

Separation of Sugars into Anomers by Partition Chromatography on Ion Exchange Resins

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Various mono- and disaccharides were separated into anomers by partition chromatography on an anion exchange resin (SO_4^{2-}) in aqueous ethanol at -10°C . With most sugars two peaks representing the α - and β -pyranoses were recorded. For some saccharides anomerization interfered with the separation. The conformation of the sugars exerts a predominant influence on their chromatographic behaviour.

Reducing sugars can be well separated into α - and β -anomers by gas chromatography of their trimethylsilyl derivatives.¹ Separate peaks corresponding to the furanoid and pyranoid forms are often obtained. Efficient separations of

methyl glycosides can be achieved by ion exchange chromatography on strongly basic anion exchange resins in their hydroxide form² and by partition chromatography on ion exchange resins in aqueous ethanol.³ In separation of sugars by paper chromatography at room temperature only one spot is obtained for each sugar although two or more forms of each sugar are present in most systems.⁴ In thin-layer chromatography single spots are obtained at $20-30^\circ\text{C}$ in contrast to the appearance of elongated or double spots at -18°C .⁵ Evidently, a partial separation of different forms occurs in favourable systems.

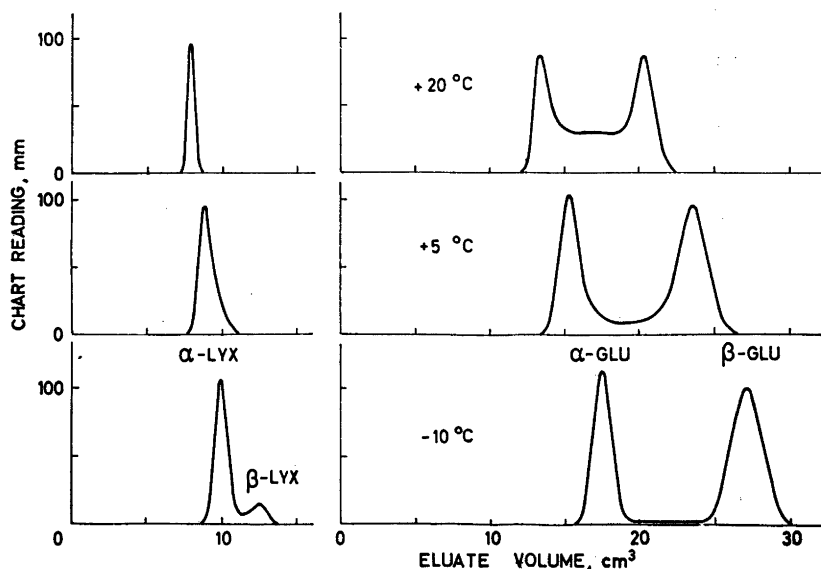


Fig. 1. Separation into anomers of D-lyxose, 20 μg (left diagrams), and D-glucose, 75 μg (right diagrams), at various temperatures.

We now report on the separation of sugars into anomers by partition chromatography on ion exchange resins in aqueous ethanol at low temperature. Separations at elevated temperature have previously been applied in analyses of complex mixtures of sugars.⁶

RESULTS AND DISCUSSION

When a mixture of α - and β -D-glucose dissolved in 75 % ethanol was eluted at 75 °C a sharp single peak was obtained. At 20 °C the mixture was resolved into two overlapping peaks [Fig. 1]. When the temperature was lowered, both peaks were eluted later which is in agreement with the earlier observation that the distribution coefficients of sugars increase with decreasing temperature.⁷ At -10 °C the curve between the two peaks approached the baseline. Separate experiments with fresh solutions of α - and β -D-glucose gave sharp single

Table 1. Volume distribution coefficients (D_v) of mono- and disaccharides in 75 % ethanol at various temperatures on an anion exchange resin (SO_4^{2-}).

Saccharide	-10 °C	+5 °C	+20 °C
D-Ribose I	4.6	—	—
D-Ribose II	5.3	4.2	3.6
α -D-Arabinose	9.1	7.1	6.3
β -D-Arabinose	4.9	4.1	4.1
α -D-Xylose	5.5	5.0	4.5
β -D-Xylose	9.4	8.2	7.0
α -D-Lyxose	5.6	4.9	4.4
β -D-Lyxose	7.0	—	—
α -D-Allose	6.2	—	—
β -D-Allose	10.8	—	—
α -D-Glucose	9.7	8.5	7.4
β -D-Glucose	15.1	13.1	11.3
α -D-Mannose	6.5	5.9	5.4
β -D-Mannose	10.9	9.3	7.8
α -D-Gulose	6.6	—	—
β -D-Gulose	10.6	—	—
α -D-Galactose	8.2	7.3	6.4
β -D-Galactose	15.1	13.2	11.1
α -D-Talose	4.7	—	—
β -D-Talose	7.1	—	—
β -D-Fructose	6.7	5.9	5.2
D-Fructose II	7.5	—	—
α -L-Sorbose	9.2	7.3	6.7
α -Cellobiose	22.5	20.4	15.6
β -Cellobiose	34.7	30.6	22.2
α -Lactose	19.9	17.8	14.1
β -Lactose	30.4	25.5	20.6

