LIQUID CHROMATOGRAPHY OF SUGARS AND RELATED POLYHYDRIC ALCOHOLS ON CATION EXCHANGERS

THE EFFECT OF CATION VARIATION

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SUMMARY

Variation of the counter-ion attached to a polystyrene-based, strong cation exchanger alters the chromatographic behaviour towards sugars and polyols. The process occurring is ligand exchange of the aquated cation and other processes are of minor importance if water is used as the mobile phase. The separation of many mixtures is significantly improved by the correct choice of counter-ion. The order of elution may be explained by a simple hypothesis concerning the stereochemistry of the molecule. The α- and β-anomers of certain monosaccharides are separated in the presence of several cations.

INTRODUCTION

Carbohydrate mixtures have been separated by gas–liquid chromatography (GLC) of suitable volatile derivatives[1], or without prior derivatisation by liquid chromatography (LC). The older LC methods were characterised by their low efficiency of separation, but more recently many excellent methods utilising ion exchangers as the stationary phase have come into general use. Both cation and less often anion exchangers have been used with water or aqueous ethanol as the mobile phase. Also, the borate ester "derivatives" have been chromatographed using the borate forms of strong anion-exchange resins. All of these ion-exchange methods and several less common ones have been the subject of a comparative review[2]. Within the past year separations have been demonstrated on boric oxide gel[3] and neutral alumina[4].

We are interested in the preparation of pure samples of monosaccharides and related polyhydric alcohols derived from plant origins and labelled with the radio-nuclide carbon-11 (half-life 20 min). The cation-exchange methods appealed to us since the eluent was water or aqueous ethanol and so could be used directly in biochemical investigations and in particular for *in vivo* work. This is not the case with the LC method utilising borate complexing[2], which gives purified components eluted in solutions containing solutes incompatible with such work and present at high concentrations. In the GLC method[1], the rather stable trimethylsilyl (TMS) ethers are

obtained. In both cases the preparation of a simple isotonic or hypotonic aqueous solution of the sugar would involve some time-consuming manipulations, particularly important when dealing with a radionuclide of short half-life. The boric oxide gel[3] and alumina[4] methods avoid this type of problem, but have not been developed sufficiently for our purpose.

Therefore, it was decided to improve the cation-exchange method so that separations could be carried out within a maximum time of 40 min and preferably within 20 min. In the previous work[2,5-7], the importance of the nature of the counter-ion attached to the ion-exchange resin was noted. In order to optimise this factor, a wide variety of cations, $M^{x+}(H_2O)_n$, were tested using a standard 50 × 0.28 cm glass column of Aminex A-5 (11 ± 2 μm) eluting with deionised water at 10.0 ml/min.

RESULTS

In general, well-shaped peaks without tailing were observed. Loadings of up to 1 mg of each component and a sample size of 100 μl had very little effect on the resolution using a standard column. Increase of flow-rate degraded the resolution disproportionately when compared with the time saved in carrying out the elution. A flow-rate of about 0.1 ml/min was the optimum.

The observed capacity factors, k', are given in Table I. The cyclic compounds tested were, with some exceptions, eluted in the same order regardless of the nature of the cation M^{x+}. However the k' values and the separation factors α varied over a wide range and in some cases two components had the same k' value. The position of the open-chain polyols within the elution sequence changed considerably for the different cations. When M^{x+} was an alkali metal or Mg^{2+}, all of the peaks occurred below $k' = 1.2$ but for other cations the later peaks, notably talose, were considerably retarded (Fig. 1a-e) with $k'_{max.} = 4$.

By an appropriate choice of counter-ion, or two counter-ions as necessary, rapid baseline separations could be achieved for many two- or three-component mixtures. Of importance to our ^{11}C-radiochemical work, the mixture glucose-fructose (from land plants) may be separated using Aminex A-5 (Ag^+) or Aminex A-5 (Ca^{2+}), both 50 × 0.3 cm, in about 25 min. The mixture galactose–glycerol (from *Girgartina stellata*) may be separated using Aminex A-5 (Ca^{2+}), 50 × 0.3 cm, 25 min, or Aminex A-5 (La^{3+}), 25 × 0.3 cm, 10 min. Finally the mixture sucrose–glucose–fructose may be optimally separated using dual 50 × 0.3 cm columns of Aminex A-5, either Na^+ + Ca^{2+}, 40 min, or (best for larger sample volumes, up to 300 μl) Rb^+ + Ca^{2+}, 50 min. All the times are at a flow-rate of 0.10 ml/min.

