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SEPARATION OF OLIGOMERIC ALDONIC ACIDS BY ANION-EXCHANGE CHROMATOGRAPHY

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SUMMARY

Oligomeric aldonic acids were separated by anion-exchange chromatography in sodium acetate, acetic acid and borate media. In sodium acetate, the logarithm of the distribution coefficient decreases linearly with the degree of polymerization. Calculations of the swelling pressure from the distribution coefficients gave results in good agreement with literature data. A study of the influence of the temperature showed that for each additional sugar moiety, a constant incremental change was obtained not only in free energy (ΔG^0) but also in enthalpy (ΔH^0). The entropy was found to decrease in all exchanges of oligomeric aldonates for acetate.

INTRODUCTION

In an earlier paper[1], it was shown that several aldonic and aldobionic acids can be separated by anion-exchange chromatography with sodium acetate and sodium tetraborate as eluents. The same method was used for the isolation of aldobiouronic[2,3] and aldotriouronic acids[4].

For studies of the structures of polymeric carbohydrates and of waste waters from industries handling polymeric carbohydrates, methods that permit clean-cut separations of higher oligomeric acids are highly desirable. The purpose of this work was to study the separation of xylonic, gluconic and oligomeric aldonic acids of the O-β-D-xylopyranosyl-(1,4)-[O-β-D-xylopyranosyl-(1,4)]$_n$-D-xylonic acid and O-β-D-glucopyranosyl-(1,4)-[O-β-D-glucopyranosyl-(1,4)]$_n$-D-gluconic acid series. The separations were carried out on strongly basic anion-exchange resins in sodium acetate, acetic acid and potassium tetraborate.

EXPERIMENTAL

Materials

Xylonic, gluconic and cellobionic acids were obtained from commercial sources or were the same as those reported in previous papers from this laboratory.

The other oligomeric aldonic acids were prepared by hypoiodite oxidation of the corresponding oligosaccharides according to Schaffer and Isbell[5]. The oligosac-

charides of the β-(1,4)-linked D-xylose series were prepared by acid hydrolysis of birch xylan with trifluoroacetic acid[6]. Cellotriose was prepared by phosphoric acid hydrolysis of cotton[7], whereas cellotetraose, cellopentaose and cellohexaose were kindly supplied by Dr. J. K. Hamilton (Shelton, Washington, D.C., U.S.A.).

Procedure

All chromatographic separations were performed in jacketed columns containing a strongly basic resin in acetate or borate form (Dowex 1 X8). The eluent was boiled under reflux before being fed into the column, which was conditioned with the eluent to be used in the subsequent elutions. The temperature was kept constant by circulating thermostatted water in the jacket. In all experiments with sodium acetate, the eluent was buffered at pH 5.9 by addition of acetic acid.

The aldonic acids were neutralized with sodium hydroxide, before the samples (0.5–3.0 mg) were applied to the column. The nominal flow-rate during the elution was 2.5–8.5 cm min^{-1} (in the empty part of the column).

The analysis system was the same as that described earlier[8]. The three-channel analyzer (carbazole, chromic acid and periodate–formaldehyde methods) was used, but in most of the experiments the chromic acid oxidation only was employed.

The ion-exchange capacity of the resin used in the studies of the influence of temperature on the separation of oligomeric aldonic acid was determined. The resin was converted completely into the Cl^- form by treatment with an excess of 1 *M* sodium chloride and washed with water. The chloride ions were eluted with 1 *M* sodium nitrate solution and determined by potentiometric titration with silver nitrate. The exchange capacity was 1.203 mequiv./cm^3 of the resin bed (acetate form).

CHROMATOGRAPHY IN VARIOUS MEDIA AT 30°

The peak elution volumes of the acids investigated were determined in sodium acetate, acetic acid and potassium tetraborate, both in runs with single species and with mixtures of different acids. In sodium acetate and acetic acid media, the peak positions of a single compound were, within the studied range, independent of the amount applied to the column, of the presence of other species and of the flow-rate. This means that the volume distribution coefficients, D_v, calculated from the peak elution volumes[9] represent the equilibrium values for the exchange of trace amounts in these media. The values obtained at 30° are listed in Table I.

