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PARTITION CHROMATOGRAPHY OF SUGARS ON ION-EXCHANGE RESINS

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SUMMARY

In separations of monosaccharides in aqueous ethanol on ion-exchange resins in their lithium and sulfate forms, the sugars are eluted, with few exceptions, in the order of an increased number of hydroxyl groups. With oligomers there exists a straight-line relationship between the logarithm of the distribution coefficient and the number of monomeric units. Higher saccharides of various types exhibit large individual differences.

INTRODUCTION

Partition chromatography on ion-exchange resins in aqueous ethanol is a valuable tool in the separation of various mono- and oligosaccharides. Within the range of ethanol concentration applied, the ratio water:ethanol is higher inside the resin than in the external solution. This explains why sugars are retained in the resin phase. In addition, interacting forces between the solutes and the counterions of the resin influence the uptake[1].

The method is of practical importance in analyzing monosaccharides in the hydrolyzate of plant materials[2]. The purpose of this work is to study the elution behavior of mono- and oligosaccharides not previously examined. An attempt has been made to correlate the structure of the sugars with their elution behavior.

MATERIALS

Talose was prepared by reduction of talono-1,4-lactone with 5% sodium amalgam[3]. The product was purified and isolated by partition chromatography on an anion exchanger in the sulfate form. Commercial samples of erythrose from various suppliers were found to contain a complex mixture of sugars. Erythrose, as well as threose, was therefore prepared by lead tetraacetate degradation of glucose and galactose, respectively[4]. The products were isolated as above, and the identity of the tetroses was verified by gas chromatography–mass spectrometry of the trimethylsilyl ethers of the corresponding alditols.

Cellotriose was prepared by phosphoric acid hydrolysis of cotton[5], whereas cellotetraose, cellopentaose and cellohexaose were gifts from Dr. J. K. HAMILTON

(Shelton, Wash.). Samples of 5,6-di-O-isopropylidene gulose and methyl-6-deoxy-α-D-glucoside, kindly supplied by Drs. W. MEYER ZU RECKENDORF (Münster) and T. E. TIMELL (Syracuse, N.Y.), were used for the preparation of the corresponding monosaccharides. Nystose, 1-kestose, planteose and stachyose were gifts from Dr. W. W. BINKLEY (New York), 1,6-anhydro-β-D-glucofuranose and 1,6-anhydro-β-D-glucopyranose from Dr. J. H. SLONEKER (Peoria, Ill.), allose, altrose, 3-ribo-hexulose, mannobiose, xylobiose from Dr. O. THEANDER (Stockholm) and 2,3-di-O-methyl-6-deoxyallose from Dr. MICHAEL H. B. HAYES (Birmingham).

EXPERIMENTAL

The equipment used in the chromatographic separation was the same as described previously[6]. Two kinds of resins were used: one cation exchanger in the lithium form (Dowex 50W-X8) and one strongly basic anion exchanger in the sulfate form (T5C). The particle sizes referred to below were determined in 92.4% ethanol for the H^+ and Cl^- forms, respectively.

Unless otherwise mentioned, the experiments were carried out at 75° to avoid decomposition of the saccharides. The eluate was analyzed automatically[8] with the orcinol method[7], using 3 g of orcinol per liter of 60% sulfuric acid as reagent solution. The reagent pump was made of Hastalloy C except for the cylinder and piston which were of glass and Teflon, respectively, and the bullets which were of ruby. The volume distribution coefficients (D_v) were calculated from the peak elution volumes as usual[8].

RESULTS AND DISCUSSION

Most monosaccharides studied were conveniently separated in 86–90% ethanol on an anion exchanger in the sulfate form. The separation of some monosaccharides not studied previously is illustrated in Fig. 1.

