Partition Chromatography of Sugars on Ion Exchange Resins

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As shown in earlier papers in this series,[1-3] several sugars can be separated quantitatively from each other by partition chromatography on ion exchange resins. One factor which limits the applicability of this technique is the extremely low rate of diffusion inside the resin particles in mixed solvents.[4,5] Porous resins and small resin particles are, therefore, preferable. When the particle size is decreased below a certain limit, ordinary chromatographic equipment does not withstand the high pressure which has to be applied to force the solution through the column. A high-pressure technique has, therefore, been employed to achieve improved separations.

A striking example which shows the influence of the particle size is given in Fig. 1. It is seen that with the fraction 45—75 μ the separation of glucose from sucrose is not complete, whereas a clean-cut separation is achieved with the fraction 15—40 μ. Raffinose exhibits an excessive broadening with the coarser particles. The quantitative separation of some sugars not included in earlier studies is also demonstrated in the lower curve. As might be expected, the less polar 2-deoxy-glucose appears ahead of glucose. It is worth mentioning that arabinose appears between these compounds and that the separation is complete.

A chromatogram obtained with a more complicated mixture of monosaccharides and oligosaccharides is given in Fig. 2. A comparison between this chromatogram and those reported earlier shows that the use of fine resin particles means a great improvement.

Our earlier attempts to apply partition chromatography in ethanol-water mixtures for the mutual separation of the monosaccharides formed during the hydrolysis of wood have not been successful.[1] The results given in Fig. 3 show that the separation in 82 or 86 % ethanol is incomplete even when finer resin particles

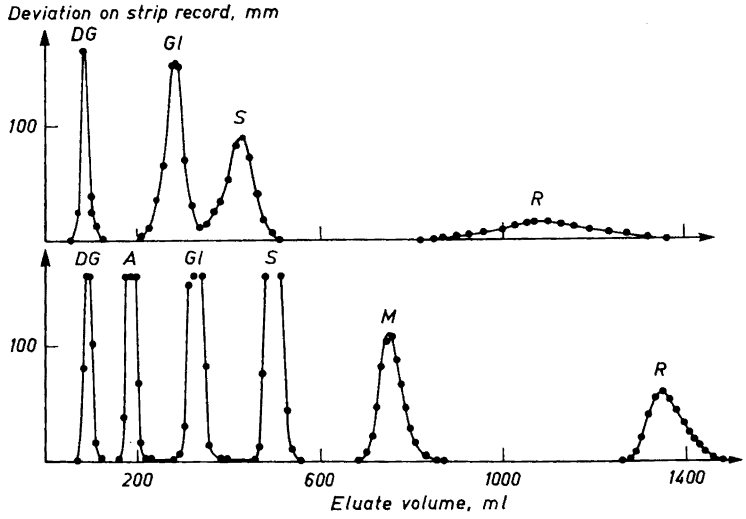

Fig. 1. Influence of the particle size upon the separation of various sugars in 74 % ethanol. Upper chromatogram 45—75 μ. Lower chromatogram 15—40 μ. DG = 2-deoxy-D-glucose; A = Arabinose; Gl = Glucose; S = Sucrose; M = Melizitose; R = Raffinose. 10 mg of each sugar.

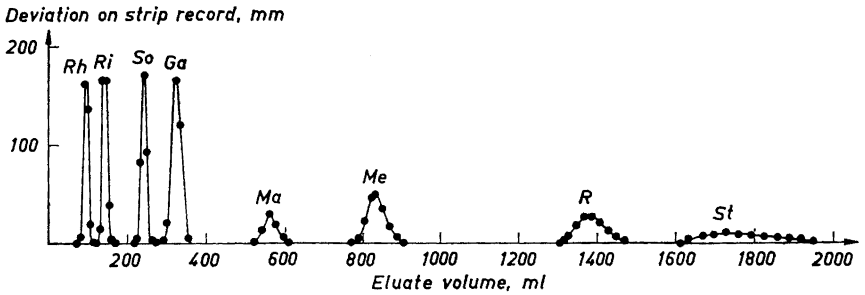

Fig. 2. Separation of various sugars from each other in 74 % ethanol. Dowex 21 K 15—40 μ. Rh = Rhamnose, 2.5 mg, Ri = Ribose, 5 mg, So = Sorbose, 2.5 mg, Ga = Galactose, 5 mg, Ma = Maltose, 2.5 mg, Me = Melibiose, 5 mg, Ra = Raffinose, 5 mg, St = Stachyose, 5 mg.

are used. It is evident that there is a marked improvement when the ethanol concentration is increased. This is explained by more favorable equilibrium distribution coefficients at the higher concentration. On the other hand, the rate of diffusion inside the resin particles is lower at a high alcohol concentration.[4,5] With the small resin particles used in the present work this factor does not offset the possibility of separating these sugars and, as can be seen in Fig. 4, a complete separation of arabinose, xylose, mannose, galactose, and glucose can be achieved when the ethanol concentration is increased to 88 %.

