# SOME ATTEMPTS To DEVELOP NEW METHODS of RESOLVING RACEMIC COMPOUNDS.

By

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Thesis for the Degree of Doctor of Philosophy.

May 1939. University of Edinburgh.

# I N D E X.

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DIAGRAMS and TABLES:

# I N T R O D U C T I O N.

Chromatographic adsorption analysis stands out amongst the developments of technique in organic chemistry of the last decade, especially as applied to the separation of mixtures of closely related organic compounds.

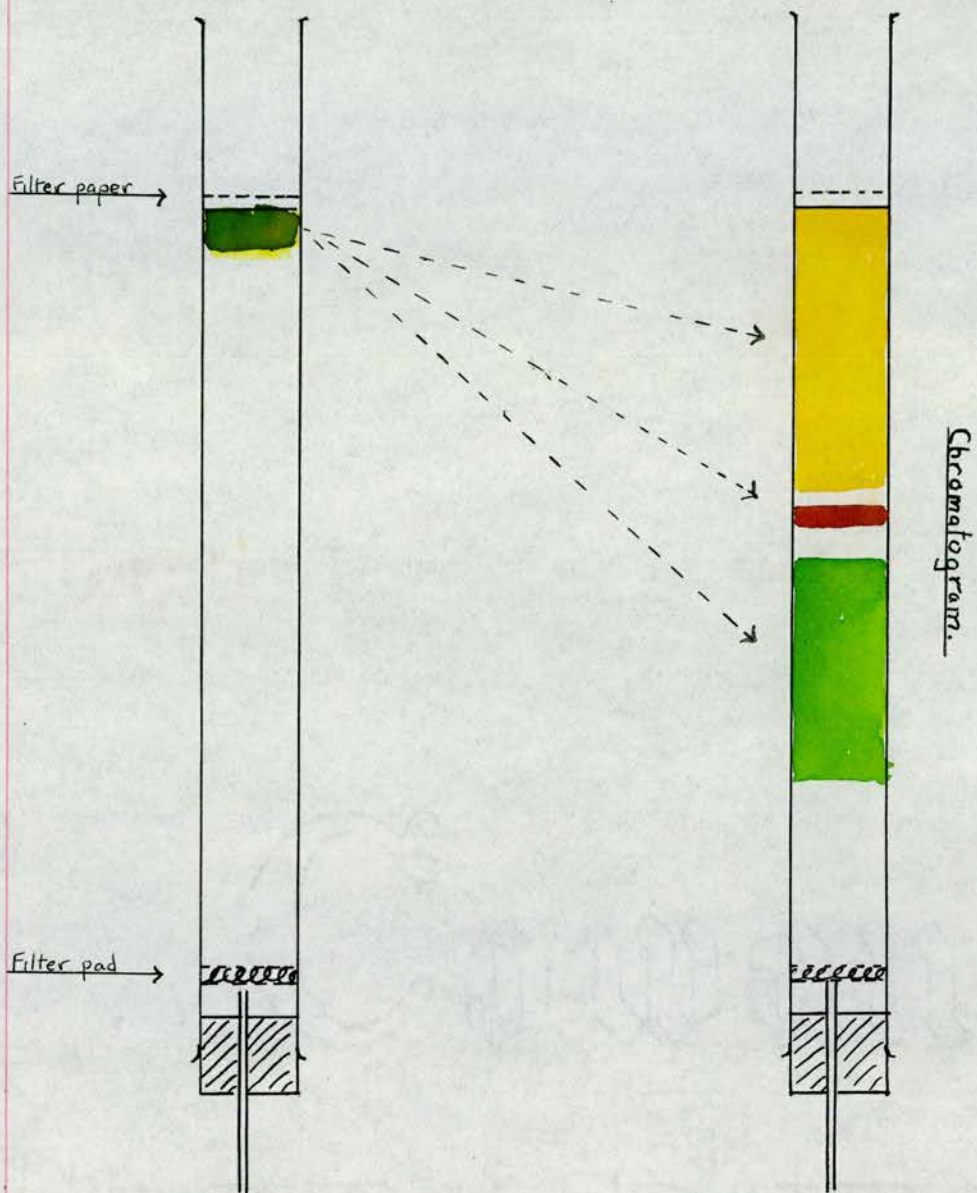
The exploitation of this invention of the Russian botanist Tswett has been occasioned by the rapid developments in micro-organic technique, which now enables chemists, especially biochemists, to work with minute quantities of material. This led to a demand for a method of isolating, in a pure state, quickly, efficiently, and with minimum loss, the constituents of natural mixtures of organic compounds, especially substances closely akin, like the plant pigments.

Tswett's chromatographic adsorption analysis, in the hands of chemists, supplied this need.

It differs from the use of charcoal in preparative organic work 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. This obscures the fact that many substances do have small differences in adsorbability and are, in general, capable of very fine differentiation.

Essentially, chromatographic analysis is a method of separating a mixture of compounds contained in a solution, by adsorbing them on to a suitable material, in a particular manner, and subjecting them to systematic treatment with solvents. The details were first published by Tswett in 1906 (Ber. Dtsch. botan. Ges., 24, 316), he having used the method in his examination of the pigments of green leaves. The method remained in obscurity until 1931, when it was revived to deal, once more, with the separation of a coloured mixture of vegetable origin, known as carotenoids, mainly by the schools of Karrer, Kuhn and Winterstein. In 1934 both Winterstein and Karrer showed that the process may be extended to colourless compounds by illuminating the chromatograms with invisible ultra-violet light, which causes many compounds to glow with a characteristic fluorescence. Karrer called these "ultrachromatograms" (P. Karrer and N. Nielson. "Trennung von Substanzgemischen im Chromatogramm und Ultrachromatogramm", Zurich: Rascher & Co., 1934).

When an adsorbing material such as alumina is shaken up in a flask with a solution of coloured compounds, the latter may be more or less completely adsorbed by the alumina. This procedure is of little value as a means of separating closely related compounds,




Adsorption. (1)

Development. (2)

which are simply deposited as a mixed coloured layer upon the surface of the alumina.

The procedure that Tswett employed, probably borrowed from capillary analysis, was to have the adsorbent in the form of a column in an upright tube, through which a dilute solution of the coloured material was allowed to flow.

Suppose we take, for example, a case in which all the colouring matter is readily adsorbed. The separation is carried out in three stages:

1) ADSORPTION.

The solution (1 - 3%), usually in non-polar solvents, runs into the tube, with the result that all the colouring matters are adsorbed by the upper layers of the adsorbent, the clear solution running out of the lower end of the tube. 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 topmost band, and the least strongly adsorbed forming the lowermost layer.

2) DEVELOPMENT.

In general, the bands overlap at this stage, and complete separation is effected by allowing a stream of pure solvent, often with the addition of some more

polar liquid, to flow down the column. The solvent tends to detach some of the coloured particles, which are carried a short distance downwards by the stream of liquid before they attach themselves once more to the adsorbent. Obviously, the smaller the adsorption coefficient of the compound, the more easily will it be detached and carried down; on the other hand, the greater the adsorption coefficient, the less easily will it be moved onwards. Hence, when we are dealing with a mixture of coloured substances, the stream of solvent leads to two changes: the coloured band moves as a whole slowly down the tube, and the band tends to spread out, those components which are strongly adsorbed travel slowly and remain at the top, while those which are weakly adsorbed are carried down faster and form a layer further down the tube.

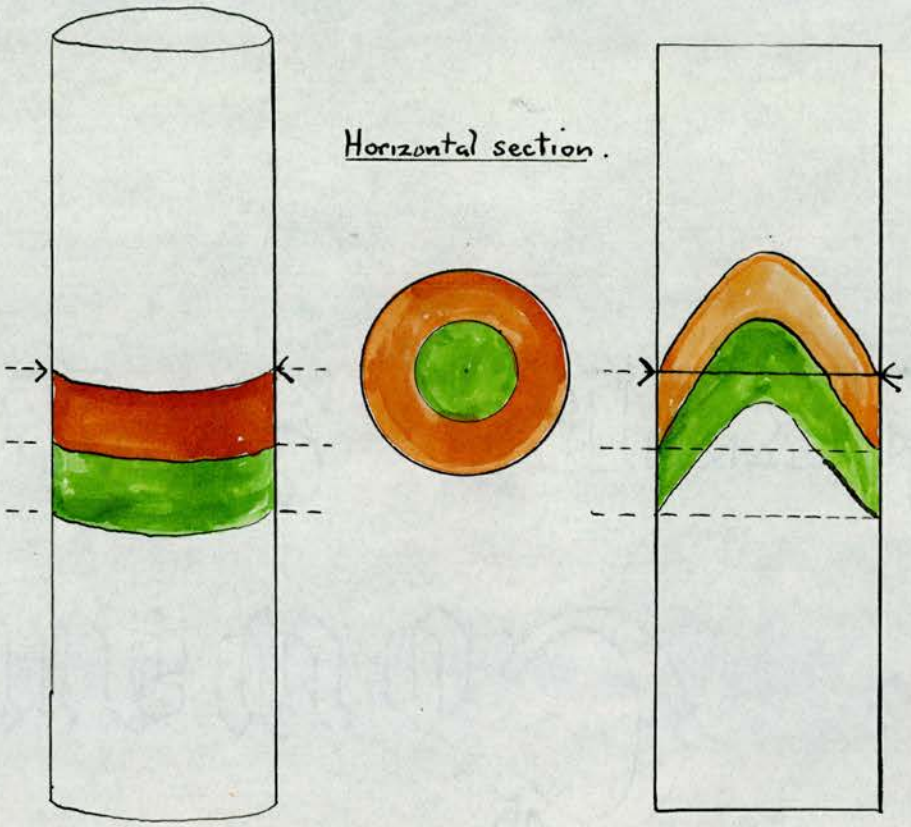
Under favourable conditions this process of development can be continued until the individual components arrange themselves in coloured zones, falling into the order of their adsorption coefficients and often separated from one another by colourless layers of adsorbent.

The series of coloured bands obtained in this way was termed a "chromatogram" by Tswett. (Diagram 3A.)

3) ELUTION.

The final separation may then be effected in one

# "Dome" formation



Horizontal section.

Outside appearance of chromatogram.

Vertical section

of two ways. Either the tube is sucked nearly dry by a pump, after which the cylinder of adsorbent is pushed out of the tubes and the coloured zones separated mechanically, the adsorbed material being then eluted with a highly polar solvent; or, alternatively, washing of the tube can be continued and each coloured product collected separately as it emerges in solution from the lower end of the tube.

The choice of adsorbents and of solvents is of vital importance for success in Tswett column analysis.

There are plenty of adsorbing materials available. One of the most useful is alumina ($Al_2O_3$) which adsorbs very strongly, often too strongly to allow satisfactory development of the chromatogram. Amongst the other adsorbents are calcium carbonate, magnesium oxide, sucrose and lactose.

Whichever adsorbent is selected, it must be filled into the tube, as a cream, in suspension with the desired solvent, and packed down evenly, often by suction and ramming with a solid rod. If this is not carefully done, the bands may develop very ragged edges or give "domes". These "domes" are formed when the band travels down the walls of the tube more quickly than in the centre, where packing may be more dense. (Diagram 5A.)

The adsorbent is usually matched with the substance to be adsorbed. Thus Winterstein recommends for the

chlorophylls, of high adsorption coefficient, the weakly adsorbing sugars. Xanthophylls are held conveniently by calcium carbonate ($CaCO_3$), whereas carotene, of low adsorption coefficient, is adsorbed most conveniently on the strongly adsorbing alumina ($Al_2O_3$).

The choice of solvents is, if anything, of even greater importance than the adsorbing material. So far, little appears to be known about the constitutional factors governing the degree of adsorption, although compounds which have a strong tendency to exist in a state of molecular association, also tend to be readily adsorbed. Thus hydroxy compounds, which associate strongly, are also strongly adsorbed. However, if the degree of association of a hydroxy compound is greatly diminished by chemical change of the hydroxy group (-OH), say by converting it into a methoxy group ($-OCH_3$), the resulting compound is much less strongly adsorbed.

It is almost certain that polar forces are involved, and to judge from published data, it appears that in any given case the adsorption takes place most readily from non-polar solvents of low dielectric constant, as would be expected if the forces involved are of the nature of an electrical attraction.

Light petroleum stands at the head of the list, adsorption from this being very marked. Then follow the more polar solvents.

**Solvents in Order of their Promotion of Adsorption.**

| Solvent | Dipole Moment | Dielectric Const. |
|---|---|---|
| a) Light Petroleum | 0 | 2·0 |
| b) $C_6H_6$ | 0 | 2·2 |
| b) $CCl_4$ | 0 | 2·3 |
| b) $CS_2$ | 0 | 2·6 |
| c) $CHCl_3$ | 1·05 - 1·2 | 5·2 |
| d) Alcohols | 1·7 | 25 - 30 |

For this reason it is usual to allow coloured compounds to be adsorbed from light petroleum or a mixture of this and benzene, or benzene containing a small quantity of alcohol. The addition of a more polar solvent in small quantity to the bulk of less polar solvent is often occasioned by the necessity of dissolving reasonable amounts of the colour mixture to be analysed. Much, of course, depends upon the solubility of the mixture of compounds to be examined, and a considerable amount of experiment is often necessary before the optimum conditions can be found.

It sometimes happens that a single adsorption and development does not result in a complete separation

of the components, even though the various coloured zones appear to the observer to be clearly differentiated. In such cases, each individual zone may be cut out and re-treated separately until it undergoes no further change.