All aldoses studied in earlier work were eluted as expected, in the order of

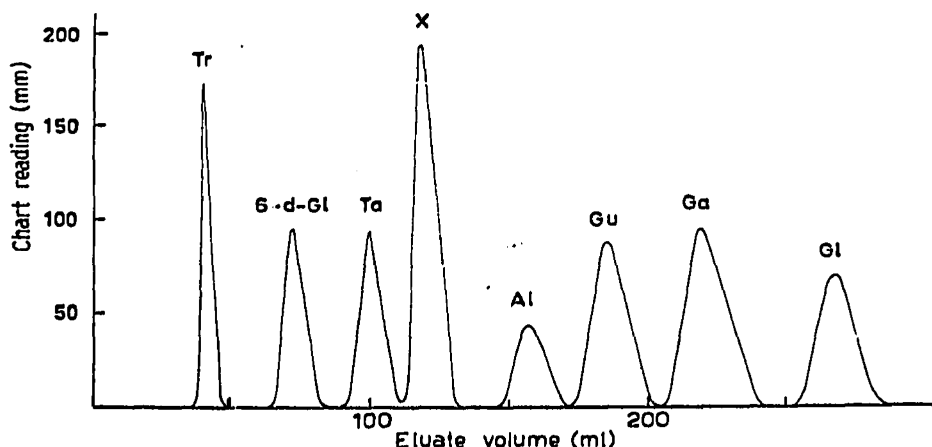

Fig. 1. Partition chromatography in 88% (w/w) ethanol. Resin bed: 4 × 550 mm, T5C, SO_4^{2-}, 14–17 μ. Flow rate: 2.2 ml·cm^{-2}·min^{-1}. Tr = threose, 500 μg; 6-d-Gl = 6-deoxyglucose, 250 μg; Ta = talose, 744 μg; X = xylose, 400 μg; Al = allose, 300 μg; Gu = gulose, 850 μg; Ga = galactose, 500 μg; Gl = glucose, 1000 μg.

TABLE I

VOLUME DISTRIBUTION COEFFICIENTS OF SOME MONOSACCHARIDES AND DISACCHARIDES AT 75°

Saccharides	*SO_4^{2-} resin (14–17 μ) 88% ethanol*	*Li^+ resin (17–21 μ) 92.4% ethanol*	*Li^+ resin (17–21 μ) 96% ethanol*
Erythrose	3.1	1.9	2.3
Threose	3.8	1.4	1.8
Arabinose	10.1	3.5	4.2
Lyxose	8.9	2.7	3.2
Ribose	6.6		4.6
Xylose	12.5	2.7	3.2
Allose	16.6	6.0	8.3
Altrose	16.6	4.2	5.6
Galactose	23.4		7.6
Glucose	28.1	4.8	6.0
Gulose	20.4	4.9	6.3
Mannose	16.4		5.9
Talose	10.7	5.8	8.3
Fructose	13.5		6.8
3-Ribohexulose	9.8	4.7	6.6
6-Deoxy-D-glucose	7.3	0.9	1.1
Rhamnose	4.8	1.4	1.6
1,6-Anhydro-β-D-glucofuranose	6.3	1.4	1.6
1,6-Anhydro-β-D-glucopyranose	3.8	2.3	2.9
2,3-Di-O-methyl-6-deoxyallose (mycinose)	0.53	0.4	0.4
Mannobiose	41.6		
Xylobiose	35.8	5.4	7.3

increasing number of hydroxyl groups. The distribution coefficients of some monosaccharides, most of which have not been studied previously, are listed in Table I. The positions of erythrose and threose, as well as those of allose and gulose, are in agreement with this rule, whereas talose constitutes an exception and appears before xylose. Another exception is 3-ribohexulose which was eluted at a position close to that of arabinose. Interestingly, it was eluted much earlier than fructose and other hexuloses studied previously. Allose could not be separated from altrose under these conditions (Table I). As expected 6-deoxyglucose exhibited a low D_v value which, however, was much larger than that of 6-deoxymannose. The two isomeric forms of 1,6-anhydro-glucose appeared well separated much earlier than glucose.

As found in previous work, a few monosaccharides which could not be separated on the sulfate resin were eluted as discrete bands from a column filled with cation-exchange resin in the lithium form, whereas some species which were well-separated on the sulfate column overlapped seriously on the lithium column. With few exceptions the correlation between number of hydroxyl groups and elution order was found to hold true for this system as well. Among the species listed in Table I no exceptions were observed. Interestingly, talose was eluted later than the other hexoses with the exception of allose. Likewise a reversed order of elution occurred with other isomers, *e.g.* with the tetroses and with the two forms of 1,6-anhydroglucose. These results confirm that not only are the differences in solubility in aqueous ethanol of the species to be separated of importance, but also interacting forces with the counterions inside the resin.

TABLE II

VOLUME DISTRIBUTION COEFFICIENTS OF SOME MONOSACCHARIDES AND OLIGOSACCHARIDES AT 75°

	SO_4^{2-} resin (14–17 μ) 70% ethanol	*Li^+ resin (17–24 μ) 85% ethanol*
Monosaccharides		
Xylose	2.3	1.9
Glucose	3.1	3.0
Disaccharides		
Cellobiose	4.4	4.9
Isomaltose	6.5	8.6
Mannobiose	2.9	7.8
Trehalose	6.8	8.2
Xylobiose	3.0	2.0
Trisaccharides		
Cellotriose	5.8	8.7
Gentianose	9.6	12.3
1-Kestose	8.7	16.2
Maltotriose	6.6	7.7
Planteose	9.1	19.3
Higher saccharides		
Cellotetraose	7.9	15.4
Nystose	14.9	27.8
Stachyose	18.5	64.7
Cellopentaose	10.9	27.4
Cellohexaose	15.2	44.4

From a practical point of view, it is important that allose and altrose are well separated on the lithium resin. In practice most separations of mixtures of monosaccharides can be carried out either on a sulfate or on a lithium resin, but it is seen that with some very complex mixtures it is necessary to apply both types of resin.