In sodium acetate the D_v values were determined both in 0.02 and 0.08 *M* solutions and in agreement with the simplified theory[10] the values recorded at the higher concentration were close to those calculated from determinations at the lower concentration by dividing by four. This means that the ratio between the activity coefficients of the eluted ions and acetate ions in the external solution was almost constant.

In sodium acetate medium, the ionic size of the anions is the factor which has the largest influence upon the separation of strongly hydrophilic species. For various polyhydroxy acids, it was observed that those with a large ionic size exhibited lower D_v values than smaller anions[11]. In agreement with this rule, it was found that within both oligomeric series the compounds appeared in order of decreasing molecular size. Moreover, all members of the xylonic acid series exhibited higher D_v values than

TABLE I

RETENTION DATA RECORDED IN SODIUM ACETATE, ACETIC ACID AND POTASSIUM TETRABORATE AT 30°

Acid	*Volume distribution coefficients, D_v*				*B value*
	NaAc (pH 5.90)		*HAc, 0.5 M*	*$K_2B_4O_7$, 0.15 M*	
	0.02 M	*0.08 M*			
Xylonic	33.4	8.19	15.7	10.7	1.28
Xylobionic	23.8	5.85	9.40	7.81	1.31
Xylotrionic	11.7	2.88	4.26	5.44	1.86
Xylotetraonic	5.85	1.48	2.05	4.0	2.7
Xylopentaonic	2.92	0.77	1.17	3.0	4.1
Xylohexaonic	1.47	0.38	0.80	2.3	6.2
Gluconic	30.2	7.26	12.5	21.3	2.82
Cellobionic	16.6	3.85	6.20	12.9	3.11
Cellotrionic	8.00	1.86	2.82	6.52	3.26
Cellotetraonic	3.86	0.89	1.33	4.0	4.1
Cellopentaonic	1.90	0.44	0.72	2.7	5.7
Cellohexaonic	0.96	0.21	0.43	1.5	6.3

the corresponding species belonging to the gluconic acid series.

A plot of the logarithm of the D_v values against the number of monomeric units in the oligomers (*DP*) is reproduced in Fig. 1. It can be seen that, starting with the aldobionic acids (*DP* = 2), straight lines were obtained. This means that for each additional sugar moiety a constant incremental change is obtained in log D_v, which in its turn is proportional to the free energy change (ΔG^0) of the ion-exchange process. The observation that this relationship is not valid for the monomeric acids was ex-

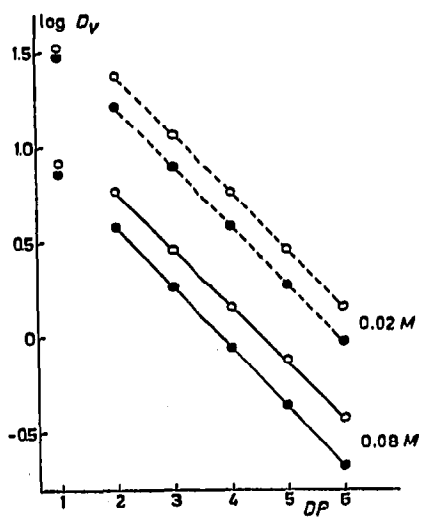

Fig. 1. Relationship between log D_v and the number of monomeric units (*DP*) in the oligomeric aldonic acids. Eluent: 0.02 *M* (dashed lines) and 0.08 *M* (solid lines) sodium acetate (pH 5.9). Resin bed: Dowex 1 X8, 25–30 μm, 30°. ○, Xylonic acid oligomers; ●, gluconic acid oligomers.