peaks and showed that the first peak contained the α -anomer and the second peak the β -anomer. The retention time of the β -anomer at -10 °C was 6 h. The anomerization of the compounds was negligible at this temperature whereas at +5 °C the anomerization interfered with the separation. Evidently, the α - and β -forms can be separated by partition chromatography on ion exchange resins at low temperatures. Favourable aspects in this connection are that the mutarotation is slower in ethanol than in water⁸ and that the low freezing point of the eluent permits separations at low temperature. A prerequisite for an efficient separation is that fine resin particles be used so that the sorption equilibrium can be approached during the chromatographic run. The volume distribution coefficients, D_v , of the anomers are given in Table 1.

Equally good resolution into two compounds was obtained at -10 °C with solutions of D-allose, D-mannose, and D-xylose. Aqueous ethanol solutions were kept at room temperature so that equilibrium was approached. One of the peaks was identified from the position of the single peak recorded in a separate run with a fresh solution of an authentic crystalline sample. The proportion of furanose forms of D-glucose, D-mannose, and D-xylose in equilibria in aqueous solutions is less than 1 %⁹ which means that the two peaks recorded must represent the pyranose forms. The relative amounts of the anomers determined from the peak areas, were in good agreement with literature data.⁹ The proportion of furanose of D-allose in aqueous solution is about 10 %⁹ but for unknown reasons no indication of its presence was obtained in the chromatograms.

D-Galactose as well as D- and L-arabinose gave rise to two well separated main peaks which were identified as α - and β -pyranose by the same technique. With both sugars a minor, seriously overlapping compound appeared after the first main peak. The experiments with D-galactose were repeated on an overloaded column to make it possible to isolate this compound. The eluate was analysed by both the orcinol and the periodate-formaldehyde methods. The results given in Fig. 2 show that the compound present in the distorted tail of the first peak gave a much stronger relative response in the periodate-formaldehyde channel

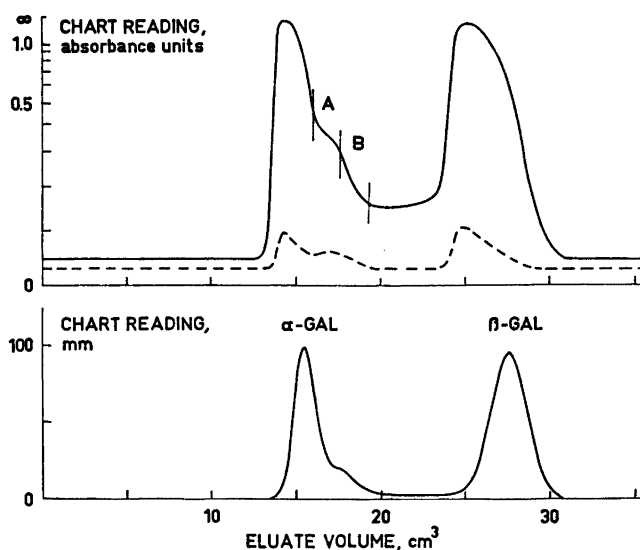


Fig. 2. Separation into anomers of D-galactose at -10°C . Analysis by the orcinol method (full line) and periodate-formaldehyde method (broken line). Loading: $3500\ \mu\text{g}$ (upper diagrams); $75\ \mu\text{g}$ (lower diagram).

than the major compounds. This suggests that the compound is either a furanose or a ketohexose. D-Tagatose, which can be formed from D-galactose by isomerization, is eluted as a single peak at -10°C at about the same position as the unknown compound. Since the elution volume of D-tagatose would probably be affected by the presence of large amounts of D-galactose, two fractions, A and B, were cut as indicated in the figure and rechromatographed on analytical columns at -10°C and 75°C . The chromatograms from the experiments at -10°C could not be distinguished from that recorded with the original galactose sample [Fig. 2]. The eluate from the runs at 75°C was analysed by the orcinol method at high amplification. The separation of D-tagatose from D-galactose is very favourable at this temperature. In addition to D-galactose only minor amounts of D-tagatose (totally in both fractions 0.5% of the starting material) were detected. The concentration of D-tagatose in fraction B was about 5 times greater than in fraction A, which means that the unknown compound cannot be D-tagatose. The results indicate that one or both furanose forms of D-galactose were present in fraction A.

