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ANION-EXCHANGE CHROMATOGRAPHY OF MONOCARBOXYLIC HYDROXY-ACIDS IN BORATE MEDIUM

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ABSTRACT

The formation of borate complexes contributes significantly to the distribution coefficients obtained in the separation of polyhydroxy acids in borate medium. A study of a large number of straight-chain hydroxy acids shows that these contributions tend to increase with the number of vicinal hydroxyl groups and with their distance from the carboxylate group. Vicinal hydroxyl groups in *gauche* conformation have the ability to form strong complexes, provided that the steric conditions are favourable in other respects. The contributions of vicinal hydroxyl groups in *anti* conformation are much less.

INTRODUCTION

Anion-exchange chromatography in tetraborate solution is a valuable tool for the separation of polyhydroxy acids[1–3]. A higher selectivity is quite often obtained in this medium than, for example, in sodium acetate, this fact can be attributed to the formation of a complex between borate ions and the polyhydroxycarboxylate anions.

In the present work, the distribution coefficients (D_v) of a large number of acyclic hydroxy acids have been determined in potassium tetraborate solution to demonstrate the usefulness and limitations of this technique in practical separations. An attempt has also been made to correlate the structure of individual species and their chromatographic behaviour.

EXPERIMENTAL

For all experiments, the column (usually 4.4×880 mm) was packed with strongly basic, anion-exchange resin (Dowex 1-X8, 25–27 μm). The eluate was analyzed automatically by chromic acid oxidation[4]. When acetic acid was present, silver sulphate (10 g/l) was added to effect oxidation.

The volume distribution coefficients (D_v) were calculated from the peak elution volumes ($\bar{v}$) according to the equation $D_v = \bar{v}/X - \varepsilon_I$, where X is the total bed volume and ε_I the relative interstitial volume ($\varepsilon_I = 0.4$). Experiments carried out with

D-gluconic acid in 0.15M potassium tetraborate showed that the D_v values differed by less than $\pm 1\%$ from the mean when the amount applied to the column varied within the range 0.15–1.5 mg. Moreover, changes in the flow rate had no detectable influence. The highest, nominal, linear flow was 5.1 $cm.min^{-1}$ (in the empty column above the resin bed). Most experiments were made at a flow rate of 3.8 $cm.min^{-1}$.

The acids were applied to the column as their sodium salts in aqueous solution (pH 8). Lactones and esters present in the original samples were saponified before the sample was introduced on to the column. Variations in the volume of the sample (1–8 ml) had no detectable influence on the distribution coefficients. The acid samples were the same as those previously studied[5,6], except for 2-*C*-methylarabinonic acid, which was kindly supplied by Professor Olof Theander, and the 3,5-dideoxypentonic acids which were prepared by cyanohydrin synthesis followed by hydrolysis[7].

DISCUSSION

Influence of the eluent concentration. — In principle, it should be possible to predict the influence of the eluent concentration on the distribution coefficients if the stability constants of the borate complexes are known[8]. Calculations of this type are, however, complicated by the fact that several species of borate ions are present in tetraborate solutions[9,10].

In non-complexing media, it is common practice[11] to illustrate the influence of eluent concentration (c) on the distribution coefficients by plots of $\log D_v$ against $-\log c$. If no complexes are present, a straight line is approached. The slope is equal to the ratio of the charge of the eluted ion to that of the eluent ion.

In tetraborate solutions of very low concentration, ions with a charge of -1 preponderate, but, at higher concentrations, appreciable amounts of polymerized anions with a charge of -2 are present[9,10]. For this reason, a straight-line relationship can only be expected within a narrow range of concentration, even for species which do not give any borate complexes. For acids which do not interact with the tetraborate solution, the slope should be less than unity and approach unity in very dilute solution. The results given in Fig. 1 show that the slope is dependent on the borate concentration and decreases with increasing concentration of borate. This can be explained by the increasing proportion of divalent borate ions in the eluent.

The results show that for species which cannot give any bidentate complexes, such as 3-hydroxypropanoic and glycolic acids and even acetic and formic acids, the slope is much greater than 1, indicating that there are strong interactions with the carboxylate ions in tetraborate medium. No attempt will therefore be made to apply the distribution coefficients for calculation of the stability constants and the ligand numbers of the complexes between borate and carboxylate anions containing several hydroxyl groups.

