperature (pure compound 360-365°) is due to the fact that we are here dealing with a mixture of d- and l-forms.
A point to be noted is that much of the pure compound is lost during the process. The total weight of the six fractions that were purified only amounts to 10.6 mg. Assuming that the three intermediate solutions from the adsorption band (numbers 7 to 9) contain similar quantities, then the weight of pure azo compound recovered will be about 16 mg. whereas 75 mg. was started with. Part of this loss is due to deep-seated adsorption; this view is supported by the fact that strongly coloured solutions are obtained from sections 1 to 3 of the column. This absorption also explains why the positive rotations are larger than the negative. The negative fractions are in the upper part of the adsorption band where the band is more attenuated, with the result that a greater amount of lactose has to be extracted to obtain similar quantities of partially resolved azo compound as compared with the more concentrated lower, positive fractions. Thus more absorbed inactive dye is eluted with the adsorbed active compound with a resultant drop in the specific rotation.

Fourth Adsorption of the Tetrazo Compound.

By means of small pilot tubes, the effect of using benzene with a small percentage of a more polar liquid as solvent was investigated. It was found that even one
quarter percent of alcohol prevented any appreciable adsorption and that the band washed away cleanly without spreading. Using one quarter of acetone, adsorption was very good but the band did not have much tendency to spread. Carbon tetrachloride had practically the same effect as light petroleum. Of all the solvents tried, it was found that "Analar" benzene was best since the dye was strongly adsorbed from it and the band, though very slow in moving down the column, spread more than from any other medium. It was therefore decided to run another large tower using benzene as solvent.

The large glass tube (5 feet by 3.25 inches) was filled with a paste of lactose in benzene and onto this was run five litres of a benzene solution of the azo compound (containing 0.075 gm.). The orange dye was adsorbed on the top fifth of the column.

Washing, which required 160 hours during which 50 litres was run through, was carried out with the same solvent. It might be noted here that the temperature of the room has a very marked effect on the running of an adsorption column. The higher the temperature, the less will be the viscosity of the solvent so that the rate of flow will be greater. Also, at the higher temperature, the solvent will redissolve the adsorbed compound more readily, both of these conditions causing the band to
move more quickly down the tube. During the course of this experiment, the room temperature was fairly low, being about 8-10° during the day and lower through the night. To prevent the benzene from freezing overnight, two Davy lamps were left burning beside the tube.

When the bottom of the yellow band reached the lower end of the tube, the band itself having spread to one third of the length of the column, the tube was drained and the lactose cut into sections. The upper two-thirds containing only absorbed material was cut into three portions (1 to 3) whilst the band itself was divided into nine sections (4 to 12 reading downwards), smaller fractions again being taken from the lower end where the colour was more intense.

Each fraction was extracted with alcohol, the solutions evaporated to dryness, washed with hot distilled water and dissolved in alcohol as in previous tubes. The solutions were then filtered and examined for optical activity in the polarimeter. It was found necessary in these filtrations to use "hardened" filter papers since the ordinary papers tended to let particles into the solution which, though seemingly negligible in a flask, greatly hindered the passage of light when in a 4 dcm. polarimeter tube.

Many of the solutions proved to be too reddish for
use in a 4 dcm. polarimeter tube, so the preliminary readings were taken in a 2 dcm. one using the mercury yellow line (wavelength = 5780 Å). The rotations observed varied progressively from -0.122° to +0.341°, fractions 1 to 3 having no rotation as before.

One of the most active fractions (number 11) was evaporated to dryness. A small portion was heated to find its decomposition point. This was 280-300°. The remainder was weighed, made up to 10 ml. with alcohol and its rotation retaken. It was then evaporated again, taken up in benzene and run down a column of calcium carbonate when a red band was left on the adsorbent. The pure compound which washed out had still the same decomposition temperature. It was then weighed and dissolved in 8 ml. of alcohol. The rotation of this solution was now observed using a 4 dcm. polarimeter tube and the same wavelength as before. Before purification, its weight was 3.43 mg. and its specific rotation 388°. After this treatment, its weight was only 1.52 mg. but its specific rotation had increased to 471°.

Other fractions were therefore purified as above and, after weighing on a semi-micro balance, dissolved in 8 ml. of alcohol. In all of these cases, it was now possible to use the 4 dcm. polarimeter tube and the mercury yellow line.
<table>
<thead>
<tr>
<th>Fraction</th>
<th>$\alpha_{5780}$ of crude dye.</th>
<th>$\alpha_{5780}$ of pure dye.</th>
<th>Weight of pure dye.</th>
<th>Specific Rotation, $[\alpha]_{5780}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-0.073°</td>
<td>-0.332°</td>
<td>3.34 mg.</td>
<td>-199°</td>
</tr>
<tr>
<td>4</td>
<td>-0.122°</td>
<td>-0.157°</td>
<td>2.77 mg.</td>
<td>-113°</td>
</tr>
<tr>
<td>5</td>
<td>-0.066°</td>
<td>-0.113°</td>
<td>3.43 mg.</td>
<td>-70°</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>+0.043°</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>+0.163°</td>
<td>+0.339°</td>
<td>3.28 mg.</td>
<td>+207°</td>
</tr>
<tr>
<td>9</td>
<td>+0.341°</td>
<td>+0.632°</td>
<td>2.19 mg.</td>
<td>+578°</td>
</tr>
<tr>
<td>10</td>
<td>+0.268°</td>
<td>+0.358°</td>
<td>1.52 mg.</td>
<td>+471°</td>
</tr>
<tr>
<td>11</td>
<td>+0.091°</td>
<td>+0.138°</td>
<td>1.09 mg.</td>
<td>+254°</td>
</tr>
</tbody>
</table>

The results from this last experiment having shown definitely that a partial resolution had been successfully effected, it was decided to prepare other similar types of compound to determine whether the adsorption could not be improved further.
Preparation of Other Derivatives of 1:1'-Dinaphthyl-2:2'-Diamine.