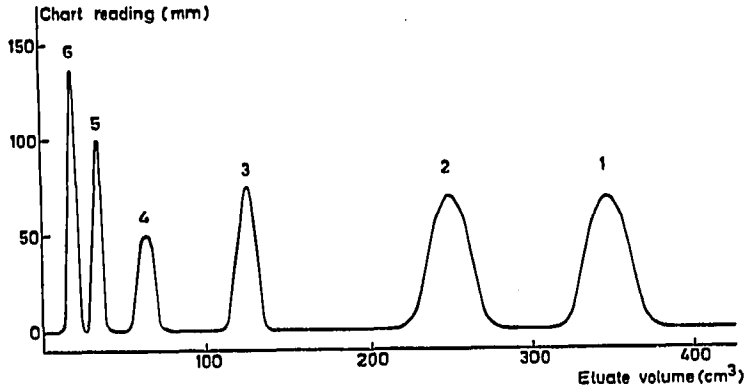

Fig. 2. Separation of oligomeric aldonic acids of xylonic acid series in 0.02 *M* sodium acetate by the chromic acid method. Nominal flow-rate: 2.8 cm min^{-1} (calculated for an unpacked column). Resin bed: 4 × 840 mm, Dowex 1 X8, 25–30 μm. 1 = Xylonic (2 mg); 2 = xylobionic (2 mg); 3 = xylotrionic (1.0 mg); 4 = xylotetraonic (0.5 mg); 5 = xylopentaonic (0.5 mg); 6 = xylohexaonic acid (0.5 mg).

pected with regard to the structure of the oligomeric aldonic acids referred to above. Plots of this type are extremely valuable for identification purposes in analyses of oligomeric compounds when authentic samples of all oligomers are not available.

The first four species within each oligomeric series can be rapidly separated in 0.08 *M* sodium acetate, but when higher oligomers are to be separated, it is recommended that a lower eluent concentration is used so as to avoid overlapping. A separation of the six members of the xylonic acid series in 0.02 *M* sodium acetate solution is illustrated in Fig .2. The conditions were chosen so that a complete separation was obtained of the species that appeared first on the chromatogram. This means that the distances between the peaks which appeared later on the chromatogram were excessively large. The elution of the last peak required about 14 h. In routine work, the separation can be speeded up by using gradient elution.

The run referred to in Fig. 2 was carried out with a resin of comparatively large particle size (25–30 μm) and to avoid broadening of the curves the flow-rate was fairly low. In agreement with earlier observations on separations of uronic acids[2], it was found that the time could be reduced appreciably when finer particles (13–18 μm) and higher flow-rates were used. With this resin, a clean-cut separation of the six oligomers was achieved within 5 h. An example of a more difficult separation, namely that of the first three members of both the xylonic and gluconic acid series, is illustrated in Fig. 3. The separation was carried out on a column with fine particles at a nominal flow-rate of 8.5 cm min^{-1} in the unpacked part of the column, which corresponds to a linear interstitial flow-rate of about 21 cm min^{-1}. The last compound was eluted within 4.5 h. It is noteworthy that, although a much higher velocity was used, the elution curves of comparable species were sharper in this run. This chromatogram demonstrates the usefulness of the application of the three-channel analyzer for identification purposes. As expected, all oligomeric species gave a response in the carbazole channel, whereas no reaction occurred with xylonic and gluconic acids. On the other hand, only these acids gave a very strong response in the periodate–formaldehyde channel. As expected, cellobionic and cellotrionic acids were recorded in this

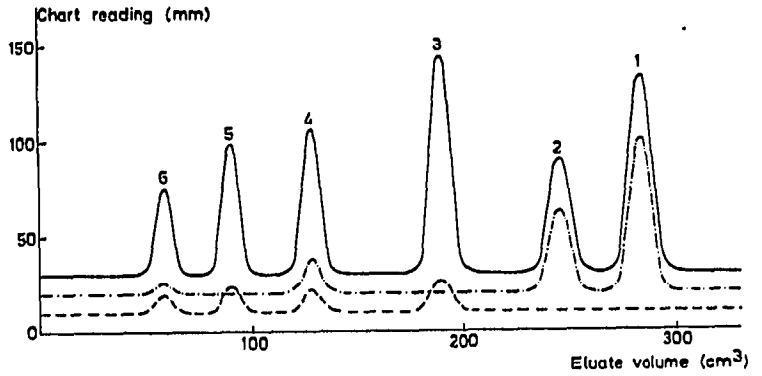

Fig. 3. Separation of 1.0 mg of xylonic (1), 0.6 mg of gluconic (2), 1.0 mg of xylobionic (3), 0.6 mg of cellobionic (4), 0.5 mg of xylotrionic (5) and 0.3 mg of cellotrionic acid (6). Channels: ———, chromic acid; — · — · — · —, periodate–formaldehyde; — — —, carbazole. Eluent: 0.02 *M* sodium acetate, pH 5.9. Nominal flow-rate: 8.5 cm min^{-1}. Resin bed: 4 × 670 mm, Dowex 1 X8, 13–18 μm.

channel, whereas the oligomeric acids belonging to the xylonic acid series, which are lacking a primary hydroxyl group *vicinal* to a free hydroxyl group, did not give rise to formaldehyde.