Too rapid development by use of a solvent in which the compounds are easily soluble must be avoided, otherwise the chromatograms will be spoiled. Sometimes, again, hydrolysis may occur in the adsorption tube, especially with esters on alumina ($Al_2O_3$). Again, actual chemical combination of the compound with adsorbent, as with certain acids on alumina, may give rise to anomalous results.

Tswett laid down the rule:

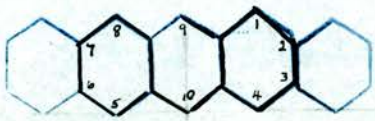
> "A compound can be considered to be homogeneous if it cannot be separated into two or more zones by adsorption and development under varying conditions." (Book, Warsaw, 1910).

Tswett was strongly convinced of the reliability and value of his method and carried out a large number of chromatographic analyses himself on naturally occurring mixtures of pigments. However, he usually contented himself with separating the product into its various coloured layers and with proving to his own satisfaction that the layers were homogeneous. He worked on a micro-scale and in general rarely isolated

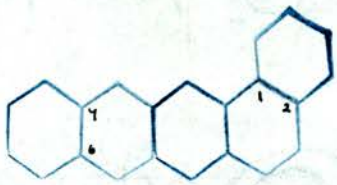
or analysed chemically the individual components of the mixtures. His work was neglected consequently, partly because of this fact, and partly because of many of his results being published in the Russian language. Nevertheless, at a time when very little of importance was known about the colouring matters of leaves, we find Tswett stating definitely that chlorophyll was not a single substance ("Chromofilli w rastitelnom i schwotnom Mirje". Warschau, 1910) but a mixture of two differently coloured chlorophylls, and he defended this belief against strong criticism. Willstätter much later proved this to be the case, in a much more laborious, if orthodox, fashion.

Highly complex substances, necessarily of high molecular weight, are of course more readily adsorbed than simpler ones, and are also more likely to be coloured. Coloured substances are usually more readily adsorbed than colourless ones, since unsaturation, which is the primary cause of colour, generally leads to a high adsorption coefficient. Thus it is only natural that the method should have been applied most vigorously to the complicated mixtures of colouring matters obtained from plant and animal sources, to sterol chemistry, and to products of a carcinogenic character prepared by synthetic means.

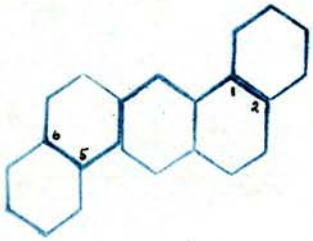
In this last connection it has been shown that the three isomeric dibenzanthracenes may be readily




2.3.6.7. dibenzanthracene




1.2.6.7




1.2.5.6.

separated chromatographically.

The 2:3:6:7 variety is more strongly adsorbed than the 1:2:6:7, and that again is more strongly adsorbed than the 1:2:5:6. (Diagram 10A.)

This process may be applied to much simpler compounds, of a relatively low order of molecular complexity. Thus a mixture of ortho-, meta- and para-nitranilines, or of ortho-, meta- and para-nitrophenols can be separated by use of calcium carbonate or alumina as the adsorbing medium. The adsorption being p-» m-» o-.

A particularly interesting case is the adsorption of the two colourless terpene liquids, cineol (b.p. 176°) and dipentene (b.p. 175°), using light petroleum as solvent, and alumina ($Al_2O_3$) in the column. In this case ultra-violet light was used to substitute the lack of colour in visible light by a fluorescence colour.

Historically, chromatographic analysis may be said to have developed from the capillary analysis of Goppelsroder (F. Goppelsroder, "Anregung zum Studium der Capillaranalyse"; Basle, 1906). In capillary analysis long strips of filter paper are suspended upright, with their ends dipping into dilute solutions of dyestuffs, and are allowed to stand for several hours. Different dyestuffs ascend up the strips under capillary attraction at different rates, forming a number of differently coloured zones at the top,

corresponding roughly to the constituents of the mixture. The individually tinted zones could then be cut out, and identification "spot" tests applied to them.

It is easy to see the connection with the Tswett technique. The paper is exchanged for a column of powdered adsorbent and capillary rise is substituted by a downward flow under gravity or suction.

Whilst the capillary analysis could handle extremely small quantities, Tswett's technique was a great improvement. The adsorbent can be varied at will, and the important process of development may be carried out.

Capillary analysis was practically restricted also to aqueous solutions, mainly of dyestuffs, for obvious reasons; many organic solvents being too volatile or having insufficient capillary rise.

Capillary analysis is really only of diagnostic value, and is quite useless in preparative work.

The successes of Tswett's chromatographic adsorption analysis in the separation of closely related and isomeric substances are of special interest to the organic chemist and must raise the question, "To what limit, if any, may the application of Tswett analysis to closely related compounds be taken?" It is natural to ask if it may be applied to the separation of optically isomeric compounds, using suitable conditions

and adsorbents.

The lack in differentiation in colour or fluorescence between any possible dextro- or laevo-zones is obviously compensated by a positive or negative activity in polarised light, thus yielding a sensitive test of separation.

If we remember Tswett's well founded empirical rule

> "A compound can be considered homogeneous if it cannot be separated into two or more zones . . . "

and then state this inversely

> "Any compound which is not homogeneous may be separated into two zones . . ."

we may see a method of resolving racemic mixtures.

Whilst optical enantiomorphs are chemically the same, they are still not <u>physically homogeneous</u>. Resolutions might be expected if we are able to introduce an asymmetric steric factor into the adsorption surface.

Thus it was proposed in this research to examine the possibility of effecting a partial or complete separation of the dextro- and laevo-rotatory components of racemic mixtures by allowing dilute solutions of racemic compounds, in non-polar solvents, to flow through upright tubes packed with an insoluble or sparingly soluble asymmetric adsorbent.

It was hoped in this way to demonstrate, by optical examination of eluted fractions, if any preferential adsorption of one component of the racemate on the optically asymmetric surface of the adsorbent took place.

Another possible method of separating racemates by means of a Tswett column was also considered. If one dissolves a racemate in an optically active solution, Willard Gibbs' theories suggest that if one isomer lowers the surface tension of the solution more than the other, it will be more highly concentrated at the surface of the solution. Thus we might theoretically expect a partial resolution to be effected in the surface layer or interfaces under these circumstances. By pouring a solution of such a racemate in an optically active solvent over the largely developed surface of a highly adsorbing powder such as alumina ($Al_2O_3$) in a Tswett column, one might expect any surface concentration to be progressively adsorbed, yielding an adsorbate containing an excess of one component of the racemate and a residual solution containing an excess of the other component.

The classical methods of resolving racemic mixtures nearly all involve the formation of a chemical compound, and the recrystallization of diastereomers. It was proposed, if possible, to attempt to carry out resolutions on a compound incapable of yielding to the

normal methods of resolution, and to depend entirely on adsorption forces. This, of course, would be the great advantage of any type of resolution carried out by a modified Tswett adsorption analysis.

Earlier attempts to demonstrate asymmetric adsorption have been markedly unsuccessful, although one may quote a passage from Frankland's Pasteur Memorial Lecture to the Chemical Society, bearing in mind that taste and smell have been demonstrated to involve adsorption phenomena.

> "The asymmetric nature of living matter was ever present to Pasteur's mind and he was always ready to discern applications of this fertile idea. Thus many years later, in 1886, in commenting on a paper by Piutti (Compt. rend., 1886, 103, 138) on the isomeric asparagines, read before the Academy, he calls attention to the fact that one asparagine (dextro) is sweet, whilst its antipode is insipid, and refers this difference to the different action of these two asymmetric isomers on the asymmetric matters present in the tissue of the gustatory nerve."

In the light of modern knowledge we may say that this case represents a simple biological demonstration of differential adsorption.

R. Willstätter has described attempts (Ber., 1904,

37, 3758) to demonstrate a preferential adsorption of the enantiomorphs in racemic alkaloids, using silk and wool as the optically active adsorbents. He found no positive evidence however.

C. W. Porter and Hirst (J.A.C.S., 1919, 41, 1264) mention in a preliminary paper on optically active dyestuffs that they have effected a partial resolution of such a racemate by adsorption on wool. They, however, gave no figures or data, and no confirmation of this statement has been forthcoming.

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

C. W. Porter and Ihrig (J.A.C.S.,1923, 45, 1990) claimed to have effected an appreciable partial resolution of a racemic dyestuff, 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 dyestuff.

G. T. Morgan and Skinner (J.C.S., 1925, 127, 1731) showed that certain "tartaric acid configuration" stereoisomeric azo-dyestuffs 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 dyeing experiment, similar to those employed by Porter and Ihrig.

R. Adams and W. R. 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, p. 39) some experiments which may have some relation to asymmetric adsorption, in the light of later work.

In essential, a warm saturated solution of chlorobisdimethylglyoximoammine-cobalt $(Codg_2(NH_3)Cl)$ 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 laevorotatory 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 in the original Japanese paper (in Japanese only: J. Chem. Soc. Japan, Vol. 56, p. 1339 (1935) ) the same type of asymmetric adsorption with a sample of synthetic, racemic, adrenaline hydrochloride. So far as can be calculated from data in the Japanese paper, the specific rotations they attained were only of the order of $[\alpha]_C = 6°$ for the cobalt complex, and in the region of $[\alpha]_D = 2°$ for the adrenaline, which when fully resolved gives $[\alpha]_D = 53°$. At no point do they, however, work out the specific rotations.

The partial resolutions claimed in all the cases referred to in the foregoing pages are very small, 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 attaches to them. Nearly all the other partial resolutions, claimed to have been detected by non-polarimetric methods, just fall without the limits of normal error.

## EXPERIMENTAL and DISCUSSION.

The aim of this research being the finding and developing of any new methods of resolving racemic compounds, it was thought best to work directly on the racemates instead of measuring any differences which might exist in the rates of adsorption of the pure dextro- and laevo-rotatory forms separately on adsorbents with asymmetric surfaces. The latter method was the one employed by Adams and co-workers (J.A.C.S., 44, 2930; 48, 2193, 2202 ), and by Morgan and Skinner ( J.C.S., 127, 1731 ). It was thought that even if any demonstrable differences in adsorption rates were found by these methods, it might still fail to lead to a satisfactory method of resolving racemates. On the other hand, differences in adsorption, possibly so subtle in character as to escape this direct rate-measurement, might by a Tswett analysis, lead to the separation of enantiomorphs. Again, in view of the notorious difficulty of preparing batches of adsorbents which can be assured of having identical powers of adsorption, it is preferable in our view, to adsorb the racemate directly on to the asymmetric surface, a condition fulfilled in our sketched Tswett adsorptions, as under these circumstances any differential adsorption will be utilised over the identical surface, under identical conditions.

The adoption of such a scheme leaves one the polarimeter as the sole instrument for the measurement and detection of any asymmetric adsorption. It is just possible that the irradiation of the chromatogram with circularly polarised light might also lead to the detection of asymmetric adsorption under favourable circumstances. One might then distinguish the zones containing a predominance of dextro and laevo-rotatory molecules respectively in different tints, through the agency of dichroic phenomena.

Choice of racemate and asymmetric adsorbent.

A little thought suggests that one should choose as a suitable racemic substance, a stable, coloured compound of high rotation when fully resolved. The colour, which is useful in developing the chromatogram, must not however be so intense as to render polarimetric readings difficult. The racemate would ideally be easily adsorbed from non-polar solutions, and be easily eluted by more polar solvents. It would also be a compound incapable of resolution by normal methods, in order to demonstrate in a striking manner the scope of the Tswett adsorption technique. Probably a colourless compound which fluoresced strongly in ultraviolet light to give clear ultra-chromatograms would be more satisfactory from the polarimetric standpoint.

