CHROM. 9737

ANION-EXCHANGE CHROMATOGRAPHY OF DICARBOXYLIC HYDROXY ACIDS

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SUMMARY

In the anion-exchange chromatography of dicarboxylic acids with one uronic acid and one aldonic acid moiety, the distribution coefficients (D_v) decrease markedly with increasing molar volume when sodium acetate is used as the eluent. This decrease in an oligomeric series can be predicted from the Gibbs–Donnan equation. For isomers, D_v increases with decreasing distance between the carboxyl groups. The shorter this distance, the higher is the acid strength, which strongly affects D_v in acetic acid.

INTRODUCTION

Anion-exchange chromatography with automatic analysis of the eluate is a useful method for the analysis of uronic and oligomeric uronic acids[1,2]. In addition to these acids, spent bleach liquors from the production of wood pulps contain dicarboxylic acids with one uronic acid and one aldonic acid moiety. We report here the chromatographic separation of acids of this type and on relationships between structure and distribution coefficients.

EXPERIMENTAL

Preparation and preparative chromatography

Birch xylan (83.3 g/l) was treated with an aqueous solution (1.6 l) of chlorine (5.1 g/l) for 8 h at 27.8° in darkness. The excess of chlorine was destroyed with sulphurous acid. Ethanol was added and the precipitated xylan removed by centrifugation and washing with ethanol and finally with diethyl ether. After evaporation to a small volume, the solution was subjected to ultrafiltration (Amicon UM-05) to remove chloride, sulphate and organic compounds of low molecular weight.

The retentate was washed with water, neutralized with sodium hydroxide and kept at pH 8.5 at room temperature for 6 h in order to split lactones. The solution was applied to an anion-exchange column (Dowex 1-X2, acetate). The sugars were washed out with water and the monocarboxylic acids with 15 column volumes of 0.08 M so-

dium acetate solution. The dicarboxylic acid fraction, eluted with 15 column volumes of 0.3 M sodium acetate in 2 M acetic acid, contained acids of the type of interest in this work (III–VIII in Table I) together with small amounts of several aldaric acids. After removal of the sodium acetate by ion exchange and evaporation, the dicarboxylic acid fraction was separated by chromatography on Dowex 1-X8 with 0.2 M sodium acetate solution with acetic acid added to pH 5.9. The fractions were rechromatographed in 3 M acetic acid to obtain a complete resolution.

The structure of the isolated oligomeric acids was established by methylation according to Hakomori[3], followed by acid hydrolysis and analysis of the hydrolysis products as their trimethylsilyl derivatives by gas chromatography–mass spectrometry[4].

Chromatography for analytical purposes

All experiments were carried out with the acetate form of a strongly basic anion exchanger (Dowex 1-X8). The 18–24-μm fraction was used in all experiments with sodium acetate solution as eluent and the 14–17-μm fraction in the runs with acetic acid. The column was thermostatically controlled at 30° and the eluate analyzed automatically, either by chromic acid oxidation[1] or with a three-channel analyzer[5]. The amounts of individual acids (0.2–2 mg) and the nominal linear flow-rate (3.0–6.2 cm/min calculated for an empty column) had no effect on the peak elution volume ($\bar{V}$). In experiments with single acids and mixtures, the volume distribution coefficient was calculated from the equation $D_v = \bar{V}/X - \varepsilon$, where X is the column volume and ε the interstitial fraction ($\varepsilon = 0.39$).

RESULTS AND DISCUSSION

Sodium acetate solution as eluent

In most previous chromatographic studies of monocarboxylic acids in sodium acetate, the eluent was acidified with acetic acid to pH 5.9 to maintain a constant pH and to avoid alkaline rearrangement of unstable compounds. For the dicarboxylic acids investigated here (Table I), this addition led to a significant decrease in D_v for compounds that were held strongly by the resin, while compounds with low D_v were virtually unaffected.

In agreement with the Donnan theory, D_v decreased markedly with an increase in the concentration of the sodium acetate solution. The D_v values in 0.3 M sodium acetate solution at pH 5.9 calculated from those determined in 0.2 and 0.08 M solution under the assumption that the selectivity coefficient[6] was constant differed by 1–7% from the observed values. These results indicate that the activity coefficient ratio in the external solution was only slightly affected by the change in eluent concentration.

