332. *A New Method of resolving a Racemic Compound.*

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dl-p-Phenylenebisiminocamphor is shown to be resolvable by taking advantage of the difference in the adsorption coefficients of the *d*- and the *l*-form for solid lactose. The racemic compound is adsorbed in the upper part of an upright tube filled with lactose, and the adsorbed layer is washed with solvent until it has expanded to the full length of the tube. Material recovered from the upper portion of the tube is found to be dextrorotatory and that from the lower portion lævorotatory. The degree of rotation can be increased by repeating the process until the optically pure product is obtained. The process is obviously limited to racemic compounds which can be adsorbed on an optically active adsorbent and whose active components possess different adsorption coefficients.

THE literature contains numerous references to the possibility of resolving racemic compounds by use of adsorption methods. In 1904 Willstätter (*Ber.*, **37**, 3758) attempted unsuccessfully to demonstrate a selective adsorption of one of the active components of a racemic alkaloid on wool or silk. Some years later Porter and Ihrig (*J. Amer. Chem. Soc.*, 1923, **45**, 1990) claimed to have effected an almost complete resolution of *dl-m*-β-naphthol-

azomandelic acid by preferential adsorption of the *d*-form on wool, but this resolution could not be confirmed either by Brode and Adams (*ibid.*, 1926, **48**, 2193, 2202) or by the present authors. Very small partial resolutions of dyes by use of wool have been described in one or two isolated cases by Ingersoll and Adams (*ibid.*, 1922, **44**, 2930) and by Morgan and Skinner (J., 1925, **127**, 1731).

Experiments with racemic metallo-organic complexes, *e.g.*, *dl*-chlorobis(dimethylglyoxime)diamminocobalt, were made by Tsuchida, Kobayashi, and Nakamura (*Bull. Chem. Soc. Japan*, 1936, II, 38), in which a warm saturated solution of the salt was allowed to cool over powdered *d*- or *l*-quartz. The residual solutions after decantation were generally, although not invariably, faintly active, the sign of activity in any given case varying with that of the quartz employed. Still more recently, Karagunis and Coumoulos (*Praktika*, 1938, **13**, 414; *Nature*, 1938, **142**, 162), adopting the same technique of chromatographic adsorption analysis as that used by the authors (*ibid.*, **141**, 917), obtained evidence of the differential adsorption of the *d*- and the *l*-component of the racemic chromium complex, $[Cr(en)_3]Cl_3$, upon powdered active quartz. With the exception of one unconfirmed result recorded by Porter and Ihrig, none of the above cases represents more than an extremely small partial resolution, no specific rotations are calculated, and all polarimetric readings fall within the range 0·01—0·12°, the two largest being 0·11° and 0·12° observed by Karagunis and Coumoulos.

In the present investigation use was made of the racemic compounds β-naphtholazomandelic acid and *p*-phenylenebisiminocamphor; among the adsorbents examined were calcium *d*-tartrate, powdered active quartz, emulsin, maltose, glucose, sucrose, and lactose. Indications of small partial resolutions of the above order of magnitude were obtained with the azo-compound on *d*-lactose and details of these experiments are given in the experimental section.

A complete resolution of *dl*-*p*-phenylenebisiminocamphor, $C_6H_4(N{:}C_{10}H_{14}O)_2$, was effected by using lactose. The general method of procedure was to allow a solution of the iminocamphor in light petroleum containing 12% or 25% of benzene to flow through an upright adsorption tube packed with lactose under the same solvent mixture. As soon as the pale yellow adsorbed layer occupied a sufficient length ($\frac{1}{4}$ to $\frac{1}{8}$ of the tube according to circumstances) it was developed by washing with the pure solvent mixture until it expanded to the foot of the tube. Towards the end of the development the yellow colour of the adsorbate became very faint, and it was found advisable to view the tube in a diffused north light; also it was usually necessary to estimate the time at which the process was complete by measuring the rate of expansion of the band while it was clearly visible in the upper three fourths of the tube. Excess liquid was then drained from the tube and the pasty contents extruded, the top and the bottom section (generally quarters or eighths) being collected separately. On extraction by agitation with warm chloroform, the crude *p*-phenylenebisiminocamphor was recovered. The *d*-iminocamphor is more strongly adsorbed on *d*-lactose than the *l*-iminocamphor; hence the product obtained from the top section is dextrorotatory and that from the bottom section lævorotatory. Greatly improved efficiency was gained by treating the lactose prior to use and by submitting the recovered iminocamphor to purification before polarimetric examination.