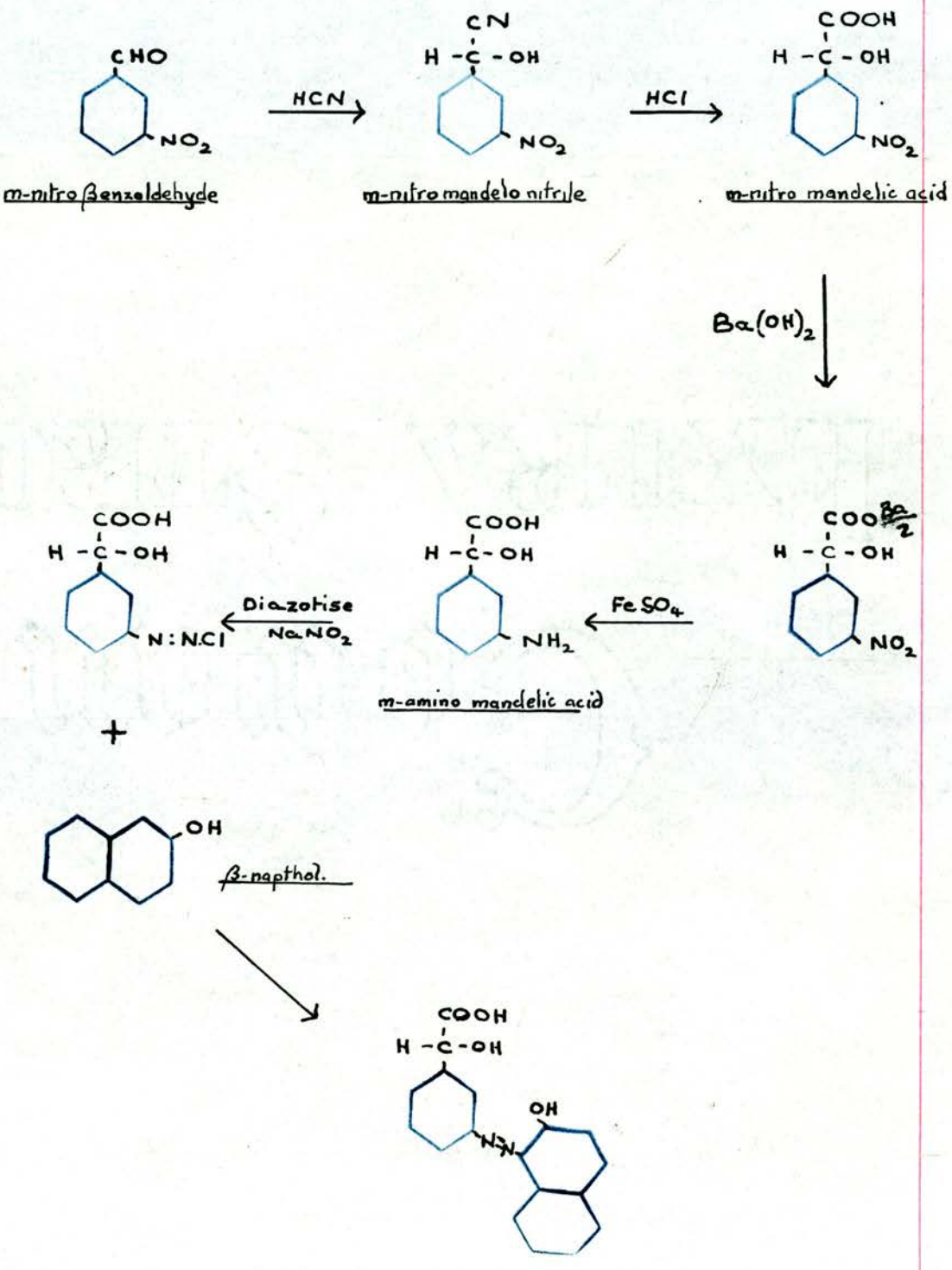
The first racemate actually taken was far from

ideal, since it was chosen for historical reasons. It was racemic m-azo-β-naphthol-mandelic acid, the red dyestuff employed by Porter and Ihrig in their adsorption experiments over wool.

The first choice of asymmetric adsorbent was calcium d-tartrate. It offers analogies with other successful adsorbents such as calcium carbonate, both being salts of weak dibasic organic acids. It can be obtained easily in a pure dry powdered form (B.D.H.) and it was thought quite probable that it would present an asymmetric surface for adsorption.

Concerning the last statement, if one accepts that optical activity is a criterion of asymmetry, then a crystal showing optical activity will have an asymmetric structure. This structural asymmetry will necessarily lead to a surface asymmetry, at least on some of the crystal faces, dependent on whether the crystal is isotropic, uniaxial or biaxial.

At the present time no exception is known to the following rule. "Substances, which are optically active in solution, also exhibit optical activity in the crystalline state." (Lowry, "Optical Rotatory Power", 1935).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NH2
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N:N.Cl
m-amino mandelic acid
+
OH
β-napthol.
COOH
H -C- OH
OH
N=N
m-azo β-napthol mandelic acid

Preparation of racemic m-azo-β-naphthol mandelic acid.

After much experiment with published data, in which difficulties were encountered in dealing with the nitrile stage and nitro group reduction, a scheme was finally devised for the preparation of the acid.

Starting with m-nitrobenzaldehyde, this was converted into m-nitro-mandelo-nitrile and then into m-nitro-mandelic acid, essentially by the method of Brode and Adams (J.A.C.S., 48, 2204). The m-nitro mandelic acid was reduced by the method of McKenzie (J.C.S., 1935, p. 106), and the m-amino mandelic acid was finally diazotised in the classical manner and coupled with β-naphthol to give the required racemic dyestuff

Typical preparation.

60 g. m-nitro benzaldehyde was converted to the nitrile in glacial acetic acid solution. The process of Brode and Adams was modified at the separation of the nitrile from the glacial acetic acid. This was accomplished by pouring the reaction mixture into twice its volume of water and separating the oily nitrile by decantation. The supernatant milky liquid was only then neutralised with strong sodium carbonate solution, when more of the oily nitrile separated.

The rest of the process followed as described in

the literature, yielding after hydrolysis with concentrated hydrochloric acid (Heller, Ber., 46, 280), the yellow crystals of m-nitromandelic acid (30 g.), m.p. 118-120°.

In the reduction of the m-nitro-mandelic acid according to McKenzie's method, the acid is reduced in the form of its barium salt with barium hydroxide and ferrous sulphate. The final stages can be simplified, in that barium m-amino-mandelate may be converted directly into m-amino mandelic acid by treatment with sulphuric acid.

In McKenzie's original instructions for the preparation of o-amino mandelic acid this could not be done, as cyclisation occurred on direct treatment with acid.

Finally, the amino acid was induced to crystallise with some difficulty and then recrystallised from hot water. Brown crystals, m.p. 128-130°, with decomposition; yield 16 g.

This m-amino mandelic acid was coupled up with β-naphthol (Various volumes "Organic Syntheses"), care being taken to avoid any excess of nitrile or β-naphthol being used. A scarlet dyestuff was obtained which, upon repeated recrystallisation from glacial acetic acid, gave crystals melting at 205-7° in a yield of 10 g. Porter and Ihrig (loc. cit.) give the specific rotation of the optically active dyestuff as $[\alpha]_{6100}$ = 47° ± 0·5° in glacial acetic acid.

Repetition of Porter and Ihrig's Experiments.

Prior to carrying out the adsorption experiments by the modified Tswett procedure, it was decided to attempt to repeat the resolution of racemic m-azo-β-naphthol mandelic acid claimed by Porter, but later regarded as dubious by Adams.

The magnitude of the observed rotation claimed by Porter and Ihrig (-0·91°) is such as to rule out any probability of optical illusion, and their technique is apparently so simple as to exclude the possibility of later workers being unable to repeat their standardised conditions. Nevertheless in the present work we were unable to confirm any such resolutions, partial or whole. Adams' findings were thus confirmed.

Experiment with natural fleece.

Natural sheep's fleece, after washing with soapy water and rinsing, was dried. It was defatted with carbon tetrachloride, and then after treatment with alcohol and ether, finally dried.

Porter's directions (J.A.C.S., 45, 1990) were carried out as far as possible, although the dye bath these workers used, 1 g. of dye in 75 c.c. glacial acetic acid, was found to be out of the question, as 1 g. of dye could only be dissolved in about 150 c.c.

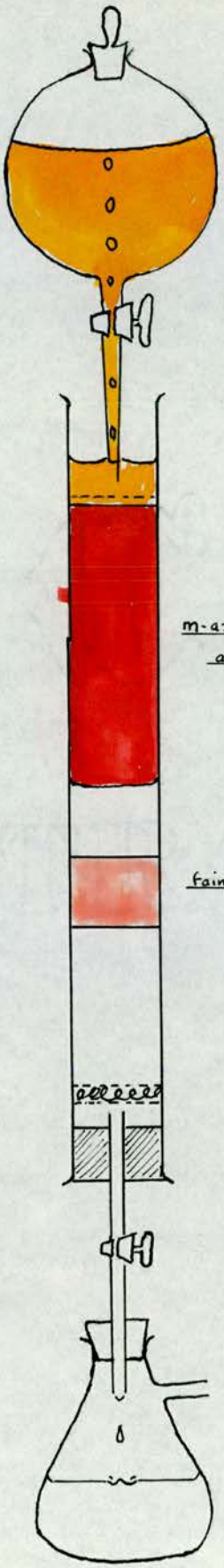
of warm glacial acetic acid.

75 c.c. of this saturated solution was placed in a conical flask and 2·5 g. of the pure fleece immersed in it. Polarimetric readings were taken on the solutions every 24 hours for 3 days, using the red 610 mμ light. Although the wool was brilliantly dyed scarlet, and the solution appreciably reduced in concentration, no rotation could be observed at any point, using a 2 dcm. polarimeter tube. This experiment was twice repeated, once at 5°C. and again at 15°C.

After 72 hours in each case, the wool was encrusted in crystals of the dyestuff, and the whole solution clogged with crystals.

This whole series of experiments was repeated with wool once more, using this time a piece of woven, natural-coloured blanket, some 20 years of age. This sample which must have undergone countless washings, should represent the other extreme from fresh fleece. Exhaustive experiments, conducted at 25-30°C. once more gave negative results, even though the adsorption of dye from the solution was carried almost to the point of exhaustion.

Taking into account the negative results obtained by Adams (J.A.C.S., 48, 2204) in similar experiments at 90°C., it would appear that the resolutions of Porter and Ihrig, produced by this method, are, to say the least of it, extremely difficult to reproduce.



m-azo β napthol mandelic acid adsorption band

faint leading band (p-isomer)

## CHROMATOGRAPHIC ADSORPTION Of RACEMATES On ASYMMETRIC SURFACES.

Having completed the work described above, the major experimental scheme of the research was now started.

### Adsorption of racemic m-azo-β-naphthol-mandelic acid on calcium d-tartrate.

The adsorbents used in the experiments, except when otherwise stated, were all dried by being heated in vacuo at 90-100°C. for three to six hours.

The solution of the dyestuff was made up in benzene (analar) at saturation point. Concentration 0·012 g. in 100 c.c.

A glass tower, 30 cm. by 2·5 cm., open at the top and stoppered at the lower end, was packed with a paste of the dry, finely-powdered adsorbent, calcium d-tartrate in benzene. Over the stopper at the lower end was placed a filter pad of paper and cotton-wool, which prevented the tartrate passing down the glass tube and stopcock. The latter led to a Buchner flask capable of being evacuated. (Diagram 25A.)

The dyestuff solution was now added to the top of the column and gentle suction applied at the foot. The dyestuff formed a bright red adsorption band at the top of the column, whilst the clear benzene emerged

at the foot at the rate of about one drop per second.

In order to maintain a steady supply of solution to be adsorbed at the top of the column, the following device was employed. A separating tap-funnel, stoppered at the top, with its tap open and its bulb three-quarters full of solution, was placed with its stem projecting into the top of the Tswett column (Diagram 25A. ). When the solution in the top of the column falls below the level of the end of the stem, air bubbles up into the bulb and more solution flows down to maintain the level at the top of the Tswett column.

After the addition of 150 c.c. of the dyestuff solution, the red adsorption band filled two-thirds of the column of tartrate. Fresh solution was now no longer added, and "development" commenced with pure benzene until the adsorption band had expanded to the full length of the column. The column of adsorbent, after partial draining from solvent, was now pushed out with a flat-ended glass rod. The top thirds and lower thirds of the extended column of tartrate were taken and reserved in separate vessels for elution.

Six such columns were run through, and 1 liter in all of racemic dyestuff solution, containing 0·12 g. dye, were thus adsorbed. The top and bottom thirds were now separately eluted by warming the pastes with acetone. The acetone extracts were then filtered and gently evaporated to dryness.

Dye recovered from top third: 0·029 g.

Dye recovered from lower third: 0·031 g.

These figures suggest that the elution was incomplete, a fact borne out by the condition of the eluted calcium d-tartrate, which was still pink, possibly due to the calcium salt of m-azo-β-naphthol mandelic acid remaining behind and displacing d-tartaric acid. Treatment with alkali effects a complete elution, along with some tartrate, however.

The recovered fractions of dyestuff were dissolved separately in 5 c.c. of glacial acetic acid, and examined for activity in a 2 dcm. capillary polarimeter tube, using the red 610 mμ radiation as illuminant.

No activity whatsoever could be detected.

Presence of other isomeric dyestuffs.

It was noted in all these adsorption experiments that, besides the main red adsorption band in the developed chromatogram, there was always present a faint, short, more disperse, leading red band. ( Diagram 25A.). Considering how the racemic m-azo-β-naphthol-mandelic acid is prepared from m-nitro-benzaldehyde, and that this starting material commonly contains traces of o- and p-nitro isomers as well, it seemed likely that the leading band might be due to the presence of p-azo-β-naphthol-mandelic acid. It could not be the ortho-isomer, as this undergoes

cyclization at the amino acid stage (McKenzie, loc.cit.) and should have been eliminated during the preparation.

Accordingly some racemic p-azo-β-naphthol-mandelic acid was prepared, and upon adding successive traces of it to our original dyestuff solution, this leading band became successively expanded and enlarged, a fact which tends to confirm the above suggestion.

Discussion of results.

It is suggested that in view of the fact that the racemic dyestuff under examination is acidic, the dyestuff may be adsorbed on to the calcium ions of the lattice frame-work in the calcium d-tartrate crystals.

An ionic salt, such as our adsorbent, may be regarded as being built up of two interpenetrating lattices, one of the calcium ions (symmetric nature) and one of the d-tartrate ions (asymmetric nature). Adsorption on to the calcium ion may thus quite possibly rule out the chance of differential adsorption of the optical isomers. This supposition tends to be borne out by the fact that we never detected any shade of resolution in this instance, although we later were able to do so on other adsorbents, and also by the fact that final complete elution required an alkaline medium.

For this reason, the use of a powdered sugar was suggested as an adsorbent, since here we have no ionic lattices to complicate the issue, and at the same time

can be reasonably sure of the asymmetric structure and surface of the crystal.

Adsorptions of racemic m-azo-β-naphthol-mandelic acid on to powdered lactose.