In the experiments with sodium acetate solution as eluent, the aldaric acids (I and II) studied for comparison exhibited much higher D_v values than the other species. 2-O-(4-O-Methyl-α-D-glucopyranosyluronic acid)-D-xylonic acid (III) exhibited the third largest D_v value. The D_v values of the two isomeric acids which contained in addition one xylose moiety (IV and V) were much lower, while the three isomers containing two xylose moieties (VI, VII and VIII) exhibited extremely low values. These results are in agreement with the observation made with strongly polar mono-

carboxylic acids, *i.e.*, that the ion-exchange affinity decreases markedly with increase in the partial molar volume[7].

For oligomeric aldonic acids, it has been shown that the D_v values of higher oligomers can be roughly predicted from the D_v of the aldobionic acid and the incremental change in partial molar volume of each sugar moiety ($\Delta\bar{v}$), by applying the Gibbs–Donnan equation. Prerequisites for the calculation are that the swelling pressure (π) is known, that the effect of the activity coefficients can be disregarded and that the resin phase is homogeneous. Although none of these requirements is strictly fulfilled, and it is likely that the activity coefficients and inhomogenieties in the resin exert a greater effect on the exchange of divalent anions than on the exchange of monovalent anions discussed previously[8], an attempt was made to calculate the D_v value of compound VIII from that of its lower oligomer, V. With the approximations already mentioned, the difference in $\ln D_v$ should be obtained from the equation $\Delta\ln D_v = -\pi\Delta\bar{v}/RT$. With the assumption[8] that π is 200 atm and $\Delta\bar{v}$ is the same as determined for β-(1,4)-linked xylose oligomers[9] (0.085 dm³/mole), the predicted D_v of compound VIII is 44 in 0.08 M, 6.8 in 0.2 M and 3.0 in 0.3 M sodium acetate solution. The results are in good agreement with the experimental results given in Table I.

It has previously been shown that the pressure–volume term in the Gibbs–Donnan equation has a large effect on the distribution coefficients of polyols[10] lacking hydrophobic interactions and oligomeric sugars[11,12]. Similarly, this term exerts a large effect on the ion-exchange equilibrium of oligomeric aldonic acids[8]. The results discussed above show that the same holds true for strongly polar dicarboxylic acids of high molar volume.

Fig. 1 shows that sodium acetate solution is an excellent eluent in separations according to the molecular size of strongly polar dicarboxylic acids. As shown in Table I, the separation factors for the isomers are less favourable. For those which contain one and two xylose moieties the distribution coefficients increase with decreasing distance between the carboxylic acid groups[13]. This chromatogram illustrates the usefulness of a three-channel analyzer[6] for analyses of complex mixtures. All species were recorded in the non-specific chromic acid channel. The last compound, II, which lacks uronic acid and sugar moieties, was not recorded with carbazole, while as expected the other acids gave a strong colour reaction. Only compound III contained a terminal vicinal diol group, and accordingly it was the only compound which, after periodate oxidation at pH 2, gave rise to formaldehyde.

Acetic acid as eluent

By carrying out the separations in acetic acid, one takes advantage of differences in the dissociation constants of isomers. The D_v values listed in Table I and the chromatogram in Fig. 2 show that excellent separations of the isomers can be achieved.

The elution order of the oligomeric acids, VIII < V < III, which must differ only slightly in acid strength ,shows that also in this medium the molar volume has a great influence on D_v. As expected, from the predicted strength of the acids[14] the isomers are eluted in order of decreasing distance between the carboxylic acid groups. The observations that in acetic acid VIII is held more strongly than IV and that III exhibits a higher D_v value than the aldaric acids (I and II) show that under the ap-