At the temperature used, 75°, no interfering decomposition was recorded. Experiments carried out with erythrose showed, however, that an appreciable decomposition occurred at higher temperatures. At 90° about 65% of the added erythrose was lost during the run.

At an ethanol concentration of 88%, the disaccharides appear much later on a sulfate resin than do the monosaccharides. As shown in Table I xylobiose and mannobiose were eluted after the hexoses and in the order predicted from the rule discussed above. At 70% ethanol the distribution coefficients of xylobiose and mannobiose were very similar, however, and both were slightly lower than that of glucose (Table II). This reversal can be ascribed to the competition between the factors mentioned in the introduction which contribute to an accumulation of the sugars in the resin phase and factors which tend to exclude the sugars from the resin. The importance of the latter increases with a lowered ethanol concentration and in pure water the exclusion effect increases with an increased partial molar volume[9]. Evidently, an ethanol concentration of 70% is too low for separations of complicated mixtures in which mono- and disaccharides are involved. This ethanol solution was, however, suitable for a complete separation of the oligosaccharides in the glucose–cellohexaose series.

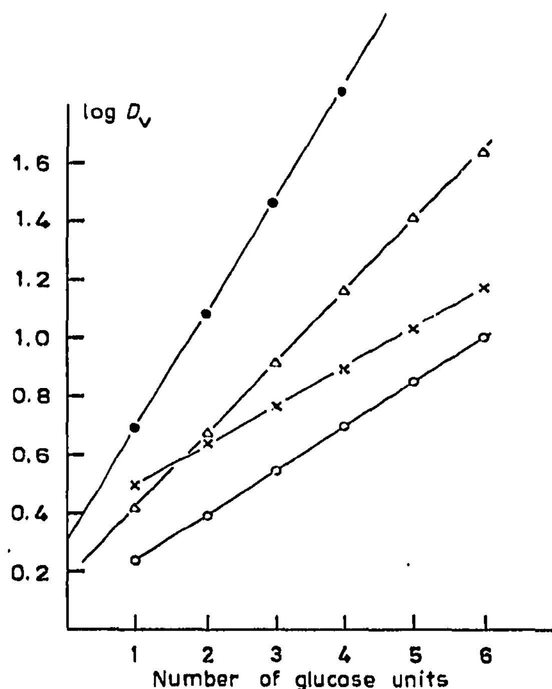

Fig. 2. Relationship between log D_v and the number of glucose units in the oligosaccharides. ×, Sulfate resin (14–17 μ) in 70% ethanol. Lithium resin (17–21 μ) at various ethanol concentrations: ○, 80%; △, 85%; ●, 92.4%.

Paper chromatographic studies[10] have shown that MARTIN's rule[11] which predicts a straight-line relationship between log $R_F/(1 - R_F)$ and the chain length holds true for several types of oligosaccharides. It could be expected therefore that a plot of the logarithm of the D_V value against the number of hexose units would result in a straight line. As can be seen from Fig. 2, this holds true for the glucose–cellohexaose series, both for the runs on the sulfate and the lithium columns. On both columns the oligomers appeared well-separated in the order of increasing

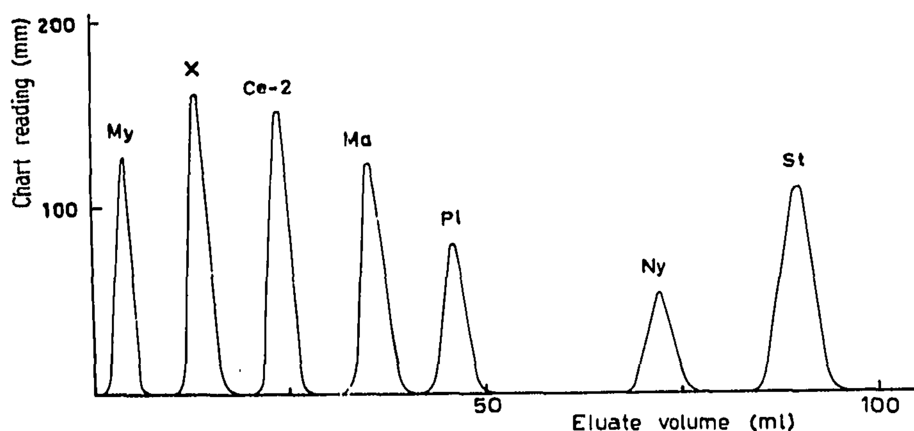

Fig. 3. Partition chromatography in 70% (w/w) ethanol. Resin bed: 4 × 550 mm, T5C, SO_4^{2-}, 14–17 μ. Flow rate: 1.9 ml·cm^{-2}·min^{-1}. My = mycinose, 88 μg; X = xylose, 50 μg; Ce-2 = cellobiose, 112 μg; Ma = maltotriose, 100 μg; Pl = planteose, 82 μg; Ny = nystose, 96 μg; St = stachyose, 200 μg.

molecular weight. Despite the fact that the swelling of the resin is much lower in ethanol than in water, no interfering screening effect[8] was observed. The results show that the method is extremely useful in separations of oligomers.