Experiments were also carried out with highly purified lactose ("AnalaR"). This adsorbed the iminocamphor strongly, but was unsatisfactory in practice because after some hours (and before the development could be completed) the material adsorbed in the upper part of the tube underwent change and became dark. Qualitative tests indicate that this dark product, which cannot be developed or extracted with chloroform, is probably a *p*-phenylenebisimino-derivative of lactose; it was observed on some occasions when "activated" Lactosum, B.P., was used and the adsorbed material had been allowed to remain in prolonged contact with the adsorbent.

Activation of Adsorbent.—Test experiments showed that the untreated Lactosum, B.P., when dried for 3 hours at 40° in a vacuum, adsorbed the racemic compound weakly and that the final elution of the lactose with chloroform removed not only *p*-phenylenebisiminocamphor but also a yellow oily material, together with traces of yellow and brown dyes. Although these impurities were only extracted in exceedingly small quantities, the

oil, at least, was comparable in amount with that of the accompanying iminocamphor. The oily material was soluble in light petroleum; it resinified on heating and was present in the original lactose, from which it could be largely removed by preliminary treatment with chloroform. For this purpose the lactose (4 kg.) was boiled for about 5 minutes with chloroform (2 l.). After filtration with the aid of a pump, the extraction was repeated, and the lactose washed with warm chloroform on the filter. Finally, it was dried in a current of warm air and placed in a vacuum chest in the cold for 3 hours to remove the last traces of chloroform, which, if allowed to remain, seriously diminished the adsorptive powers. Activated lactose so prepared not only adsorbed the racemic *p*-phenylenebisiminocamphor three or four times as strongly as the original, crude, heat-dried lactose, but the resulting resolutions gave products of greatly increased optical activity (see summary on p. 1571).

Purification of Recovered p-*Phenylenebisiminocamphor.*—The above-mentioned impurities could be eliminated by evaporating the chloroform, dissolving the residue in benzene (40 c.c.), and allowing the solution to flow down a short tube (15 × 1 cm.) packed with Merck's aluminium oxide immersed in the same solvent. The resulting brown adsorption layer at the top of the tube was developed by washing with benzene containing up to 20% by volume of chloroform. In this manner the single brown layer was expanded into a narrow upper brown band merging lower down into a wider yellow band, the lowest part of which was somewhat deeper in tint than the remainder. Still further down the tube, and divided from the above bands by a layer of colourless alumina, was a faint pink band. When viewed under ultra-violet light, the lower part of the central yellow layer, which alone contained the iminocamphor, appeared as a dark zone. Working under ultra-violet illumination the column of aluminium oxide could therefore be pushed out of the tube, and the dark zone cut out and extracted separately with chloroform. Subsequent evaporation of the solvent generally gave the pure iminocamphor as a clear yellow crystalline solid. In a few cases final washing with cold light petroleum was employed in order to remove traces of oily matter.

Typical Separations.—I. About 0·05 g. of racemic *p*-phenylenebisiminocamphor, dissolved in 8 l. of petroleum–benzene (8 : 1), was adsorbed on dried, untreated Lactosum B.P. (about 10 kg.) contained in 12 tubes, so that the yellow adsorbed layers constituted one fourth of the tube lengths. After development (30 hours) the upper and the lower fourths were separated and extracted. The upper fourths gave 0·0050 g. of the bisiminocamphor, $[\alpha]_D^{16°}$ + 90° (α + 0·082° in chloroform,* $l = 2$, $c = 0·05$); the lower fourths gave 0·0060 g., $[\alpha]_D$ − 50° (α − 0·063°, $l = 2$, $c = 0·06$). The lævorotatory fraction was also examined in light of the mercury green line, $[\alpha]_{5461}^{16°}$ − 66° (α − 0·13°, $l = 4$, $c = 0·05$ in chloroform). The optically pure product in chloroform has $[\alpha]_D$ ± 1500°, $[\alpha]_{5461}$ ± 1975°.