TABLE I

VOLUME DISTRIBUTION COEFFICIENTS (D_v) OF DICARBOXYLIC HYDROXY ACIDS IN ACETATE MEDIA AT 30°

No.	Carboxylic acid	Eluent						
		0.08 M NaOAc, pH 5.9	0.2 M NaOAc, pH 5.9	0.3 M NaOAc, pH 5.9*	0.3 M NaOAc–2 M HOAc	1 M HOAc	3 M HOAc	5 M HOAc
I	Galactaric	—	83.9	37.8 (43.5)	12.5	—	48	~16.3
II	4-O-Methylglucaric (3-O-methylgularic)	—	61.4	29.0 (30.6)	8.5	—	35.1	11.6
III	2-O-(4-O-Methyl-α-D-glucopyranosyluronic acid)-D-xylonic	—	23.4	9.7 (9.9)	4.1	—	73	22.2
IV	O-(4-O-Methyl-α-D-glucopyranosyluronic acid)-(1→2)-O-β-D-xylopyranosyl-(1→4)-D-xylonic	81	12.6	5.5 (5.6)	1.7	96.7	8.0	2.2
V	O-β-D-Xylopyranosyl-(1→4)-[O-(4-O-methyl-α-D-glucopyranosyluronic acid)-(1→2)]-D-xylonic	88	13.5	5.9 (6.0)	—	—	33.2	8.7
VI	O-(4-O-Methyl-α-D-glucopyranosyluronic acid)-(1→2)-O-β-D-xylopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1 >4)-D-xylonic	32.9	5.6	2.6 (2.6)	1.0	43.9	3.2	0.73
VII	O-β-D-Xylopyranosyl-(1→4)-[O-(4-O-methyl-α-D-glucopyranosyluronic acid)-(1→2)]-O-β-D-xylopyranosyl-(1→4)-D-xylonic	—	6.2	2.9 (2.9)	—	—	4.7	1.05
VIII	O-β-D-Xylopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→4)-[O-(4-O-methyl-α-D-glucopyranosyluronic acid)-(1→2)]-D-xylonic	38.2	6.6	3.1 (3.1)	—	—	12.2	3.0

* The D_v values within parentheses refer to 0.3 M sodium acetate solution without addition of acetic acid.
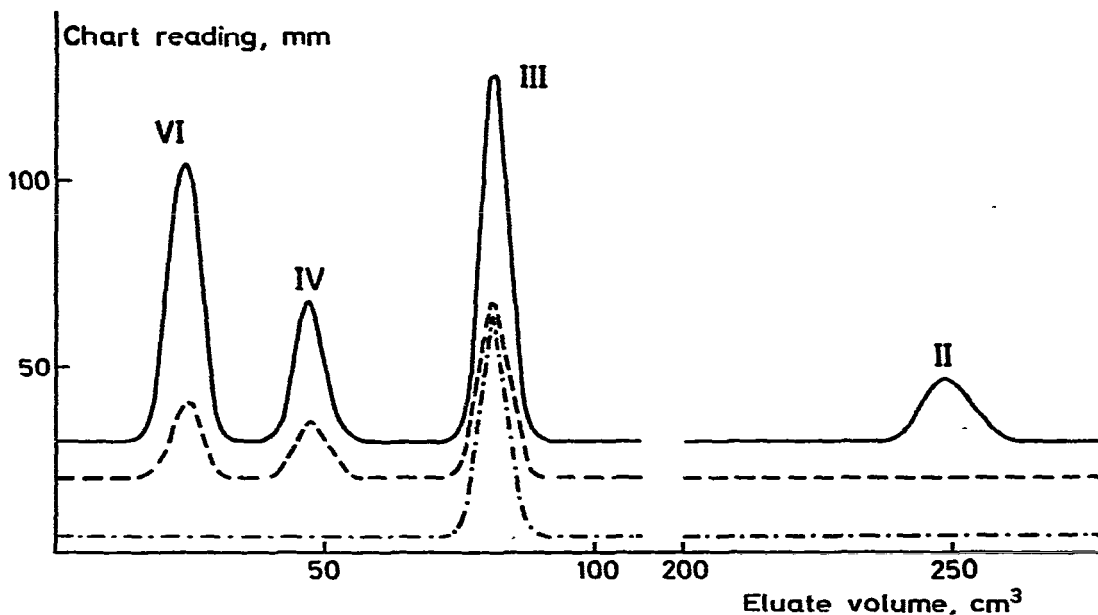
Fig. 1. Separation of oligomeric dicarboxylic hydroxy acids in 0.3 M sodium acetate solution, pH 5.9. Column (685 × 4 mm) packed with Dowex 1-X8, 18–24 μm. Nominal linear flow-rate: 3.9 cm/min. Channels: ———, chromic acid; — — —, carbazole; —·—·—·, periodate–formaldehyde.
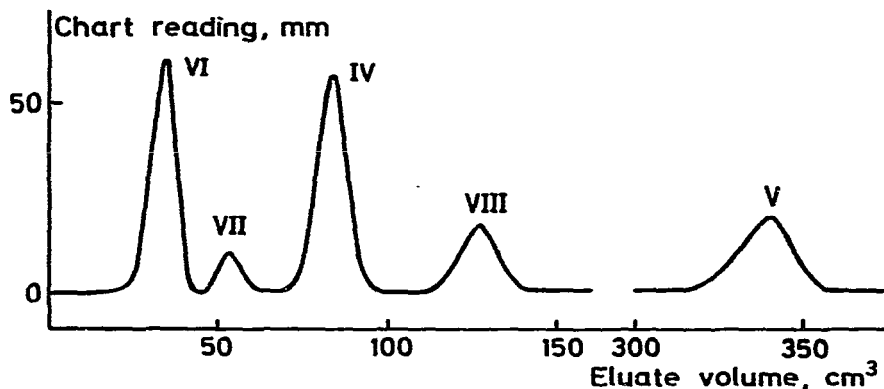