A solution of diazotised dinaphthyl diamine was poured into an alkaline solution of β-naphthol, the mixture being alkaline. This immediately yielded a brick red precipitate, probably of a sodium salt. After acidification, the red compound was filtered off and washed and, after drying, was extracted with hot light petroleum which removed a small amount of a red-violet material. The residue was further extracted with a little benzene in which it is fairly soluble and then crystallised from chlorobenzene. The compound as thus obtained has melting-point 324-327°.

This bright red compound is insoluble in alcohol and ligroin and probably has the following structure:

\[
\begin{align*}
\text{As is shown below, this compound did not prove suitable for adsorption work and was not obtained in analytical purity.}
\end{align*}
\]

A solution of this red dye in benzene was run down
a short column of lactose but there was no apparent adsorption, and washing with the solvent left the adsorbent white. There was also no appreciable adsorption from 20% benzene in light petroleum, a mixture in which the dye is only slightly soluble. It was also found that this compound was not adsorbed on quartz or calcium d-tartrate from benzene or benzene-petroleum mixtures. Even on alumina it was only adsorbed to a very small extent.

These trial tubes showed that it was very unlikely that this compound would yield any appreciable resolutions by adsorption methods. No further work was therefore carried out with it.

B A solution of dinaphthyl diamine in moderately concentrated hydrochloric acid was diazotised as before and excess nitrous acid destroyed with urea. The diazo solution was then added to an alkaline solution of phenol resulting in the formation of a deep red solution which yielded a reddish-purple precipitate on acidification.

This substance proved to be fairly soluble in benzene and alcohol but only slightly soluble in light petroleum. A solution of it in benzene was run down a column of calcium carbonate to purify it, but no appreciable adsorption was observed and the band washed out of the lower end of the tube practically unchanged. It was,
however, readily adsorbed onto alumina from benzene and washing with benzene removed small quantities of a yellow and a purple material which were discarded. The coloured band at the top of the column showed very little change on washing with benzene but if a more polar solvent such as alcohol was added, it contracted in depth and washed out. On washing for two weeks with pure benzene, however, it could be separated into layers sufficiently removed for the column to be cut up. The fractions were eluted by alkali.

The light brown upper band yielded a yellow compound of melting-point 273-275°.

The product from the lower band which was also light brown was dissolved in the minimum of alcohol and a drop of water added. This yielded a brown substance of melting-point 165-170°. On adding more water to the mother liquor of the latter compound, a red compound was obtained which melted at 257-263°.

These three compounds were only obtained in small yield and since they all were much less strongly adsorbing than the resorcinol compound, no further time was spent on them.

The third compound to be made differed from the previous two in that the azo groups were exchanged for
The compound synthesised had the following formula:

![Chemical structure](image)

This should be very similar to the resorcinol tetrazo compound, having the same geometric isomers.

(a) Resorcylic Aldehyde.

(Shoesmith and Haldane, J.C.S., 1923, 123, 2704.)

A mixture of 22 gm. resorcinol, 24.2 gm. formanilide, (made by the action of formic acid on aniline) and 10.5 gm. phosphorus oxychloride was dissolved in 65 ml. of dry ether. The solution was warmed to start the reaction and then allowed to stand overnight.

Next day, the ether was evaporated off and the residue digested with a solution of 45 gm. sodium hydroxide in 400 ml. water. Half of the solution was slowly distilled away, taking liberated aniline with it. After diluting the product with 100 ml. water and cooling, it was acidified with 30 ml. sulphuric acid in 200 ml. water. The resorcylic aldehyde was filtered off and extracted with ether. The mother liquor was saturated with salt and also extracted
with ether. The two ether solutions were mixed and evaporated. The aldehyde was then crystallised from water and benzene.

Yield: 8 gm. m.p. 134-135°.

(b) **Condensation of Resorcylic Aldehyde with Dinaphthyl Diamine.**

An attempt to carry out the condensation in alcohol using sodium ethoxide as agent was unsuccessful. A mixture of the two in the proper proportion was therefore heated in a tube on an oil-bath at 200° for 10 minutes. Steam was given off and the mixture darkened. On cooling, the product was dissolved out in sodium hydroxide solution in which it gives a strong red colour, filtered and precipitated with acid.

The compound thus obtained is readily soluble in pyridine and alkali, fairly soluble in alcohol but insoluble in the other common solvents. It does not even colour benzene, ligroin or chlorobenzene.

It decomposes about 300°.

From alcohol, it adsorbs onto alumina but the red band thus obtained only moves extremely slowly and gives no separation on prolonged washing. It was not further examined as its behaviour with solvents did not hold out any reasonable hope of purification or successful use in the adsorption work.
Although a number of different racemic derivatives of dinaphthyl diamine had been examined up to this stage, the only one which showed definite signs of being resolvable by adsorption methods was the tetrazo compound obtained by diazotising the amine and coupling with resorcinol. A final adsorption experiment was therefore carried out with this compound in the hope of increasing the degree of resolution previously effected.

Fifth Adsorption of the Tetrazo Compound.

On this occasion, instead of using a single large column (5 feet by 3.25 inches) which contains nearly six kilos of lactose, a number of smaller tubes arranged in series were used, each 5 feet by 2 inches and containing two and a quarter kilos of lactose. The main purpose of this variation was to find if the degree of resolution was influenced greatly by the length of the column.