Fig. 1 shows that those polyhydroxy acids which exhibit the largest distribution coefficients also exhibit the highest slopes at low concentration of borate. The results for xylonic acid coincide with those obtained with talonic acid and, for clarity, are not

included. The slopes depend both upon the stability constants and on the ligand number[8]. The fact that the values determined for arabinonic and galactonic acid are above 2 indicates that higher complexes with a charge of -3 are formed with these species.

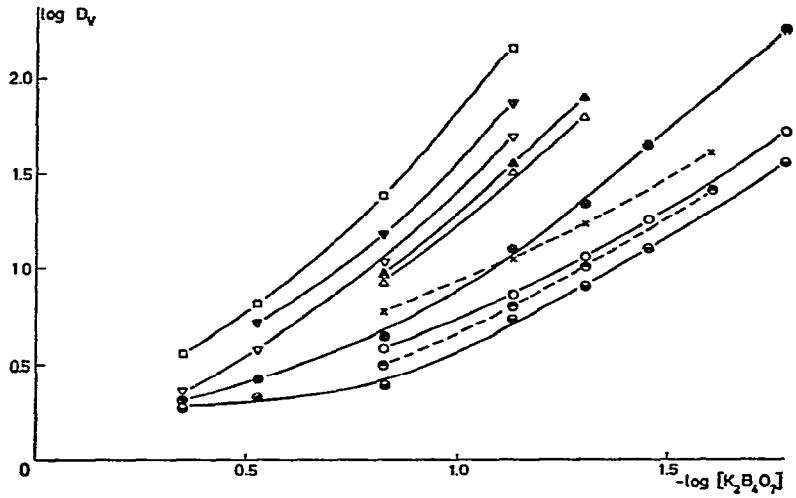

Fig. 1. Influence of the tetraborate concentration on the volume distribution coefficients. The values given below represent the slopes of the curves at low concentration. ◒ 3-Hydroxypropanoic, 1.40; ◓ Acetic, 1.38; ○ Glycolic, 1.64; ● Glyceric, 1.82; × Formic, 1.29; △ Lyxonic, 1.90; ▲ Ribonic, 2.04; ▽ Talonic, 2.18; ▼ Arabinonic, 2.44; □ Galactonic, 2.64.

From a practical point of view, it is noteworthy that the curves representing the hydroxy acids do not intersect within the investigated range of concentration, which is greater than that of interest in practical chromatographic work. The difference between log D_v of various species tends to increase at low concentration. This is in agreement with the observations that improved separations are obtained at low concentration of borate[1].

Correlation between the structure and the distribution coefficients. — In non-complexing media, *e.g.*, sodium acetate, the distribution coefficients of strongly polar anions tend to decrease with increasing ionic size[6]. This observation, which is in agreement with the Gibbs–Donnan theory[11] is valid also in borate medium. Another factor that greatly influences the selectivity is the strength of the parent acid. For anions of comparable size, the higher the strength of the parent acid[12], the more strongly they are held.

The D_v values in borate medium cannot, therefore, be used as criteria of the complexing ability of the anions. A better measure of the contributions of the complex formation to the distribution coefficients is the ratio of the distribution coefficients in borate medium to that in a non-complexing medium. All acids included in the present work have previously been studied in 0.08M sodium acetate[5,6]. Since a straight-line

relationship exists between $\log D_v$ and $-\log c$ with slopes approximately equal to unity, this medium was chosen as reference. The ratio of the D_v value in 0.15M potassium tetraborate to that in 0.08M sodium acetate is designated by B. These values will be used in comparing the contributions of the complex formation to the ion-exchange affinities. A high B-value reflects a strong ability of an anion to form borate complexes, but it should be stressed that both the ligand number and the stability constants will affect the B-values[8]. For strongly polar, dicarboxylic acids, the positions of the carboxylic acid groups influence the D_v values in non-complexing media, and it can therefore be expected that the distances between the ionic groups in borate–polyhydroxycarboxylate complexes also have an influence and that, for this reason, the B-values may differ even for compounds which exhibit the same ligand numbers and stability constants.

