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SEPARATION OF DICARBOXYLIC HYDROXY ACIDS BY ANION-EXCHANGE CHROMATOGRAPHY AND GAS CHROMATOGRAPHY

LENA JANSÉN AND OLOF SAMUELSON
Department of Engineering Chemistry, Chalmers University of Technology, Gothenburg (Sweden)
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SUMMARY

Various aldaric and deoxyaldaric acids were prepared and isolated by anion-exchange chromatography on a preparative scale. Their elution behaviour in magnesium acetate, sodium sulphate and sodium phosphate media was studied on analytical columns coupled with automatic analysis of the eluates. Most species can be well separated by this technique.

Gas chromatography of the fully trimethylsilylated trimethylsilyl esters showed that on polar stationary phases the separation factors are by far superior to those obtained with trimethylsilylated alditols.

INTRODUCTION

Anion-exchange chromatography of monocarboxylic acids coupled with automatic colorimetric analysis of the eluate has been studied systematically in previous work from this laboratory, whereas comparatively little work has been devoted to the separation and determination of dicarboxylic acids by this technique[1–3]. It was therefore deemed of interest to carry out a study on the behaviour of aldaric acids and deoxyaldaric acids upon elution in various media, chosen so that no interference occurred during the colorimetric analysis by chromic acid oxidation. For the purposes of comparison a few other dicarboxylic acids were included.

With very complex mixtures of diastereomeric acids a complete resolution could not be obtained and therefore the application of gas chromatography (GC) was also studied.

CHROMATOGRAPHIC TECHNIQUES

Chromatography on strongly basic anion-exchange resins was used both for the isolation of the dicarboxylic acids and in studies of their chromatographic behaviour. The experimental details are given in the legends to the figures. The eluates were analysed automatically by chromic acid oxidation[4], and in some runs by spectrophotometric determination of the periodate consumption as well[5]. The pH of the periodate reagent was lowered by the addition of sulphuric acid (7.5 ml conc. acid per l) to prevent crystallisation after mixing with the magnesium acetate solution.

From experiments with single acids, the adjusted retention volumes (peak elution volume *minus* interstitial volume) were calculated in column volumes. With species which exhibit a linear exchange isotherm the values correspond to the volume distribution coefficients, D_v (ref. 6). Although some elution curves exhibited tailing, indicating that the isotherms were non-linear, the term D_v value is used for these species as well.

Several of the dicarboxylic acids contained appreciable amounts of lactones formed during the isolation of the fractions. To avoid complications both during anion-exchange chromatography and GC, the acids were converted to their sodium salts by treatment with sodium hydroxide at pH 10 for 2 h at 60°. The sodium salts were used to prepare fully trimethylsilyl (TMS) substituted trimethylsilyl esters[7] which were studied by GC and, if not otherwise mentioned, the acids were applied to the anion-exchange columns as their sodium salts.

A Perkin-Elmer 900 gas chromatograph equipped with a flame ionisation detector was employed in the study of the TMS-derivatives. Steel columns of dimensions 0.2 × 200 cm were used with nitrogen as the carrier gas (30 ml/min). The temperature of the injection block was 240–260° and that of the manifold 260–270°. The following stationary phases from Appl. Science Lab. were employed: OV-1: 0.5 % OV-1 methyl silicone gum on Chromosorb G (AW-DMCS); OV-17: 0.5 % OV-17 methyl-phenyl silicone fluid on Chromosorb G (AW-DMCS); QF-1: 3 % DC QF-1 silicone fluid on Gas-Chrom Q; XE-60: 1 % GE XE-60 silicone gum on Gas-Chrom Q.

PREPARATION AND ISOLATION OF THE DICARBOXYLIC ACIDS

The dibasic acids were prepared by oxidation of the corresponding aldoses, deoxyaldoses, aldonic and deoxyaldonic acids with nitric acid. After addition of water and evaporation, the residue was dissolved in water and treated with a strongly basic anion-exchange resin in the acetate form to remove the remaining nitric acid and some brownish material which was held very strongly by the resin in the subsequent elution step. Magnesium acetate (0.2 *M*, with acetic acid added to pH 7) was used to elute the organic acids. The total effluent from the column was treated with a cation exchanger in the H^+ form and evaporated to dryness to remove the acetic acid. The residue was neutralised with sodium hydroxide and kept at pH 10 for 4 h at room temperature to split the lactones and was then applied to a preparative anion-exchange column preconditioned with 0.2 *M* magnesium acetate solution (pH 7). The same solution was used as eluent in the subsequent chromatographic elution[3].

Fig. 1 shows a chromatogram obtained with the products formed from D-arabinose after oxidation under the comparatively severe conditions as used by CHALOV[8]. The eluate fractions were passed through a cation-exchange column in the H^+ form and the acetic acid was removed by evaporation under vacuum.