Of the various sugars tried out, sucrose, glucose, maltose and lactose, lactose proved to be the best adsorbent. It is approximately two and a half times as effective as the tartrate in the same state of division. The lactose used throughout the research was "Lactosum B.P." supplied by T. and H. Smith of Edinburgh.

The adsorption experiments, as described on page 25 et seq., were repeated, but using lactose as the adsorbent in place of calcium d-tartrate.

Two liters of the benzene solution of the racemic dyestuff, containing 0·24 g. of the racemate, were adsorbed on to three columns of lactose, each containing some 200 g. of adsorbent, and 50 cm. by 2·5 cm. in dimensions, followed by development of the adsorbed layers.

From the top thirds, 0·07 g. of the dyestuff was recovered, but the acetone extract from the lower third was accidentally destroyed by fire. The top portion, however, did show a slight positive rotation over a blank extract from pure lactose, when examined in the polarimeter.

It should be noted here that this particular

batch of lactose was dried by boiling up with benzene and so distilling off adsorbed moisture. It appears that this procedure must have activated the lactose, as is suggested by certain results recorded later in this thesis. This was not realized at the time, and this method of drying, which could only conveniently be applied to small quantities of lactose, was superseded by the method originally used on calcium d-tartrate: that of drying in vacuum by heat.

Average rotation, 2 dcm. tube, light 610 mμ (corrected for instrument and lactose extracts blanks) . . +0·08°.

Concentration: 0·07 g. in 10 c.c. glacial acetic acid.

$$[\alpha]^{15^\circ}_{6100} = \frac{\alpha \times 100}{c \times l} = \frac{0{\cdot}08 \times 100}{0{\cdot}7 \times 2} = +6^\circ$$

The above extract, besides being contaminated with traces of lactose, was suspected of being otherwise impure. It was evaporated down to small bulk in glacial acetic, and precipitated with water. The dyestuff was then collected on a micro filter, washed with successive quantities of boiling water, and dried. A small quantity of the remaining substance was made up in glacial acetic acid and again examined for activity. Another small fragment gave a melting point of 199-201°.

Average rotation (corrected for instrument) .. +0·03°

Concentration .. 0·015 g. in 5 mls. glacial acetic acid.

$$[\alpha]^{15}_{6100} = \frac{0{\cdot}03 \times 100}{0{\cdot}15 \times 2} = +10^\circ$$

Thus we see here a suggestion of a partial resolution, although it will require both positive and negative rotations from top and bottom fractions before the results can be regarded as established.

This last adsorption experiment was repeated, with the difference that the method of vacuum-drying was applied to the lactose. The final dyestuff eluted was purified from water soluble components, and dissolved in glacial acetic acid as before, and examined in a 2 dcm. tube.

Upper fractions: 0·050 g. dye in 5 c.c. acetic acid.
Average rotation: +0·05°.

$$[\alpha]^{15°}_{6100} = \frac{0·05 \times 100}{1 \times 2} = +2·5°$$

Lower fractions: 0·030 g. in 5 c.c. acetic acid.
Average rotation: -0·02°

$$[\alpha]^{15°}_{6100} = \frac{0·02 \times 100}{0·6 \times 2} = -2°$$

These results are suggestive, and although the observed rotations are very small, +0·05° and -0·02°, they represent a <u>difference</u> of 0·07°, which is quite definite. It is obvious that a compound which has a higher specific rotation would give better and more accurate results, since the specific rotations are liable to large errors with the multiplying factors.

On another occasion, however, a repetition of this experiment yielded no observable rotations. In this

case apparently, the lactose was insufficiently activated by drying.

Attempted adsorption of racemic trans-acenaphthylene-glycol.

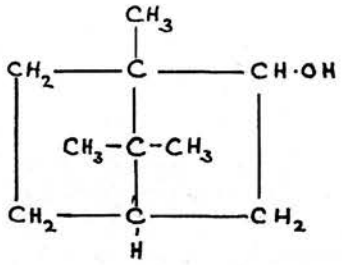
In addition to the above work, and before passing on to a compound of higher specific rotation, the adsorption of racemic trans-acenaphthylene-glycol was attempted on lactose and calcium d-tartrate, a specimen of this racemate being in the laboratory. This compound, which is colourless, did not show any appreciable adsorption.

Preparation of p-phenylene bis-imino camphor.

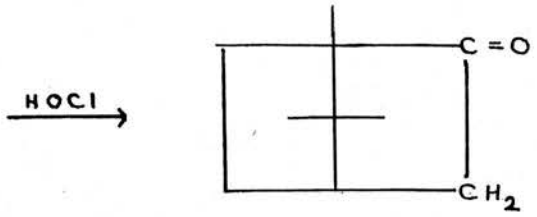
Specimens of this compound were prepared in the racemic, dextro- and laevo-rotatory forms. So far as can be ascertained, no internally compensated meso-isomer is formed when racemic camphor quinone is condensed with p-phenylenediamine to form the p-phenylene bis-imino camphor complex.

Racemic p-phenylene bis-imino camphor formed from racemic camphor appeared to be identical with that made by mixing equal weights of dextro- and laevo-p-phenylene bis-imino camphor. All attempts to form the meso compound have failed.

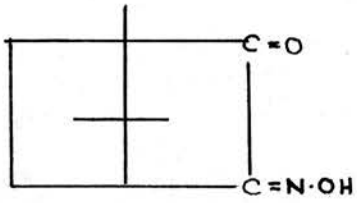
The literature upon the preparation of the racemic and active p-phenylene bis-imino camphors is quite extensive. See Forster and Thornley, J.C.S. (1909),



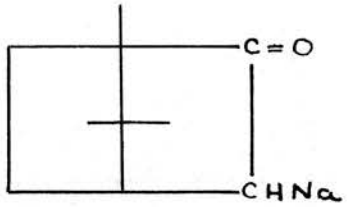
Borneol

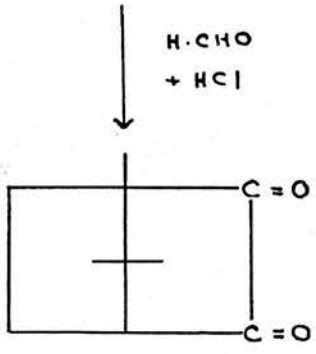


Camphor

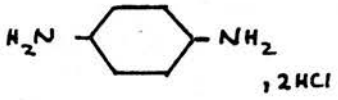


isonitroso camphor

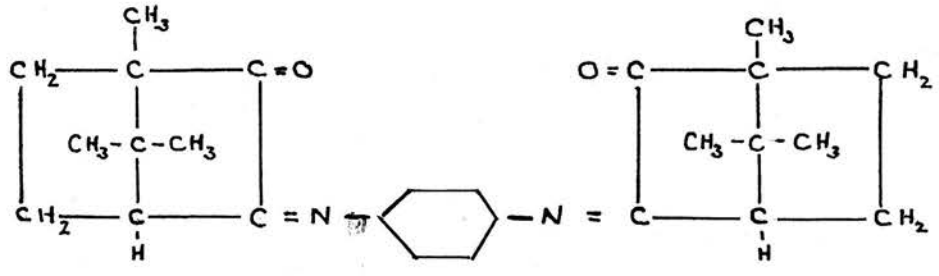




Camphor quinone
(2 mols.)



p-phenylene diamine HCl
(1 mol.)



p-phenylene bis. imino camphor

95, 942, and also Singh and Bhaduri, J. Indian Chem. Soc., (1930), 7, 555.

Essentially the method used in the present work was to convert camphor, by treatment with sodium in dry ethereal solution, into the sodium compound. This upon interaction with amyl nitrite, gave iso-nitroso camphor. The iso-nitroso camphor was then transformed into camphor quinone by means of formaldehyde. Finally, the camphor quinone was coupled with p-phenylene-diamine, by a slight modification of the literature methods, involving a compromise between the coupling in solution of Forster and the coupling by fusion of Singh.

The racemic form was first prepared from a sample of racemic synthetic camphor. The racemate was also made later from the individual derivatives prepared from dextro- and laevo-camphor respectively. Whilst dextro-camphor is easily obtained, the laevo variety had to be prepared from l-borneol by means of oxidation with chlorine water. According to the literature this oxidation had apparently been carried out only on iso-borneol. ("Unit Processes in Organic Synthesis"; Groggins, p. 342).

## Typical Preparations.

### Laevo-camphor.

Laevo-borneol, 50 g., was dissolved in the minimum amount of chloroform, and to this was added 3500 c.c.

of fresh, saturated chlorine water. After vigorous mechanical shaking for three hours, the chloroform layer was separated, washed with dilute thiosulphate solution followed by water, then dried, and finally evaporated in vacuo to a solid residue. On steam distillation this yielded 38 g. of l-camphor, m.p. 175-176°.

To confirm the preparation, apart from the having the correct melting point and specific rotation, the 2:4-dinitro phenylhydrazone derivatives of this l-camphor and of authentic d-camphor were made by Brady's method (See p. 87, "Qualitative Organic Chemistry, N. Campbell). The 2:4-dinitrophenyl-hydrazone of synthetic l-camphor melted at 176°. The 2:4-dinitrophenyl-hydrazone prepared from d-camphor melted at 176°. Mixed melting point (1:1), 164-5°.

It is interesting to note that a similar preparation to the above, only using benzene as the solvent for l-borneol, gave 9·5 g. of l-camphor from 10 g. of l-borneol.

The yield given by the oxidation, it appears, is almost quantitative, and any losses are probably those incurred during the evaporation of the solutions at the end of the preparation.

Attempts were also made to convert l-borneol directly into l-camphor quinone by means of selenium dioxide in acetic anhydride solution. These were

uniformly unsuccessful, although traces of quinone were formed when a start was made from l-camphor (Evans, Ridgion and Simonsen, J.C.S., 1934, 137).

The classical methods for the preparation of camphor quinone were found to be most effective.

Preparation of iso-nitroso camphor from racemic camphor.

The directions for this preparation, which are equally applicable to dextro- or laevo-camphor, were taken from the 1923 edition of Vanino's "Preparative Chemie", p. 362.

A solution of 102 g. of camphor in 500 c.c. of dry ether was cooled over ice, and 15·2 g. of sodium were dissolved in the solution. To this 78 g. of amyl nitrite were added. The sodium salt of iso-nitroso camphor was shaken out with water, and after freeing the aqueous extract from ether by a blast of air, isonitroso camphor was precipitated by acidification with acetic acid. The rather sticky crude substance upon crystallisation from a benzene-ligroin mixture yielded 30 g. of faintly yellow crystals of m.p. 110-111°.

In newer text-books, the metallic sodium of this preparation is substitutéd by sodium-potassium amide. We found that the slightly increased yield given by this method is more than offset by the additional

time involved and the difficulties encountered in making the fresh metalamides.

Camphorquinone.

The method given in Volume 3 of the "Die Methoden der Organischen Chemie" (3rd edition 1929), p. 784 et seq., by Houben-Weyl, was followed.

25 g. isonitrosocamphor, moistened with 7·5 c.c. of 40% formaldehyde solution, and with the addition of a drop of concentrated sulphuric acid, was refluxed for a half hour on a water-bath. Some 15 c.c. of concentrated hydrochloric acid was now added and the refluxing continued whilst a gummy oil separated. The reaction mixture was distilled in steam, and the crystals of camphor quinone recrystallised from dilute alcohol. Yield 8 g. ; m.p. 197-198°.

p-Phenylene bis-imino camphor.

The procedure used involved a departure from the published methods. Whilst Forster's method is the quickest, our best yields obtained in this manner were 40%. Singh's method claims yields of 60%, and whilst we did not find it possible to repeat his directions as published, a slight modification also gave us 60% yields.

Forster's Method.

Alcoholic camphor quinone (2 mols) is added to an aqueous solution of p-phenylenediamine hydrochloride

(1 mol) with an excess of sodium acetate present. After one half hour on the water-bath, the golden yellow crystals of p-phenylene bis-imino camphor separated on cooling. Recrystallised from alcohol Forster records m.p. 259° for the d-form. No yield is quoted.

Singh's Method.

Exactly the same proportions of reactants as above, which are however heated in the dry state on a water-bath for 8 hours. The mass was then extracted with hot alcohol, precipitated with water and crystallised as before. M.p. 259-60° (active forms); 252-53° (racemic form). Yields: 60%.

We found that the heating of the dry reactants on the water-bath did not lead to any reaction. Upon heating over a free flame, however, the reaction went forward at once.

Note on purification of p-phenylene bis-imino camphor.

p-Phenylene bis-imino camphor prepared by Forster's method gave large golden yellow needles, which formed slowly from solution, and melted at 259-60° (active form).

The same substance formed by our modified Singh fusion method crystallised as small brown needles, which formed rapidly from a smoky-brown solution, even after repeated recrystallisation. They also melted at

259-60° and showed no differences in a mixed melting point.

The trouble was suspected to arise from black colloidal material, introduced with, or arising from, the p-phenylene diamine hydrochloride.

On pouring the hot, quickly crystallising, cloudy, brown solution of p-phenylene bis-imino camphor down a short Tswett column of $Al_2O_3$ , held as a half-inch layer on the foot of a sintered glass crucible, the solution poured out of the other end, under suction, as a clear and in all respects normal solution, giving the large golden yellow crystals.

The aluminium oxide showed a short, black adsorption band, Apparently this material, which defied normal filtration, must have acted as a nucleus for crystallisation.

This possibly caused the rapid formation of small dark crystals, and at the same time caused this contaminant to be swept out of solution and so rendered recrystallisation ineffective as a means of purification.