As in sodium acetate medium, symmetrical elution peaks were recorded in 0.5 *M* acetic acid with all of the species investigated. Within each oligomeric series, the peaks appeared in order of decreasing molecular weight. In addition to the factors that determine the D_v values in sodium acetate, the strength of the eluted acid has a predominant influence upon the distribution coefficients when acetic acid is used as eluent[11]. The simplified method for calculation of the D_v value in acetic acid medium from that in sodium acetate and from the dissociation constant of the acid can be used to obtain a rough idea of the elution behaviour in acetic acid. An exact agreement between calculated and observed D_v values cannot be expected as it has been found[12] that the acetate counter ions in the resin have a strong tendency to give association compounds by hydrogen bonding with undissociated acids. The results in Table I show that the separation factor, xylonic acid–gluconic acid, is higher in acetic acid than in sodium acetate and that the same holds true for the oligomers of the same degree of polymerization (*DP*). A probable explanation is that xylonic acid and its oligomers are slightly stronger acids than the corresponding species within the gluconic acid series.

The results show that acetic acid is a more suitable eluent in separations when species belonging to both groups are present. This holds true for the lower oligomers and was confirmed in chromatographic runs with the four lowest members of each series. On the other hand, the distribution coefficient of xylohexaonic acid is fairly close to that of cellopentaonic acid (reversed order compared with sodium acetate), which means that sodium acetate of low concentration, *e.g.*, 0.01 *M*, is the more favourable eluent in separations of higher oligomers belonging to both series.

In Fig. 4, the logarithm of the distribution coefficient is plotted against the *DP* of the aldonic acids. It can be seen that curved lines were obtained. The positions of xylonic and gluconic acids relative to those of the oligomers of *DP* = 2 and 3 were expected with regard to the results obtained in sodium acetate medium, whereas

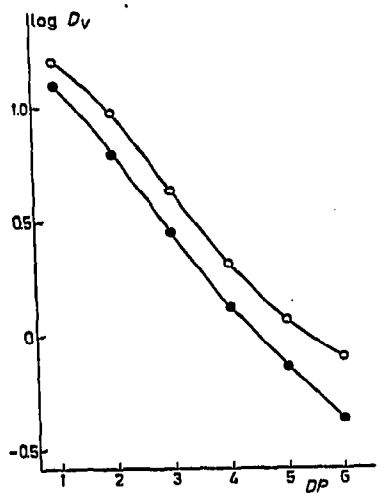

Fig. 4. Relationship between log D_v and the number of monomeric units (*DP*) in the oligomeric aldonic acids. Eluent: 0.5 *M* acetic acid. Resin bed: Dowex 1 X8, 25–30 μm, 30°. ○, Xylonic acid oligomers; ●, gluconic acid oligomers.

the D_v values corresponding to the higher oligomers were markedly higher than those corresponding to a straight-line extrapolation. These results, as well as those obtained in sodium acetate, show that the permeation of even the highest oligomers (length about 30 Å) into a resin with acetate as the predominant counter ion is not subjected to detectable restrictions. A possible explanation of the positive deviation from the straight-line relationship is that in acetic acid medium, hydrogen bonding in the resin phase has a larger influence with higher oligomers than with dimeric and trimeric species.

In tetraborate medium, complex formation between borate and the hydroxy groups in polyhydroxy compounds has a predominant influence upon the retention volume. As can be seen from Table I, the order of elution between xylonic and gluconic acids was reversed. In agreement with earlier observations, the separation factor was very large[13]. Within each oligomeric series, the aldonic acids were eluted in order of decreasing molecular weight. Symmetrical and comparatively sharp elution peaks were recorded for the three lowest oligomers, whereas the peaks that appeared first on the chromatogram and corresponded to the higher oligomers were broad and non-symmetrical. The broadest peaks were recorded with the aldohexaonic acids. Experiments carried out at different flow-rates showed that the establishment of equilibrium was very slow when higher oligomers were involved. For the higher oligomers, the D_v values reported in Table I should not be taken as true equilibrium distribution coefficients but rather as approximate measures of the adjusted retention volume calculated in column volumes.

