*The Selective Adsorption of Optical Antipodes as Revealed by the Chromatographic Techniques**

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A number of attempts for the optical resolution by adsorption have been so far made, using various optically active adsorbents such

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as protein[1], starch[2], lactose[3], alumina coated with L-(+)-tartaric acid,[4] optically active quartz powder[5], ion exchange resins[6] and so on. Most of these observations, with the use of batch technique, appear to give too small a rotation to draw any definite conclusion while some using the chromatographic one seem to give a partial yet definite resolution[2,4]. Even for the latter case there are conflicting observations and uncertainties, suggesting that the phenomenon is extremely sensitive to the environment of the adsorbate-adsorbent system, particularly to the surface structure of the adsorbent.

We undertook this problem by utilizing both gas chromatographic and Tswett type column chromatographic techniques and by using starch, (−)-quartz, cellulose, alumina coated with various optically active substances as column packings.

Particular interest was first in the gas chromatographic separation of DL-*sec*-butanol on starch due to Karagounis et al.[7] We prepared *specific starch* according to the method kindly communicated by Karagounis and followed their work.

Helium was the carrier when the thermal conductivity cell was used as the detector, while nitrogen was the carrier when the hydrogen flame ionization gauge was employed as the detector*1. In both cases we had nothing but single peaks on the chart, however. Other optically active packings were investigated without success.

On the other hand the passage of DL-mandelic acid through Tswett column packed with starch—the one first studied by Krebs et al.[2]—was found to give a distinct resolution of the racemate: the initial fractions of the eluate as well as those close to the final stage gave rise to almost 100% optical purity differing in the direction of rotation.

Experiments were made as follows. A potato starch column 149×1.6 cm. was employed. Mandelic acid was chosen as the adsorbate because of its large specific rotation. Sample of 0.25~2.00 g. mandelic acid in a 5% aqueous methanol was adsorbed on top of the column and then eluted with water through the column kept at room temperature. The eluate from the column was fractionated with the automatic fraction collector. The rotation of each fraction amounting 2.2 cc. was determined by Zeiss-Kreis polarimeter. The amount of mandelic acid in each fraction was weighed out after evaporating the solvent up to dryness.

The specific rotation $[\alpha]_D$ was now calculated according to the expression:

$$[\alpha]_D = (100 \times \alpha)/(d \times c)$$

where c is the number of grams of mandelic acid in 100 cc. of the solution, α the observed angle of rotation and d the cell length (usually 1 dm.). In Fig. 1 $[\alpha]_D$ is shown against fraction numbers.

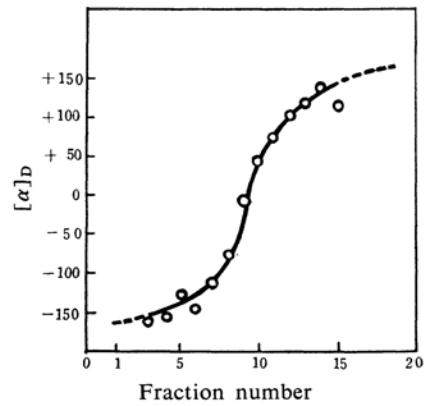

Fig. 1. The plot of the specific rotation $[\alpha]_D$ against fraction numbers.

Figure 1 shows that mandelic acid having a negative angle of rotation is eluted first, then followed by the one with the positive angle of rotation. It is apparent therefore that the latter, denoted by L-(+)-mandelic acid, is adsorbed on the starch more strongly than D-(−)-isomer. Now assuming that the additivity rule holds between the specific rotation and the composition of each optical isomer and that $[\alpha]_D$ of mandelic acid is independent of the concentration at ±157 at room temperature, the concentration of D- or L-isomer was calculated against each fraction. The chromatogram obtained in this way is shown in Fig. 2.