Physical properties and constants of p-phenylene bis-imino camphor.

| Proportion of d l -isomeride. | Proportion of d -isomeride. | M.p. |
|---|---|---|
| 100% | 0% | 252-3° |
| 90 | 10 | 253-4 |
| 50 | 50 | 253-4 |
| 15 | 85 | 254-5 |
| 5 | 95 | 257-8 |
| 0 | 100 | 259-60 |

A mixture of equal quantities of d- and l-isomerides melting at 252-3°, shows no depression of m.p. with the racemic compound made from racemic camphor.

The order of rotatory power is

| Solvent. | $[M]^{35°}$ $Na_D$ | $Hg_{yellow}$ | $Hg_{green}$. |
|---|---|---|---|
| Benzene | 6359° | 6577° | 8474° |
| Pyridine | 6292 | 6490 | 8342 |
| Chloroform | 6160 | 6395 | 8193 |
| Acetone | 5624 | 5696 | 7181 |
| Ethyl Alcohol | 5420 | 5537 | 7134 |

These figures, quoted from Singh's paper, show that p-phenylene bis-imino camphor has a specific rotation of 2000° for $Hg_{green}$ and 1500° for $Na_D$.

It is evident that small partial resolutions should be easily detectable by polarimetric methods.

Method of observing polarimetric rotations.

The polarimeter used during the work on racemic m-azo-β-naphthol mandelic acid was a Schmidt and Haensch instrument fitted with a wavelength selector (Doppelmonochromator), and deriving its illumination from a small filament lamp. This instrument only took tubes up to 2 dcm. in length, and so finally most of the work on racemic p-phenylene bis-imino camphor was performed on a Hilger instrument, taking tubes up to 4 dcm., and illuminated by a mercury vapour lamp, giving brilliant yellow (5780 A°) and green (5461 A°) lines.

In all the work, the zero blank of the instrument was carefully checked for each set of readings. In taking the rotation of each sample at least a dozen readings were taken, approaching the matching tints alternately from the positive and negative sides. The readings were then examined for consistency and averaged.

It should always be borne in mind that whilst +0·03° may be regarded as a small rotation, in most of our determinations the opposite and extreme ends of our developed chromatograms yielded results which deviated from zero in both positive and negatuve senses - for example, +0·03° and -0·03°. Thus the total difference of $\Delta$ 0·06° must be the significant magnitude considered in any arguments involving those of experimental error.

Thus if the experimental error involved in a reading is, let us say, 0·02° (it is usually less than this), we may say that any set of readings greater than ±0·01°(△ 0·02°) are entering reality. In actual experimental practice no reliance was placed upon any set of readings unless, when the instrument was set at the average negative reading, and the dextro-rotating tube subsequently placed in position, a definite difference in the half-fields was immediately apparent.

The fact that we obtain readings consistently deviating from zero in both positive and negative fashions from the opposite ends of individual chromatograms, confirms that our results are due to asymmetric adsorption, and also demonstrates that they do not arise, at least in the main, from asymmetric decomposition.

Note on taking rotations through dense solutions.

When observations were taken on concentrated and highly coloured solutions in the polarimeter, it was often found that the half-fields could be matched more easily and with less strain if the centre of vision was directed slightly away and to the side of the visual image.

This practice, known to astronomers, depends on the fact that the "yellow-spot" of the retina, in the normal centre of vision, is practically blind under circumstances

of poor illumination, whilst the surrounding area of the retina undergoes "dark adaption" and so becomes highly light-sensitive. This effect is known as the Purkinje phenomena.

## ADSORPTIONS Of RACEMIC p-PHENYLENE BIS-IMINO CAMPHOR On LACTOSE.

These adsorptions were, in general, carried out exactly as for the racemic m-azo-β-naphthol mandelic acid, and experience showed that the process is governed by several factors.

(a) p-Phenylene bis-imino camphor is insoluble in petroleum, but is readily soluble in benzene, from which solution, however, it is not adsorbed on to this particular preparation of lactose. Light petroleum containing 12% benzene is generally the best solvent. The use of this mixture limits the concentration of p-phenylene bis-imino camphor to 0·006 g. per litre, since this figure represents saturation point at room temperatures.

(b) Upon developing the original adsorption band, an expansion to four times the original length represents the maximum degree practicable, since the faint yellow adsorption band tends to invisibility about this phase. It is also suspected that the prolonged contact of p-phenylene bis-imino camphor involved in

any greater degree of development than this leads to its decomposition.

(c) Owing to the colour of the solution, a final concentration of 0·04 g./100 c.c. in chloroform represents the practical maximum at which solutions may be read with the necessary degree of accuracy in a 4 dcm. tube.

(d) The best conditions for observing the progress of development of the faintly yellow chromatograms are quite critical. The band tends to become prematurely invisible if it is illuminated by sunlight, artificial light or a "top" light. Diffuse, white, north daylight, coming from the side is found to represent the best conditions. The use of a white card also aids the eye in tracing the progress of the development, if held alongside the tube. Ultraviolet light is helpful to some extent, since the adsorption of p-phenylene bis-imino camphor quenches the bluish fluorescence of lactose, and so appears as a "shadow" on the lactose column, but even this becomes faint upon continued development.

(e) Before rotations were taken on the lactose extracts, these had to be purified by means of chromatographic adsorption upon a short column of aluminium oxide. Various other colouring matters were present in small quantities, and whilst they were

demonstrated to be optically inactive, their presence interfered with the polarimetric readings by increasing the density of colour present in the solution.

## TYPICAL EXPERIMENTS.

### Preliminary Test.

Twelve tubes, each 30 cm. by 2·5 cm. and carrying 150 g. charges of lactose, were employed. In all, two litres of a solution of 0·008 g. of racemic p-phenylene bis-imino camphor, in light petroleum (b.p. 80-100°) containing 6% of benzene, were used. This faintly yellow solution was added to the top of each column and allowed to trickle down under gravity until the weak yellow adsorption band filled the upper half of the lactose column. Pure solvent was now added and development continued until the adsorption band filled the whole tube.

The upper and lower halves of the fully developed lactose column were then extruded into separate vessels and their adsorbed material eluted with chloroform after draining off the original petroleum-benzene solvent. The elution was carried out by simply stirring the lactose paste several times with warm chloroform and filtering from suspended lactose.

The yellow chloroform extracts from the upper and lower halves respectively were concentrated to 5 mils.

each, and their rotations taken in 2 dcm. capillary tubes, using the $Na_D$ line. Since 0·008 g. of material was originally employed, and as it was seen to be practically equally partitioned between the fractions, the concentration in the final extract will be of the order 0·004 g./5 c.c. at the maximum - that is,

C = 0·08 g./100 c.c.

In this preliminary experiment the quantity of p-phenylene bis-imino camphor was estimated colorimetrically by comparison with a standard solution in chloroform, in which manner it was adjudged to be C = 0·04 g./100 c.c. in each fraction. The actual weights of material present were not in this instance determined directly by evaporation of the extracts, since possible interference from traces of dissolved lactose and other foreign materials was anticipated. To allow for this contingency, a blank lactose chloroform extract was made up under exactly the same conditions as those obtaining in the above extractions upon the upper and lower halves of the lactose column, and parallel rotations taken upon it to correct for any possible error arising from this source. It seemed inconceivable that any possible decomposition product of p-phenylene bis-imino camphor should have a greater $[\alpha]_D$ than 1500°.

Average rotation of upper halves - +0·04(6)°

Average rotation of lactose blank - +0·00(5)°

Average rotation of lower halves - -0·04(0)°

By difference the corrected rotations are thus:

Upper halves: +0·04(1)°

Lower halves: -0·04(5)°

The extreme difference in visual rotation $\Delta \alpha = 0{\cdot}086°$ should be noted. The convention $\Delta \alpha$ will be used to signify this in further experiments.

The approximate specific rotations may now be estimated:

Upper halves:

Angular rotation $\alpha = +0{\cdot}04(1)°$

Concentration $c = 0{\cdot}04$ g./100 c.c.

Length of tube $l = 2$ dcm.

$$[\alpha]_D^{15} = \frac{\alpha \times 100}{c \times l} = +51°$$

Lower halves:

$\alpha = -0{\cdot}04(5)°$

$c = 0{\cdot}04$ g./100 c.c.

$l = 2$

$$[\alpha]_D^{15} = \frac{\alpha \times 100}{c \times l} = -56°$$

It should be noted that even if the colorimetric estimation were faulty, the minimum possible $[\alpha]_D$ is of the order of $\pm 25°$, provided that a 100% recovery of p-phenylene bis-imino camphor had been made. Actually the colorimetric estimation of concentration in this case was probably too high, owing to the presence of extra colouring matters, possibly resulting from decomposition of the bis-imino compound. Thus the calculated $[\alpha]_D$ of $\pm 50°$ was probably too low, a fact

borne out by later experiments. The original solutions, diluted each to half their original concentrations with chloroform from the lactose blank, still gave appreciable rotations.

Upper: $\alpha = +0{\cdot}025^{\circ}$

Lower: $\alpha = -0{\cdot}015^{\circ}$

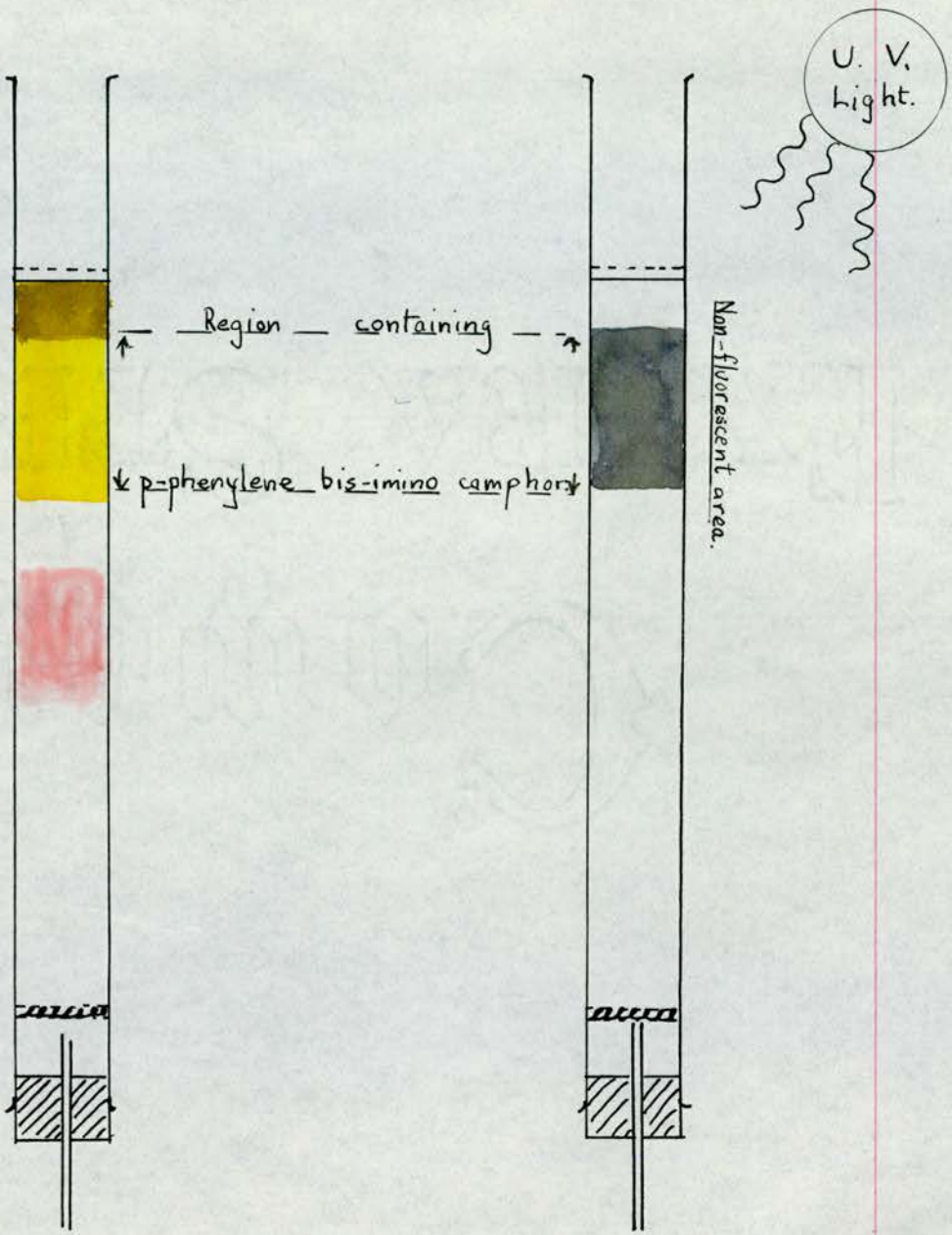
$\Delta\alpha = 0{\cdot}040^{\circ}$

This compares well with half of the original $\Delta\alpha$, $\frac{0{\cdot}086^{\circ}}{2}$.

This preliminary experiment, whilst giving encouraging results, led us to try a repeat experiment on a slightly larger scale, in order to allow of sufficient material to undergo purification at the end of the adsorption. In this way we hoped to be able to weigh and check the melting points of the partially active p-phenylene bis-imino camphors, thus leading to indisputable values for the specific rotations.

Repeat Experiment.

Thirteen tubes, each 60 cm. by 2·5 cm. and carrying 250 g. charges of lactose, were treated with 6 litres of a solution of 0·02 g. of the racemate in light petroleum containing 6% benzene. The adsorption in this instance was carried out on the top third of the tube, before developing to the whole length. The upper and lower thirds were eventually reserved for elution in the same manner as in the last experiment.