In addition, non-polar interactions contribute markedly to the ion-exchange affinities of non-polar species such as 2-hydroxypentanoic acid in non-complexing media[6]. An increased size of the hydrocarbon portion of the anion is reflected not only in increased D_v values but also in a tailing of the elution peaks.

Table I shows that the D_v values in tetraborate solution of the 2-hydroxy acids become markedly higher with an increasing number of methylene groups. Starting with 2-hydroxybutanoic acid, an increasingly severe tailing occurred. The observation that the B-values are much higher for the least polar species than for the more polar anions cannot be attributed to increased complex formation but rather to larger non-polar contributions in borate medium than in sodium acetate.

It has been demonstrated that the anions corresponding to 2-hydroxycarboxylic acids have the ability to form weak complexes in a borate medium[13,14]. Since the charge of the anions is unaffected, the influence of this type of complex formation on the ion-exchange affinities of hydroxy acids can hardly be predicted. The observation (Table I) that 2-hydroxypropanoic acid is retained more strongly than 3-hydroxypropanoic acid, both in acetate and borate media, can be explained by its higher acid strength. Their B-values differ only slightly and cannot be taken as an indication that the complexing with borate increases the ion-exchange affinity of the 2-hydroxypropanoate anions. Similarly, the observation that 2-hydroxybutanoic acid is held much more strongly than 3-hydroxybutanoic acid is explained by its higher acid strength and by larger non-polar interactions. Therefore, the contribution of complexes with the carboxylate group will be disregarded in the discussion of the chromatographic behaviour of the polyhydroxy acids. Moreover, the polyhydroxy acids studied (with one exception) lacked large non-polar groups. Accordingly, the non-polar interactions should be small and often negligible.

The results obtained with species containing two or more hydroxyl groups show that both their number and location have a great influence. With all comparable species, the introduction of a methylene group next to the carboxylate groups results in an increased B-value. The results indicate that, with species of otherwise identical configuration, the complex formation is favoured by an increased distance between the carboxylate group and the hydroxyl group involved in the complexing. This

TABLE I

VOLUME DISTRIBUTION COEFFICIENTS (D_v) AND B-VALUES IN 0.15M $K_2B_4O_7$ AT 25°

Acid	D_v	*B*	*Acid*	D_v	*B*
Formic	6.63	0.249	3,6-Dideoxy-*ribo*-hexonic (sf)	4.57	0.573
Acetic	3.66	—	3,6-Dideoxy-*arabino*-hexonic (pz)	5.40	0.631
Glycolic	3.74	0.253	3-Deoxy-2-*C*-hydroxymethyl-tetronic	4.26	0.501
2-Hydroxypropanoic	3.99	0.290	3-*O*-Methyl-*ribo*-4-hexulosonic	11.0	1.10
2-Hydroxybutanoic	6.72 (t)	0.381	Ribonic (sf)	9.08	0.982
2-Hydroxypentanoic	28.1 (t)	1.01	Arabinonic (pz)	15.0	1.68
2-Hydroxyhexanoic	39–44 (t)	—	Xylonic (sf)	11.0	1.35
2-Hydroxy-2-methylbutanoic	7.75 (t)	0.435	Lyxonic (pz)	8.08	0.783
2-Hydroxy-3-methylbutanoic	14.02 (t)	0.537	2-Deoxy-*arabino*-hexonic (pz)	12.6	1.94
2-Hydroxy-3-methylpentanoic	31.3 (t)	0.700	2-Deoxy-*lyxo*-hexonic (pz)	8.00	1.19
3-Hydroxypropanoic	2.85	0.246	3-Deoxy-*ribo*-hexonic (sf)	7.91	1.11
3-Hydroxybutanoic	3.27 (t)	0.292	3-Deoxy-*arabino*-hexonic (pz)	7.10	0.936
3-Hydroxy-2,2-dimethylpropanoic	6.94 (t)	0.460	3-Deoxy-*xylo*-hexonic (pz)	7.54	1.10
4-Hydroxybutanoic	2.84	0.268	3-Deoxy-*lyxo*-hexonic (sf)	6.91	1.05
4-Hydroxypentanoic	3.39 (t)	0.320	6-Deoxymannonic (pz)	8.75	0.802
Glyceric (pz)[a]	4.80	0.403	6-Deoxygalactonic (pz)	17.9	2.15
3,4-Dihydroxybutanoic (pz)	4.14	0.444	2-*C*-Methylribonic	7.54	1.12
2,4-Dihydroxybutanoic (pz)	3.43	0.294	2-*C*-Methylarabinonic	9.24	1.05
4-Deoxyerythronic (pz)	4.39	0.381	3-Deoxy-2-*C*-hydroxymethyl-*erythro*-pentonic	5.18	0.855
4-Deoxythreonic (pz)	6.24	0.521	3-Deoxy-2-*C*-hydroxymethyl-*threo*-pentonic	5.28	0.817
3,5-Dideoxy-*erythro*-pentonic (sf)	4.93	0.457	3-*O*-Methylallonic (sf)	6.81	1.00
3,5-Dideoxy-*threo*-pentonic (pz)	4.25	0.346	3-*O*-Methylmannonic (pz)	7.51	1.19
2-*C*-Methylglyceric	4.08	0.368	3-*O*-Methylgulonic (sf)	5.54	1.14
2,4-Dideoxy-3-*C*-methylpentonic	5.62 (t)	0.476	3-*O*-Methyltalonic (sf)	6.35	1.24
Erythronic (pz)	6.53	0.628	*xylo*-4-Hexulosonic	29.9	2.23
Threonic (pz)	7.61	0.720	Galacturonic acid dimethyl acetal (pz)	14.9	2.88
2-Deoxy-*erythro*-pentonic (pz)	6.35	0.751			
2-Deoxy-*threo*-pentonic (pz)	6.31	0.853			
3-Deoxy-*erythro*-pentonic (sf)	4.83	0.582			
3-Deoxy-*threo*-pentonic (pz)	5.34	0.583			
2,6-Dideoxy-*ribo*-hexonic (sf)	6.76	0.800			