II. By using lactose activated by previous extraction with chloroform, about 0·03 g. of the racemic bisiminocamphor, dissolved in 5 l. of the solvent mixture, was adsorbed on the upper eighth of a single tube of dimensions 130 × 10 cm. and holding 6 kg. of lactose. Adsorption and development required 60 hours. Upper and lower fourths were collected as before, but the uppermost fraction was found to contain very little adsorbed material and its extract was therefore united to that from the quarter immediately below it. The two upper fourths gave 0·00232 g., m. p. 252—254°, $[\alpha]_{5461}^{15°}$ + 485° in chloroform (α + 0·450°, $l = 4$, $c = 0·0232$); the lowest fourth gave 0·00180 g., m. p. 252—254°, $[\alpha]_{5461}^{15°}$ − 728° (α − 0·524°, $l = 4$, $c = 0·0180$). The pure active forms melt at 259—260°, and the racemic product at 252—253° (see p. 1572).

The above two experiments are representative of a large number, in all of which an excess of *d*-*p*-phenylenebisiminocamphor was recovered from the upper part of the tube and an excess of the *l*-form from the lower part. It must therefore be concluded that the partial resolutions thus effected are due to the adsorption coefficient of *d*-lactose for the *d*-iminocamphor being greater than that for the *l*-isomeride. According to Tswett's principles of chromatographic analysis, it should be possible to continue the process of separation until a complete resolution has been achieved, and confirmatory evidence on this point was first obtained by collecting the partly resolved material from several experiments and submitting

* Each value of α given in this paper is the average of numerous readings, in order to obtain an approximation for the third decimal figure.

it to a second treatment over lactose. In this way a dextrorotatory mixture after adsorption and development was treated by selecting a fraction from the upper part of the tube; with a lævorotatory mixture, on the other hand, a bottom fraction was collected. In both cases the recovered products showed enhanced rotations.

Unfortunately, *p*-phenylenebisiminocamphor is so sparingly soluble and so weakly adsorbed on lactose that a complete resolution could only be carried out in the above manner by an altogether disproportionate expenditure of time and material. The principle at issue was therefore established more simply by making up a synthetic mixture of the pure *d*- and *l*-forms having an optical activity which corresponded to the lævorotatory mixture actually isolated from experiment II. This was adsorbed, developed, and a lower fraction worked up; a synthetic mixture corresponding to the recovered material was then compounded and the process repeated. After four adsorptions over lactose, including that given under II, an optically pure sample of *l*-*p*-phenylenebisiminocamphor was obtained. With the experience gained, it is probable that the same result could now be attained with only three treatments.

The following details refer to separations carried out as in II above, except that it was found preferable to use a solvent mixture containing 25% of benzene. In this way more of the iminocamphor could be handled in a given volume of solution, and the development process was considerably expedited. The latter point is of importance, as too prolonged adsorption on the lactose leads to decomposition of the iminocamphor, a change indicated by the pale yellow colour of the adsorbate passing into a deeper brown tint.

III. A synthetic mixture, 0·03 g. ($[\alpha]_{5461}$ — 660°), was dissolved in 2 l. of petroleum–benzene (4 : 1) and adsorbed in a single tube of dimensions 130 × 6 cm., holding 2·5 kg. of activated Lactosum B.P. The adsorbed layer occupied the upper fourth of the tube. After rapid development with the same solvent mixture (time, 16—18 hours) the top and the bottom fourth were separated and extracted: top fourth, 0·00160 g., m. p. 248—252°, $[\alpha]_{5461}$ — 162° (α — 0·104°, $l = 4$, $c = 0{\cdot}016$ in chloroform); bottom fourth, 0·00262 g., m. p. 253—256°, $[\alpha]_{5461}$ — 1389° (α — 1·456°, $l = 4$, $c = 0{\cdot}0262$).