The theoretical treatment of the elution behaviour in tetraborate medium[14] is complicated by the fact that various species of borate ions are present in the eluent. A rough, relative estimate of the contribution of the complex formation to the ion-exchange affinities of various species can be obtained from the *B* value, defined as the ratio of the D_v value in borate medium to that in acetate medium at an arbitrarily

chosen acetate concentration. As in previous work, 0.08 *M* sodium acetate was chosen as a reference, but as the values obtained for the highest oligomers may be uncertain, the applied D_v in sodium acetate was that calculated from the runs in 0.02 *M* sodium acetate. The *B* values are listed in the last column in Table I. The behaviour of various monomeric aldonic acids will be discussed elsewhere[15], and only a few comments on the D_v and *B* values of the oligomeric species will therefore be made here.

The observation that, within both series, the D_v values decrease markedly with increase in the *DP* of the oligomers strongly indicates that, as in acetate medium, the ionic size has a predominant influence upon the retention volume in borate medium and that for species of equal complexing ability those of larger size are eluted ahead of smaller ions.

It is noteworthy that the *B* values increase within both oligomeric series, but that within the xylonic acid series the difference between xylonic and xylobionic acids is hardly significant. Xylonic acid contains four hydroxyl groups on adjacent carbon atoms and can, therefore, theoretically add two borate ions at the most. The comparatively large differences between the *B* values of the higher oligomers strongly indicate that not only the aldonic acid group but also the sugar moieties contribute to the complex formation. Xylobionic acid contains only three hydroxyl groups in its aldonic acid moiety, which means that only one borate ion can be added to the xylonic acid moiety. This loss in complexing ability is compensated for by the presence of an additional xylose unit and explains why xylonic and xylobionic acids exhibit almost the same *B* values.

The fact that cellobionic acid exhibits a larger *B* value than gluconic acid can not be ascribed to a significantly larger effect of the glucose moiety than of a xylose unit, as the results obtained for the higher oligomers indicate that a glucose moiety contributes less than a xylose unit to the complex formation. A more likely explanation is that the loss in complexing ability of the gluconic acid moiety is less important, as two pairs of *vicinal* hydroxyl groups, which have the ability to participate in the complex formation, are present in the gluconic acid moiety of cellobionic acid.

The *B* values, which can be calculated for the higher oligomers, are less accurate and as already mentioned the size of the ions seems to have a predominant influence upon the distribution coefficients. For these reasons, the reported *B* values will give only a very crude picture of the complexing ability of the higher oligomers and no attempt will therefore be made to interpret the results in detail.

From a practical point of view, the application of tetraborate solution as eluent can be advantageous in analyses of complex mixtures of different aldonic acids and their lower oligomers in the presence or absence of additional acids. When only species of the xylonic and gluconic acid series are involved, the separation can equally well be carried out in sodium acetate or acetic acid. For the highest oligomers, the broadening of the elution curves in borate medium is so serious that this eluent is unsuitable.

INFLUENCE OF THE TEMPERATURE UPON SEPARATIONS IN SODIUM ACETATE MEDIUM

As shown above, in sodium acetate there exists a linear relationship between log D_v and the *DP* of the oligomeric acids in both series. This means that the change in

free energy (ΔG^0) is an additive property within each of the oligomeric series. For this reason, it was of interest to study the temperature dependence of the distribution coefficients in order to obtain information about the contributions of enthalpy and entropy changes to ΔG^0. The experiments were carried out in 0.02 *M* sodium acetate solution.

As already mentioned, the D_v values in 0.08 *M* sodium acetate solution are close to those calculated from the values in a 0.02 *M* solution by dividing by four. It can therefore be concluded that the ratio of the activity coefficients of the eluted anions to those of acetate ions in the external solution is independent of the acetate concentration within the investigated range. Because, within this range, sodium acetate exhibits a behaviour typical of alkali salts, which give a negligible ion pairing and formation of association compounds, it can be concluded that the activity coefficient ratio in the external solution can be taken as unity and that the selectivity is entirely dependent upon the conditions inside the resin phase. The observed selectivity coefficient, $k_{A/Ac}$, determined in 0.02 *M* sodium acetate solution can therefore be taken as a measure of the corrected selectivity coefficient, k^a.