Although Aminex A-5 (Ag^+) gives good separations it has certain disadvantages in use. It decomposes in the presence of light and reacts electrochemically with stainless-steel components, in both cases to produce metallic silver.

When glucose, galactose, xylose and mannose were chromatographed on columns of Aminex (Ca^{2+} or Sr^{2+}), a closely spaced double peak was obtained for each sugar (Fig. 1d), and this was shown to be caused by separation of the α- and β-anomers as follows. If a fresh solution of either the pure α- or β-anomer was used, a single major peak was obtained (a small amount, 5-10%, of the other form was always observed). The anomer that eluted first was identified as β-glucose, β-galactose, β-xylose or α-mannose. If serial injections were now made from this stock solution,

TABLE I

CAPACITY FACTORS (k') OF VARIOUS SACCHARIDES, GLYCEROL AND MANNITOL ON AMINEX A-5 (M^{x+} aq.) ELUTING WITH WATER

Accuracy of values in most cases: ±0.05 units. Tris$^+$ = $^+NH_3C(CH_2OH)_3$.

Counter-ion	Sucrose	Glucose	Galactose	Mannose	Talose	Fructose	Glycerol	Mannitol
Tris$^+$	0.10	0.35	0.35	— [i]	—	0.35	0.45	—
Li$^+$	0.20	0.35	0.50	0.45	—	0.45	0.90	1.05
Na$^+$	0.20	0.40	0.55	0.70	0.50	0.65	0.65	0.75
K$^+$	0.40	0.70	{0.80* {1.10*	—	—	1.05	0.80	—
Rb$^+$	0.40	0.75**	{0.75* {1.00*	{0.75* {1.25*	0.95**	0.90	0.75	0.55
Ag$^+$	0.45	0.75	0.85	0.95**	1.7	1.15	1.10	0.85
Tl$^+$	0.45	0.70	0.85	—	—	1.10	1.00	—
Mg^{2+}	0.15	0.30	0.40	0.40	—	0.35	0.70	0.40
Ca^{2+}	0.15	{0.30* {0.45*	{0.45* {0.65*	{0.45* {0.65*	~3.8	1.15***	1.05	1.40
Sr^{2+}	0.20	{0.30* {0.45*	{0.40* {0.55*	—	—	1.10***	0.90	—
Ba^{2+}	0.25	0.40**	0.45**	0.70**	—	1.00***	0.60	1.00
Cd^{2+}	0.15	0.25	0.35	—	—	0.50	0.80	—
La^{3+}	0.10	0.15	0.25	0.30	~4.0	0.35	0.80	1.70

Additional results

Gulose: k' = 1.25 (Rb$^+$), 1.15 (Ag$^+$), 1.3 (Ca^{2+}), 1.0 (La^{3+}).
Galactitol (sorbitol): k' = 0.60 (Rb$^+$), 2.1 (Ca^{2+}).
Xylose: k' = 0.45*, 0.60* (Ca^{2+}).
α-D-Galactosyl-(1,2)-glycerol: k' = 0.50 (Li$^+$), 0.30 (Na$^+$), 0.40 (Rb$^+$).

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* α/β forms.
** Asymmetric peak, partial separation.
*** Skew peak.
[i] — = not measured.

the minor peak grew at a rate consistent with that of mutarotation[8]. The ratio of areas at equilibrium, assigned to α/β-glucose, α/β-xylose and α/β-galactose, calculated from the overlapping peaks was A_α:A_β = 0.6:0.4, in agreement with the equilibrium composition of the sugars in aqueous solution*. The double peaks were not resolved on the Ba^{2+} form of the resin but the peak envelope was asymmetric in each case.