Since these four towers were to be connected in series, it was first necessary to find some means of eliminating rubber stoppers at the lower end of each in order to prevent impurities dissolved from the rubber being washed into the following columns. This was accomplished by placing a Buchner funnel over the lower end of each tube and sealing it to the glass with a cement made by mixing yellow lead oxide with glycerol.
Onto one of these columns of lactose packed in benzene was run five litres of a nearly saturated benzene solution of the resorcinol azo compound, the same quantity as was used in the two previous adsorptions. This formed an adsorbed layer occupying the top third of the column. Washing throughout was carried out with benzene.

After seven days' continuous washing, the lower end of the adsorption band reached the bottom of the tower. A second similar tower was now connected on to the bottom of the first by passing the stem of the Buchner funnel through a one-hole rubber stopper which tightly fitted the top of the second tower. This made an air-tight joint between the two tubes so that both tubes had to run at the same speed and without permitting direct contact between rubber and benzene. The height of the apparatus including the receiver at the bottom and the five-litre reservoir funnel at the top was now approximately thirteen feet and it was supported by a long stand (10 feet) resting on a table.

The yellow-red band washed out of the first tube into and down the second. The upper tower was now removed and a third one connected onto the lower end of the second. By this method, the band was washed through four tubes of total length nearly twenty feet containing altogether nine kilos of lactose. In this process, which
Method of Fixing a Buchner Funnel to the Adsorption Tube and of Changing the Receiver Overnight.
required thirty-three days of continuous washing, the band spread from less than two feet in length to seven and a half feet, the lower part being bright yellow whilst the upper part faded away to the cream colour of the used lactose. During the running of these columns, the temperature varied greatly (February to March 1940) with the result that while on some days the band would move about ten inches, on other colder days it only moved five or six inches. The speed of flow also varied from 100 ml. per hour to 175 ml. per hour for the same reasons.

Considering that the band was spread over a tube and a half and that the upper part of the band was very weak, it was decided to allow the band to wash out of the towers instead of draining, cutting into sections and extracting with alcohol as in previous towers. This required a further fortnight’s washing with benzene. At first, the fractions were caught in five hour runnings, but when they became more dilute, eight hour runnings and finally twenty-four ones were collected. Since the majority of the nineteen fractions caught were eight hour ones, a device was used whereby the receiver was automatically changed once overnight (see sketch). The fractions were numbered 1 to 19 in order of their collection.

Each fraction was evaporated to dryness, washed well with hot distilled water, dissolved in 10 ml. of alcohol
and examined for optical activity in the polarimeter, using a 4 dm. tube and the mercury yellow line. It was hoped that, since the band was washed off the lactose with benzene instead of being extracted with alcohol, the fractions would be free of the red impurity that accompanied them in the earlier experiments. This expectation was not borne out and it was discovered that the impurity was still present and in greater proportion than in previous experiments.

The rotations observed with the solutions of the crude product were as follows:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Rotation</th>
<th>Fraction</th>
<th>Rotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>11</td>
<td>-0.083°</td>
</tr>
<tr>
<td>2</td>
<td>+0.195°</td>
<td>12</td>
<td>-0.128°</td>
</tr>
<tr>
<td>3</td>
<td>+0.332°</td>
<td>13</td>
<td>-0.095°</td>
</tr>
<tr>
<td>4</td>
<td>+0.299°</td>
<td>14</td>
<td>-0.136°</td>
</tr>
<tr>
<td>5</td>
<td>+0.198°</td>
<td>15</td>
<td>-0.110°</td>
</tr>
<tr>
<td>6</td>
<td>+0.092°</td>
<td>16</td>
<td>-0.140°</td>
</tr>
<tr>
<td>7</td>
<td>+0.044°</td>
<td>17</td>
<td>-0.164°</td>
</tr>
<tr>
<td>8</td>
<td>+0.017°</td>
<td>18</td>
<td>-0.066°</td>
</tr>
<tr>
<td>9</td>
<td>-0.029°</td>
<td>19</td>
<td>-0.025°</td>
</tr>
<tr>
<td>10</td>
<td>-0.050°</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fractions 2, 3 and 4 were each weighed, their weights being respectively 1.51 mg., 2.10 mg., 2.97 mg. These corresponded to specific rotations of 323°, 395° and 252°.

Fraction 4 was then dissolved in benzene and run down a column of calcium carbonate. A slight trace of a pale yellow forerunner was observed, but of the yellow band characteristic of the pure dye there was no sign, the reddish-yellow band adsorbed on the upper layers of the carbonate being unaffected by washing with benzene. On adding alcohol to the wash liquid, all the coloured material was eluted from the column and, it was found, this yellow solution had still a positive rotation of +0.095°. It is highly probable that, this being a trial purification column, too much carbonate was used with the result that the whole of the very small amount of active dye remained adsorbed on the column while benzene was employed as wash liquid, but was nearly all eluted when the highly polar alcohol was added.

To find if there was any appreciable quantity of the original azo compound left, fractions 5, 6 and 7 were mixed, dissolved in benzene and run down a column of calcium carbonate. In this case, a yellow band washed down the tube with benzene as required of the pure compound. Since the activity proved to be very slight,
the weight of this fraction was not determined.

The remaining two good positive fractions, 2 and 3, were now mixed and treated as above and the yellow band which washed out in the normal manner was dissolved in alcohol and examined in the polarimeter. The rotation observed in a 4 dcm. tube was 0.169° with the Hg yellow line and 0.255° with the Hg green. On evaporating 9.4 ml. of this solution, its weight was found to be 0.45 mg. Its specific rotation is therefore 883° for the Hg yellow or 1332° for the Hg green line.

