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ANION-EXCHANGE CHROMATOGRAPHY OF ALDITOLS IN BORATE MEDIUM

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ABSTRACT

The retention data for anion-exchange chromatography in tetraborate solution were determined for 33 alditols and deoxyalditols. The order of elution of 1-deoxyalditols is related to chromatographic data for the corresponding aldonic acids. For all types of polyhydroxy compounds studied, the distribution coefficient increases with increasing stability and charge of the borate complex. 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

The method devised by Zill, Khym, and Cheniae[1] for the separation of polyols as their borate complexes on anion-exchange resins is widely used for the analysis of samples of biological origin[2,3]. We now report retention data for a large number of polyols in borate medium; most of these have not been studied previously.

In a previous study[4] of monocarboxylic hydroxy-acids, it was found that the preferred conformation of the non-complexed solutes has a predominant effect on the contribution of complex formation to the distribution coefficients in borate medium. The over-all change in free energy for the complex formation can be considered as the sum of the energy required to bring the hydroxyl groups to positions necessary for the formation of a complex and the change in free energy for its formation. The results permit the conclusion that the differences in thermodynamic stability of the borate complexes are mainly determined by the first term. The assumption was made that, in aqueous solution, the preferred conformations of the non-complexed solutes are the same as for acyclic sugar derivatives in organic solvents[5,6]. Recent studies by Angyal, Greeves, and Mills[7] confirm that this assumption is justified and show that, in aqueous solution, the alditols mainly take up an extended planar-zigzag conformation, provided that there are no oxygen atoms involved in parallel 1,3-interactions. Whenever such interactions are present, a preponderant portion of the

molecules will be sickle conformers. An approach similar to that used previously will be adopted in the discussion of the retention data for the alditols.

EXPERIMENTAL

A jacketed column (2.4 × 840 mm) packed with strongly basic, anion-exchange resin (Dowex 1-X8, 14–17 μm) was preconditioned with the eluent. The substances (25–100 μg) were applied to the column in aqueous solution (0.1–0.2 ml) and were eluted with potassium tetraborate solution at 25° at a nominal, linear flow-rate of 5.5 cm.min^{-1}, calculated for the empty part of the column. The eluate was analyzed automatically by chromic acid oxidation[8] or by periodate oxidation with subsequent determination of formaldehyde[9].

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 ($\varepsilon_I = 0.39$). Electrophoretic-mobility data determined by Frahn and Mills[10] were used for comparison (M_G = migration relative to glucose).

The 1-deoxypentitols and 1-deoxyglucitol were prepared by reductive desulphuration of the corresponding diethyl dithioacetals using Raney nickel as catalyst[11]. The 1,2-dideoxypentitols were side-products in the synthesis of the 1-deoxypentitols and were identified from the fragmentation products obtained on periodate oxidation. The *erythro* form was produced from both ribose and arabinose, whereas xylose and lyxose gave the *threo* form. The 1-deoxytetritols were prepared by borohydride reduction of the ethyl esters of the corresponding 4-deoxytetronic acids, and threitol by borohydride reduction of the diethyl ester of threaric acid. The other test substances came either from commercial sources or were obtained by borohydride reduction of the corresponding sugars or aldonolactones. All alditols were converted into their Me_3Si-ethers and identified by g.l.c.–m.s.[12].

RESULTS AND DISCUSSION

Influence of the eluent concentration. — In agreement with the results reported by Spencer[3], excellent separations were obtained in tetraborate media. With the small amounts of alditols applied to the column, the elution curves were symmetrical. Since borate was present in large excess, 2:1 complexes (alditol–borate) can be disregarded. In agreement with the results with aldonic acids, the D_v values increase with increasing numbers of hydroxyl groups in positions suitable for the formation of bidentate complexes. The large increase in D_v with increasing number of hydroxyl groups (Table I) strongly suggests that alditols containing four and five hydroxyl groups will form 1:2 complexes whenever possible, whereas those with six and seven hydroxyl groups will form 1:3 complexes[13] when the steric conditions are favourable. In addition, the slopes of the curves obtained by plotting log D_v *versus* −log [borate], which mainly reflect the charge of the complex[4,14], increase with increasing D_v (Fig. 1). In no case is a reversed order of elution observed when the concentration is