The very small peak (I) contained unreacted sugar, whereas peaks II and III contained mainly D-arabinonic and oxalic acids. The minor peak IV was not identified, whereas the main band (V) contained, as expected, D-arabinaric acid. The yield of the main product was about 35 %

D-Arabinaric acid was prepared in much better yield (85 %) by oxidation of D-arabinuronic acid with 0.2 *M* sodium chlorite in 1 *M* acetic acid for 67 h at room temperature, after which time nitrogen was bubbled through the solution, which was

then passed through a cation exchanger in the H^+ form and evaporated. D-Arabinaric acid was isolated by anion-exchange chromatography as described above. Anion-exchange chromatography, GC and GC–mass spectrometry (MS) showed that the acids prepared by the two methods were identical. The same technique was used for the preparation of D-glucaric, *meso*-galactaric, D-mannaric and D-talaric acids from the corresponding uronic acids.

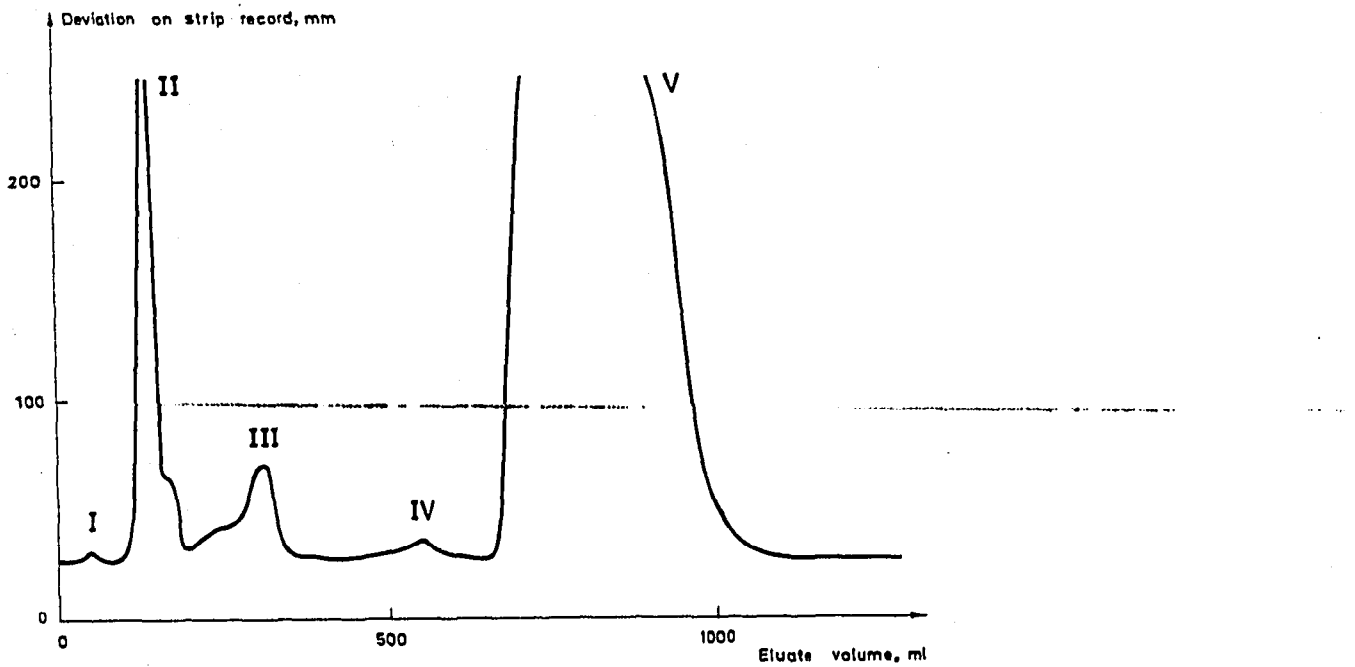

Fig. 1. Chromatographic separation of the reaction mixture obtained by nitric acid oxidation of D-arabinose. Oxidation conditions: D-Arabinose (2 g) dissolved in nitric acid (5.8 ml, $d = 1.36$) was heated at 60° for 3 h, whereupon the temperature was raised to 90° over a 20-min period and kept at 90° for 15 min. Chromatography: 20% of the total reaction mixture was applied to an anion-exchange column (10 × 845 mm, Dowex 1 X8, 40–60 μm) and eluted with 0.2 *M* magnesium acetate at a flow rate of 0.65 ml cm^{-2} min^{-1}.