Since the rotations observed for the negative end of the band were relatively small, the last four solutions were mixed and run down a column of the carbonate, the column requiring to be much larger than for the previous ones due to there being a greater volume of solution and a stronger colour in it. A large amount of yellow fore-runner was obtained from this fraction but it had no activity. The yellow band containing the pure dye was washed out and, after dissolving in alcohol, was examined for activity. Using a 4 dcm tube, the rotation observed was -0.147° with the Hg yellow and -0.233° with the Hg green line. The weight of the dye in the 10 ml. of alcohol was found to be 1.25 mg., so that the specific rotation was -284° for the Hg yellow or -466° for the Hg green line.
Putting these results into tabular form, we obtain the following:

<table>
<thead>
<tr>
<th></th>
<th>Positive Fraction</th>
<th>Negative Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>0.48 mg./10 ml.</td>
<td>1.25 mg./10 ml.</td>
</tr>
<tr>
<td><strong>Observed (\alpha_{5770})</strong></td>
<td>+0.169°.</td>
<td>-0.147°.</td>
</tr>
<tr>
<td><strong>Specific Rotation</strong></td>
<td>+ 883°.</td>
<td>- 284°.</td>
</tr>
<tr>
<td><strong>Observed (\alpha_{5461})</strong></td>
<td>+0.255°.</td>
<td>-0.233°.</td>
</tr>
<tr>
<td><strong>Specific Rotation</strong></td>
<td>+ 1332°.</td>
<td>- 466°.</td>
</tr>
</tbody>
</table>

On attempting to find the decomposition temperatures of the active fractions for comparison with the starting material, it was found that these were only semi-solid, being semi-transparent "glasses". When a sample of one of these was heated in the melting-point apparatus, it lost its tackiness and gradually decomposed at 280-320°. Suspecting that there might be lactosides present, the laevo-rotatory fraction was heated on the steam-bath with dilute hydrochloric acid for three hours and was then washed and dissolved in alcohol. The solution was examined in the polarimeter and its concentration found.

It was found that the specific rotation for Hg yellow light was \(-280°\). Thus treatment with hydrochloric acid has not affected the optical activity but the treated fraction was still only semi-solid.
The lactose from the first of the four adsorption columns was extracted with alcohol after the band had been washed out. The alcohol solution was concentrated and examined for optical activity but none could be detected. The lactose from the fourth column was also extracted. The product was run through a column of calcium carbonate when the reddish compound was readily adsorbed. After washing with benzene, the adsorbed band was eluted with alcohol and examined in the polarimeter. No rotation could be detected and on evaporation, the product proved to be only semi-solid. This substance was heated on the steam-bath for two hours with dilute hydrochloric acid in case it might be a hydrolysable lactoside, but no appreciable change could be detected after treatment.

Discussion of the foregoing results will be deferred until a later section.
The partial resolution of a racemic compound by adsorption on wool has been claimed by several workers (see page 10), the most definite claim being that of Porter and Ihrig using m-azo-naphthol mandelic acid. These workers report that they recovered the almost pure laevo-rotatory dye-stuff from their exhausted dye-bath. Attempts to repeat this work by other experimenters have, however, failed and, since the optical rotations obtained by other chemists using different compounds differ very little from the experimental error, it is very doubtful if any real partial resolution by adsorption on wool has yet been accomplished.

First Adsorption on Wool.

For the present work, the compound used was the resorcinol tetrazo derivative which has been employed successfully for adsorptions on lactose. In this experiment, the wool consisted of a piece of woven natural-coloured blanket, some twenty years of age, which must have undergone extensive washing in its time. Before use, it was given a final washing with soap and water and well rinsed. After having been wrung out thrice with alcohol and dried, it was extracted with carbon tetrachloride in
a Sohxlet to defat it.

Small scale experiments showed that the wool did not adsorb the dye-stuff appreciably from benzene or alcohol, but that if the dye was dissolved in sodium hydroxide solution and the solution treated with acid until a very slight precipitation occurred, the wool was then dyed a strong yellow colour.

Accordingly, 0.075 gm. of the azo compound was dissolved in about 3½ litres of very weak alkali and the alkalinity reduced to the minimum necessary to keep the compound in solution. Twelve pieces of wool (total weight 200 gm.) were each steeped for 24 hours in the solution by which time the colour of the solution was reduced from a yellow-red to a very pale yellow. The solution was concentrated and acidified when a small amount of precipitate was obtained. This showed no activity in the polarimeter and, from its appearance, was not the original compound.

The last two pieces of wool to be dyed were separately treated with acid, dried and extracted with alcohol and the concentrated solutions examined in the polarimeter. Both solutions gave rotations of approximately -0.09°. The solutions were then mixed and run down a column of calcium carbonate in benzene. The whole coloured solution formed no band but washed through the column without
adsorption. None of the original dye-stuff had thus been extracted from the wool.

A piece of the original wool was extracted in a Sohxlet with alcohol when a yellow solution was obtained which, on evaporation, yielded a large amount of a yellow solid. This probably indicates slow decomposition of the wool over many years, hence, in a second experiment with wool, fresh natural sheep's fleece was used after washing, defatting with carbon tetrachloride and extraction with alcohol.

**Second Adsorption on Wool.**

Pieces of this fleece (5 gm. at a time) were each steeped for a day in a solution of 0.022 gm. of the azo compound in 500 ml. of very weak alkali. After eight lots had been dyed, the filtered solution was acidified and the brownish precipitate thus obtained examined for optical activity. None was detected.

The first piece of wool to be dyed was extracted with alcohol and this solution also examined in the polarimeter. Again there was no optical activity.

This work, coupled with that of others, strongly suggests that wool is unsuited for use as an asymmetric adsorbent and no further experiments were carried out with it.
Quartz Crystals.

Left-handed

(Ideal)

Right-handed

Right-handed

common form (etched)
ADSORPTIONS ON POWDERED OPTICALLY ACTIVE QUARTZ.

Quartz owes its optical activity to the presence of an asymmetric crystal unit in which three silica molecules form a right or left-handed spiral. If we regard a crystal of quartz as a giant molecule, then it is obvious that the surface of powdered active quartz is closely akin to that of a powdered active sugar such as lactose. Powdered quartz should therefore be utilisable for resolving racemic compounds by adsorption methods.