The selectivity coefficient is calculated from the D_v values by means of the equation $k_{A/Ac} = 0.02\ D_v/[Ac]_r$. As the electrolyte invasion can be neglected and the eluted ions were present in trace amounts, $[Ac]_r$ is equal to the exchange capacity of the resin calculated per cubic centimetre of the bed volume[9]. Determinations of the D_v values were carried out within the temperature range 0–95° with all of the acids investigated in this work. The selectivity coefficients are listed in Tables II and III together with the changes in free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0) calculated[16] from the equations

$$\Delta G^0 = -RT \ln k^a$$

$$\frac{\mathrm{d} \ln k^a}{\mathrm{d}(1/T)} = -\frac{\Delta H^0}{R}$$

$$\Delta S^0 = (\Delta H^0 - \Delta G^0)/T$$

At all temperatures, plots of $\ln k^a$ against *DP* for the oligomers gave straight lines within the range $DP = 2$–6, demonstrating that the change in free energy of the ion exchange is an additive property of the oligomeric compounds within the whole temperature range (*cf.*, Fig. 1). Plots of $\ln k^a$ against $1/T$ resulted in curved lines at all temperatures and with all of the investigated species. ΔH^0 was determined from the slope of the curves.

As can be seen from Tables II and III, all of the selectivity coefficients were less than unity, which means that acetate ions were held more firmly than all of the aldonate ions. The ΔH^0 values depended largely upon the temperature, an observation made previously for many other exchanges of ions of equal charge[17]. At high temperatures, ΔH^0 was positive in all exchanges and for the highest oligomers ΔH^0 was positive also at low temperatures. At a given temperature, the values increased with increase in ionic size, *i.e.*, with decrease in ion-exchange affinity. This holds true for both oligomeric series. Observations that the uptake of an ion of low affinity is an endothermic reaction and that ΔH^0 increases with decrease in ion-exchange affinity have previously been made for exchanges of alkali metal ions on sulphonic

TABLE II

SELECTIVITY COEFFICIENTS AND THERMODYNAMIC FUNCTIONS OF ANION-EXCHANGE EQUILIBRIA AT VARIOUS TEMPERATURES FOR OLIGOMERS OF XYLONIC ACID

Acid	*Temperature (°C)*	*Selectivity coefficient,* $k_{A/Ac}$	$\Delta H°$ *(kcal mole*$^{-1}$*)*	$\Delta G°$ *(kcal mole*$^{-1}$*)*	$\Delta S°$ *(cal mole*$^{-1}$ *deg*$^{-1}$*)*
Xylonic	0	0.615	−0.70	0.26	−3.5
	30	0.554	−0.32	0.36	−2.2
	60	0.548	0.18	0.40	−0.7
	95	0.578	0.68	0.40	0.7
Xylobionic	0	0.459	−0.97	0.42	−5.1
	30	0.396	−0.54	0.56	−3.6
	60	0.375	−0.02	0.65	−2.0
	95	0.391	0.40	0.69	−0.8
Xylotrionic	0	0.221	−0.77	0.82	−5.8
	30	0.195	−0.36	0.99	−4.4
	60	0.191	0.18	1.10	−2.8
	95	0.201	0.48	1.17	−1.9
Xylotetraonic	0	0.105	−0.44	1.22	−6.1
	30	0.097	−0.10	1.40	−5.0
	60	0.100	0.32	1.53	−3.6
	95	0.108	0.87	1.63	−2.1
Xylopentaonic	0	0.049	−0.22	1.63	−6.8
	30	0.048	0.16	1.82	−5.5
	60	0.053	0.66	1.95	−3.9
	95	0.059	1.05	2.08	−2.8
Xylohexaonic	0	0.024	0.00	2.03	−7.4
	30	0.025	0.46	2.24	−5.9
	60	0.028	0.87	2.38	−4.5
	95	0.032	1.31	2.51	−3.3

acid resins[17–19] and halide ions on resins containing quaternary ammonium ions[16,18].