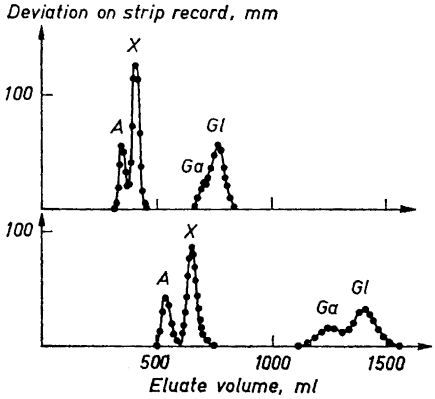

Fig. 3. Influence of the ethanol concentration upon the separation of 2.5 mg arabinose (A), 5 mg xylose (X), 2.5 mg galactose (Ga), and 5 mg glucose (Gl). Upper chromatogram 82 % ethanol. Lower chromatogram 86 % ethanol.

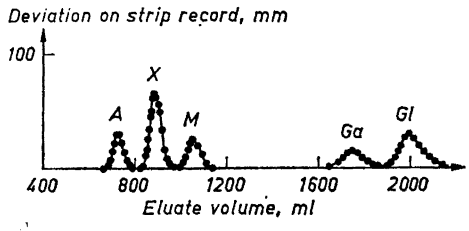

Fig. 4. Separation of 2.5 mg arabinose (A), 5 mg xylose (X), 2.5 mg mannose (M), 2.5 mg galactose (Ga), and 5 mg glucose (Gl). Ethanol concentration: 88 %.

The examples given in this paper show that partition chromatography on ion exchange resins in mixed solvents offers great possibilities in separations of monosaccharides as well as lower oligosaccharides. The particle size here is, however, a much more critical factor than in ion exchange separations in water solutions. The broadening of the elution curves is largely determined by the coarser fraction, whereas the pressure drop in the column is chiefly caused by the finest particles. The fractionation of fine resin particles into more uniform fractions than those used in this work results in further improvements, but the fractionation is extremely tedious. A modified technique, which has been used successfully, involves the application of extremely fine resin particles mixed with Celite. Excessively high pressures can be avoided in this way.

Experimental. The ion exchange column consisted of nylon tubing with an outer dia-

meter of 10 n.m and an inner diameter of 8 mm. The resin bed was supported by a perforated plate of stainless steel covered with glass wool and Celite. The dimensions of the resin bed were 8 × 750 mm. The resin (Dowex 21 K) was classified as usual and used in its sulfate form. The solution was fed onto the top of the column by means of a high-pressure piston-type pump. The pressure drop in the column was about 15 kp/cm². The flow rate was 0.8 ml cm^{-2} min^{-1}. In other respects the working conditions were the same as reported earlier.[2]

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The Configuration of N-Methyl-*p*-chlorobenzaldoxime

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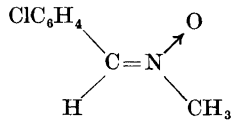
The configuration of the N-methyl derivative of oximes has been previously studied by chemical methods. Methylation by use of dimethyl sulfate, which has never yielded both of the theoretically possible N-methyl isomerides has been reported to yield the isomers with the "anti" configuration.[1,2] On the other hand, use of diazomethane as a methylating agent is stated to yield the same N-methyl isomers from the "syn" oximes, but different N-methyl isomers from the "anti" oximes.[3]

We have prepared N-methyl-*p*-chlorobenzaldoxime by methylating "syn" *p*-chlorobenzaldoxime with dimethyl sulfate;[4] after repeated crystallization from benzene the m.p. was 128–129.5°, and the formula ClC_8H_8NO was confirmed by microanalysis.

The crystal structure of the compound was determined by a three dimensional X-ray diffraction study, the result of which showed conclusively that the N-methyl-*p*-chlorobenzaldoxime has the "anti" configuration,



The positions of the hydrogen atoms were revealed by a threedimensional difference Fourier synthesis. The molecular dimensions as found after least-squares refine-

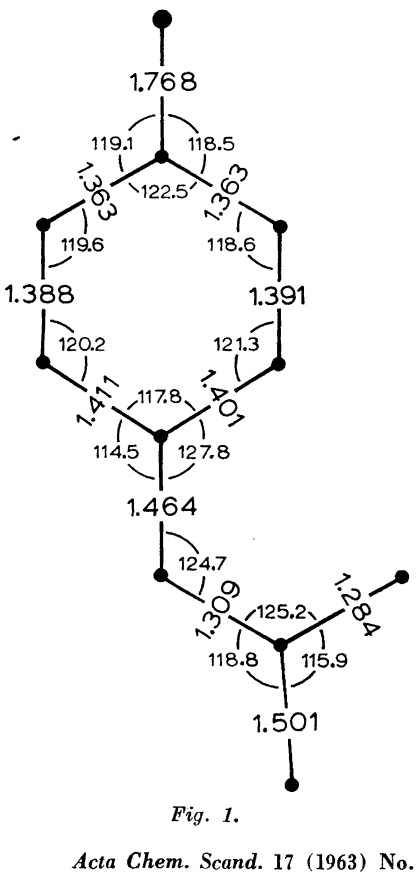


Fig. 1.