Tsuchida and co-workers allowed warm saturated solutions of certain metallo-organic complexes to cool and crystallise out over powdered active quartz. They found that their residual solutions were often slightly optically active which, they suggested, was due to asymmetric adsorption. Similar results were obtained when they used synthetic racemic adrenaline hydrochloride as adsorbate.

Henderson (Thesis, Edinburgh, 1939.) attempted the resolution of potassium cobalti-oxalate \( [K_3 Co(Ox)_3] \) by running an aqueous solution of it over powdered quartz. He found, however, that adsorption was weak and that the compound was unstable with the result that he could find no evidence of resolution. He also used p-phenylene bis-imino camphor in a Tswett column containing l-quartz in
benzene. Again he could find no traces of activity.

Karagunis and Coumoulos (Praktika de l'Academie d'Athens, 13, 1938, page 414; Nature, July 23, 1938.) employed a dry column of freshly-heated active quartz powder. Down this they ran a 10% aqueous solution of a racemic chromium complex \[ \text{[Cr (en) \text{Cl}_3]} \] and eluted it with aqueous alcohol. As a result, they were able to obtain slightly active fractions (up to 0.12°) but since no information was given as to length of polarimeter tube or concentration of solution, it is not possible to estimate the degree of resolution. This cannot, however, have been appreciable since the specific rotation of the complex used was 3400°.

Production of Powdered Active Quartz.

In the first instance, it is necessary to separate the natural quartz crystals (about 3 inches long) into the right and left-handed types. In the ideal case, this is readily done by observing the hemihedral facets (see sketch). The majority of the crystals do not, however, show these facets and differentiation is obtained by etching them with hydrofluoric acid when the two crystal forms may be distinguished by examination of the arrow-headed indentations thus produced.

The distinguishing properties of a right-handed
crystal as opposed to a left-handed one with which it is enantiomorphous, are as follows:
(a) Hemihedral facets pass off to the top right when the crystal is held upright.
(b) The normal facets show arrow-shaped pits pointing to the left when etched.
(c) The crystal rotates polarised light in a negative fashion when viewed in the polarimeter.

Industrialy, quartz is powdered by heating it to red heat and quenching in cold water. This method is, however, useless for our purpose since the quartz crystal unit is destroyed in the process.

To obtain the active powder, the large crystals were first broken up on an anvil and then powdered in a hard steel-jawed stone crusher. This was followed by milling between toothed steel plates which sheared the particles. The powder was then sieved to remove large particles and steel filings removed by use of a powerful electro-magnet. The final grinding was carried out in a granite, power-driven mortar with a loaded, idling, eccentric pestle.

It was considered likely that the quartz powder thus obtained could be further "activated" by etching the particles with hydrofluoric acid. The powder was therefore treated for two hours with this acid in a lead dish on the steam-bath. It was then filtered off, washed well with
water and then boiled out twice with distilled water to remove traces of acid. It was dried in vacuo at 115° for four hours.

**Adsorption of p-Phenylenes Bis-imino Camphor.**

The first adsorption to be tried on quartz was a repetition of one carried out by Henderson (Thesis, loc. cit.) using p-phenylene bis-imino camphor. A column (150 by 1\(\frac{1}{4}\) cm.) containing 400 gm. of the powdered 1-quartz suspended in 25% benzene in petroleum ether was set up. Onto this was run 35 ml. of a saturated solution of the compound in the same solvent mixture. This solution is yellow but it cannot be seen against the dark grey colour assumed by quartz in presence of benzene. Washing was carried out with the same solvent and after about 175 ml. had run through, a faintly yellow solution started to emerge from the lower end of the tube. This was collected in 100 ml. portions and concentrated to 7.5 ml., nine such fractions being obtained. These solutions were examined for optical activity but no definite rotations could be detected. This confirms the results reported by Henderson and Rule.

**Adsorption of the Tetrazo Compound prepared from Dinaphthyl Diamine and Resorcinol.**
The powdered quartz used in the previous experiment was purified by boiling out with alcohol, benzene and chloroform and drying in vacuo at 115°. It was found in the previous column that the adsorbent packed down so well that the flow of solvent was very slow, about 125 ml. per day. To try and speed the flow, the quartz was this time filled into two separate tubes (each 50 by 1.9 cm.) connected in series, but this did not permit more rapid washing. The solvent used was 5% light petroleum in benzene.

Onto the quartz was run 835 ml. of a saturated solution of the azo dye in the solvent mixture, containing 15 or 16 mg. of the pure compound. The dye adsorbed on the top nine inches of the column. Washing was then begun with the pure solvent. After ten days (125 ml./day), the band had scarcely moved, so the mixed solvent was changed to "analar" benzene. After a further seven days, the band had still not moved appreciably, therefore a quarter percent of alcohol was added to the wash liquor. Two days later, the benzene in the reservoir from which the wash liquid flowed was unfortunately frozen. This had the effect of increasing the percentage of alcohol in the small amount of benzene left liquid with the result that the band was collapsed and washed completely into the top of the second tube.
However, washing was continued, but it was found that even a quarter percent of alcohol did not move the band very quickly so, after nineteen days' washing with that mixture, the percentage of alcohol was increased to a half. Five days later, coloured solution started to emerge from the lower end of the column and this was collected in portions of 100 mls. When all the colour had emerged, successive fractions were mixed so as to produce six reasonably strong solutions. These were evaporated to dryness when it became obvious that they were badly contaminated with rubber and decomposition products from the azo compound. Without further treatment, they were each dissolved in alcohol and examined in the polarimeter. The rotations observed were:

\[
\begin{align*}
1 & \quad 2 & \quad 3 & \quad 4 & \quad 5 & \quad 6 \\
-0.025^\circ & \quad -0.006^\circ & +0.032^\circ & +0.005^\circ & +0.025^\circ & +0.015^\circ & +0.054^\circ
\end{align*}
\]