Chromatogram.

Ultrachromatogram.

Purification of partially active extracts.

The chloroform extracts of the partially active p-phenylene bis-imino camphor were concentrated by distillation to small bulk. Three times their volume of pure benzene was now added and the distillation once more continued to small bulk. In this way benzene solutions of the extracts were obtained, each about 40 c.c. in volume. These were then adsorbed in a straightforward manner on to a 15 cm. by 1 cm. column of aluminium oxide, where they adsorbed as a short brown band. Development with benzene containing 20% chloroform gave a chromatogram consisting of a series of bands (diagram 48A. ), from which a yellow band containing the p-phenylene bis-imino-camphor was separated. It was noticed that the whole of this yellow band appeared under ultraviolet illumination as a black band (Compare p. 56 and diagram 56A.)

The partially active material was easily eluted from the aluminium oxide by means of chloroform. The chemically pure chloroform extracts were now evaporated to dryness in weighed glass vessels at 35°, and after checking the melting points on a small fragment of material by means of an electric micro-melting point apparatus attached to a microscope, the fractions were carefully weighed on a micro-balance. The whole material from each fraction was then dissolved in 5 mils. of chloroform, and the rotation taken in

a 2 dcm. capillary tube, using the $Na_D$ line.

Upper Thirds: $l = 2.$ $C = 0{\cdot}0364$ g./100 c.c.

0·00182 g. (m.p. 245-50°) dissolved in 5 mils.

Rotation = +0·03(8) $[\alpha]_D = +41°$

Lower Thirds: $l = 2$ $C = 0{\cdot}0396$ g./100 c.c.

0·00198 g. (m.p. 240-50°) dissolved in 5 mils.

Rotation = -0·02(2)° $[\alpha]_D = -28°$

$\Delta\alpha = 0{\cdot}05°$

It is interesting to note that when these solutions were estimated colorimetrically by comparison with a standard, the concentrations obtained in this way agreed to within ±5% with the above figures.

It now appeared to have been established beyond doubt that a partial resolution had been accomplished using the above adsorption technique. The question remained whether this partial resolution could be improved upon, by further treatment of the partially active materials over fresh columns of lactose. Could the resolution thus be made to grow progressively?

To carry out such a programme it was realised that the above experiment would have to be repeated many times on a large scale in order to accumulate a sufficient quantity of partially active starting materials. Other variations under consideration as possible means of enhancing the separation, were proved

to be impracticable. Thus initial adsorption to a fraction of less than one quarter of the column so as to obtain greater subsequent development was shown to be useless (see page 42). Mechanical difficulties were met with in trying to use tubes longer than five feet, owing to the high pressure on the stopper at the lower end of the columns, and difficulties in manipulating the column for fractionation before elution. In one attempt, several tubes were placed in series, one above the other, but this again was abandoned as the lower tubes often tended to drain before the upper ones could pass sufficient liquid down to them.

The solution to these difficulties lay in the use of larger diameter glass tubes (five feet long and four inches in diameter) which we accordingly had specially made for us. These had stout walls of 1/8-inch glass to withstand the expansion of the rubber being in contact with the solvents at the lower end. Such a tube could hold between 10 and 14 lbs. (about 6 kg.) of lactose at one charge. The stout walls, however, introduced another minor difficulty, as the slight yellow tint in the glass tended to obscure the delicate tint of the faintly yellow adsorption band of p-phenylene bis-imino camphor.

In this respect, the position of the head of the band was noted on the outside of the tube at frequent intervals during development, by means of timed referance marks. If the band became invisible during the last

stages of development, its approximate position could then be extrapolated. This procedure proved quite successful.

Whilst awaiting delivery of the large 150 cm. by 10 cm. towers, some further experiments were made, using several 150 cm. by 4 cm. tubes which were found to hand. This tube held 600-800 gm. of lactose, and some 8 litres of a solution of 0·05 g. of the racemate in light petroleum, containing this time 12% benzene, were adsorbed on to the top quarters for 15 successive fillings of these tubes, before development with the same solvent. Altogether 10 kilos of lactose were used in this experiment.

The upper and lower quarters were eluted with chloroform, and purified over Tswett columns of aluminium oxide as before. The rotations were taken in a 2 dcm. capillary tube, using the $Na_D$ line. The concentrations in this instance were estimated colorimetrically after being adjusted to the maximum possible concentration compatible with good visibility in the polarimeter.

Upper quarters: $C = 0{\cdot}050$ g./100 c.c. $l = 2$.

$\alpha = +0{\cdot}082^\circ$ $\quad [\alpha]_D = +90^\circ$

Lower quarters: $C = 0{\cdot}060$ g./100 c.c. $l = 2$.

$\alpha = -0{\cdot}063^\circ$ $\quad [\alpha]_D = -50^\circ$.

$\Delta\alpha = 0{\cdot}145^\circ$

Whilst the magnitude of the partial resolution is no greater than before, the observed angles are larger, and thus make this experiment valuable in confirming the earlier conclusions. Out of the 0·05 g. racemic starting material, some 0·01 g. of partially active material was recovered in a chemically pure state. These fractions were reserved for further readsorption over lactose.

It was also recorded in this experiment that rotations could be taken in 4 dcm. capillary tubes on the available Hilger instrument, using the $Hg_{green}$ (5461 A°) line. Not only will a 4 dcm. tube double the rotation as compared with a 2 dcm. tube, using the same concentration, but owing to dispersion phenomena, the specific rotation of the pure active compound is increased from $[\alpha]_D$ = 1500° to $[\alpha]_{5461}$ = 2000°.

For example, upon diluting the chloroform solution of the lower quarters of the above experiment slightly to C = 0·05 g./100 c.c. in order to fill the 4 dcm. tube, the rotation obtained was -0·13°, corresponding to $[\alpha]_{5461}$ = -66° which is in excellent agreement with $[\alpha]_D$ = -50°. In future it was decided to use such a tube and the mercury green line for rotations. The above results were recorded in Nature, 141, 997 (May 21, 1938).

**Adsorption of partially active p-phenylene bis-imino camphor on lactose.**

The last experiment, described above, was repeated several times, until in all some 0·20 g. of the racemic material had been partially resolved into two active portions obtained from the upper and lower quarters of the tubes respectively. In all, these extensive operations involved the use of about 50 kilos of lactose (about one hundredweight) during the nine weeks in which the adsorptions were running.

Finally, samples about 0·05 g. of each of the partially active dextro and laevo materials, $[\alpha]_{}$ ±60°, were obtained, and these were dissolved separately in 8 litres of the mixed petroleum -12% benzene solvent. The new solutions were passed down separate columns of fresh lactose in exactly the same fashion as the original racemic solution. However, after development, only the top quarter of the column containing the partially active dextrorotatory product and the lower quarter of the column containing the laevorotatory product were reserved for polarimetric examination.

These quarters were purified over short columns of aluminium oxide and rotations taken in a 4 dcm. tube, using the Hg yellow line (5780 A°). It was found in this case that the Hg green (5461 A°) line was obscured for a reason to be discussed later.

Upper quarters: (twice adsorbed product)

$l$ = 4 dcm. C = 0·004 g./100 c.c. estimated colorimetrically

$\alpha = +0·018°$

$[\alpha]^{18°}_{5780} = +113°$

Lower quarters: (twice adsorbed product)

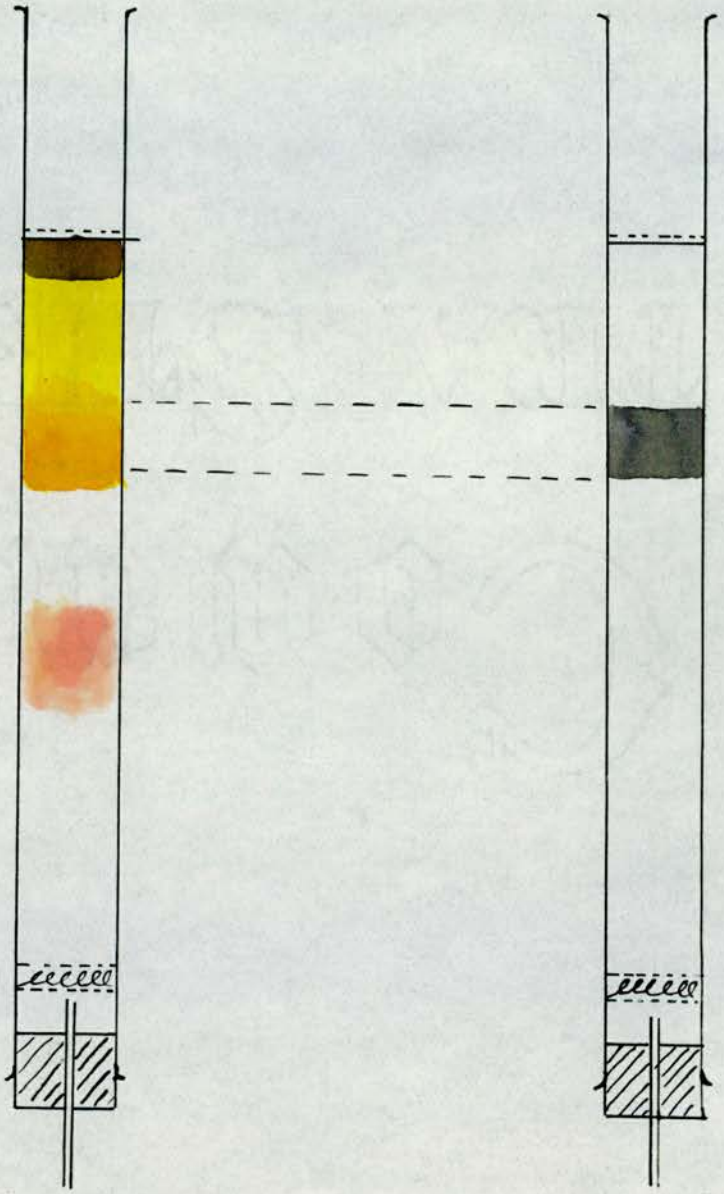
$l$ = 4 dcm. C = 0·030 g./100 c.c. estimated colorimetrically

$\alpha = -0·103°$

$[\alpha]^{18°}_{5780} = -86°$

These results seem to suggest that a progressive resolution is possible by the readsorption, over fresh lactose, of partially active material, but on the whole they are disappointing in view of the small increases after the application of so much labour and time.

The time factor involved is suspected to have had some effect upon the final yields of partially active material. Sometimes, during this work, the p-phenylene bis-imino camphor remained adsorbed on the lactose for long periods before elution. As small recovery yields were recorded along with large quantities of dark brown unknown foreign materials, we were driven to the conclusion that some decomposition had taken place. It is a fairly widely established fact that many substances, after prolonged adsorption, undergo constitutional changes.



Chromatogram.

Ultrachromatogram.

It must also be recorded that the elutions with chloroform upon the portions of the chromatograms were carried out, in this instance, in large copper soxhlets, capable of holding about 2 kilos of lactose at one charge.

From later work also, it appears certain that these continuous extractors are so efficient that many other "foreign" materials are extracted from the lactose, formerly untouched by the milder original method of extraction (p.44.).

A blank extraction of B.P. lactose showed an appreciable yellow colouring of the chloroform extract. Further, these foreign yellow materials of lactose origin take up their position in the purification chromatogram over aluminium oxide adjacent to the yellow band due to p-phenylene bis-imino camphor, from which they are nearly indistinguishable in daylight, and so were included, in this instance, unwittingly in the final solutions for polarimetric examination. This led to the following phenomena:

(a) The extinction of the Hg. green (5461 A°) line.

(b) Lowering of the estimated $\alpha_{\text{Hg.yellow}}$ (5791 A°) when concentration is determined colorimetrically.

(c) Difficulty of obtaining a chemically pure solid product upon evaporation of the final solutions.

It was later noticed by examining the yellow band of the aluminium oxide chromatograms under ultraviolet

light that only the lower portion of this band appeared as the typical black (non-fluorescent) band associated with p-phenylene bis-imino camphor. When this part alone was separated, rotations using $\alpha$ $Hg_{5461}$ could then be determined with ease. (Diagram 56A.)

Adsorption of a synthetic racemic mixture of d- and l- p-phenylene bis-imino camphors on lactose.

This experiment is essentially a repetition of the experiment on page 47 , which made use of racemic p-phenylene bis-imino camphor prepared directly from racemic camphor. It was primarily carried out to confirm the suggestion that no internally compensated meso-isomer arose when the racemic bis-imino derivative was made from racemic camphor. Equal weights of pure dextro and laevo p-phenylene bis-imino camphors (m.p. 259-60°) were mixed together and, after four recrystallisations, fine golden yellow needles, of m.p. 252-53°, were obtained (see p.39·), whose solution was optically quite inactive.

Should there have been present some meso-isomer in the racemic mixture used formerly, we should have expected in this latest experiment to have reached a slightly greater degree of resolution. Actually the resolution obtained, whilst at first sight lower, was probably of the same order.

Upper quarters:

Weight of material: 0·0072 g. dissolved in 10 c.c. chloroform.

C = 0·072 g./100 c.c. $l$ = 4 dcm. using $Hg_{5461}$

Rotation: $\alpha$ = +0·061°

$$[\alpha]^{18°}_{5461} = +21°$$

Lower Quarters:

Weight of material: 0·0184 g. dissolved in 10 c.c. chloroform.