When galactose and mannose were chromatographed on columns of Aminex (K$^+$ or Rb$^+$), widely spaced double peaks were produced whereas glucose gave a single peak (Fig. 1b). Again it was shown as above that the separation effected was that of the α- and β-anomers, but this time α-galactose eluted ahead of the β-form and β-mannose ahead of the α-form, the reverse of the behaviour on the Ca^{2+} form resin. For galactose, there was baseline separation between the two peaks, and it was possible (using a column in the Rb$^+$ form in this particular experiment) to collect them as separate fractions which were subjected to further analysis. Also the ratio of areas under these two peaks was, for an equilibrated sample, A_α:A_β = 0.32:0.68.

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* For general information on structure, conformation and aqueous equilibria, *cf.* ref. 8.
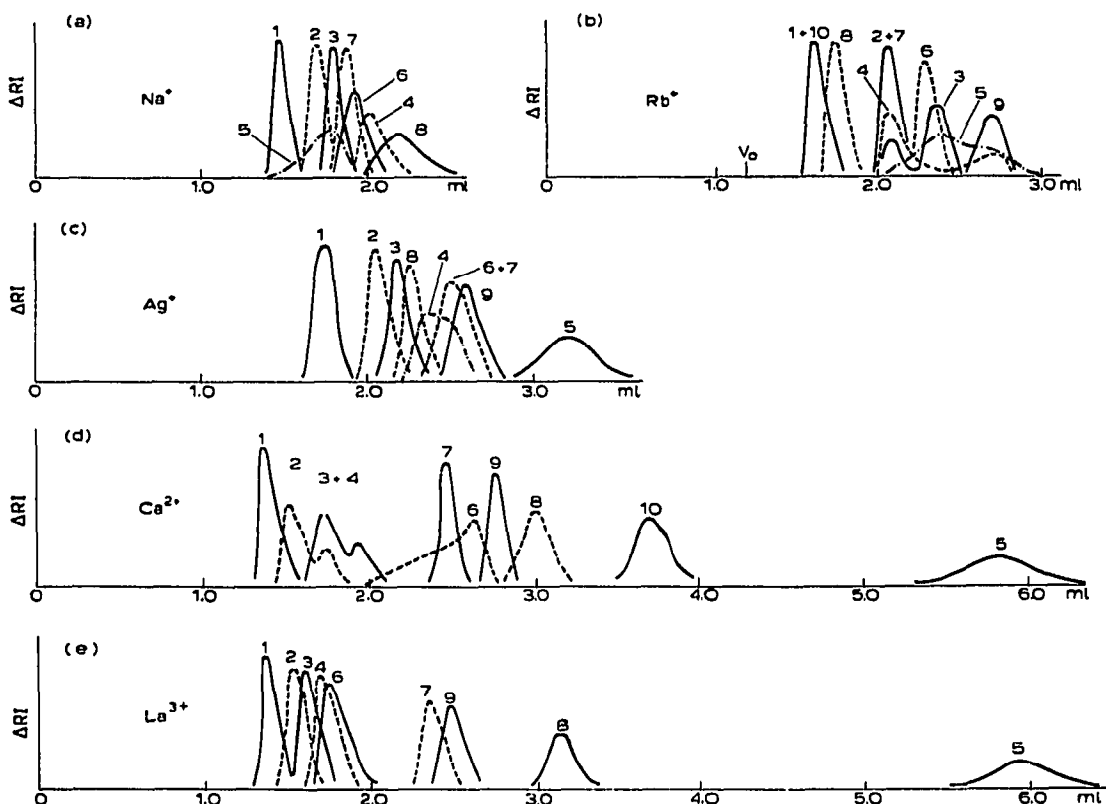
Fig. 1a–e. Behaviour of selected polyols on a 50 × 0.28 cm column of Aminex A-5 (M^{x+} aq.), eluting with deionised water at 0.10 ml/min. Composite chromatograms of sucrose (1), glucose (2), galactose (3), mannose (4), talose (5), fructose (6), glycerol (7), mannitol (8), gulose (9), and galactitol (10).