Portions 4 and 5, which were more dilute, were combined with 3 to give a rotation of +0.054°. When the two active fractions were weighed, it was found that they were unexpectedly heavy. Among other things, sulphur was detected in these fractions and was removed by boiling with copper wire in acetone. Attempts to isolate the pure compound from the bulkier impurities were unsuccessful, so the experiment was discontinued at this stage.
These rotations, though small, are not unsatisfactory when the size of the column is taken into consideration. The main difficulty was due to the use of rubber stoppers for the connection between the two parts of the column since the benzene attacked the rubber considerably during the month it was in contact with it. For future experiments, a glass tap was sealed onto the end of the tube.

**Adsorption on d-quartz.**

Having obtained a quantity of powdered d-quartz, it was treated in the same way as the l-product. It was found to be, however, coarser than the l-quartz that was used with the result that when the resorcinol azo dye was run onto it, the flow of solvent was faster but the adsorption was very much less. Within two days, a coloured solution started to drip from the lower end of the column (all-glass) and was caught in portions. None of these fractions, however, showed any signs of optical activity.
SUMMARY AND GENERAL DISCUSSION.

Adsorption Experiments on Lactose.

As has already been stated, the results obtained by Rule and Henderson (loc. cit.) who examined a large number of possible asymmetric adsorbing media, led to the decision to employ lactose as our chief adsorbent since it adsorbed more strongly than the other agents tested and was readily obtained cheaply, in quantity, and in such form that the particle size did not vary appreciably from one batch to another. This last point was of importance if any comparative conclusions were to be drawn from the results of the different adsorptions. Lactose is practically insoluble in benzene and light petroleum, the two solvents of which most use is made, but is readily soluble in water, thus making the elimination of traces of it from partially resolved organic compounds very simple.

Dinaphthyl Diamine Condensation Compounds.

The first part of the work consisted of attempts to effect partial resolutions of the three polycyclic compounds 1 to 3 in each of which there is the same eight-membered heterocyclic ring whose "warping" leads
to the existence of mirror image forms. The first of these experiments, using the benzil derivative of 1:1'-dinaphthyl-2:2'-diamine (compound 1), only yielded a small partial resolution of 36° as against a possible 1900° for the known optically pure compound. The other two compounds (2 and 3) did not lead to any definite positive results. The relative lack of success in these cases is almost certainly due to the extreme weakness of the adsorption on lactose which, in turn, is possibly related to the lack of polar groupings in the molecule.

In the hope of improving the adsorbability of this type of compound, it was decided to introduce polar groups, preferably of such a kind that they could not be used to separate the optical isomers by standard methods. Nitro groups are very suitable for this purpose, generally greatly increasing the polarity of the molecule to which they are added. Accordingly, 3:3'-dinitrobenzil was prepared and condensed with dinaphthyl diamine to yield compound 4. Test experiments showed, however, that while the adsorption was slightly improved, it was still not such as to lead us to expect that any appreciable degree of separation would occur. No large scale effort at resolution was therefore attempted with this compound.

At this stage, we turned our attention to a different type of compound which was much more strongly adsorbed.
Dinaphthyl Diamine Diazotised and Coupled with Resorcinol.

The remainder and major portion of the work was carried out with the compound (5) obtained by diazotising dinaphthyl diamine and coupling with resorcinol. The molecule of this substance contains four hydroxyl groups, the presence of which, no doubt, accounts in part for the high degree of adsorbability shown by it. Some difficulty was experienced in the purification due, possibly, to the simultaneous formation of geometric isomers and, since it was not possible to check the purity of the compound by melting-point – it decomposes over a range about 300°, two adsorptions had been carried out on lactose before it was realised that the product was not homogeneous. For the second of these, the compound had been sufficiently purified for definite results to be obtained. These indicated that the compound, when obtained pure, might prove suitable for resolution by adsorption methods.

Various means were next examined for the purpose of purifying the azo compound. After much experiment, it was found that this could be achieved by chromatographic adsorption methods, using a column of "Analar" calcium carbonate as adsorbent and benzene as solvent.

Employing the thus purified compound, three further resolutions were attempted. In all three cases, approximately the same quantity of azo compound was used. The main difference between the first two was that the
solvent in one was a mixture of 5% light petroleum in benzene, whilst in the other, pure benzene was adopted. The experimental conditions such as quantities of lactose, amount and duration of washing were very nearly the same in both cases. Since the addition of petroleum usually increases the degree of adsorption of a polar compound on a polar adsorbing medium, it was expected that the column with the mixed solvent would lead to the better results. It was found, however, that the contrary was the case, the column with benzene yielding specific rotations nearly four times as great as those obtained from the column containing the mixed solvent. The only other variable that might account for these differences is the temperature. During the running of the mixed solvent column, the room temperature was about normal - 14 or 15° but the other adsorption was carried out during an extremely cold spell, the day temperature in the room never rising above 10° whilst the night one was a great deal lower. This would tend to bring about a relatively stronger adsorption from the benzene solution and the most feasible explanation of the results obtained is that the difference between the degrees of adsorption of the d- and the l- forms of the azo compound upon lactose is enhanced at lower temperatures.

At this stage, it is convenient to summarise the
results obtained in these three experiments using the coupled product from diazotised dinaphthyl diamine and resorcinol. The main facts are given in tabular form below.