TABLE I (*Continued*)

Acid	D_v	*B*	*Acid*	D_v	*B*
Allonic (sf)	14.3	1.72	Talonic (sf)	11.0	1.49
Altronic (sf)	22.3	2.98			
Gluconic (sf)	20.7	2.87	D-*glycero*-L-*manno*-		
Mannonic (pz)	16.9	1.78	Heptonic (pz)	25.7	3.34
Gulonic (sf)	12.5	1.62	D-*glycero*-D-*gulo*-		
Idonic (sf)	19.8	2.90	Heptonic (sf)	19.9	3.00
Galactonic (pz)	23.5	3.14			

[a]Key: pz, planar zigzag; sf, sickle form; t, tailing.

distance rule is supported by the following order between the B-values: 3,4-dihydroxybutanoic > glyceric; 2-deoxy-*erythro*-pentonic > erythronic; 2-deoxy-*threo*-pentonic > threonic; 2-deoxy-*lyxo*-hexonic > lyxonic; 2-deoxy-*arabino*-hexonic > arabinonic.

Previously published data[15] regarding the complex formation of acyclic polyhydroxy compounds show that 1:1 complexes are formed with vicinal diols, and that their stability, reflected in the electrophoretic migration rate, is dependent on the configuration[16]. Vicinal *threo* diols give the most stable complexes since the preferred conformation is *gauche*. The distances between the hydroxyl groups are favourable for the formation of a bidentate borate complex[16,17]. With vicinal *erythro* diols in the planar zigzag form, the hydroxyl groups are *anti* and, accordingly, the situation is less favourable for complex formation. Terminal vicinal diols take an intermediate position, as far as the stability of the complexes is concerned[16,17].

It can, therefore, be predicted that the B-values should increase in the order: 4-deoxyerythronic < glyceric < 4-deoxythreonic acid. The results given in Table I are in agreement with this prediction and show that the D_v values also increase in this order. The results with other dihydroxy acids show, however, that there is no generally valid correlation between B and D_v. With the first two dihydroxy acids listed in Table I, the order is reversed. The fact that glyceric acid is retained more strongly than 3,4-dihydroxybutanoic acid, although its B-value is lower, is explained by the smaller size of the anion and the greater acid strength of the parent acid.

