3MM). Elution was with phenol–water (3 : 1, w/w). After colouring with the periodate–Schiff reagent we found a single spot in each fraction. In the GDG-fraction, a spot with R_F 0.68 (= monogalactosyglycerol) was found and in the GGDG-fraction the spot had R_F 0.51 (= digalactosylglycerol).

This demonstrated that:

(1) The phospholipid fraction contained no glycolipids.

(2) The GDG-fraction contained no phospholipids or GGDG.

(3) The GGDG-fraction was free of GDG and phospholipids.

Most of the plant pigments were removed by this method. However, the glycolipid fractions contained some yellow-brown pigments.

Acknowledgements

The author appreciates the helpful suggestions of Dr. J. F. G. M. WINTERMANS. This work was supported by the Netherlands Organization of Pure Scientific Research (ZWO-SON).

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Received October 31st, 1966

J. Chromatog., 28 (1967) 131–132

Partial resolution of mandelic acid with Sephadex gels

Racemic mandelic acid and other racemic substances have been resolved, at least partially, with optically inactive eluents and with starch[1–3] and cellulose[4–8] as the stationary phase. Since Sephadex is a crosslinked polymer of glucose, it seemed likely that it could also serve as the immobile phase in chromatographic resolutions.

Experimental

Sephadex G-10 and G-25 were obtained from Pharmacia Fine Chemicals and pretreated according to the company literature. Research grade DL-mandelic acid (Aldrich Chemical Co., Inc., Milwaukee 10, Wisc.) was found to be optically inactive and was used without further purification. De-ionized water was used. The other chemicals were of reagent grade and were used without further treatment.

The column of Sephadex G-10 was 306 cm × 0.785 cm^2 and had an interstitial volume of 88.1 ml. The column of G-25 was 310 cm × 0.785 cm^2 with an interstitial volume of 94.8 ml. Eluent was forced through the columns with a Minipump (Milton Roy Co., 1300 E. Mermaid Lane, Philadelphia, Pa.) at a rate of 0.092 cm/min. The effluent was monitored spectrophotometrically at 257 mμ with a 1-cm flow cell. Fractions of 5.54 ml of eluate were collected automatically, and the rotation of each was measured with either a Rudolph Model 80 or a Perkin Elmer Model 141 automatic polarimeter after acidification with hydrochloric acid. Ultraviolet and infrared spectra of some fractions were determined. For the latter, the solute was extracted with ether and mulled with Nujol.

Results and discussion

A preliminary elution of 0.68 mmole of racemic mandelic acid with water as eluent and Sephadex G-10 as stationary phase gave an elution graph (absorbance *vs.* volume of eluate) with two peaks. The first peak was about $^1/_{10}$ as high as the second, had a maximum at 180 ml, and tailed badly. These fractions had no detectable rotation but showed the absorption spectra of mandelic acid. The second peak with a maximum at 510 ml was approximately Gaussian in shape. These fractions also had the absorption spectra of mandelic acid. The fractions on the ascending part of this peak had distinct positive rotations, the largest being +0.028° in a 2-dm tube; those on the descending portion had negative rotations, the largest being —0.079°. The sum of the negative rotations was 2.6 times the sum of the positive rotations. The authors are unable to offer a satisfactory explanation of the first peak. They suggest that the excess of laevo rotations is due to the presence in the Sephadex of bacteria that destroy the (+)-mandelic acid faster than the (—)-isomer.

Several changes were made in subsequent elutions: (1) Aqueous sodium chloride was used as the eluent instead of pure water. This increased not only the sorption of both isomers but also the specificity of sorption[9], thus improving the separation at the expense of time. (2) In order to compensate in part for the greater adsorption of mandelic acid in the presence of sodium chloride, a gel of lesser crosslinking (G-25) was used. (3) The column was pretreated with aqueous ethanol to decrease the bacterial population. (4) The eluent was passed through membrane filters (0.45 and/or 0.20 μ) to eliminate or impede the entrance of bacteria with the eluent. The last two precautions were partly successful; in one elution, they decreased to 1.3 the ratio of the cumulative negative rotations to the cumulative positive rotations. On the other hand, no method of eliminating the small, first peak was found.

The data of the second peak of an elution are given in Table I. This was performed after pretreatment of the column with ethanol. The sample was 2.00 ml of 0.355 *M* racemic mandelic acid in 3.0 *M* sodium chloride. In this and in all other elutions, the sign of the rotation changed at the peak. The data of columns 2 and 3 were used to calculate the figures in the last two columns. The elution graphs of each isomer were drawn from these data. Although these curves departed rather markedly from the ideal Gaussian shape, they were used to calculate[10] the number of plates per cm of column (9.0 and 9.4 for the (+) and (—)-isomers, respectively) and the length of column required for a quantitative separation ($6.7 \cdot 10^5$ cm).

Although these data indicate that a quantitative resolution of mandelic acid with Sephadex G-25 is not practicable, modest resolutions were obtained with very

TABLE I

PARTIAL CHROMATOGRAPHIC RESOLUTION OF MANDELIC ACID WITH SEPHADEX G-25

Volume (ml)	*Total concn. (mmole/ml)*	*Rotation* × 1000 (degrees)*	*Concn. of enantiomers × 1000 (mmole/ml)*	
			(+)	(—)
310.7	2.2	+ 10	1.2	1.0
316.2	2.1	+ 12	1.2	0.9
321.8	1.8	+ 9	1.0	0.8
327.3	8.5	+ 61	4.9	3.6
332.8	20.0	+ 119	11.3	8.7
338.4	37.6	+ 90	19.8	17.8
343.9	52.2	— 202	24.0	28.2
349.5	18.1	— 142	7.5	10.6
355.0	5.4	— 40	2.3	3.1
360.6	2.5	— 12	1.1	1.4

* In a 2-dm tube.

little difficulty, and surely other racemic substances may be more easily separable by this method.

Conclusion

Partial resolution of racemic acid has been accomplished by elution through Sephadex with water or preferably aqueous sodium chloride. The elution graphs (concentration of mandelic acid *vs.* volume) have two peaks. The first peak is much smaller, shows no rotation and tails badly. The second peak is approximately Gaussian. The fractions on the ascending slope have positive rotations; those on the descending slope, negative. An impracticably long column would be required for quantitative resolution of this particular compound.

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Received August 29th, 1966

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