<table>
<thead>
<tr>
<th></th>
<th>Third Adsorption</th>
<th>Fourth Adsorption</th>
<th>Fifth Adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of lactose used.</td>
<td>5$\frac{1}{2}$-6 Kgm.</td>
<td>5$\frac{1}{2}$-6 Kgm.</td>
<td>9 Kgm.</td>
</tr>
<tr>
<td>Size of tube or tubes.</td>
<td>5' by 3.25&quot;</td>
<td>5' by 3.25&quot;</td>
<td>18' by 2&quot;</td>
</tr>
<tr>
<td>Weight of azo compound.</td>
<td>0.075 gm.</td>
<td>0.075 gm.</td>
<td>0.075 gm.</td>
</tr>
<tr>
<td>Solvent.</td>
<td>5% light petroleum in benzene.</td>
<td>benzene.</td>
<td>benzene.</td>
</tr>
<tr>
<td>Duration of washing.</td>
<td>7 days.</td>
<td>7 days.</td>
<td>40 days.</td>
</tr>
<tr>
<td>Highest negative specific rotation.</td>
<td>-61°</td>
<td>-199°</td>
<td>-284°</td>
</tr>
<tr>
<td>Highest positive specific rotation.</td>
<td>+172°</td>
<td>+578°</td>
<td>+883°</td>
</tr>
</tbody>
</table>

The specific rotations are determined in alcoholic solution using the mercury yellow line.

It is to be noted that the laevo-rotatory form of the azo compound is more strongly adsorbed on lactose than the dextro form. A minor point is that although the highest negative rotations were always obtained from the extreme top of the adsorption band, the highest positive
ones seldom corresponded to the extreme lower end. This is caused by the liquid flowing at a slightly faster rate down the walls of the tube than in the middle of the column where the packing is more complete and so denser. The result is a "dome" formation which leaves the most active part of the compound situated in about the second or third section to be cut off from the bottom.

Though lack of time has prevented us from obtaining confirmatory evidence, two main conclusions may be drawn from the figures given in the table:

(a) By comparing adsorptions 4 and 5, it is seen that where the solvent and quantity of azo compound used are the same, the degree of partial resolution depends on the quantity of lactose present rather than on the dimensions of the column. Thus the highest specific rotations obtained from tube 5 which contained half as much lactose again as 4, are one and a half times as great as those obtained from tube 4.

(b) In all cases, the negative specific rotation is much smaller than the positive one, being usually about one third in magnitude.

The first of these conclusions is somewhat surprising since, according to the accepted theory of adsorption, a particle of adsorbent from which a molecule of adsorbate has just been eluted should be capable of adsorbing
another molecule of adsorbate in its place. This being so, the degree of resolution should depend to some extent on the length of the column of adsorbent over which the band of adsorbate has been washed. However, a number of minor factors may have played a part in reducing the expected degree of resolution.

(1) During the running of column 5, the temperature varied greatly and, as was seen from a comparison of tubes 3 and 4, the temperature may play an important part in determining the degree of resolution effected.

(2) Benzene, though immiscible with water, will nevertheless absorb a minute amount of it if left standing in the open air. The top of an adsorption tube being open, moisture may thus be brought into contact with the lactose and adsorbed by it. This will have the effect of seriously decreasing the adsorptive power of the lactose with a resultant decrease in the degree of resolution. The amount of moisture thus introduced will depend on atmospheric conditions and the duration of the experiment.

(3) A third factor, which is somewhat similar to the previous one, has to do with decomposition in the adsorbed state. If any decomposition products are formed during the adsorption and these remain fixed on the lactose, then the efficiency of the lactose will again be decreased.

Using the resorcinol tetrazo derivative of dinaphthyl
diamine, decomposition did take place and the product remained fixed on the lactose unless eluted with alcohol.

At this stage, therefore, we cannot form any definite opinion as to how the degree of resolution varies with the quantity of adsorbent and the length of the column.

With regard to the second conclusion (b) drawn from the figures given in the table, the smallness of the negative specific rotation may possibly be explained in part by the occurrence of asymmetric decomposition. If there is any tendency for the compound to decompose during the time it is adsorbed, then it is practically certain that the more strongly adsorbed isomer will suffer more decomposition.

It is well known that hydrolytic and other chemical changes sometimes occur with remarkable ease when a suitable chemical compound is allowed to remain in the adsorbed state over a prolonged period. In the successful partial resolution of p-phenylene bis-imino camphor carried out by Rule and Henderson (loc. cit.), the washing down of the adsorbed band was completed in a time varying from 8 to 60 hours. Prolonged treatment on the lactose led in this case to decomposition of the adsorbate, probably with liberation of camphor and formation of a p-phenylene bis-imino derivative of lactose.
However, it had not been anticipated that an azo compound prepared from dinaphthyl diamine and resorcinol would readily undergo decomposition under the mild experimental conditions employed, except possibly to form lactosides. No evidence pointing to the production of glycosides was observed in the course of this work, but there is no doubt that a proportion of the azo compound originally introduced into the tube did undergo some chemical change. In this connection, it should be emphasised that the duration of the adsorption experiments using benzene or hydrocarbon solvents was much greater than in the case of the previous experiments of Rule and Henderson. In most cases, washing could not be completed under 7 days, and in one instance it was prolonged to over 40 days. Various attempts were made to reduce this time by adding small proportions of a polar solvent such as alcohol to the solvent medium.

From pilot tubes it was seen that even small changes of this kind led to a greatly increased speed in the movement of the band down the column. At the same time, however, the band showed no signs of being actually adsorbed and it washed down the column cleanly and without spreading. From these observations, it was considered extremely unlikely that the addition of a polar solvent would increase the degree of resolution
and so no large scale experiments were carried out under such conditions.