TABLE I

VOLUME DISTRIBUTION COEFFICIENTS (D_v) OF POLYOLS IN 0.075M AND 0.0375M $K_2B_4O_7$, MIGRATION RATES RELATIVE TO GLUCOSE (M_G) IN 0.05M $Na_2B_4O_7$, AND B-VALUES FOR THE CORRESPONDING ALDONIC ACIDS

	D_v 0.075M	D_v 0.0375M	B-value	M_G
Propane-1,2-diol	0.56		0.403	0.16
Glycerol	0.97			0.49
1-Deoxythreitol (pz)[a]	1.28		0.720	
1-Deoxyerythritol (pz)	1.05		0.628	
1,2-Dideoxy-*threo*-pentitol (pz)	2.06		0.853	
1,2-Dideoxy-*erythro*-pentitol (pz)	1.68		0.751	
Erythritol (pz)	2.76	8.14		0.75
Threitol (pz)	1.60	3.50		0.75
1-Deoxyarabinitol (pz)	2.98		1.68	
1-Deoxyribitol (sf)	2.31		0.982	
1-Deoxyxylitol (sf)	1.90		1.35	
1-Deoxylyxitol (pz)	1.45	3.00	0.783	
2-Deoxy-*erythro*-pentitol (pz)	1.28	2.65		
1,4-Dideoxy-*ribo*-hexitol (sf)	1.05			
1,4-Dideoxy-*lyxo*-hexitol (pz)	0.92			
1,5-Dideoxy-*ribo*-hexitol (sf)	1.10			
Ribitol (sf)	5.03	16.2		0.85
Arabinitol (pz)	4.44	14.1		0.87
Xylitol (sf)	3.01	8.83		0.79
1-Deoxygalactitol (pz)	5.38	20.1	3.14	
1-Deoxymannitol (pz)	4.38	13.3	1.78	
1-Deoxyglucitol (sf)	3.65		2.87	
1-Deoxygulitol (sf)	2.15		1.62	
2-Deoxy-*arabino*-hexitol (pz)	3.03	9.10		
2-Deoxy-*lyxo*-hexitol (pz)	1.81	4.48		
3-Deoxy-*ribo*-hexitol (sf)	2.44			
3-Deoxy-*arabino*-hexitol (pz)	2.03			
Mannitol (pz)	9.51	44.2		0.91
Galactitol (pz)	7.19	31.3		0.97
Allitol (sf)	6.68	28.1		0.90
Glucitol (sf)	6.31	24.2		0.83
Altritol (sf)	6.29	24.3		0.89
Iditol (sf)	5.85	23.3		0.81
D-*glycero*-L-*manno*-Heptitol (pz)	17.5			0.98
meso-*glycero*-*gulo*-Heptitol (sf)	12.6			0.85

[a]Key: pz, planar zigzag conformation favoured; sf, sickle conformation favoured.

varied within the range of analytical interest. Since tetraborate solutions contain a complex mixture of anions[15,16], reliable, quantitative determinations of the stability constants and ligand numbers cannot be derived from the chromatographic data.

Correlation between the retention data of alditols and aldonic acids. — In a comparison of the D_v values of 1-deoxyalditols with the complexing ability of the corresponding aldonic acids, we will use the B-values, defined as the ratio between the D_v value at a given borate concentration and that in a non-complexing eluent[4].

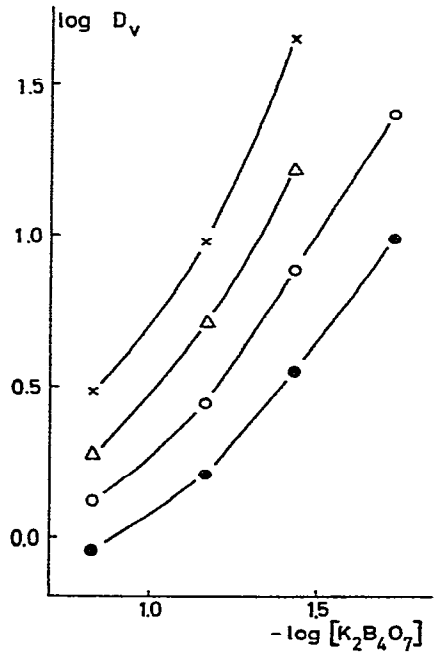

Fig. 1. Influence of the tetraborate concentration on the volume distribution coefficients: ×, mannitol; △, ribitol; ○, erythritol; and ●, threitol.