Exceptions to this rule were found at low temperatures for the lower oligomers within both series. In these cases, heat was evolved during their uptake although acetate was the preferred ion. Evidently, no correlation exists of general validity between the ion-exchange affinities and enthalpy changes. On the other hand, within each oligomeric series starting with the aldobionic acid, a straight-line relationship exists between ΔH^0 at a given temperature and the number of monomeric sugar units in the oligomeric aldonic acids. These results show that, as is the change in free energy, the change in enthalpy is an additive property within both series of oligomeric acids. For the xylobionic acid series, the slope of the straight lines was not affected to any detectable extent by changes in temperature from 0° to 95°. For the cellobionic acid series, the slopes of the straight lines were largely dependent upon the temperature and, as can be seen from the values in Table III, the slope was extremely high at 0°. As both ΔH^0 and ΔG^0 are additive properties, it follows that the same holds true for the entropy change.

In exchanges of alkali metals on cation exchangers as well as halide ions on

TABLE III

SELECTIVITY COEFFICIENTS AND THERMODYNAMIC FUNCTIONS OF ANION-EXCHANGE EQUILIBRIA AT VARIOUS TEMPERATURES FOR OLIGOMERS OF GLUCONIC ACID

Acid	*Temperature (°C)*	*Selectivity coefficient,* $k_{A/Ac}$	$\Delta H°$ *(kcal mole^{-1})*	$\Delta G°$ *(kcal mole^{-1})*	$\Delta S°$ *(cal mole^{-1} deg^{-1})*
Gluconic	0	0.544	—0.84	0.46	—4.7
	30	0.501	—0.34	0.42	—2.5
	60	0.501	0.12	0.46	—1.0
	95	0.515	0.22	0.49	—0.7
Cellobionic	0	0.285	—0.42	0.68	—4.0
	30	0.275	—0.12	0.78	—3.0
	60	0.275	0.16	0.85	—2.1
	95	0.286	0.36	0.92	—1.5
Cellotrionic	0	0.126	0.50	1.13	—2.3
	30	0.133	0.12	1.22	—3.6
	60	0.133	0.18	1.34	—3.5
	95	0.139	0.44	1.45	—2.7
Cellotetraonic	0	0.057	1.09	1.56	—1.7
	30	0.064	0.24	1.66	—4.7
	60	0.066	0.30	1.80	—4.5
	95	0.070	0.68	1.94	—3.4
Cellopentaonic	0	0.026	1.55	1.98	—1.6
	30	0.032	0.58	2.08	—5.0
	60	0.033	0.48	2.26	—4.8
	95	0.035	0.60	2.45	—5.0
Cellohexaonic	0	0.012	2.19	2.41	—0.8
	30	0.016	0.79	2.49	—5.6
	60	0.017	0.52	2.70	—6.6
	95	0.018	0.76	2.86	—5.7

anion exchangers, it has been shown that the entropy of the system increases with the uptake of an ion of lower affinity[17,18]. As can be seen from Tables II and III, the opposite holds true for all aldonate anions in their exchange for acetate ions (except for xylonic acid at the highest temperature). Within the xylonic acid series, the decrease in entropy is larger for the higher oligomers than for those of smaller ionic size. This holds true also for the gluconic acid series within the temperature range 30–95°, whereas at 0° the order is reversed.

Within the xylonic acid series, the decrease in entropy is much larger at low than at high temperatures, and the same holds true for gluconic acid. For cellobionic acid the temperature effect is less pronounced and for the higher oligomers the largest decrease in ΔS^0 was observed at a medium temperature.

The differences between the species of the two oligomeric series show that the ion-exchange behaviour of the strongly polar anions is not only determined by the size and the number of hydroxyl groups but also by the structure and conformation of the species. Changes in both enthalpy and entropy contribute to the anion-exchange

affinity and their relative importance at a given temperature is different when different species are exchanged for acetate ions.

The results in Tables II and III show that for several species appreciable advantages were gained, as far as the separation factors are concerned, when the separations were carried out at very low temperatures. On the other hand, it is inconvenient to use temperatures below room temperature, because of the cooling required and the increased viscosity and broadening of the elution curves when high flow-rates are used. The separation factors of the individual species, within each oligomeric series, were affected only to a slight extent by an increase in temperature from 30° to 95°. On the other hand, the elution peaks were sharper and the counter pressure lower at high temperatures. A more rapid separation can therefore be achieved at high temperatures.