That the azo compound did undergo chemical change was indicated in several ways. On distilling the benzene wash liquor, it was found that the concentrated liquid was yellow in colour. Thus a very dilute solution must have been washing out of the column during the course of the experiment. Also, after the band had washed over the lactose, the lactose remained a creamy colour. This, on elution with alcohol, yielded a small quantity of an optically inactive, dark red, gummy product. A further proof of decomposition is indicated by the decrease in the quantity of pure dye that can be isolated on increasing the duration of the adsorption. This was best seen in tube 5 where the compound was adsorbed for 6 weeks. In this case, only extremely minute active fractions could be isolated, the rest of the coloured product being due to decomposition. The total weight of pure dye recovered in 6 of the 19 fractions of the last experiment was 1.7mg.

Even allowing for 50% loss during the purification of these fractions, the amount of unchanged original compound at the end of the adsorption could not have exceeded 15% of the starting material.

Another factor leading to a comparatively small negative rotation is directly due to this decomposition,
As the band washes onto fresh lactose, the lower end of the band tends to be resolved leading to a good positive rotation. Before, however, the upper negative end of the band washes down, a certain amount of decomposition will have occurred with the result that much of the lactose is deactivated in so far as its adsorptive capacity is concerned. Thus the laevo-rotatory dye, instead of collecting in the upper end of the band, since it does not undergo adsorption to the same extent as the dextro isomer, will wash more readily down the tube with the result that, instead of the original band tending to split into two equally strong bands, there will be a concentrated portion near the lower end while the upper end will fade off gradually. This change was observed to take place. It was also noticed that only the lowest quarter or fifth of the band yielded positive rotations, the rest being negative.

**Adsorption Experiments on Wool.**

In the experiments with wool it was found that, though the tetrazo compound itself was not adsorbed appreciably from organic solvents, its sodium salt was taken up strongly from aqueous solution. No trace of preferential adsorption of one isomer could, however, be detected. This agrees with nearly all of the other work carried out with wool as adsorbent under similar
experimental conditions. The chance of an appreciable resolution by this "steeping" method is not nearly so great as in a properly conducted chromatographic adsorption, the technique of which has been so developed as to bring about a continuously increasing degree of separation of the components.

One point worth mentioning is that, after acidifying and drying the dyed wool, elution with alcohol in a Sohxlet only removed a small amount of the azo dye. The strength of the adsorption, if it is adsorption, must be much greater than on lactose or quartz and is more comparable to that on alumina.

Adsorption Experiments on Quartz.

In one case where powdered d-quartz was used as adsorbent, a very small partial resolution of the tetrazo compound was effected. Unfortunately, we did not possess sufficient of the powder to carry out a larger scale experiment. The results indicated, as would be expected, that the degree of adsorption on the quartz depends on the fineness of the powder but the more finely powdered material tends seriously to hinder the passage of solvent. From benzene, powdered quartz adsorbs the azo dye so strongly that further washing with benzene does not move the band. When a half percent of alcohol is added to the
benzene, the band moves slowly down the column but it is very difficult to follow its movement due to the extraordinarily dark colour of the adsorbent in benzene.

Thus it is apparent that powdered quartz is a much more powerful adsorbent for the tetrazo compound than lactose. That this comparative strength of adsorption is not fixed, but depends on the compound being adsorbed is realised when p-phenylene bis-imino camphor is used. This compound (see Henderson, Thesis, Edinburgh, 1939) washes down a column of quartz fairly readily but if adsorbed onto "Analar" lactose, the band is not affected by washing and decomposes within a few hours.

With quartz as adsorbent, decomposition was again noted with the tetrazo compound, similar to that occurring on lactose.

**Nature of Decomposition Products.**

The nature of this decomposition which occurred in all the adsorptions whether on lactose or quartz could not be discovered. Generally, the product was reddish and gummy, it dissolved fairly readily in benzene and was strongly adsorbed on calcium carbonate. Since heating the product with dilute hydrochloric acid had no apparent effect, it is unlikely that this red compound is a simple lactoside.
Since the molecule of the tetrazo compound has four hydroxyl groups, there is a large number of possible methods by which these could condense intra- or inter-molecularly. On the other hand, it was suspected for a long time that what was occurring was an isomeric change; if the original compound was, for example, trans-trans, then the red impurity might be a cis-cis or a cis-trans form. This change, though somewhat uncommon, is not impossible since it is known to occur in some cases under ultra-violet illumination. When it is remembered that an adsorbed molecule is almost certainly subjected to great strain, this type of change cannot be ruled out. Since, however, one red isomer was found to be a stable compound only decomposing about 300°, it is improbable that the isomers can be identified with this red tacky product.

**Other Compounds Investigated.**

Three other preparations were carried out when it became obvious that the resorcinol tetrazo compound was not leading to entirely satisfactory results. It was hoped to discover a compound which would yield good partial resolutions but would not decompose during the
adsorption.

The first compound \(1\) was obtained by diazotising dinaphthyl diamine and coupling with \(β\)-naphthol. The red compound thus obtained proved to be very weakly adsorbing, almost as weak as the benzil condensation product of the diamine.

A similar preparation in which the \(β\)-naphthol was replaced by phenol with the object of making compound \(2\) led to the production of three difficultly separable compounds whose nature was not investigated since their adsorbability, though stronger than the \(β\)-naphthol derivative, was not such as to lead us to expect any satisfactory resolutions.

The third compound \(3\), prepared by condensing dinaphthyl diamine with resorcylic aldehyde, was expected to be very similar to the resorcinol azo dye which forms the main subject of this thesis. However, it was found that this substance was insoluble in the common solvents except alcohol so that it could not be readily purified. Also, its insolubility made it unsuitable for adsorption work.
POSTSCRIPT.

In conclusion, the author wishes to express his indebtedness to Dr. H. G. Rule for valuable advice and helpful encouragement throughout the course of this work, and to make grateful acknowledgment to the Carnegie Trust for the Universities of Scotland for a Scholarship held during 1938 - 1940.