These values are given in Table I, together with the D_v values of a large number of alditols and the preferred conformations of the *free* alditols.

The theoretical approach adopted in the previous paper[4] requires that those diastereomeric 1-deoxyalditols that are preponderantly planar zigzag conformers are eluted in the order of increasing B-value of the corresponding aldonic acid. Table I shows that this holds true not only for diastereomers but also for all species for which results with corresponding aldonic acids are available. The only exception is 1,2-dideoxy-*erythro*-pentitol, which is held more strongly than 1-deoxylyxitol because of hydrophobic interactions. The rule is valid also for 1-deoxyalditols that are mainly present as sickle conformers, provided that the preferred sickle conformers of the alditol and the acid are produced from the zigzag conformers by rotation about the same carbon–carbon bond. As expected, a reversed order compared to that of the aldonic acids was obtained only with 1-deoxyribitol and 1-deoxyxylitol. With these alditols, the sickle conformer obtained by rotation about the C-2–C-3 bond will be preferred, whereas for ribonic and xylonic acid (due to the larger size of the carboxyl group compared to the methyl group), the sickle conformer produced by rotation about the C-3–C-4 bond will be favoured. These results confirm that valuable information on complex formation and chromatographic behaviour can be gained by consideration of the conformation of the non-complexed solutes.

Correlation between the structure and the distribution coefficients. — The

distribution coefficients in borate solution will be affected both by complex formation and by factors that determine the distribution of non-electrolytes in non-complexing media. As predicted by the Gibbs–Donnan theory and confirmed in experiments with solutes such as glycerol and glucitol, the distribution coefficients in such media decrease with increasing molar volume[17]. Therefore, it cannot be concluded that, for instance, 1,5-dideoxy-*ribo*-hexitol gives weaker borate complexes than 1-deoxy-threitol. The small difference may be entirely due to the larger size of the hexitol. The discussion of the complex formation will, therefore, be restricted to diastereomers and the effect of the introduction of hydroxyl groups in comparable solutes.

With solutes containing hydrophobic groupings, non-polar interactions contribute to the sorption[18], *i.e.*, they have an influence opposing that of an increased molar volume. For the solutes studied in the present work, an appreciable effect can be predicted only for the 1,2-dideoxypentitols. Their D_v values compared to those of the corresponding 1-deoxytetritols ($\Delta \ln D_v = 0.48$ for the *threo* form and 0.47 for the *erythro* form) confirm the previous observation that non-polar contributions are very strong in borate media[4]. In the other dideoxyalditols, the carbon atoms lacking hydroxyl groups are so remote that the effect should be negligible.

The results with 1-deoxytetritols and 1,2-dideoxypentitols confirm the observation made with aldonic acids that two vicinal, secondary hydroxyl-groups essentially in *gauche* conformation give larger contributions to D_v than those mainly in *anti* conformation, or than terminal, vicinal hydroxyl-groups. This result confirms observations made by several other methods that borate complexes with vicinal *threo*-diols more strongly than with vicinal *erythro*-diols or terminal diols[2,10,19].

In 1-deoxyarabinitol, HO-2 and HO-3 are mainly *gauche* and will give a strong complex, whereas HO-3 and HO-4 are mainly *anti*. The steric conditions are therefore the best possible for formation of a terminal 4,5-complex. In 1-deoxylyxitol (HO-2–HO-3 *anti* and HO-3–HO-4 *gauche*), a strong 3,4-complex will depress further complex formation. As expected, 1-deoxyarabinitol exhibits the highest D_v value among compounds containing four hydroxyl groups, whereas the *lyxo* isomer is retained less effectively than its diastereomers.