Figure 2 shows that the initial fractions as well as those close to the final stage are highly enriched or almost pure with regard to D- or L-mandelic acid respectively. None of the previous reports ever achieved such high resolution*2. The efficiency of resolution, judged

1) W. Bradley and G. C. Easty, *J. Chem. Soc.*, **1951**, 499.
2) H. Krebs, J. Diewald and J. A. Wagner, *Chem. Ber.*, **89**, 1875 (1956).
3) G. M. Henderson and H. G. Rule, *J. Chem. Soc.*, **1939**, 1568.
4) G. Karagounis, E. Charbonnier and E. Floss, *J. Chromatography*, **2**, 84 (1959).
5) G. M. Schwab and L. Rudolph, *Naturwissenschaften*, **20**, 363 (1932). R. Tsuchida, A. Nakamura and M. Kobayashi, *J. Chem. Soc. Japan*, **56**, 1339 (1935); G. Karagounis and G. Coumoulos, *Nature*, **142**, 162 (1938).
6) N. Grubhofer and L. Schleith, *Naturwissenschaften*, **40**, 508 (1953).
7) G. Karagounis and G. Lippold, ibid., **46**, 145 (1959).
*1 Karagounis used Scott type detector using hydrogen as the carrier.

*2 Using a starch column 45 cm. long, Krebs et al. were able to enrich D- or L-mandelic acid only partially. Our satisfactory results may perhaps be due partially to the comparatively long column length 149 cm., which has much more plate numbers effective in the resolution.

TABLE I. AMOUNT OF D-MANDELIC ACID (%) IN THE FIRST THREE FRACTIONS

Run	Amount of racemate g.	Recovery %	% D-Mandelic acid in fraction number			Ratio of V_R L/D
			1	2	3	
1	2.00	83	65	57	52	
2	2.00	97	96	90	87	1.04
3	1.00	99	100	99	89	1.04
4	0.50	100	93	94	100	1.04
5	0.25	96	100	100	79	1.04

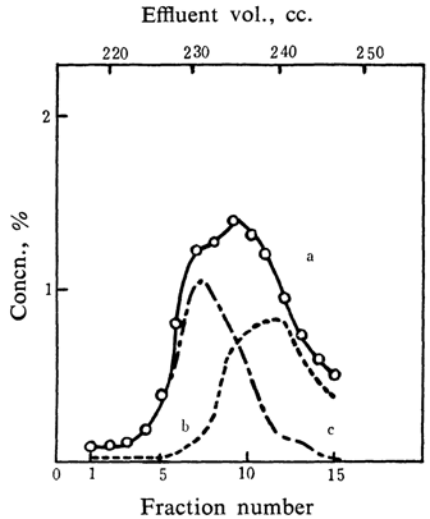

Fig. 2. Chromatograms of D- and L-mandelic acid. Flow rate of effluent: 2.5 cc./hr.

a Total amount of mandelic acid
b L-(+)-Mandelic acid
c D-(−)-Mandelic acid

from the optical rotation of the initial fractions, was found unexpectedly to be little affected by preheating the starch in the air around 110°C or by the pretreatment with ether.

Table I shows the concentration of D-mandelic acid calculated for the first three fractions respectively.

It is noted from the third column of the table that fresh starch i. e. the one used in run 1 adsorbs mandelic acid irreversibly or results in an imperfect material balance. This was not the case with the subsequent runs: run 2—5 gave almost perfect material balance. Thus, a sample of starch pretreated with dilute mandelic acid solution was packed in the column and subjected to experiment which was found to give reasonable recovery of mandelic acid with high resolution similar to those of run 2—5 of the table. The resolution of racemate on the *pretreated* starch may perhaps be associated with some physical modification of the adsorbent due to the irreversibly occluded mandelic acid molecules.

To estimate the degree of resolution of the racemate, the ratio of the retention volume V_R*3 of each isomer was calculated except run 1 and compared in the last column of the table. The ratio was invariable at 1.04 with varying the amount of mandelic acid eluted.

Several homologues of mandelic acid were also subjected to experiment for resolution on the starch column and found to be resolved definitely but to a less extent. The details of the experiments will be presented and discussed in the near future.

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*3 V_R was defined as the volume of the effluent that passed through the column until the highest concentration of L- or D-mandelic acid was collected in the fraction.