During the oxidation of D-arabinose with nitric acid, as in all similar experiments (except for those with added sodium nitrite), only one dicarboxylic acid with the same number of carbon atoms as the starting material was produced. This was established by the various chromatographic techniques described in this paper, as well as by GC–MS. The latter method clearly showed that the compound was a pentaric acid. Since all diastereomers were prepared and shown to exhibit different chromatographic properties and since nitric acid oxidation is a classical method for the determination of the absolute configuration of carbohydrates, no further identifications were carried out.

Most of the deoxyaldaric acids were prepared by nitric acid oxidation under milder conditions, similar to those used in the pioneering work by FISCHER AND PILOTY[9]. The same ion-exchange technique as described above was used for their isolation. In some of the syntheses the reaction mixture was subjected to anion-exchange chromatography in 0.2 *M* magnesium acetate (pH 7) on an analytical column coupled to an automatic two-channel analyser. The chromatogram recorded with the reaction mixture obtained by nitric acid oxidation of 2-deoxy-D-ribose is reproduced in Fig. 2.

The first peak contained unchanged starting material, whereas the second peak, which was the largest one, had the position of the expected intermediate (2-deoxy-D-ribonic acid). A significant peak with a strong periodate response was recorded at the position of *meso*-erythraric acid indicating that fragmentation occurred even under relatively mild working conditions. The last peak was the expected dicarboxylic acid (2-deoxy-D-*erythro*-pentaric acid). The yield was about 10 %. GC–MS showed that the compound

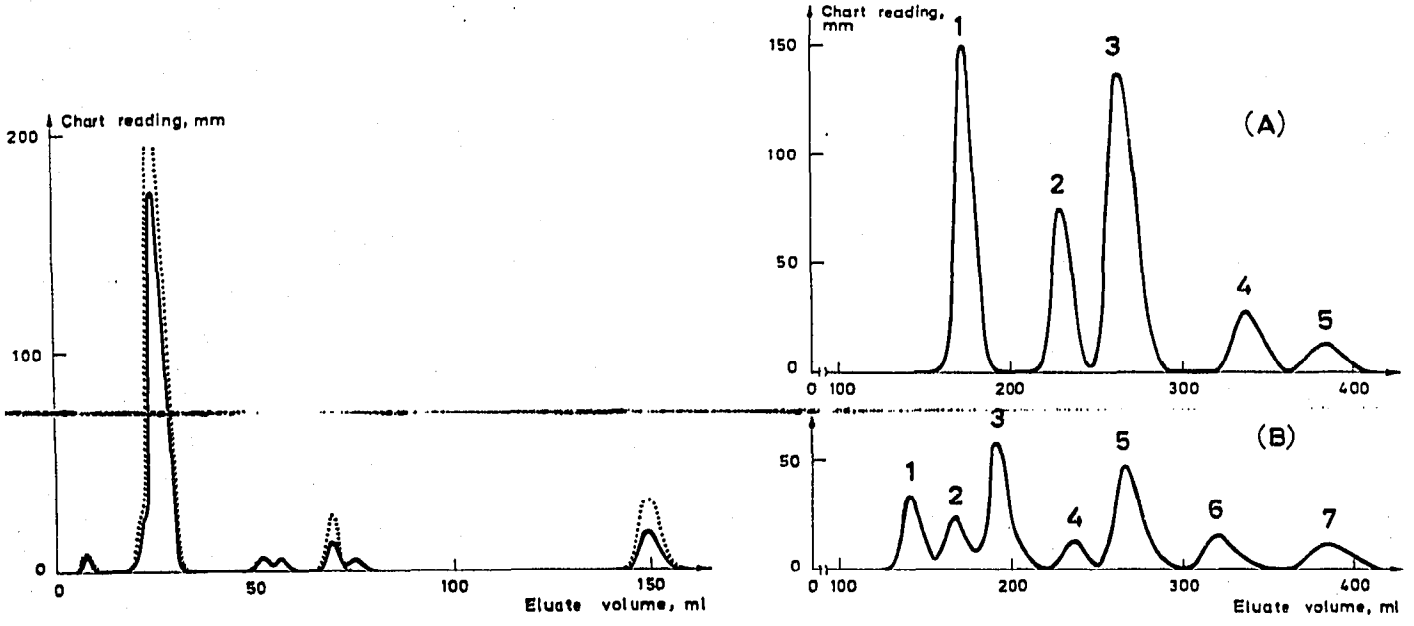

Fig. 2. Separation on an analytical column of the reaction mixture obtained by oxidation of 2-deoxy-D-ribose. Oxidation conditions: 2-deoxy-D-ribose (210 mg) was dissolved in nitric acid (1.5 ml, $d = 1.2$) and after evaporation of the nitric acid on a steam bath for about 1 h, water was repeatedly added in small portions and evaporated. Chromatography: 1.8 mg of the reaction mixture applied to an analytical column (3.2 × 1350 mm, Dowex 1 X8, 17–20 μm) and eluted at 70° with 0.2 *M* magnesium acetate. Flow rate: 4.9 ml cm^{-2} min^{-1}. (———) chromic acid channel; (· · · ·) periodate consumption channel.