The observation that 2-deoxy-*arabino*-hexitol has a D_v value close to that of 1-deoxyarabinitol confirms that 1,3-diol complexes are very weak compared to complexes with vicinal hydroxyl groups mainly in *gauche* conformation. With 2-deoxy-*lyxo*-hexitol, the situation is similar to that of 1-deoxylyxitol. Since HO-4 and HO-5 are involved in a strong complex, and HO-3 and HO-4 are essentially *anti*, contributions from a 1,3-complex explain the higher D_v for the hexitol. As expected, the elution order of these 2-deoxyhexitols is the same as that of the 1-deoxypentitols. Similarly, their D_v values were in the order of the B-values of arabinonic (1.68) and lyxonic (0.783) acids, as well as those of 2-deoxy-*arabino*-hexonic (1.94) and 2-deoxy-*lyxo*-hexonic (1.19) acids.

For 2-deoxy-*erythro*-pentitol, the situation for the formation of strong, bidentate complexes is unfavourable (HO-3–HO-4 *anti*) and, as expected, D_v is low.

The terminal 4,5-complex will be the most-favoured complex. Since C-2 does not have a hydroxyl group, the contribution of a 1,3-complex cannot be disregarded.

1-Deoxygalactitol exhibits a much higher D_v value than any other compound containing five hydroxyl groups. This is easily understood, as the conformation (HO-2–HO-3 and HO-4–HO-5 *gauche*; HO-3–HO-4 *anti*) is the best possible for the formation of a very strong 2,3:4,5-complex, free from steric hindrance. The observation that 1-deoxymannitol is held less strongly than 1-deoxygalactitol is explained by the formation of a less-stable 3,4:5,6-complex as the main product (HO-4–HO-5 *anti*). As expected, D_v is about the same as for arabinitol.

The 1-deoxyalditols discussed above are essentially present in planar zigzag conformations, whereas the sickle form is preferred for the 1-deoxyalditols discussed below. In 1-deoxyxylitol, all vicinal, secondary hydroxyl-groups will be mainly *gauche*. For steric reasons, the 2,3:4,5-complex must therefore be less abundant than with 1-deoxyarabinitol. The formation of a 3,4-complex will compete and depress the charge of the complex. In the sickle form of 1-deoxyribitol (rotation about the C-2–C-3 bond), HO-2 and HO-3 will be mainly *gauche* and HO-3 and HO-4 *anti*. The conditions for the formation of a 2,3:4,5-complex are therefore highly favourable. The arrangement obtained after rotation about the C-3–C-4 bond would favour the formation of a strong 3,4-complex only. These considerations explain why the *xylo* and *ribo* isomers take an intermediate position between the *arabino* and *lyxo* forms.

The preferred sickle conformers of 1-deoxyglucitol and 1-deoxygulitol will be those obtained by rotation about the C-2–C-3 and C-4–C-5 bond, respectively. With 1-deoxyglucitol (HO-2–HO-3 and HO-3–HO-4 *gauche*; HO-4–HO-5 *anti*), the most-stable complexes will be the 2,3:5,6 and the competing 3,4:5,6-complexes. With 1-deoxygulitol (HO-2–HO-3 *anti*; HO-3–HO-4 and HO-4–HO-5 *gauche*), the competing 3,4- and 4,5-complexes will be most abundant. The 3,4-complex can add a second borate ion in the vicinal, terminal position, but the steric conditions are unfavourable (*cf.* 1-deoxyxylitol). As expected, 1-deoxygulitol exhibits a much lower D_v than the diastereomers.

The correlations discussed above make it possible to predict the behaviour of alditols having two pairs of vicinal, terminal hydroxyl-groups. In threitol, the secondary hydroxyl groups are essentially *gauche*, which strongly favours the formation of a 2,3-complex but is unfavourable for the formation of a 1,2:3,4-complex. It can be predicted that the stable 2,3-complex dominates and that complex formation with the primary hydroxyl groups is less important. The small increase in D_v compared to 1-deoxythreitol ($\Delta \ln D_v = 0.22$) supports this conclusion.