With D-glucose two well separated peaks were recorded. No crystals were available and an

equilibrium mixture in aqueous ethanol was therefore analysed. The relative areas of the peaks obtained at -10°C were in agreement with literature data.⁹ The smaller peak was therefore ascribed to the α -pyranose form and the larger one to the β -pyranose form. No other compounds were recorded.

D-Talose, which is known to exist as both anomers of the furanose and pyranose forms and to give an extremely rapid mutarotation,¹⁰ gave only two distinct peaks. The first of these was identified as the α -pyranose form by the study of an authentic sample. A large amount of the sugar was eluted between the peaks. This confirms that a rapid interconversion of various forms occurs with D-talose.

The chromatograms from the run with D-lyxose [Fig. 1] show tailing single peaks at $+20^{\circ}\text{C}$ and $+5^{\circ}\text{C}$ and two partially separated peaks at -10°C . Authentic samples were available for both α -L- and β -D-lyxose and identification of the peaks was obtained as with D-glucose. In agreement with mutarotation data¹⁰ the chromatograms showed that the anomers were rapidly interconverted.

With D-ribose which exhibits a very rapid mutarotation, appreciable amounts of the furanose forms are present together with the pyranoses.⁹ Only two seriously overlapping

peaks were recorded at -10°C . The resolution was much lower for solutions equilibrated at $+80^{\circ}\text{C}$ and -20°C after mixing with ethanol than in the experiments with D-ribose dissolved in 75% ethanol (-20°C) or first in water ($+2^{\circ}\text{C}$) followed by addition of ethanol (-20°C) and chromatographed as soon as the sugar was dissolved. These results as well as the shape of the elution curves show that at least three forms were present at equilibrium.

Among the ketoses studied, D-fructose gave rise to two peaks which exhibited a severe overlapping at -10°C . At $+5^{\circ}\text{C}$ and $+20^{\circ}\text{C}$ only one peak was recorded. L-Sorbose was the only sugar which gave a sharp single peak even at the low temperature. This was expected since it had previously been observed that L-sorbose exists almost exclusively in the α -pyranose form.¹⁰

The two disaccharides studied, cellobiose and lactose, can exist only in the pyranose forms and as expected, two sharp peaks were recorded at low temperature with each sugar. Negligible amounts were recorded between the peaks. The mutarotation was so slow that rough estimates of the distribution coefficients could be obtained even at $+20^{\circ}\text{C}$.

The results presented above show that the method is well suited to the separation at -10°C of anomeric pyranoses both for analytical and preparative purposes. Separation at low temperature can also be of interest for identification of single sugars or simple mixtures when only trace amounts are available. With some sugars the interconversion of different anomers is so rapid at -10°C that the resolution is poor.

A previous study of methyl pyranosides of D-glucose and D-xylose by partition chromatography on a sulfate resin at high temperature showed that the α - and β -forms were well separated and appeared in that order.³ The results given in Table 1 show that for those sugars for which it was possible to determine the elution order of the anomeric pyranose forms, the α -anomer was eluted before the β -anomer, i.e. the elution order was the same as with the derivatives. This holds true for all disaccharides and monosaccharides with the exception of D-arabinose. The fact that D-arabinose constitutes an exception is not unexpected in regard to results from conformational

studies.¹¹ They show that the 1C_4 conformation is favoured for α -D-arabinopyranose and to a lesser extent for β -D-arabinopyranose whereas with the other monosaccharides studied the 4C_1 conformer is favoured. A study of ethyl- α - and β -D-arabinopyranoside on a sulfate resin [92.4% (w/w) ethanol] at 75°C showed that for the α -form the D_V value was equal to 2.0 whereas for the β -form it was 1.5. Hence, the arabinosides were eluted in the same order as the arabinose anomers. The results show that the conformation of the sugars and sugar derivatives have a predominant influence on their chromatographic behaviour.

EXPERIMENTAL

The chromatographic equipment was the same as described earlier.⁶ The eluate was automatically analysed by the orcinol method¹² and by the periodate-formaldehyde method at pH 2.¹³ A strongly basic anion exchanger in its sulfate form (Technicon T5C, $10-17\ \mu\text{m}$) was used. The eluent (75% aqueous ethanol, w/w) was cooled before entering the column. The volume of the resin bed decreased with decreasing temperature. The volume ($143 \times 4\ \text{mm}$) measured at -10°C was used in the calculation of all D_V -values. Because of high pressure in the column, the flow rate was low, $0.6\ \text{cm min}^{-1}$ (-10°C), $1.1\ \text{cm min}^{-1}$ ($+5^{\circ}\text{C}$) and $1.7\ \text{cm min}^{-1}$ ($+20^{\circ}\text{C}$). The ethanol concentration in all sugar solutions was 75%, w/w. All sugars were commercial samples except D-allose and D-gulose, which were kindly supplied by Dr. O. Theander (Stockholm) and Dr. W. Meyer zu Reckendorf (Münster).

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