A sample of pure galactose was taken, equilibrated in aqueous solution, and then analysed by GLC of the TMS derivatives using 3% SE-30 at 160° (ref. 1). This reference material gave a minor peak followed by two major peaks (γ-, α- and β-(TMS)$_5$-galactose). The two samples collected from the LC experiment were treated by exactly the same procedure when an identical result was obtained, and no further peaks were eluted when the column was programmed to 250°.

MECHANISM OF THE SEPARATION OF POLYOLS

The results may be explained if the polyol molecule $R(OH)_n$ exchanges with the water molecules held in the hydration sphere of the cation M^{x+}aq. The stability of the complex formed with the metal ion will increase in relation to the availability for coordination. The molecules in the mobile phase are uncomplexed, but are adsorbed in the complexed form, and so k' increases as the stability of the complex increases. All of the sugars show very low k' values when chromatographed on a column which carried the efficient hydrogen-bonding species Tris, $^+NH_3C(CH_2OH)_3$, as counter-

ion. Therefore hydrogen bonding must play only a minor role in the adsorption process. This conclusion also applies to interaction with the fixed sulphonate groups and hydrophobic interactions. Therefore k' will be directly related to the stability constant $K_{stab.}$ of the complex. If V_s is the volume of the stationary phase, V_m the volume of the mobile phase, and K_d the distribution constant of $R(OH)_n$ between these two phases then

$$K_{stab.} = \frac{[M^{x+} R(OH)_n \text{ aq.}]}{[R(OH)_n][M^{x+} \text{ aq.}]} = \frac{K_d}{[M^{x+} \text{ aq.}]} \quad (1)$$

The basic equation of adsorption behaviour is

$$k' = \frac{V_s}{V_m} K_d \quad (2)$$

substitution into which gives an equation relating k' and $K_{stab.}$

$$k' = \frac{V_s}{V_m} [M^{x+} \text{ aq.}] K_{stab.} \quad (3)$$

By measurement $V_m = 1.2$ ml and $V_s = 1.6$ ml for the 50×0.28 cm column. From the Bio-Rad catalogue the exchange capacity of sulphonic acid type, 8 % cross-linked, polystyrene resin is $ca.$ 1.8 mequiv./ml. The latter quantity should be increased ($\approx 30\%$) since the volume quoted is that of resin and void water. A factor x, equal to the valency of the cations, should be included to convert to units of mmole/ml (mole/litre). Then in numerical terms, the relationship is

$$k' = \frac{3.2}{x} K_{stab.} \quad (3a)$$

By using this equation*, it is possible to calculate $K_{stab.}$ values directly from the column chromatographic data.

Hypothesis

It has been shown by nuclear magnetic resonance spectroscopy[9] and electrophoresis studies[10], both carried out in the presence of metal cations, that the sequence ax–eq–ax of three adjacent hydroxyl groups in cyclohexitol or pyranose rings (generalised formula I), or the sequence *cis–cis* in furanose rings gives rise to

ax - eq - ax   ax - eq   eq - ax - eq
   (I)   (II)   (III)
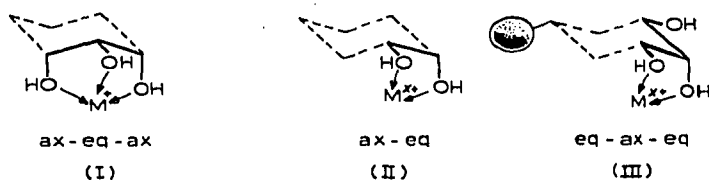
relatively strong tridentate chelates with suitable metal cations, and that the stability constant is then in the range $K_{stab.} \approx 1$–5 mole^{-1}. On the other hand, when the complex can only be formed from a pair of ax–eq (*cis*) hydroxyl groups (II), it

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* In order to obtain really accurate K_{stab} values the exchange capacity of Aminex A-5 resin should be measured for each cation considered.

is much weaker with $K_{stab.} \approx 0.1$ mole^{-1}. In pyranose sugars, if inversion (1C $\rightleftharpoons$ Cl) is prevented by the equatorial preference of hydroxyl groups, and of the 6-CH$_2$OH group especially, the sequence eq–ax–eq of three hydroxyl groups (III) can only form the weaker bidentate chelates. Thus mannose formed a weak complex but the pentose lyxose formed a strong complex in the Cl(D)-pyranose form[9]. Finally, the sequence eq–eq is less favoured than ax–eq because of the greater distance between the oxygen atoms. The sequence ax–ax or *trans* cannot form cyclic complexes.