CALCULATION OF THE SWELLING PRESSURE

According to the Gibbs–Donnan theory, the corrected selectivity coefficient of the exchange between aldonate ions (A) and acetate ions (Ac) is given by the equation

$$\ln k^a_{A/Ac} = \pi(\bar{v}_{Ac} - \bar{v}_A)/RT + \ln(\gamma_{Ac(r)}/\gamma_{A(r)}) \quad (1)$$

where π is the swelling pressure, $\bar{v}$ are the partial molal volumes and γ the activity coefficients of the exchanging ions within the resin phase.

In most of the exchanges studied previously, the contribution of the pressure–volume term to k^a was fairly small[20]. On the other hand, it has been shown that the pressure–volume term has a predominant influence upon the distribution of polyalcohols between ion-exchange resins and an external aqueous solution[21].

In dilute aqueous solutions of oligosaccharides[22], a linear relationship exists between the partial molal volume and the number of monosaccharide moieties in the oligomer (DP). It is reasonable to assume that, starting from the aldobionic acids (aldobionate anions; $DP = 2$), the same holds true for the oligomeric aldonate ions and that the incremental change in partial molal volume, $\Delta\bar{v}$, obtained for each additional sugar moiety, is the same as that obtained for the corresponding sugar. Eqn. 1 can therefore be written as

$$\ln k^a_{A/Ac} = \frac{\pi[\bar{v}_{Ac} - \bar{v}_2 - \Delta\bar{v}(DP - 2)]}{RT} + \ln\frac{\gamma_{Ac(r)}}{\gamma_{A(r)}} \quad (2)$$

($DP \geqslant 2$), or

$$\ln k^a_{2/Ac} - \ln k^a_{A/Ac} = \frac{\pi}{RT}\Delta\bar{v}(DP - 2) + \ln\frac{\gamma_{A(r)}}{\gamma_{2(r)}} \quad (3)$$

where the subscript 2 refers to aldobionate ions.

As can be seen from eqn. 3, the activity coefficient term should be either very small or proportional to $(DP - 2)$. Numerical values of $\Delta\bar{v}$ can be obtained from a recent investigation by Brown and Chitombo[22], who found that, for oligosaccharides of the β-(1,4)-D-glucose series, $\Delta\bar{v}$ is 100 cm^3 mole^{-1}, whereas for the xylose series it is 85. By inserting these $\Delta\bar{v}$ values and a value of the swelling pressure obtained by interpolation of the data reported by Boyd and Soldano[24] (see also ref. 23) (π = 200 atm),

it can be seen that in the systems studied the influence of the activity coefficient term is small and that the pressure–volume term predominates.

It should therefore be possible to obtain a rough estimate of the swelling pressure from the correlation between the selectivity coefficients and the *DP* of strongly polar compounds under the assumption that the ratio between the activity coefficients within a series of oligomers is unity. Sufficient amounts of oligomeric acids were not available for determinations of the $\Delta\bar{v}$ values to be carried out, and in the present work the values determined by Brown and Chitombo[22] for aqueous solutions of sugars at 20° were taken as the $\Delta\bar{v}$ values of the aldonate ions in the resin phase at 30°. With these approximations, a calculation according to eqn. 3 gave a swelling pressure of 175 atm when the calculations were based on the determinations for the gluconic acid series, and 200 atm with the data for the xylonic acid series. With regard to the approximations introduced, the mutual agreement can be considered as good and so can the agreement with the value taken from the paper by Boyd and Soldano[24], based upon isopiestic measurements. Once the swelling pressure of a given resin is known, eqn. 3 can be used in order to predict roughly the slope of the lines in plots of log D_v (or log k^a) against partial molal volume.

The fact that it seems reasonable to disregard the activity coefficient term in eqn. 3 does not necessarily mean that the same holds true for the activity coefficient terms in eqns. 1 and 2. Because, as a rule, small ions of very similar size exhibit significant differences in their ion-exchange affinities, it would be a fortuitous effect if this term should be negligible for the monomers and lowest oligomers. A calculation shows, however, that the relative importance of the pressure–volume term increases very rapidly with increase in *DP* and that the error obtained by disregarding the interaction term is very small for the higher oligomers. Eqns. 1 and 2 can therefore be applied conveniently in order to predict the selectivity coefficients and hence the peak positions on chromatograms for higher oligomeric acids, provided that relevant partial molar volumes are known or can be estimated from data for related substances.

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