Fig. 3. Chromatographic anion-exchange separation in 0.04 *M* sodium sulphate at 30°. Column: 4.4 × 1120 mm, Dowex 1 X8, 15–18 μm. Flow rate: 6.1 ml $cm^{-2}min^{-1}$. (A) 1.8 mg D,L-malic (1), 1.5 mg *meso*-erythraric (2), 2.2 mg L-threaric (3), 0.8 mg tartronic (4), and 2.2 mg oxalic acids (5). (B) 0.3 mg *meso*-galactaric (1), 0.4 mg L-talaric (2), 0.9 mg *meso*-allaric (3), 0.3 mg *meso*-ribaric (4), 1.0 mg L-threaric (5), 1.3 mg D-mannaric (6), and 3.6 mg oxalic acids (7). (Lower photometer amplification than in run A).

was a 2-deoxypentaric acid and since its chromatographic behaviour differed from that of the diastereomer no further attempts at identification were made.

In the experiments with the 3-deoxypentonic acids it was found that no detectable amounts of nitrous gases were evolved during the treatment with nitric acid. A chromatographic study of the organic material revealed that in these cases the starting material was virtually unchanged. In one of these syntheses the treatment with nitric acid was repeated until appreciable amounts of nitrous gases were evolved. An attempt was also made to prepare 3-deoxy-*meso*-*erythro*-pentaric acid under the conditions suggested by PIGMAN *et al.*[10], *i.e.* by use of a small amount of sodium nitrite (0.27 moles per mole of starting acid) together with concentrated nitric acid ($d = 1.39$). The reaction was allowed to proceed at a lower temperature for a longer time (0° for 4 h

followed by 20 h at 20°). Under these conditions oxidation took place, but the reaction product contained 3-deoxy-D-*threo*-pentaric acid together with the expected 3-deoxy-*meso*-*erythro*-pentaric acid. When the same oxidation method was used with 3-deoxy-D-*arabino*-hexonic acid, isomerisation also occurred giving some 3-deoxy-D-*ribo*-hexaric acid together with the 3-deoxy-D-*arabino*-hexaric aicd. No traces of isomerisation products were found when 3-deoxy-D-*arabino*-hexonic acid was oxidised with nitric acid in the absence of nitrite.

The following sugars and acids were oxidised with nitric acid: D-xylose, D-arabinose, D-mannose, D-ribonolactone (from commercial sources), D-idonic, D-altronic and D-allonic acids (prepared by cyanohydrin synthesis[11]), 2-deoxy-D-ribose, 2-deoxy-D-glucose, 2-deoxy-D-galactose (from commercial sources), 3-deoxy-D-*erythro*- and *threo*-pentaric acids (isolated in earlier work) and the *arabino*- and *ribo*-forms of 3-deoxy-D-hexonic acid (prepared from laminaran[12]) as well as the diastereomeric *lyxo*- and *xylo*-forms (prepared from D-galactose[13]).

ANION-EXCHANGE CHROMATOGRAPHY IN SODIUM SULPHATE AND SODIUM PHOSPHATE SOLUTIONS

Sodium sulphate solution was chosen as an example of an unbuffered eluent without complex forming properties. Sulphate ions are held strongly by anion-exchange resins and for this reason a fairly low eluent concentration could be chosen. As can be seen from Fig. 3, several dicarboxylic acids can be separated well in this medium. The D_v values are listed in Table I.

Several diastereomers were well separated from each other. Compounds with D_v values differing by less than 10 % exhibited serious overlapping. In agreement with results recorded in sodium acetate medium[2,3], oxalic acid was held very strongly in sodium sulphate medium. No simple correlation seems to exist between the structure of the acids and the D_v values. Upon elution of strongly polar monocarboxylic hydroxy acids in non-complexing media, the anions with higher molecular weight are as a rule eluted earlier than those with a lower molecular weight[14]. This rule does not seem to be valid for the dicarboxylic acids. The fact that D-mannaric acid is held much more strongly than any of the other hexaric acids indicates that the conformation of the corresponding anions has a predominant influence upon the ion-exchange affinity. In this connection it is worth mentioning that in sodium acetate mannonic acid exhibits a much higher D_v value than the other hexonic acids.