In aqueous solutions of unbranched, acyclic, polyhydroxy compounds, the planar zigzag (pz) form is preferred unless there are *syn*-axial interactions between the oxygen atoms. Whenever such 1,3-interactions are present in the zigzag form, a large proportion of the molecules is found to be in the sickle (sf) form[18,19]. When two hydroxyl groups separated by a methylene group are in the *erythro* configuration, a complex may sometimes be formed, indicating that the sickle form can invert to enable the formation of a 1,3-borate complex. The distances between the hydroxyl groups in 1,3-*threo* and terminal 1,3-diols are too large for the formation of stable borate complexes[16]. Among the dihydroxy acids studied, 3,5-dideoxy-*erythro*-pentonic acid belongs to the group which, in borate-free medium, is present preferentially as a sickle conformer. Its B-value is larger than that of the *threo* form and

that of 2,4-dihydroxybutanoic acid. The predicted conformer (pz or sf) of the polyhydroxy acid in borate-free medium is given in Table I.

As already mentioned, the non-polar contributions are larger in borate than in acetate medium, and various observations[17] indicate that, under comparable conditions, complexing enhances these contributions. A comparison between the observed B-values and the migration rates in electrophoresis of the corresponding alditols in borate medium indicates that, although the non-polar interactions are weak with 3,5-dideoxy-*erythro*-pentonic acid in acetate medium, the interactions in borate medium contribute significantly to its B-value. The presence of two non-polar groups close together on one side of the zigzag conformer and a borate group on the opposite side may explain the high B-value.

The observation that both D_v and B are large with 2,4-dideoxy-3-*C*-methylpentonic acid can be explained by non-polar interactions. The tailing of the peak supports this conclusion[6].

The tetronic and 2-deoxypentonic acids must be assumed to have a planar zigzag form, and by reference to the results already mentioned it can be predicted that the strongest complex would be formed with the vicinal *threo* hydroxyl groups. The B-values confirm this conclusion. The observations that the B-values of threonic and erythronic acids are larger than those for the corresponding 4-deoxytetronic acids show that terminal hydroxyl groups are involved in complex formation even when the structure is such that there is a competition with non-terminal hydroxyl groups.

It can be predicted that 3-deoxy-*erythro*-pentonic acid takes the sickle form by rotation about the C-3–C-4 bond. The situation is, therefore, unfavourable for the formation of the 2,4-complex. The 4,5-complex should therefore preponderate. The fact that the B-value is low and practically identical with that of the *threo* isomer supports these conclusions.

A rotation about the C-4–C-5 bond will give rise to the sickle form of 2,6-dideoxy-*ribo*-hexonic acid. As a result of this change, OH-4 and OH-5 will be *gauche* and thus give a strong complex which will stabilize the sickle conformation. The 3,5-complexes should therefore be of little importance, and so should the complex formation between OH-3 and OH-4 which are *anti*. In agreement with these assumptions, the B-value was slightly higher than that of 2-deoxy-*erythro*-pentonic acid.

The planar zigzag form is the most stable conformation of 3,6-dideoxy-*arabino*-hexonic acid, whereas with the *ribo* isomer, the sickle form is preferred. Evidently, only weak complexes can be formed, and in agreement with these conclusions the B-values are low with both diastereomers.

With 3-deoxy-2-*C*-hydroxymethyltetronic acid, the situation favours the formation of a terminal vicinal-diol complex in which the 2-*C*-hydroxymethyl group is involved. The B-value suggests that, in agreement with the results reported above (*cf.* 2,4-dihydroxybutanoic acid), the 2,4-complex is of little or no importance.

Among the trihydroxy acids studied, 3-*O*-methyl-*ribo*-4-hexulosonic acid exhibited the largest B-value although the configuration is favourable only for the

terminal vicinal-diol complex. This suggests that the keto group is hydrated and participates in the complex formation.

In contrast to the acids discussed, the tetrahydroxy acids can, in principle, form complex anions with a charge of -3. The highest B-value can be predicted for arabinonic acid since OH-2 and OH-3 are *gauche* and since, in addition, the conformation is favourable (OH-3 and OH-4 *anti*) for the formation of a terminal vicinal-diol complex. With lyxonic acid, OH-3 and OH-4 are *gauche* and the strong complex will inhibit the formation of complexes in other positions. This explains why the B-value is only slightly higher than that of threonic acid.