In a complex of low stability, R(OH)$_n$ exists in the same conformation(s) which is (are) present in the absence of the cation. Change of conformation and pyranose-to-furanose interconversion can occur only if a sufficiently large gain in stability of the new complex can be achieved when compared with the complex produced without such change. Also, for a low $K_{stab.}$ value, most of the molecules of R(OH)$_n$ are present in the uncomplexed state. When there are two independent bidentate complexing sites present, the number of molecules complexed to the cation, hence k', will be approximately doubled.

The formation of the types of complex described above with ax–eq and ax–eq–ax sequences of hydroxyl groups requires a cation which coordinates octahedrally or, as with group 1a cations, does not have a perfectly ordered array of ligands when aquated. Small cations give rise to bidentate complexes and medium-sized cations to tridentate complexes. Over-sized cations are not able to form tridentate complexes but instead coordinate with eq–eq and 1,3-ax–ax (*cis*) diol sequences to form bidentate complexes.

Transition metal cations in particular have specific spatial coordination requirements and, when compared with non-transition metals, generally form more stable complexes. They may be expected to cause distortion of the polyol molecule in the process of complexation.

The favoured conformation[8,11] of long-chain acyclic polyols has an anti-parallel disposition of hydroxyl groups about a planar zig-zag backbone of carbon atoms (IV). If 1,3-OH interactions thereby occur, a deformation into a sickle (bent) form occurs. For complexation with the metal cation, a *syn* orientation of adjacent hydroxyl groups is required. The necessary conformational change will bring about C–H eclipsing (V) or C–C eclipsing (VI). The former is energetically more favourable and so the greater number of such arrangements per molecule, the stronger the complex formed.
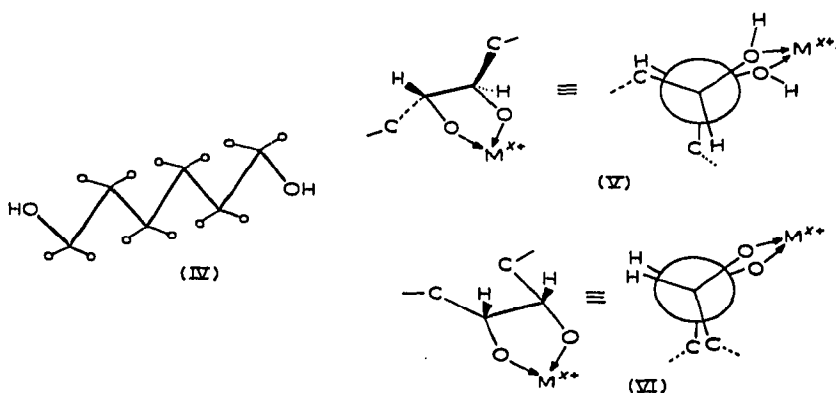
Thus in the column chromatography experiment, the retention should increase with the number of pairs of favourably orientated hydroxyl groups present. When tridentate complexing of a component can occur, the retention should be greatly increased. Metal ions which form strong oxygen-linked complexes should give increased retention of all components when used as counter-ion.

DISCUSSION

The nature of the polyol —interaction with Li^+, Na^+, Ca^{2+} and La^{3+}

The results of Jones and Wall[7] have been used where appropriate to supplement our own results and so extend the argument.