C = 0·184 g./100 c.c. $l$ = 4 dcm. using $Hg_{5461}$

Rotation: $\alpha$ = -0·180°

$$[\alpha]^{18°}_{5461} = -25°$$

$$\Delta\ \alpha = 0·24°$$

The specific rotations recorded are certainly too low, for in spite of purification of the product over a column of aluminium oxide, the weighed material consisted of yellow crystals of p-phenylene bis-imino camphor embedded in a non-volatile clear yellow liquid of an oily character. Long treatment of this residue with warmth in vacuum only converted the mixture to an insoluble resin. However, a rough colorimetric determination of the concentration had been made before this stage, and this had suggested specific rotations of ±50°, comparable with the results obtained with the ~~results obtained with the~~ derivative made from racemic

camphor.

Once again this difficulty was traced to the too highly efficient soxhlet extractors used during the elution of the lactose chromatogram with chloroform, since the same yellow oil could be obtained from "pure Lactosum B.P." by direct extraction. Even the careful mechanical separation of the ultrachromatogram obtained by adsorption of the partially active fractions on aluminium oxide sometimes does not achieve efficient separation of the imino camphor from the oil. This incomplete separation arises simply because it is extremely difficult to cut away an area of a chromatogram containing the camphor derivative from surrounding areas demonstrated to be highly charged with the oil. It is here that the "dome" formation described on page 5. is very troublesome, especially if suction is applied to hasten the process of purification over aluminium oxide.

The foreign material extracted by chloroform from lactose consists mainly of three substances, namely, some colourless feathery needles embedded in a faintly yellow oil which contains a volatile fraction with a distinctive odour. These foreign materials, which no doubt arise from the milk used as the source of lactose, were demonstrated to be optically inactive, at least in the concentrations in which they were present in the bis-imino-camphor extracts already examined. Yet another source of contamination of the adsorbent lactose

was traced to the heating at first used in drying the lactose. Even mild heating at 90° for three hours was found to produce slight traces of a brown material, probably of a caramel nature.

## SUGGESTED IMPROVEMENTS In EXPERIMENTAL TECHNIQUE.

(a) To purify all lactose before use by extraction with the elutant to be employed.

(b) To avoid heating the lactose as far as is practicable.

(c) To carry out adsorption, development and elution as rapidly as possible.

(d) To avoid the soxhlet extraction process and return to the milder conditions of simple extractions with warm chloroform.

(e) To purify the product carefully over aluminium oxide, cutting the column under ultraviolet illumination and drastically rejecting any regions of the main band bordering upon other components of the ultrachromatogram.

(f) To repurify this product by a second adsorption on aluminium oxide in cases of difficulty.

(g) To wash the partially active residues of p-phenylene bis-imino camphor obtained after final evaporation from chloroform with light petroleum (b.p. 40-60°) if they appear still oily. It was found that the oil, before prolonged heating led to its resinification, was miscible with this solvent. The p-phenylene bis-imino camphor is, of course, insoluble in this medium.

The schemes (a), (b), (c), (e) and (g) were incorporated in a new experiment which otherwise repeated the technique used before. A definite improvement followed the primary extraction of the lactose.

Purification of lactose by primary extraction with chloroform.

Since chloroform was the elutant employed at the end of all the adsorptions, which resulted in the co-extraction of foreign materials along with the p-phenylene bis-imino camphor, it was thought wise to extract all the lactose with this solvent before use. Alcohol and carbon tetrachloride were demonstrated to be less effective as solvents.

The warm chloroform used, we imagined, in addition to freeing the surface of the lactose from any oily materials, would also tend to remove any surface moisture and thus superficial drying might result. In this

respect, it must be borne in mind that we are concerned almost entirely with the surface of the lactose hydrate crystals, and not with the interior of the crystals which would certainly need much more drastic treatment by heating in vacuo before producing appreciable dehydration.

The lactose was extracted by warming it, four kilos at one time, in a clean enamelled bucket with two litres of pure, dry chloroform. After gentle simmering, with stirring, for about five minutes on a water-bath, the lactose was filtered off by suction. This extraction was carried out twice on each batch, and after further washing with several quantities of hot chloroform on the filter, the lactose was air-dried in the warm air from a steam radiator, before being placed in a vacuum chest for three hours in the cold, in order to remove the last traces of chloroform.

It is very important to remove the last traces of chloroform, as these appear to be most strongly attached, and if allowed to remain, seriously diminish the adsorptive powers of the lactose.

Lactose prepared in this manner proved to possess greatly enhanced powers of adsorption, as compared with the original raw heat-dried material. Not only did it adsorb the racemic camphor derivative at least three times as strongly, but the resulting resolutions gave products of greatly increased optical activity. The

lactose thus prepared will be referred to as "activated lactose".

## ADSORPTION Of The RACEMATE On "ACTIVATED LACTOSE".

This experiment is a repetition of those already described, in most details, except in the use of activated lactose. In five litres of light petroleum containing 12% benzene, 0·030 g. of racemic p-phenylene bis-imino camphor were dissolved, and adsorbed on to the top 1/8th of the column of activated lactose, 6 kilos of which were held in a tube 130 cm. by 10 cm. The tube, after complete development which lasted about 60 hours (compared with the 30 hours required for non-activated lactose), was quartered. The results are best expressed in a tabular form. The quarters of the tube were numbered 1 (top), 2, 3 and 4 (foot) from the top downwards. The "crude" rotations recorded are those taken after purification upon aluminium oxide, but before washing the partially active residues, obtained by evaporation of the chloroform solutions, with light petroleum (b.p. 40-60°), to rid them of any possible oil. The samples were then carefully, but without difficulty, worked up into dry crystalline powders. The melting points of each fraction were taken and the materials carefully weighed before being each dissolved in 10 c.c. of chloroform in graduated flasks. The final

rotations were then taken in a 4 dcm. capillary tube, using the $Hg_{5461}$ line. The top quarter (1) proved to have very little material in it, and was therefore added to the next fraction (2).

| | 1 (top) | 2 | 3 | 4 (foot) |
|---|---|---|---|---|
| "Crude" $\alpha_{5461}$ | +0·100° | +0·453° | +0·448° | -1·042° |
| $\Delta\ \alpha$ | 1.495° | | | |

| | (1 + 2) | 3 | 4 |
|---|---|---|---|
| Pure, dissolved in 10 c.c. chloroform | 0·00232 g. | 0·00542 g. | 0·00178 g. |
| M.p. | 252 - 54° | 250-53° | 252-54° |
| Pure $\alpha_{5461}$ | +0.450° | +0.332° | -0.524° |
| $[\alpha]^{18°}_{5461}$ | +485° | +154° | -737° |
| $\Delta\ \alpha$ | 0.974° | | |

For comparison and reference, the following figures may be quoted:-

Pure racemic mixture: m.p. 252-53°

Pure active compound: m.p. 259-60°

Pure active compound $[\alpha]^{18°}_{5461}$ = 1975°.

At the best we have therefore attained an active product containing about two parts *l*-isomer to one

part d-isomer. Considering that we started with only 0·030 g. of a material incapable of direct resolution by normal methods, and that we have demonstrated it to exist in optically active forms, before recovering one third of the material in a chemically pure and partly active state, this experiment suggests that the adsorption method of resolution may prove of practical value in certain selected cases.

Attempts to enhance the partial resolution by readsorption over activated lactose.

The question once more arises, can this resolving process be made progressive so as to result finally in an optically pure material.

To avoid the tedious work and excessive time required to work up, naturally, sufficient material for readsorption experiments (see page 54), it was decided to make up synthetic partially-resolved mixtures from the optically pure materials corresponding to the product obtained at the end of each previous resolution experiment. This procedure is a strictly logical one designed to save time and labour.

The optically pure materials we found to have specific rotations of

$[\alpha]_{5461}^{15^\circ}$ -1980° and +1970°

Average 1975°.

Under the conditions of our experiment we have to

assume a possible error of 0·01° in the polarimetric readings and 0.00002 g. in the weighings at the maximum. This suggests that in the region of complete resolution a tolerance of ±20° must be allowed for on the figures for the specific rotations.

Typical readsorptions:

A mixture of 0·020 g. of pure l-bis-imino camphor and 0·010 g. of pure d-bis-imino camphor ($[\alpha]_{5461}$ = -650°) was dissolved in 5 litres of light petroleum containing 12% of benzene. On attempting to adsorb this on to the spent activated lactose from the last experiment, it was adsorbed very strongly as a narrow yellow band. This band scarcely moved upon development, and after several hours decomposed into a brown tinted substance which certainly contained no bis-imino camphor.

It had been hoped, by using spent lactose, which had been continuously extracted with hot chloroform at the end of the previous experiment, to save much time and labour. Possibly, however, it was over-activated after this treatment and was possessed of too high an adsorption coefficient.

Two courses are open:

(a) To use the above lactose in conjunction with a solvent containing more benzene, so making the adsorption milder.

(b) To return to lactose activated by one simple treatment with chloroform.

In further experiments a combination of these two methods was adopted, along with some modifications previously indicated (page 60 (d) ). Finally the best conditions were found to be those where the simply activated lactose is used in conjunction with light petroleum containing 25% benzene. The enhanced adsorption of the lactose and the greater powers of solvency of the 25% benzene allow of a much smaller experimental set-up to be used, as illustrated in the following experiment.

First stage of readsorption:

0·02034 g. of laevo-isomer

0·01010 g. of dextro-isomer.

The above mixed materials, $[\alpha]_{5461} = -660°$, were dissolved 500 c.c. of benzene and 1500 c.c. of light petroleum added (25% benzene) to the solution.

The bis-imino camphor was adsorbed on to the upper quarter of a comparatively small tube, 130 cm. by 6 cm., containing 2·5 kilos of activated lactose. After a rapid development with the same solvent, the upper and lower quarters were cut and examined as before. The time involved in this experiment was reduced to between sixteen and twenty hours.

Upper fraction:

0·00160 g. material, mp. 248-52°,

which was dissolved in 10 c.c. chloroform.

$\alpha = -0·104°$ $\quad l = 4$

$[\alpha]_{5461} = -162°$

Lower fraction:

0·00262 g., m.p. 253-56°, which
was dissolved in 10 c.c. chloroform.

$\alpha = -1·456°$ $\quad l = 4$

$[\alpha]_{5461} = \underline{-1389°}$.

Second stage of readsorption.

0·02037 g. l -isomer
0·00398 g. d-isomer $\quad [\alpha]_{5461} = -1330°$

Treated as in previous experiment.

Lower fraction:

0·00218 g., m.p. 254-56°, which
was dissolved in 10 c.c. chloroform.

$\alpha = -1·565°$ $\quad l = 4$

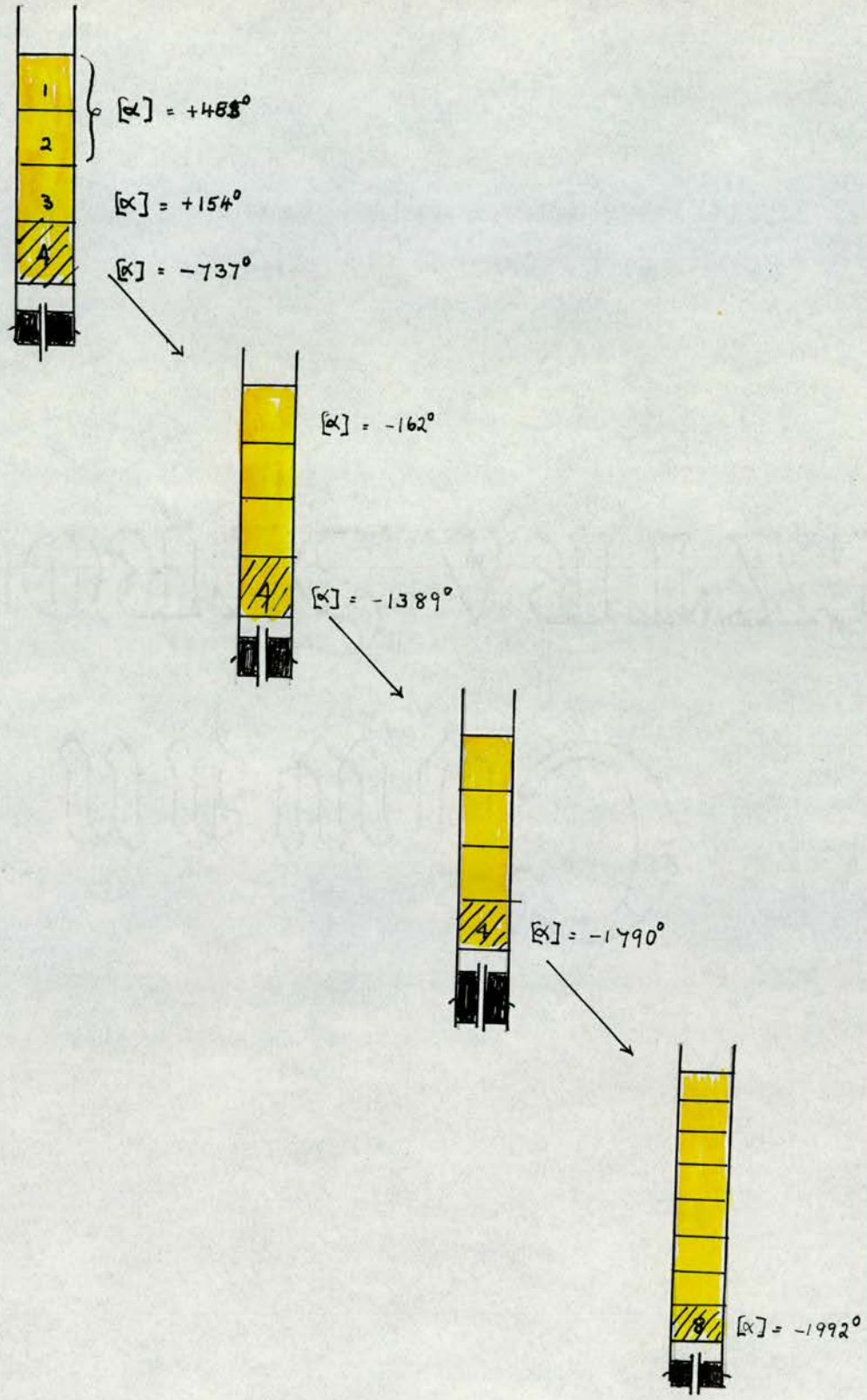
$[\alpha]_{5461} = \underline{-1790°}$

In this case 30% benzene in the solvent had been used, but adsorption was markedly poorer, so a return to 25% was decided upon in the next stage.