Xylonic acid takes an intermediate position. This is understandable, since all secondary hydroxyl groups are *gauche*. The positions are favourable for the formation of a 2,3:4,5-complex but, on the other hand, the formation of the 3,4-complex will compete and depress the B-value. In addition, the steric conditions (OH-3 and OH-4 *gauche*) are less favourable for complexing with two borate ions than for arabinonic acid.

If the sickle form of ribonic acid is derived from the planar zigzag form by rotation about the C-2–C-3 bond, OH-2 and OH-3 will be *gauche* and give a strong complex. The formation of an additional borate complex in terminal position is favoured. Rotation about the C-3–C-4 bond will favour the formation of the 3,4-complex only. The observed B-value was lower than that of xylonic acid, indicating that the 3,4-complex is of great importance.

The B-values of the 2-deoxyhexonic acids can be predicted from those of the corresponding pentonic acids, remembering that the value increases as the borate and carboxylate groups become more distant.

As expected, the 3-deoxyhexonic acids exhibited comparatively low B-values. The *xylo* form will give a strong 4,5-complex, and the formation of the terminal vicinal complex should therefore be less important. With the *lyxo* form, the sickle conformation is preferred and the same complex as with the *xylo* isomer will preponderate. The *arabino* and *ribo* isomers have no secondary hydroxyl groups in *gauche* conformation. For this reason, terminal vicinal complexes will preponderate with both species. The formation of a weak 2,4-complex in the *ribo* form may explain its somewhat higher B-value.

A higher B-value than for any other aldonic acid containing four hydroxyl groups can be predicted for 6-deoxygalactonic acid since OH-2 and OH-3, as well as OH-4 and OH-5, are *gauche*, while OH-3 and OH-4 are *anti*. These conditions are the best possible for formation of a strong 2,3:4,5-complex. An analogous behaviour can be predicted for the dimethyl acetal of galacturonic acid. With 6-deoxymannonic acid, the situation is favourable for a strong 3,4-complex and it can be predicted that the B-value should be about the same as that of lyxonic acid. The experiments corroborate these predictions.

Analogously, it can be predicted that *xylo*-4-hexulosonic acid should give a 2,3:5,6-complex. If the effect of the keto group could be neglected, the B-value should

be about the same as that of arabinonic acid. The observed value supports the conclusion that the keto group exerts a significant effect.

From the results already discussed, it can be concluded that, among the hexonic acids, galactonic acid should exhibit the largest B-value. The fact that the value is much higher than that of 6-deoxygalactonic acid indicates that the terminal hydroxyl group is involved in the complex formation.

Altronic acid has vicinal, secondary hydroxyl groups in the same relative positions as galactonic acid but, since it is a sickle conformer, a slightly lower B-value can be predicted. When OH-4 and OH-5 are complexed, the hydroxymethyl group and the substituent at C-4 will be *cis* in the complex, which is less favourable than for galactonic acid where the corresponding groupings are *trans*. In idonic acid, all secondary hydroxyl groups are *gauche*. Not only the 2,3:4,5- and the 2,3:5,6-complexes but also the 3,4:5,6-complex can be formed with this diastereomer. Interactions between the borate groups can be suspected and offer an explanation for its somewhat lower B-value[17].

In gluconic acid, OH-2 and OH-3 as well as OH-3 and OH-4 are *gauche*, while OH-4 and OH-5 are *anti*. The conditions are favourable for the formation of 2,3:5,6- and 3,4:5,6-complexes. Since the stability of the terminal complex is less than that of the vicinal, secondary hydroxyl groups in *gauche* conformation, a somewhat lower B-value should be obtained than with galactonic, altronic, and idonic acids.

With the remaining four diastereomers, the B-values are much lower. With mannonic acid, only OH-3 and OH-4 are *gauche*. The 3,4:5,6-complex is therefore the only complex of importance. A B-value which is only slightly higher than that of arabinonic acid (distance rule) can be predicted. The much lower value obtained with 3-*O*-methylmannonic acid supports the existence of a strong complex in which OH-3 is involved.

Gulonic, allonic, and talonic acids exhibited the lowest B-values among the hexonic acids. The differences were small. A comparison between these values and those of the corresponding 3-*O*-methylated species indicates that complexing with OH-3 is important for allonic and gulonic acids, but of little importance with talonic acid.