With erythritol, the situation is reversed. The secondary hydroxyl groups are mainly *anti*, which is unfavourable for the formation of a bidentate complex with these groups. On the other hand, the preferred conformations of both pairs of terminal hydroxyl groups are favourable for the formation of 1,2:3,4-complexes. Therefore, a complex having a charge of -2 with lower stability constants than that of the most-stable threitol complex can be predicted. The large increase in D_v compared to 1-deoxyerythritol ($\Delta \ln D_v = 0.97$), and the higher slope of the curve

$\log D_v$ *versus* $-\log [K_2B_4O_7]$, given in Fig. 1, support the conclusion that the charge of the erythritol complex is higher than that of the threitol complex. The charge has a greater influence on the ion-exchange equilibrium than on the electrophoretic migration. This explains why D_v is much larger for erythritol although the M_G values are identical.

With arabinitol, a stable 2,3:4,5-complex can be predicted (HO-2–HO-3 *gauche* and HO-3–HO-4 *anti*). The large increase in D_v compared to 1-deoxylyxitol ($\Delta \ln D_v = 1.12$) and the small increase compared to 1-deoxyarabinitol ($\Delta \ln D_v = 0.40$) confirm that the conditions are most favourable for the terminal 4,5-complex, while the effect of the presence of a hydroxyl group at C-1 is small (*cf.*, threitol). In its sickle conformer, the arrangement of the hydroxyl groups in ribitol will be similar to that in arabinitol. The large increase in D_v compared to 1-deoxyribitol ($\Delta \ln D_v = 0.78$) confirms that complexing in terminal positions makes a significant contribution.

In the sickle conformer of xylitol, HO-2–HO-3 and HO-3–HO-4 are mainly *gauche*. The formation of a bidentate complex in either position is therefore strongly favoured. The increase in D_v compared to 1-deoxyxylitol ($\Delta \ln D_v = 0.46$) shows that terminal complexes contribute to the sorption but are less important than with the diastereomers. As expected, the D_v and M_G values for xylitol are lower than for the diastereomers.

In both 3-deoxyhexitols (HO-4–HO-5 *anti*), the conformation is favourable for 1,2:5,6-complexes and, as expected, the D_v values are similar and much lower than that for 1-deoxygalactitol, and also somewhat lower than that for 2-deoxy-*arabino*-hexitol. The results confirm that, when no steric hindrance exists, the secondary, vicinal, *gauche* hydroxyl-groups contribute more to the D_v value than the terminal, vicinal hydroxyl-groups. The somewhat higher D_v value of 3-deoxy-*ribo*-hexitol (compared to the *arabino* form) is anticipated from the elution order of ribitol and arabinitol.

Among the hexitols, only mannitol and galactitol are planar zigzag conformers. As expected, the D_v value of mannitol (HO-2–HO-3 *anti*) is much larger than that of 1-deoxymannitol ($\Delta \ln D_v = 0.77$). These results permit the conclusion that, in addition to the strong complex with HO-3 and HO-4 (*gauche*), vicinal, terminal complexes are formed at both ends.

The conformation of galactitol (*cf.*, 1-deoxygalactitol) is the best possible for a very strong 2,3:4,5-complex which will depress complexing in terminal positions. The small increase in D_v compared to 1-deoxygalactitol ($\Delta \ln D_v = 0.29$) confirms this conclusion. Both mannitol and galactitol should give high D_v and M_G values. The experiments show that mannitol exhibits the highest D_v and the second highest M_G among the hexitols, and galactitol the highest M_G and the second highest D_v. This reversal is explained by the greater tendency of mannitol to form complexes with three borate ions[13] and by the higher stability of the second complex of galactitol compared to the second and third complexes with mannitol (*cf.* threitol and erythritol).

In glucitol (rotation about the C-2–C-3 bond), HO-2–HO-3 and HO-3–HO-4 are mainly *gauche*, while HO-4–HO-5 are essentially *anti*. The formation of rather

stable 2,3:5,6 and 3,4:5,6 complexes should therefore be highly favoured. The large increase in D_v compared to 1-deoxygulitol ($\Delta \ln D_v = 1.08$) supports this conclusion. The increase compared to 1-deoxyglucitol ($\Delta \ln D_v = 0.55$) indicates that complexing with HO-1 and HO-2 cannot be disregarded.

For the other hexitols, no results with the corresponding 1-deoxyhexitols are available and the prediction of the favoured complexes must await further investigations.

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