Third stage of readsorption.

0·02140 g. l
0·00155 g. d $\quad [\alpha]_{5461} = -1708°$

Treated as in previous experiment.


1
2
3
A
[α] = +488°
[α] = +154°
[α] = −737°
[α] = −162°
A
[α] = −1389°
A
[α] = −1790°
B
[α] = −1992°

Lower eighth:

0·00190 g., m.p. 257-60°, which was dissolved in 10 c.c.

$\alpha = 1{\cdot}515° \qquad l = 4$

$$[\alpha]^{15°}_{5461} = \underline{-1992°} \pm 20°$$

This last figure indicates that a full resolution has been reached after four successive adsorptions.

A new method of resolving certain types of racemic compounds is thus made possible by these results, especially when the general features had been confirmed in some independent work recorded in the next few pages (page 76.).

Quite apart from the intrinsic utility of the method, it suggests a new sensitive tool which may be used in probing the nature of adsorption forces. The method also opens up the possibility of resolving racemic hydrocarbons incapable of direct resolution by normal methods.

## FURTHER ATTEMPTS To Develop NEW METHODS Of Resolving RACEMIC COMPOUNDS.

As outlined in the Introduction (p. 13), a consideration of Willard Gibbs' theories suggest that if one dissolves a racemic substance in an optically active medium, the stereoisomer which causes the greatest lowering of the free surface energy must be concentrated at any interface.

### Attempted resolution from active solvent.

d-Pinene was chosen as the active solvent. This, however, required the addition of some 20% benzene before any appreciable amount of racemic p-phenylene bis-imino camphor would dissolve in it.

In 400 c.c. of these mixed solvents, some 0·05 g. of the racemate was dissolved, and the solution allowed to flow down a column of aluminium oxide contained in a tube, 100 cm. by 2 cm., when a yellow adsorption band was seen to form in the upper third of the column. The chromatogram was developed with the same solvents until coloured fractions of liquid began to pass out of the lower end of the column. Unfortunately the fractions defied purification, since the d-pinene appeared to have polymerised into a gummy substance. Rotations could thus not be observed with any shade of accuracy.

The principle remains yet to be carried out under better conditions.

Attempted resolution involving known intermediate complex formation.

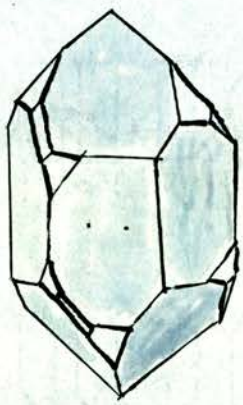
Eisenlohr and Meier (Berichte, 71, 1005 (1938) ) report successful resolutions of racemates in some cases by causing the racemate to form a loose molecular complex with an active substance, which on crystallisation led to the separation of the racemate into its active forms. In many cases the existence of such complexes may only be deduced from a study of the melting point curves. The formation of the complexes does not depend upon the normal valency forces, and it was thought that they might be regarded as having some relation to adsorption forces.

These authors reported that whilst amygdalin and racemic menthol formed a well-defined complex, it was however too unstable to allow of the crystallisation of the diastereomers.

We decided to pack a Tswett column with dry powdered amygdalin, and to pass a 6% racemic menthol solution in light petroleum (b.p. 40-60°) down it. The effluent fractions we found to be possessed of a slight negative rotation which was eventually traced to dissolved amygdalin. It appears that instead of the menthol being adsorbed on to the surface of the amygdalin

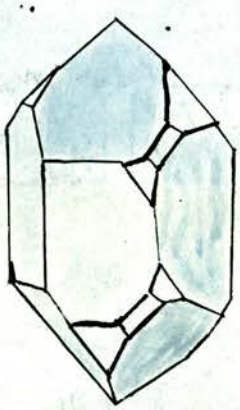
the surface of the amygdalin is removed by the menthol solution and is so carried down the column in solution. Suitable modifications of this experiment might give interesting results, however.

# Quartz Crystals.



Left-handed

(ideal)



Right-handed.

Right-handed
common form (etched)

## ADSORPTIONS Using RIGHT And LEFT-HANDED QUARTZ CRYSTALS.

When it became apparent that we were indeed producing partial resolutions of racemic substances over lactose, we reviewed in a more favourable light the work of Tsuchida on quartz (p. 16). It occurred to us that his simple technique could be much improved by using the quartz powder in a Tswett column.

Quartz owes its activity to the presence of an asymmetric crystal unit, in which three silica ($SiO_2$) molecules form a right or left-handed spiral. Since a crystal of quartz may be regarded as a giant molecule, we thus see that the surface of powdered active quartz is closely akin to that of a powdered active sugar-like lactose.

### Selection of right and left-handed quartz crystals.

Out of a batch of quartz crystals, those having well-formed facets and regular transparent bodies were selected. From these, crystals showing the hemihedral facets, as in the diagram 73A , were placed into separate batches as right and left-handed. The remaining and major portion of the selected crystals were etched with hydrofluoric acid and examined under a lens for the arrow-headed indentations by which the right and left-handed varieties may be distinguished

(see diagram 73 A, foot.)

A good deal of confusion exists in the literature over these conventions which may be summarised for the right-handed crystal, the left being enantiomorphous.

(a) Hemihedral facets pass off to top-right when held upright as in diagram.

(b) The normal facets show arrow-shaped pits pointing to the left ( < ) when etched, as illustrated in diagram.

(c) Crystal rotates polarised light in a negative fashion when viewed in a polarimeter.

(d) To a physicist the crystal rotates light in a positive fashion, since he views it in the direction of propagation of the light.

Reduction of quartz to powder.

For this important process, no reasonable account is available in the literature; the fact that quartz is almost as hard as diamond makes it rather a difficult subject. Fortunately it is relatively brittle, rendering it vulnerable to crushing.

Industrially quartz is easily powdered by heating it to red-heat and then quenching in cold water. This is useless for our purposes as it destroys the quartz crystal unit.

The separate batches of right and left-handed crystals were powdered, after smashing on an anvil to

chips, 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 the larger particles and the steel filings removed magnetically. The final grinding to a fine sand was carried out in a granite, powder-driven mortar, with a loaded, idling, eccentric pestel. In this way a kilo of each variety was worked up into a fine powder, contaminated slightly with granite.

Adsorptions on quartz.

We selected for resolution the highly active racemic potassium cobalti-oxalate $K_3Co(OX)_3$, which is soluble in water and has a reputed specific rotation $[M] = 50{,}000°$. This salt was easily prepared in a straightforward manner according to text-book methods. After one trial adsorption, the work was abandoned for a variety of reasons.

(a) The salt did not adsorb strongly from aqueous solutions.

(b) The salt not only racemises rapidly but is unstable in light.

(c) Immediately after starting this experiment, a communication from Professor Karagunis of Athens was received, stating that he had seen our letter in Nature 141, 997 (1938) and that he had just read a paper before the Athens Academy describing small partial resolutions carried out with a

racemic chromium complex salt $[Cr(en)_3]Cl_3$ over quartz powder used in a Tswett column.

His results were summarised in a letter to Nature (July 23, 1938) and published in the "Praktika de l'Academie d'Athens", 13, 1938, p. 414.

Karagunis' Results.

By pouring a 10% aqueous solution of his racemic complex down a dry column of freshly-heated active quartz powder, and finally eluting the adsorbed salt through the tower by developing with aqueous alcohol, the filtrate and subsequent washings through the column showed regular variations in activity.

| Adsorbent | Direct Filtrate | Progressive Elution | | | |
|---|---|---|---|---|---|
| | | 1st Fraction | 2nd | 3rd | 4th |
| l-Quartz | -0·023° | -0·041° | +0·020° | +0·108° | - |
| l-Quartz | -0·060° | -0·121° | -0·018° | +0·050° | +0·034° |
| d-Quartz | +0·023° | -0·020° | -0·050° | - | - |

These small rotations, about which no relevant details of tube length, concentration and so on are given, indicate a partial resolution and so support in principle our results with lactose. Since the specific rotation of the complex used is 3400°, these figures can only represent a small partial resolution.

In addition to stating these results, Karagunis

also criticises the results of Tsuchida (p. 16) and denies that these were due to asymmetric adsorption over quartz. He compares them with the results of Ostromisslensky (Ber., 41, 3035 (1908) ) in support of his views. In this way he implies that he is the first to demonstrate asymmetric adsorption.

It will be remembered that Tsuchida stirred quartz with a warm saturated solution of his racemic complexes, and upon cooling, he found the supernatant liquid to be slightly active. Karagunis suggests that this involved an asymmetric crystallisation, implying that the isomers were apparently being unevenly seeded out in the presence of active quartz powder.

We incline to the view that all these results, including those of Ostromisslensky, are at least primarily due to asymmetric adsorption.

Theoretical Example.

If the presence of d-quartz in a saturated solution of a racemate induces the ?-isomer to crystallise out of solution preferentially, this crystallisation must have at least had its start on the surface of the quartz, whatever secondary processes may later intervene.

The quartz cannot act by its mere presence, and as in the catalysis of surfaces, it is unreasonable

to postulate extra-surface forces.

The l-isomer must then have first crystallised on the surface of the d-quartz. Subsequent deposition upon this original layer will involve the ordinary effects of "seeding out" a component from solution.

Any preferential crystal growth on the surface of the quartz must then have been due to adsorption, since adsorption simply means any force leading to a concentration at an interface. If the crystal growth is composed of one enantiomorph of the racemate only, then we have an example of asymmetric adsorption.

One may go a step further with the above reasoning and state that crystallisation, in general, is only a special case of adsorption, where the adsorbent and adsorbate are one and the same substance.

In the case of ordinary adsorption a layer of adsorbate forms on the surface of a foreign adsorbent material. In crystallisation the solid phase may be regarded as auto-adsorbing, each adsorbed layer of molecules forming a fresh adsorbing surface.

We may thus say that a perfect example of asymmetric adsorption is afforded by Pasteur's original method of resolution.

When a saturated solution of sodium ammonium racemate is allowed to crystallise below 27° it appears as both left and right-handed crystals in equal weights. These crystals have the property, in presence of the

saturated solution, of selecting only the isomer of which they are composed. Thus a d-crystal only adsorbs on to its surface d-molecules and rejects the l-molecules. It is, in fact, a case of 100% asymmetry in adsorption.

Adsorption of p-phenylene bis-imino camphor over quartz.

The resolution of p-phenylene bis-imino camphor was attempted over l-quartz in a Tswett column from benzene solution, and although slight adsorption took place, the filtrates at no time showed activity.

Kieselguhr.

Whilst considering these experiments with quartz, it occurred to us that any silica mineral, laid down under the agency of vital forces, might bear a twisted structure or surface. Thus it was thought that the siliceous skeletons of certain minute protozoa, found in the mineral Kieselguhr, might quite possibly have an asymmetric surface. In a picturesque way one could imagine the fossilised imprints of the living tissue which laid down these skeletons leaving their traces. These skeletons must have been built up in intimate contact with living tissues, most probably of anasymmetric nature. Whilst it was found that p-phenylene bis-imino camphor is exceedingly strongly adsorbed from benzene on to Kieselguhr,

no practical method of applying this to a Tswett analysis has yet been devised, owing to the fineness of the Kieselguhr powder, which packs to such an extent as to stop the flow of liquid in a column.

## SUMMARY OF THESIS.

Tswett's chromatographic analysis method is outlined and a possible extension to the separation of optical isomers devised (pp. 1 - 17).

The results using racemic p-phenylene bis-imino camphor over lactose result in a partial resolution (p. 44 ).

A means of activating commercial lactose is discovered (p. 61. ) and this leads to a complete resolution (p. 69. ). This constitutes a new method and is the first case of complete resolution to be effected by asymmetric adsorption.

The work is confirmed in principle by the independent work of Karagunis on quartz (p. 76. ).

A general theory uniting asymmetric crystallisation and asymmetric adsorption is outlined (p. 74. ).

Several other experimental schemes, not as yet applied successfully, have been indicated.

# P O S T S C R I P T.

The author wishes to express his debt to the Carnegie Trust for the scholarship which enabled him to carry out the work described in this thesis. He was also in receipt of the Mackay Smith Scholarship in Chemistry.

To his Director, Dr H. Gordon Rule, the author owes inexpressible thanks for the advice, encouragement and help liberally given throughout the period of research.

The research could not easily have been carried out without the facilities for large-scale preparations made available by Dr D. Bain of the Technical Chemistry Department.