IV. The separation was repeated as in III, 0·024 g. of a synthetic mixture, $[\alpha]_{5461}$ — 1330°, being used: bottom quarter, 0·00218 g., m. p. 254—256°, $[\alpha]_{5461}$ — 1790° (α — 1·565°, $l = 4$, $c = 0{\cdot}0218$ in chloroform). In this case 30% benzene in petroleum was employed as solvent, but adsorption was notably poorer and the increase in rotation less pronounced. In the following experiment a return was therefore made to a solvent mixture containing 25% benzene.

V. From 0·023 g. of a mixture, $[\alpha]_{5461}$ — 1708°, the lowest eighth of the developed column being extracted, there was recovered 0·00190 g. of optically pure *l*-*p*-phenylenebisiminocamphor, m. p. 257—260°, $[\alpha]_{5461}$ — 1992° (α — 1·513°, $l = 4$, $c = 0{\cdot}0190$).

	Mg. taken.	[α].	Lactose, kg.	Adsorbed layer.	Layer extracted.	Mg. recovered.	α.	[α].
I *	50	± 0°	10	1, 2	1, 2	5·0	±0·11°	+ 117°
					7, 8	6·0	−0·13	− 66
II	30	± 0	6	1	1, 2, 3, 4	2·32	+0·450	+ 485
					5, 6	5·40	+0·332	+ 154
					7, 8	1·80	−0·524	− 728
III	30	— 660	2·5	1, 2	1, 2	1·60	−0·104	− 162
					7, 8	2·62	−1·456	−1389
IV	24	−1330	2·5	1, 2	7, 8	2·18	−1·565	−1790
V	23	−1708	2·5	1, 2	8	1·90	−1·513	−1992

* Unactivated lactose used. The two dextrorotations in this experiment are calculated from the value actually observed for the D line, as recorded on p. 1570. In the remaining experiments activated lactose was employed. The final value in V is subject to an error of about ±20°.

The significance of these data is more readily appreciated from the preceding tabular summary, in which the column of lactose in the adsorption tube is regarded as being divided into eight equal sections, numbered 1 to 8 from the top downwards; α and $[\alpha]$ refer to the green line λ 5461.

Experiment showed that by a similar process involving the uppermost fractions of

the adsorbate the rotation of a dextrorotatory mixture could be increased in the same manner, but no attempt was made to isolate the pure *d*-form.

Discussion.—The foregoing results demonstrate clearly that under suitable conditions a racemic mixture may be completely resolved into its optically active forms by taking advantage of the difference in their adsorption coefficients on a given optically active adsorbent. Obviously the essential factor is the asymmetric character of the adsorbing surface, which causes it to react differently towards the enantiomorphous components of the racemic compound, a point which is emphasised by the work of Tsuchida, Kobayashi, and Nakamura (*loc. cit.*) and more especially of Karagunis and Coumoulos (*loc. cit.*) on active quartz. The forces involved may be identical with those operative in bringing about the growth of a crystal in contact with its mother-liquor. If this view is correct, it would imply that no fundamental distinction in principle exists between separations effected in this manner and those carried out by Ostromisslensky (*Ber.*, 1908, **41**, 3035), in which a supersaturated solution of a racemate was seeded out by the addition of an asymmetric crystal such as that of sodium chlorate. Indeed, the historical experiment of Pasteur on sodium ammonium racemate crystals may fall into the same category, the one-sided attraction of each type of active molecule in solution for its related crystal form leading to a complete differentiation. In other words, in 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 layer of molecules on being deposited on the interface forming a fresh adsorptive surface of the same nature as the original.