Chromatograms illustrating the application of sulfate resins and lithium resins in separations of complex mixtures including higher saccharides of various types are given in Figs. 3 and 4. As can be seen from these chromatograms, as well as from the results given in the table, some species are more easily separated on a sulfate column, whereas with others a lithium column is preferred. On both types of resins the elution curves were virtually symmetric. It is seen that the structure of the individual disaccharides and higher saccharides has a great influence upon the retention volume. As an example, it can be mentioned that isomaltose is held much

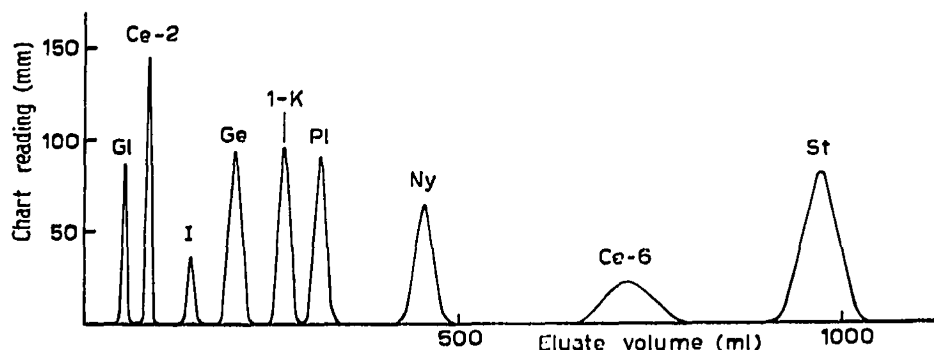

Fig. 4. Partition chromatography in 85% (w/w) ethanol. Resin bed: 4 × 1050 mm, Dowex 50W-X8, Li^+, 17–24 μ. Flow rate: 4.4 $ml \cdot cm^{-2} \cdot min^{-1}$. Gl = glucose, 20 μg; Ce-2 = cellobiose, 56 μg; I = isomaltose, 480 μg; Ge = gentianose, 80 μg; 1-K = 1-kestose, 135 μg; Pl = planteose, 82 μg; Ny = nystose, 160 μg; Ce-6 = cellohexaose, 180 μg; St = stachyose, 300 μg.

more strongly than cellobiose, both on the sulfate column and on the lithium column. Another striking example is the tetrasaccharide stachyose which is held more strongly on both columns than cellohexaose.

In agreement with the results published with various monosaccharides the method was found to be well suited to quantitative analyses. It is extremely important that the analyzing system is kept clean and that no gas bubbles are permitted to enter the system. In addition care must be exercised to avoid leakage in the pumps. When these precautions were taken, the deviations in the peak areas in successive runs were less than $\pm$ 2% from the mean.

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REFERENCES

1 H. RÜCKERT AND O. SAMUELSON, *Acta Chem. Scand.*, 11 (1957) 315.
2 L.-I. LARSSON AND O. SAMUELSON, *Svensk Papperstidn.*, 70 (1967) 571.
3 F. L. HUMOLLER, *Methods in Carbohydrate Chemistry II*, Academic Press, New York, 1963, p. 105.

4 A. S. Perlin and C. Brice, *Can. J. Chem.*, 33 (1955) 1216.
5 E. S. Becker, J. K. Hamilton and W. E. Lucke, *Tappi*, 48 (1965) 60.
6 P. Jonsson and O. Samuelson, *Science Tools*, 13 (1966) 17.
7 L.-I. Larsson and O. Samuelson, *Mikrochim. Acta*, (1967) 328.
8 O. Samuelson, *Ion Exchange Separations in Analytical Chemistry*, Almqvist and Wiksell, Stockholm, and Wiley, New York, 1963.
9 M. Mattisson and O. Samuelson, *Acta Chem. Scand.*, 12 (1958) 1386.
10 D. French and G. M. Wild, *J. Am. Chem. Soc.*, 75 (1953) 2612.
11 A. J. P. Martin, *Biochem. Soc. Symp.*, 3 (1949) 4.