For aldohexoses, it is possible to predict the order of elution from a cation-exchange column using the data in Table II. Also, α- and β-anomers should be separable under optimum conditions if the rate of mutarotation is slow compared with the speed of of separation by the column. In the event, for Aminex A-5 (Ca^{2+} and La^{3+}), the order of retention observed is sucrose < glucose < galactose < mannose < talose. This order tends to occur for all the other cations investigated. The ratio k'(talose)/k'(glucose) is of the order of unity for group 1a cations but in the range 10–20 for Aminex A-5 (Ca^{2+}). Hence talose, which has the ax–eq–a xtriol sequence, must form a bidentate complex with the group 1a cations but a tridentate complex with Ca^{2+} in agreement with the previous studies[9]. The anomers of certain aldohexoses are separated on Aminex A-5 (Ca^{2+}) when the order, which agrees exactly with the hypothesis concerning pairs of ax–eq hydroxyls, is β-glucose (0 pair) < α-glucose, α-mannose, β-galactose (1 pair) < α-galactose, β-mannose (2 pairs) < gulose (1 pair or 1 triplet) < talose (1 triplet). Gulose elutes ahead of talose (both as single peaks) apparently because the ability to chelate as a tridentate ligand exists in one anomer of gulose, but in both anomers of talose. Allose on this basis (2 pairs or 1

TABLE II

NUMBER OF AX–EQ PAIRS (b), AND AX–EQ–AX TRIPLETS (t) OF ADJACENT HYDROXYL GROUPS PRESENT IN THE PYRANOSE FORMS OF MONOSACCHARIDES

Unless otherwise indicated, the ring exists in the Cl(D) conformation. O–O distances: ax–eq, 2.67 Å; eq–eq and 1,3-ax–ax, 2.71 Å. Conformational data from refs. 8 and 12.

Pyranose	α	β	Pyranose	α	β
Aldopentoses			*Aldohexoses*		
Ribopyranose	1 × t	2 × b	Talopyranose	1 × t	1 × t
Arabinopyranose	1 × b*	2 × b*	Allopyranose	1 × t	2 × b
Lyxopyranose	2 × b*	1 × b*	Gulopyranose	1 × t	1 × b
Xylopyranose	1 × b	—	Galactopyranose	2 × b	1 × b
			Mannopyranose	1 × b	2 × b
Ketohexoses			Altropyranose	1 × b**	2 × b**
Psicopyranose	1 × t	1 × t*	Idopyranose	—*	1 × b*
Tagatopyranose	1 × b	·1 × t*	Glucopyranose	1 × b	—
Fructopyranose	1 × b*	2 × b*			
Sorbopyranose	1 × b**	—			

* The preferred form in aqueous solution is 1C(D).
** Both forms, Cl(D) and 1C(D) occur in significant amounts.

triplet) would be expected to show intermediate retention compared with gulose and talose.

Eqn. 3a, relating k' and $K_{stab.}$, makes it possible to calculate a set of self-consistent $K_{stab.}$ values from Table I. As examples may be quoted: for the complex Ag^+–talose, $K_{stab.} = 0.55$ mole^{-1}; for Ca^{2+}–talose, $K_{stab.} = 2.4$ mole^{-1}; and for La^{3+}–talose, $K_{stab.} = 3.7$ mole^{-1}.

The aldopentoses have been chromatographed on Dowex 50-X8 $(Ba^{2+})^7$. These molecules in aqueous solution exist mainly in pyranose forms[8]. From the reported chromatographic data[7] it is possible to estimate the k' values which may be compared with the potential chelation sites (Table II). The elution sequence observed was xylose $(k' = 0.5$, 1 pair) arabinose and xylose $(k' = 0.8$, 2 pairs), ribose $(k' = 2.7$, 1 triplet). Apparently, lyxose does not interact in the less stable Cl(D) conformation (1 triplet) with Ba^{2+}. When xylose was chromatographed on Aminex A-5 (Ca^{2+}) the anomeric forms were resolved. For Ba^{2+}–ribose complex, calculation as before gives $K_{stab.} = 1.7$ mole^{-1}.

Fructose generally elutes after mannose and the usual value of k' observed (0.7–1.0) indicates bidentate complex formation. Hence, β-fructopyranose (VII), the major form normally present in solution[12], is the one involved in chelation and contains two ax–eq diol groupings. The k' value is rather high and so the exocyclic 1-OH group may also contribute when bonding occurs through O(2)O(3). Sorbose on Dowex 50-X8 $(Ba^{2+})^7$ elutes before fructose $(k' = 0.5$ and $k' = 1.0$, respectively). The molecule has one ax–eq diol grouping in the two α-pyranose forms and none in the β-form preferred, the Cl(D) pyranose (Table II). Tagatose was strongly retarded but eluted before talose[7]; the elution volume was not given, however. Psicose, not chromatographed, should have a k' value on Aminex A-5 (Ca^{2+} or Ba^{2+}) similar to ribose and talose since all three possess the triol sequence ax–eq–ax in both anomeric forms (Table II).