In some experiments free acids instead of sodium salts were applied to the column. Complications occurred not only with acids containing lactones[3], but also with species which do not give rise to any lactones. For example, when 2.4 mg of oxalic acid was applied to the column, the adjusted retention volume was 18.8 column volumes, whereas when the amount was increased to 5.3 mg the corresponding value was 15.2. With neutralised samples of oxalic acid (1.3–5.5 mg) the peak appeared at $D_v = 22$. The results are explained by a broadening of the loading band and a more rapid elution in the non-buffered medium as a result of the presence of monovalent anions and free acid in the sample solution. The results clearly show that with unbuffered eluents and columns it is advisable to neutralise the acids before application of the sample to the column even if no lactones are present.

A systematic study of the use of orthophosphate solutions for the elution of

TABLE I

ION-EXCHANGE CHROMATOGRAPHY IN FOUR MEDIA AND GAS CHROMATOGRAPHY OF TMS-DERIVATIVES ON FOUR STATIONARY PHASES

Acid[a]	*D_v in ion-exchange chromatography*				*Relative retention in gas chromatography*			
	0.2 M Mg-acetate	*0.04 M Na-sulphate*	*0.3 M Na-phosphate*	*0.3 M NaAc–2 M HAc*	*OV-1 160°*	*OV-17 160°*	*QF-1 120°*	*XE-60 120°*
Oxalic	2.1	22.0	10.1	47				
Tartronic	4.5	19.4	8.0	25	0.064	0.152	0.190	0.194
meso-Erythraric	5.8	13.2	6.5	14.2	0.174	0.313	0.354	0.369
L-Threaric	16.5	15.0	7.7	23	0.212	0.432	0.471	0.526
D-Arabinaric (C)	10.2	13.9	7.8	18.0	0.522	0.864	0.945	0.998
meso-Ribaric (C,F)	9.7	12.9	7.8	18.1	0.532	0.873	1.037	1.055
meso-Xylaric (C)	9.6	10.6	6.2	15.0	0.545	0.978	1.031	1.162
meso-Allaric (C)	9.7	10.5	6.8	11.4	1.242	1.669	1.90	1.993
meso-Galactaric	10.8	7.7	5.3	12.3	1.500	2.272	2.31	2.618
D-Glucaric	9.9	11.3	6.5	13.4	1.295	1.814	1.920	2.097
D-Idaric (C)	9.8	9.8	5.8	12.3	1.602	2.518	2.589	3.083
D-Mannaric (C)	15.1	18.3	9.2	18.3	1.074	1.346	1.401	1.485
D-Talaric (C)	9.0	9.4	6.0	11.2	1.419	2.098	2.195	2.411
D,L-Deoxytetraric	6.4	9.7	6.3	9.6	0.103	0.212	0.283	0.287
2-Deoxy-D-*erythro*-pentaric (F)	13.1	9.9	6.6	8.7	0.278	0.517	0.655	0.673
2-Deoxy-D-*threo*-pentaric (F)	12.0	8.3	6.1	8.8	0.291	0.573	0.736	0.747
2-Deoxy-D-*arabino*-hexaric (C,F)	13.2	9.8	6.4	7.6	0.777	1.277	1.565	1.632
2-Deoxy-D-*lyxo*-hexaric (F)	12.2	7.4	5.4	6.5	0.847	1.467	1.751	1.942
3-Deoxy-*meso*-*erythro*-pentaric (P)	7.0	9.6	6.8	11.0	0.280	0.538	0.673	0.719
3-Deoxy-D-*threo*-pentaric (F)	8.5	10.3	7.4	14.8	0.295	0.593	0.774	0.782
3-Deoxy-D-*arabino*-hexaric (C,P)	10.7	10.4	7.0	13.3	0.732	1.290	1.550	1.650
3-Deoxy-D-*lyxo*-hexaric (F)	8.2	9.4	6.4	10.7	0.767	1.425	1.675	1.916
3-Deoxy-D-*ribo*-hexaric (F)	8.3	9.5	6.6	11.1	0.761	1.317	1.639	1.814
3-Deoxy-D-*xylo*-hexaric (F)	9.5	9.3	6.5	12.2	0.789	1.470	1.713	1.938

[a] C, F and P denote acids prepared according to the methods suggested by CHALOV[8], FISCHER AND PILOTY[9], and PIGMAN *et al.*[10], respectively.

various dicarboxylic and tricarboxylic acids has been carried out recently[15]. In the present work the elution behaviour of aldaric and deoxyaldaric acids was studied in a buffered sodium orthophosphate solution (0.3 *M*, pH 7). This means that the eluent contained both HPO_4^{2-} and $H_2PO_4^-$. The experimental details are given in the legend to Fig. 4. All species gave some tailing in this medium, but in agreement with earlier observations[15] it was found that with most species the tailing was largely eliminated when the amount applied to the column was decreased to about 0.5 mg of each acid.