Of the two investigated heptonic acids, D-*glycero*-L-*manno*-heptonic acid exhibited a larger B-value than galactonic acid. The distance rule and contributions from OH-2 explain this behaviour.

Practical applications. — The previous discussion shows that it is often possible to predict the elution order of hydroxy acids from their structure. Moreover, the results given in Fig. 1 show that the order of elution of the hydroxy acids is independent of the eluent concentration. On the other hand, the elution order will be affected by the type of resin, since, for instance, non-polar contributions are affected by the structure of the resin matrix and the effect of the ionic size depends on the swelling pressure, which decreases markedly with decreasing degree of cross-linking[11]. For acids with large differences in structure, these effects must be more pronounced than when diastereomers are separated. From a practical point of view, the observed D_v

would be of limited interest unless the order of elution was unchanged from batch to batch.

Experience gained in our laboratory on separations of hydroxy acids by anion-exchange chromatography shows that a reversal of the elution order is not observed unless the resin has suffered severe degradation. On the other hand, small changes in the relative positions have been observed from one batch to another[20]. To study whether there are appreciable differences between resins of the same type from different manufacturers, experiments were made with Aminex A-25 which, according to the manufacturer (Bio-Rad), is of the same type as Dowex 1-X8.

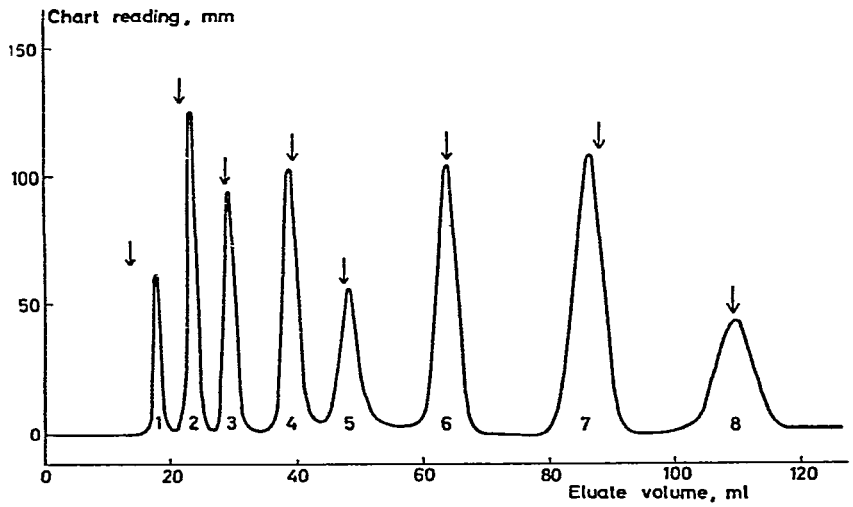

Fig. 2. Separation of 0.10 mg of 3-hydroxypropanoic (1), 0.11 mg of glyceric (2), 0.11 mg of erythronic (3), 0.10 mg of ribonic (4), 0.11 mg of xylonic (5), 0.15 mg of arabinonic (6), 0.15 mg of gluconic (7), and 0.15 mg of D-*glycero*-L-*manno*-heptonic acid (8). Eluent: 0.15M $K_2B_4O_7$. Nominal, linear flow: 4.2 cm.min^{-1}. Resin bed: 2.6 × 830 mm, Aminex A-25, 17.5 ±2 μm.

In the run represented in Fig. 2, arabinonic acid was used as a marker, which means that the bed volume was calculated from the D_v of arabinonic acid determined for the Dowex 1-X8 column. The value calculated by this method differed from the observed volume by ~5%. The peak positions recorded for the same substances on the Dowex resin are indicated by arrows. By definition, the peak positions of arabinonic acid coincide but it is seen that, with the exception of the two first acids on the chromatograms, the differences between the resins are not significant. A very large difference was observed with 4-hydroxybutanoic acid (not included in the run reported in Fig. 2). With the Aminex resin, the D_v was 4.5 compared to 2.8 with Dowex 1-X8. All investigated polyhydroxy acids were, however, affected so little that distribution coefficients determined for one resin can be used for a tentative identification even when another resin is employed.

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