Some di- and trisaccharides have been chromatographed on Dowex 50-X8 $(Li^+)^7$. If the results are compared with the behaviour of galactose and glucose on Aminex A-5 (Li^+), the order of elution was raffinose (trisaccharide, 1 pair) < sucrose (0 pair), maltose (1 pair) < lactose (2 pairs), glucose (1 pair) < galactose (2 pairs). Disaccharides elute before monosaccharides with the same number of ax–eq pairs of hydroxyls because the ratio of a pair of hydroxyls to the total number of hydroxyls in the molecule is halved compared with monosaccharides. Also, the larger molecules may have greater difficulty in entering the pores of the resin and steric crowding particularly adjacent to the saccharide linkage(s) may make it difficult for the metal ion to chelate with the molecule.

The three cyclitols which contain the ax–eq–ax triol sequence, cis-, allo- and epi-inositol, form strong complexes in solution with Ba^{2+} (refs. 9 and 10). These compounds have only been chromatographed using resin in the Li^+ form with aqueous ethanol as eluent[13]. Then tridentate complexing does not occur. Chromatography on the Ca^{2+} or La^{3+} forms should give an entirely different elution sequence for the various cyclitols.

Several alditols have been chromatographed on Dowex 50-X8 $(Ba^{2+})^7$ when the elution order was glycerol (Table I, $k' = 0.60$) < mannitol $(k' = 1.0)$ < glucitol $(k' = 1.5)$ < galactitol $(k' = 1.6)$ < xylitol $(k' = 1.7)$. When the argument derived in the previous section is applied to each compound, it is found that glycerol can form

a bidentate complex from any of two pairs of hydroxyls, mannitol from any of three pairs, and the other compounds from any of four pairs. The same order was observed in electrophoretic work[10] using Na^+ as complexing ion. When we chromatographed the glycoside O-α-D-galactosyl-(1,2)-glycerol(VIII) *, the k' value was almost identical to that of galactose except on Aminex A-5 (Na^+, K^+ and Rb^+). In this case chelation can occur through the 1,3-OH groups of the glycerol moiety because of a favourable steric constraint. ˑ

β-D-fructopyranose-1C

(VII)

α-D-galactosyl-(1,2)-glycerol-C1

(VIII)

D-galacturonic acid-C1

(IX)
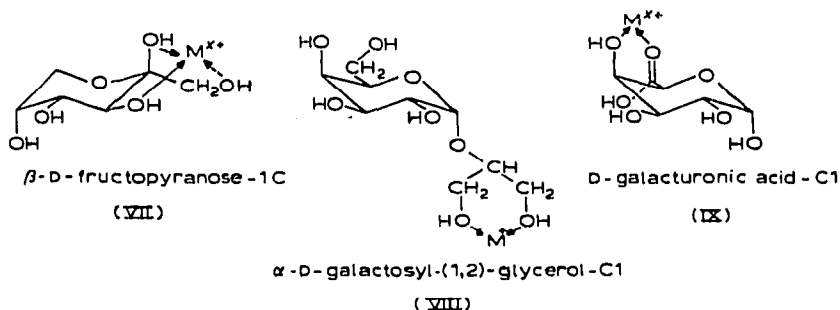
Finally, galacturonic acid on Dowex 50-X8 (Ba^{2+}) showed a surprisingly large retention ($k' = 2.7$)[7], which can be explained by chelation through the 6-COOH and the axial 4-OH groups (IX). Glucuronic acid on this basis would be expected to show a normal k' value since O(4) is equatorial in the preferred C1(D) form.