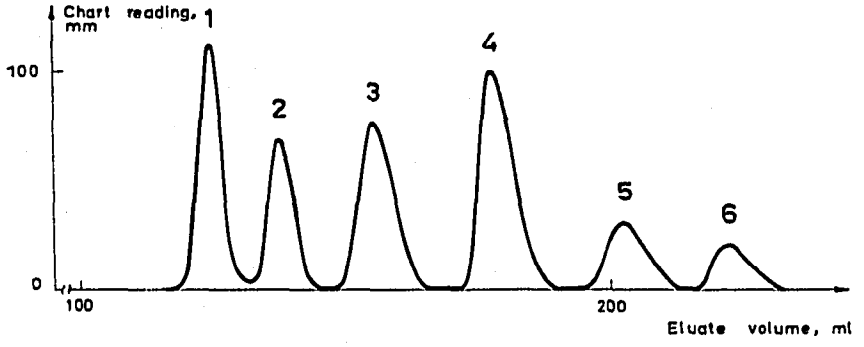

Fig. 4. Separation in 0.3 *M* sodium orthophosphate at 80° of 0.4 mg *meso*-galactaric (1), 0.3 mg L-talaric (2), 0.4 mg *meso*-allaric (3), 0.6 mg D-arabinaric (4), 0.5 mg D-mannaric (5) and 0.9 mg oxalic acids (6). Column: 4.2 × 1420 mm, Dowex 1 X10, 11–14 μm. Flow rate: 4.8 ml cm^{-2} min^{-1}.

The D_v values listed in Table I were determined in experiments with added amounts within the range 0.5–1 mg and do not differ much from the true equilibrium distribution coefficients (*cf.* ref. 15).

With few exceptions the order of elution was the same as that obtained in sodium sulphate solution indicating that specific interactions between the anions in the eluents and the eluted anions are of minor importance with most eluted species.

ANION-EXCHANGE CHROMATOGRAPHY IN SODIUM ACETATE–ACETIC ACID SOLUTION

In acid eluents the peak position is largely determined by the acid strength of the eluted species, whereas other factors exert a predominant influence at a high pH[14]. Acid mixtures which are not completely resolved in neutral eluents can quite often be separated well in acid medium.

In the present work 0.3 *M* sodium acetate in 2 *M* acetic acid (pH 3.8) was used as eluent in runs carried out at 70°. The formation of lactones during the course of the elution resulted in serious complications. Fig. 5 shows the chromatograms obtained in two runs with 2-deoxy-D-*erythro*-pentaric acid. In one of the runs (A) a mixture of the lactone and the sodium salt was applied to the column. The mixture was obtained by neutralisation of the corresponding acid fraction from the preparative column to pH 10 at room temperature. It is seen that two well-separated peaks are recorded, but that the curve between these did not drop to the base line. The results indicate that interconversion between the anions and lactones occurred during the run. In the other run (B) the sodium salt was added to the column and, as expected, the main peak had the same position as the second peak on the first chromatogram. The experiments show that this peak corresponds to the non-lactonised acid. The elution band recorded in run B exhibited serious fronting, however, which clearly shows that lactone formation occurs during the chromatographic run. Similar chromatograms were recorded

with D-glucaric and *meso*-galactaric acids, whereas with *meso*-allaric acid the formation of lactone was so rapid that most of the acid (applied as sodium salt) was converted to the lactone during the elution.

The D_v values listed in Table I are those corresponding to the non-lactonised acid. The dissociation constants of five of the hexaric acids have been determined by MAI[16] and it is interesting to note that the species are eluted in the order of increasing acid strength. Moreover, the 2-deoxyaldaric acids are eluted before the corresponding