Fig. 2. Separation of oligomeric dicarboxylic hydroxy acids in 3.0 M acetic acid. Column (835 × 4 mm) packed with Dowex 1-X8, 14–17 μm. Nominal linear flow-rate: 6.2 cm/min. Analysis by chromic acid oxidation.

plied conditions D_v is affected more strongly by an increase in acid strength than by a decrease in molar volume. The higher D_v of III compared with the aldaric acids can be related to the strongly acid properties of uronic acids.

The distribution coefficients show that it is possible to separate all dicarboxylic acids listed in Table I in a single run in 5 M acetic acid. Larger amounts can be separated if the mixture is first chromatographed in sodium acetate solution and the

fractions that contain isomers are re-chromatographed in acetic acid. This procedure is recommended also in analyses of unknown compounds, which require identification by additional methods. In most practical work, dicarboxylic acids of the types discussed above are obtained together with sugars and oligomeric aldonic acids. In the procedure applied for the isolation of the acids, a group separation was first made on an anion-exchange resin with a low degree of crosslinking to permit a quantitative-uptake of the oligomeric aldonic acids[15]. The sugars were washed out with water and the monocarboxylic acids eluted with 0.08 M sodium acetate solution before the elution of the dicarboxylic acids as a group.

REFERENCES

1 S. Johnson and O. Samuelson, *Anal. Chim. Acta*, 36 (1966) 1.
2 K. Shimizu and O. Samuelson, *Sv. Papperstidn.*, 76 (1973) 150.
3 S. Hakomori, *J. Biochem.*, 55 (1964) 205.
4 G. Petersson, *Tetrahedron*, 26 (1970) 3413.
5 B. Carlsson, T. Isaksson and O. Samuelson, *Anal. Chim. Acta*, 43 (1968) 47.
6 O. Samuelson, *Ion Exchange Separations in Analytical Chemistry*, Almqvist and Wiksell, Stockholm, Wiley, New York, 1963.
7 O. Samuelson and L. Thede, *J. Chromatogr.*, 30 (1967) 556.
8 J. Havlicek and O. Samuelson, *J. Chromatogr.*, 83 (1973) 45.
9 W. Brown and K. Chitombo, *Chem. Scr.*, 2 (1972) 88.
10 M. Mattisson and O. Samuelson, *Acta Chem. Scand.*, 12 (1958) 1386.
11 B. Z. Ginzburg and D. Cohen, *Trans. Faraday Soc.*, 60 (1964) 185.
12 J. Havlicek and O. Samuelson, *Carbohyd. Res.*, 22 (1972) 307.
13 L. Bengtsson and O. Samuelson, *Anal. Chim. Acta*, 57 (1971) 93.
14 J. Robert and M. Saszrio, *Basic Principles of Organic Chemistry*, W. A. Benjamin, New York, Amsterdam, 1965.
15 J. Havlicek and O. Samuelson, *J. Chromatogr.*, 114 (1975) 383.