In the case of *p*-phenylenebisiminocamphor and lactose the quantity of racemic material bears a very small ratio to that of the lactose required for resolution. This ratio must depend upon the difference between the adsorption coefficients of the two forms for the lactose surface, and other examples may soon be brought to light in which the resolution can be completed with less difficulty. A salient characteristic of the method is that it is not subject to the presence of a chemically active group, such as carboxyl or amino-, in the molecule of the racemic compound to be resolved, and there is no reason why it should not be applied with success to a hydrocarbon. In this connexion it may be pointed out that the carbonyl groups in *p*-phenylenebisiminocamphor do not appear to react with phenylhydrazine or its dinitro-derivative.

The other carbohydrates mentioned on p. 1569 adsorbed the iminocamphor less strongly than lactose and were not further investigated.

p-Phenylenebisiminocamphor is also adsorbed extremely weakly upon powdered active quartz, but no optical activity could be detected by treatment involving this material.

Experimental.

dl-p-Phenylenebisiminocamphor was prepared from *dl*-camphorquinone (2 mols.), *p*-phenylenediamine hydrochloride (1 mol.), and excess of sodium acetate. The mixture was heated carefully over a free flame, and the brownish needles obtained on crystallising the resulting mass from alcohol were clarified by dissolution in hot alcohol and rapid filtration through a ½-in. layer of alumina held in a sintered-glass funnel. Large golden-yellow crystals were deposited; m. p. 252—253°, yield 60% (Forster and Thornley, J., 1909, **95**, 942, obtained only a 40% yield by carrying out the reaction in alcoholic solution). We failed to obtain any reaction by heating the dry mixture for 8 hours on a water-bath (cf. Singh, *J. Indian Chem. Soc.*, 1930, **7**, 555).

The corresponding *d*- and *l*-bisiminocamphors were prepared from the pure active *d*- and *l*-camphors by way of the quinones. They melted at 259—260°, and the racemic form obtained by mixing equal weights was identical with that prepared as above. There is therefore no evidence for the presence of any internally compensated *meso*-derivative in the latter product (cf. Singh, *loc. cit.*).

dl-m-β-Naphtholazomandelic acid was prepared by the known method (Brode and Adams, *loc. cit.*; McKenzie, J., 1935, 106; Porter and Ihrig, *loc. cit.*). Attempts were made to effect a resolution by means of wool according to the directions of the last-named authors. As a cold acetic acid solution of the dye in the concentration (1 g. in 75 c.c.) recommended by them could not be used owing to rapid deposition from the supersaturated liquid, a solution of 0·5 g. in 75 c.c. was treated at 30° with successive quantities (each 2·5 g.) of wool, each lot being withdrawn

after 2 days and replaced by a fresh one. No measurable rotation ($l = 2$, $\lambda = 6100$) could be detected in the residual liquor at any stage, even though the process was continued until the bulk of the dye had been adsorbed. These tests were conducted with use of (*a*) natural fleece which had been defatted by washing, followed by extraction with carbon tetrachloride and drying, (*b*) a piece of old woollen blanket which had been washed many times.

dl-m-β-Naphtholazomandelic acid in benzene solution was found to be adsorbed by lactose (Lactosum B.P.). 2 L. of a benzene solution containing 0·24 g. of the dye were adsorbed in three tubes (50 × 2·5 cm.) each holding about 200 g. of lactose. The adsorbate occupied the upper thirds of the tubes, and development by washing with benzene was continued until the red layer expanded to fill the tube. Top and bottom thirds were then removed, and extracted with acetone. The recovered dye was washed with boiling water to remove traces of lactose, and dried. Upper thirds gave 0·050 g., $[\alpha]_{6100} + 2{\cdot}5°$ in acetic acid ($\alpha + 0{\cdot}05°$, $l = 2$, $c = 1$); lower thirds gave 0·030 g., $[\alpha]_{6100} - 2°$ in acetic acid ($\alpha - 0{\cdot}02°$, $l = 2$, $c = 0{\cdot}6$). Although these rotations do not greatly exceed the probable error, the partial separation could be repeated. This part of the work was carried out with untreated Lactosum B.P. The pure forms of the active dye are stated to have $[\alpha]_{6100}$ $\pm 47°$ in acetic acid.

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