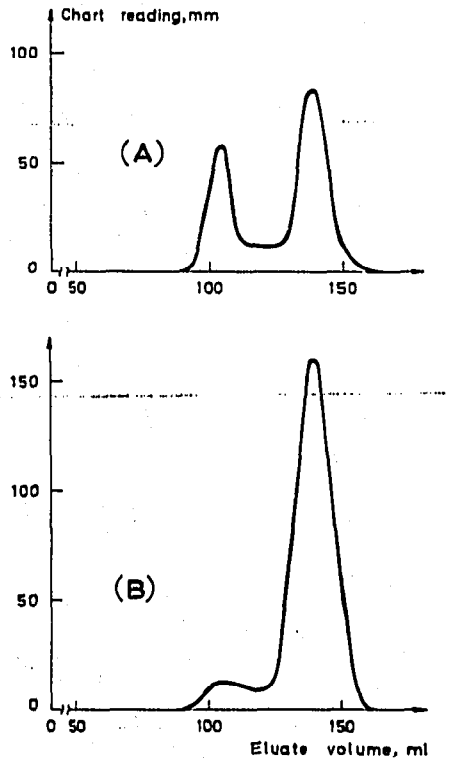

Fig. 5. Elution of 2-deoxy-D-*erythro*-pentaric acid with 0.3 *M* sodium acetate in 2 *M* acetic acid at 70°. Column: 4.2 × 920 mm, Dowex 1 X8, 18–27 μm. Flow rate: 7.0 ml $cm^{-2}min^{-1}$. (A) 0.8 mg acid applied to the column after neutralisation at pH 10 for 1/2 h at room temperature. (B) 1.4 mg acid applied to the column after neutralisation at pH 10 for 2 h at 60°.

aldaric and 3-deoxyaldaric acids, which is in agreement with the fact that the presence of a hydroxyl group in an α-position with respect to a carboxyl group results in markedly increased acid strength.

The separation conditions applied in the experiments referred to in this section are unsuitable for analytical purposes with solutions containing several aldaric acids, and more systematic investigations are necessary before advantage can be taken of the differences in acid strength of the acids in separations of complicated mixtures of aldaric acids.

ANION-EXCHANGE CHROMATOGRAPHY IN MAGNESIUM ACETATE

In chromatographic separations in magnesium acetate advantage is taken of the differences in the complex equilibria between magnesium ions and the species to be separated[2,3]. To avoid complications as a result of lactone formation the experiments were carried out at pH 7. The experimental conditions are given in the legend to Fig. 2.

In agreement with earlier observations[3], the elution bands showed some tailing, especially when amounts larger than about 1 mg of each acid were applied to the column. The bands corresponding to the deoxyaldaric acids were more symmetrical than those of the aldaric acids.

A comparison of the D_v values determined in magnesium acetate with those obtained in sodium sulphate shows that with some species the differences were small, whereas other species exhibited much lower D_v values in magnesium acetate than in sodium sulphate. The greatest difference was observed with oxalic acid, but great differences were recorded also with tartronic and *meso*-erythraric acids which shows that the formation of magnesium complexes with little or no affinity for the resin is favoured with these acids. The diastereomeric tetraric acid (L-threaric acid) is held more strongly in magnesium acetate than in the sodium sulphate solution which shows that small differences in the structure can exert a great influence. Galactaric, 2-deoxypentaric and 2-deoxyhexaric acids are also eluted later in 0.2 *M* magnesium acetate than in 0.04 *M* sodium sulphate, whereas the other species are either eluted somewhat earlier in magnesium acetate or at about the same positions. Evidently, galactaric acid has less ability than the other hexaric acids to form complexes of low affinity for the anion exchanger. As expected, the same holds true for the 2-deoxyaldaric acids.

As can be seen from Table I some species exhibit more favourable separation factors in magnesium acetate than in sodium sulphate, whereas with others the opposite holds true. With complex mixtures it is often useful to apply one of these eluents for a first separation and to rechromatograph the fractions in the other eluent.

GAS CHROMATOGRAPHY OF TMS-DERIVATIVES

A GC study of three fully TMS-substituted aldaric acids has been carried out by RAUNHARDT *et al.*[7]. The elution order mannaric acid < glucaric acid < galactaric acid was the same as observed in the present investigation.

In the present work fully TMS-substituted D-glucitol was used as a marker in the study of the TMS-substituted TMS-esters of the dicarboxylic acids investigated. The retention was calculated relative to that of D-glucitol. The adjusted retention times of the D-glucitol derivative were 12.5 min on OV-1 at 160°, 6.1 min on OV-17 at 160°, 15.8 min on QF-1 at 120° and 12.9 min on XE-60 at 120°.

The results given in Table I show that the retention of all of the TMS-derivatives investigated relative to that of D-glucitol increases with increased polarity of the stationary phase. This can be ascribed to polar interactions between the carbonyl groups of the ester groupings and the stationary phase. The retention data obtained with D-glucaric acid indicate that these contributions are quite large especially with the most polar phases (QF-1 and XE-60). Within the aldaric acid series and among the deoxyaldaric acids the TMS-derivatives were eluted in groups which appeared in the order of increasing number of carbon atoms. On the non-polar OV-1 phase the deoxyhexaric acids are held less strongly than D-glucitol which is explained by the fact that they contain one TMS-group less, whereas on the more polar stationary phases the deoxyhexaric acids are held more firmly than D-glucitol. This reversal in order is explained by the strong contribution of the carbonyl groups. D-Mannaric acid is held much more strongly than the deoxyhexaric acids on OV-1 whereas the opposite holds true for the phase of highest polarity.