The nature of the stationary phase —extension to other cations

The chromatographic behaviour on Aminex A-5 (Ca^{2+}, Sr^{2+} and Ba^{2+}) (Fig. 1d), when the cation has ionic radius $r_+ = 1.0$–1.4 Å, shows the best agreement with the basic hypothesis. Cyclic compounds which act as bidentate ligands elute ahead of acyclic polyols. Gulose, ribose, tagatose and talose have large k' values in agreement with their ability to act as tridentate ligands. The α/β-anomers of glucose, galactose and mannose elute in the order expected from Table II. On Aminex A-5 (Cd^{2+} and La^{3+}) (Fig. 1e, $r_+ = 1.1$ Å), the components which behave as bidentate ligands are more closely spaced. The behaviour resembles Ca^{2+} except that the α/β-anomers are not resolved. On Aminex (Li^+, Na^+ and Mg^{2+}) (Fig. 1a, $r_+ = 0.6$–0.9 Å), talose and gulose show relatively low k' values, which may be explained by the assumption that they form bidentate rather than tridentate complexes[9,10].

On Aminex A-5 (Rb^+) (Fig. 1b, $r_+ = 1.5$ Å), all the compounds investigated elute in the range $0.4 < k' < 1.3$, with the more strongly complexed anomers of galactose and mannose, gulose, and talose eluting at the highest k' values. It appears that here again tridentate complexing does not occur. The anomalous behaviour of α/β-galactose and α/β-mannose may be explained by the assumption that the cation is sufficiently large to complex preferentially with an eq–eq sequence, in particular at the anomeric centre[14]. Sucrose, in which the hydroxyl groups are all equatorial, has a rather large k' value compared with say Na^+ or Ca^{2+}.

* Ex *G. stellata*, storage sugar.

Therefore, the triol sequence ax–eq–ax forms a bidentate complex through the outer 1,3-diaxial hydroxyls. The sequence ax–ax–ax which occurs in altrose and idose should form the same kind of complex. Acyclic polyols have low k' values, indicating that the large cation encounters steric repulsion when attempting to chelate with these molecules. Aminex A-5 (K^+), as far as investigated, resembles the Rb^+ form.

Aminex A-5 (Tl^+ and Ag^+) (Fig. 1c, $r_+ = 1.3$–1.4 Å) can be considered to be intermediate between the Rb^+ and Ba^{2+} forms. Glucose, galactose and mannose have relatively large k' values (similar to Rb^+) and glycerol fructose and talose have large k' values (similar to the Ba^{2+} group). Amberlite IR-120 (Cu^{2+}), not investigated by us, strongly retarded acyclic polyols, xylose, gulose, and ribose. Glucose and galactose were eluted rapidly[15].

Because of the Jahn–Teller distortion, Ag^+ and Cu^{2+} do not exhibit octahedral coordination but instead linear and square planar coordination, respectively[16]. Since octahedral coordination cannot occur and also because of the large size of the cation, in the case of Aminex A-5 (Ag^+) relatively strong monodentate complexing with the polyol is indicated.

Complex formation between monosaccharides and $Cu(NH_3)_4^{2+}$aq. has been studied in solution[17], when complexing through ax–eq and eq–eq diol sequences occurred along with distortion of the O–C–C–O bond angles, and through the 1,3-ax–ax diol sequence without measurable distortion. $Cu(OH_2)_4^{2+}$aq. should complex in an analogous manner. From the column chromatography results[15], it would appear that the 1,3-ax–ax diol sequence produced a strong complex and that acyclic polyols probably coordinated through 1,3-*cis* diol groups, the arrangement normally unfavoured. We intend to extend the scope of our experiments to cover this area, which seems to give unique separations.

Monosaccharides have been chromatographed on neutral alumina with water as the eluent[4]. The elution sequence was very similar to that observed on Aminex A-5 (Ba^{2+}). The main difference was that altrose, fructose, sorbose and idose exhibited a similar magnitude of retention as allose, talose and ribose, which indicated that these components were adsorbed in normally unfavoured conformations or ring forms which contain the ax–eq–ax triol sequence. Aluminium ions are presumably responsible for the separation.

Most separations of organic compounds using ion exchangers are carried out on columns of the Na^+ or Li^+ form (cation exchangers) or Cl^- or acetate form (anion exchangers) with modification of the mobile phase to optimise the separations. A more systemic investigation of the effect of less common counter-ions might allow better separations between the critical components of the mixture.

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