The elution order of different diastereomers is the same on all investigated column packings, but a comparison of the retention data of diastereomeric compounds (*e.g.* mannaric and idaric acids) on different phases indicates that the polar interaction forces are much more important with those species which are held more strongly in the stationary phase. It is interesting to note that a relationship can be traced between the stereochemical structure of the derivatives and their affinity for the stationary phase and that, among the column packings studied, this relationship is independent of the stationary phase.

In a GC study of the TMS-ethers of aldono-1,4-lactones on a large number of stationary phases PETERSSON *et al.*[17] observed that lactones with their TMSO-groups in a *trans* (*threo*) position on the number 2 and 3 carbon atoms have less affinity for the stationary phase than the corresponding *cis* (*erythro*) isomers. The validity of this rule has been confirmed in later investigations with the lactones corresponding to tetronic, pentonic and hexonic acids[18]. A plausible interpretation of this rule is that the retention is favoured if the TMSO-groups are close together.

It is reasonable to assume that within the aldaric acid series the polar interaction forces between the carbonyl groups and the stationary phase are larger with those species which exhibit large interactions between their TMSO-groups in the α-positions and neighbouring TMSO-groups. In acyclic compounds such as aldaric acids the substituents at the adjacent carbon atoms in *threo* position are closer together than those in the *erythro* position and it could therefore be expected that L-threaric acid should be held more strongly than erythraric acid and that among the 2-deoxypentaric acids the *threo*-form should exhibit the highest retention. This *α-threo*-rule is confirmed by the results given in Table I.

In the following an *α-threo* grouping is defined as a group having *threo* configuration at the two carbon atoms adjacent to the carboxylic acid groups. Since idaric and galactaric acids contain two *α-threo* groupings these should exhibit larger affinities for the stationary phase, according to the above rule, than talaric and glucaric acids, each with one *α-threo* grouping, and mannaric and allaric acids which contain no *α-threo* grouping. The experimental results are in agreement with the rule.

The *α-threo* rule does not permit a differentiation between the two individual hexaric acids within each of these three groups. There must, however, exist appreciable interactions between the groupings present at carbon atoms 2 and 4 as well as those at carbon atoms 5 and 3, provided that these are in *erythro* position. The additional contributions to the relative retention due to the interaction between these groupings will be denoted as β_E-*erythro* contributions when the configuration adjacent to the carboxylic acid group is *erythro* and β_T-*erythro* contribution when this configuration is *threo*. Recalling that the differences in the affinities of the diastereomers on polar stationary phases are largely explained by the interactions between the carbonyl groups and the stationary phase, it is reasonable to assume that β_E-*erythro* contributions are larger than β_T-*erythro* contributions.

Idaric acid contains two β_T-*erythro* groupings whereas galactaric acid contains no *β-erythro* groupings. This explains the fact that idaric acid is held much more strongly than galactaric acid. Talaric acid contains one β_E-*erythro* grouping which explains why it is held more strongly than glucaric acid which contains one β_T-*erythro* grouping. Allaric acid is held much more strongly than mannaric acid. This can be

explained by the *β-erythro* rule since allaric acid contains two β_E-*erythro* groupings whereas no *β-erythro* groupings are present in mannaric acid.

An application of the above rules to the six deoxyhexaric acids studied in this work leads to predictions of the elution order of the diastereomers in agreement with the experimental results. One exception is 3-deoxy-D-*xylo*-hexaric acid which exhibited a relative retention slightly higher than that of the *lyxo* isomer.

For the pentaric acids these rules predict that xylaric acid is held more strongly than its diastereomers. The order for arabinaric and ribaric acids cannot be predicted by the rules given above. The presence of one *α-threo* grouping close to one of the carbonyl groups will favour the retention of arabinaric acid, but on the other hand, the β_E-*erythro* contributions in ribaric acid will contribute to increased interactions between the stationary phase and both carbonyl groups. The experiments show that ribaric acid is held more strongly than arabinaric acid indicating that the latter contributions are larger.

With most of the acids investigated the best separation was achieved on XE-60 which is the most polar of the stationary phases studied in this work. None of the stationary phases tested was capable of separating all the deoxyaldaric acids, however. The results clearly show that gas chromatography of the TMS-derivatives is a valuable tool in separations of aldaric acids. The separation factors are as a rule more favourable than those recorded with TMS-ethers of alditols[19]. It is believed that in analysis of complicated mixtures of sugars, monocarboxylic acids and aldaric acids such as are present in various technical liquors, *e.g.* effluents from pulp mills, and in solutions of biological interest, a combination of ion-exchange chromatography and gas chromatography coupled with mass spectrometry is the method of choice. The mass spectrometric part of the present investigation, carried out by GÖRAN PETERSSON, will be published in a separate paper.

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