ANION EXCHANGE SEPARATIONS OF ALDOBIONIC AND ALDONIC ACIDS

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An anion exchange method for the separation of aldonic acids in borate medium described in earlier papers[1,2], has been applied to the analysis of sulfite spent liquors[3,4] and to the determination of aldonic acid end groups in cellulose[5] and hemicellulose[6]. In connection with this work it was found desirable to develop methods which permit separations in systems involving aldobionic acids. The present work deals with the separation of aldobionic acids and aldonic acids both in borate and acetate media.

EXPERIMENTAL

Preparation of barium aldobionates

Cellobionic, maltobionic, lactobionic and melibionic acids have been prepared by electrolyte oxidation of the corresponding disaccharides. The conditions were, with a few exceptions, the same as those described by ISBELL AND FRUSH[7]. The reaction mixture consisted of 0.1 mol disaccharide, 10 g $CaCO_3$, and 4 g $CaBr_2 \cdot 2H_2O$ in 400 ml water. The distance between the graphite anode and the platinum cathode was kept as small as possible in order to obtain a low potential (3–4 V). The reaction was carried out in the dark[8] and was allowed to proceed until 85–90 % of the sugar had been oxidized.

After the oxidation step, nitrogen gas was bubbled through the solution. A slight excess of silver carbonate was added and the solution was stirred in the dark for a few minutes. After filtration, the solution was tested for bromide. The solution was then cooled with ice and stirred with 100 ml of a cation exchanger in its free-acid form (Dowex 50 X-4; 50–100 mesh) for 2 min. After filtration, the acid solution was brought in contact with 100 ml of a strongly basic anion exchange resin in its bicarbonate form (Dowex 1 X-2; 60–140 mesh). After stirring for 15 min, the resin was transferred to a column and washed with water until the anthrone test was negative. Nitrogen pressure was applied to increase the flow rate. The column was emptied and the resin stirred with 100 ml of ice-cooled 0.5 *M* sulfuric acid to remove carbon dioxide. The acid washed resin was then transferred to a column and the elution of aldobionic acid was completed by treating with 150 ml of the cold acid and washing with 500 ml water. The time of elution was 10–15 min; the washing required about 30 min. The eluate and washings were collected in a beaker containing barium carbonate. The solution was heated to boiling and the precipitate removed by centrifugation. The solution was evaporated under vacuum (30°) to a small

volume and added to a tenfold volume of absolute ethanol in a mortar. The barium salt appeared as a white powder which was separated from the solution by centrifugation, washed with ethanol, and dried between filter papers over phosphorus pentoxide. The yield was 70–75%.

Chromatographic procedures

The separations in acetate medium were carried out with a strongly basic anion exchange resin in the acetate form (Dowex 1 X-8; 40–60 μ). The column, which had a diameter of 10 mm and a length of 920 mm, was loaded with a solution of barium salts in about 5 ml of water. After washing with water, elution was carried out at a flow rate of 1.3 ml.cm^{-2}.min^{-1}. The temperature was kept at 25°. The standard technique with a motor-driven pump for feeding the eluant onto the column and a time-actuated fraction collector were employed[9]. The eluate fractions were analyzed by chromic acid oxidation using the Technicon AutoAnalyzer[3].

The experiments with the borate resin were carried out analogously. If not otherwise mentioned, the column dimensions were 10 × 1460 mm. The temperature was 30°. Under the elution conditions used in this work, the peak elution volumes are higher in borate medium. This results in broader elution bands, and in order to make the analysis of the eluate as simple as possible larger amounts of the acids were used in these experiments than in the separations in acetate medium.

RESULTS AND DISCUSSION

Separations in acetate medium

In an earlier paper it was shown that several hydroxy acids can be easily separated by elution with sodium acetate solution[10]. Acids with a higher molecular weight, such as glucosaccharinic acid, appeared ahead of those with a lower molecular weight, such as lactic and glycolic acids. It could, therefore, be expected that at the same

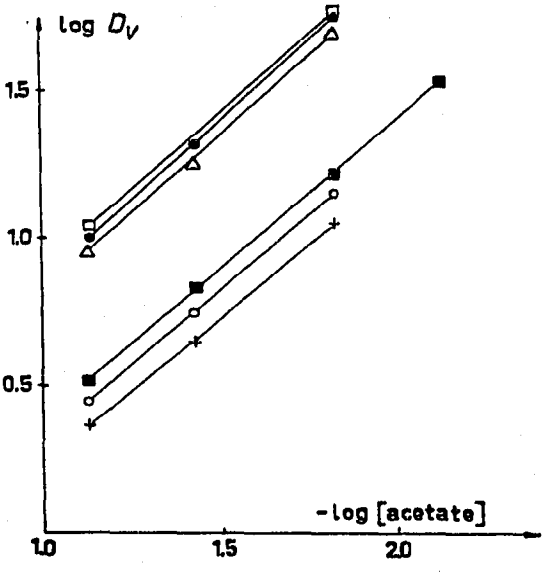

Fig. 1. Influence of the sodium acetate concentration upon the volume distribution coefficient, D_V. □ rhamnonic acid; ● mannonic acid; △ xylonic acid; ■ cellobionic and maltobionic acids; ○ lactobionic acid; + melibionic acid.

eluant concentration the aldobionic acids would appear before any of the acids studied in the previous investigation. This was confirmed experimentally. The peak elution volumes have been determined at various eluant concentrations and the volume distribution coefficients (D_v) calculated as usual[9]. In Fig. 1 log D_v has been plotted against −log [acetate]. Straight lines were obtained with a slope of + 1 (*cf.* ref. 9), which is in agreement with theory. There were significant differences between the distribution coefficients of melibionic, lactobionic and cellobionic acids, whereas the difference between cellobionic and maltobionic acid is, over the whole range of concentration, within the limits of experimental error. Sodium acetate is unsuitable as an eluant for the separation of cellobionic and maltobionic acids. An experiment with both acids on the same column showed that only one elution peak was obtained even at a very low eluant concentration (0.0075 *M*).

The elution behavior of some simple aldonic acids in acetate medium is also demonstrated in Fig. 1. It is seen that the aldonic acids are held much more strongly by the resin than the aldobionic acids, which is in agreement with the previously mentioned observation that many hydroxy acids appear in the order of decreasing molecular weight. This behavior may serve as a preliminary indication when identifying unknown mixtures, but xylonic acid, which contains one carbon atom less than mannonic acid, is held less firmly, which shows that this rule is not always valid.

From the experimental data it can be concluded that a group separation of aldonic acids from aldobionic acids can be carried out very easily in acetate medium. In the experiment presented in Fig. 2 cellobionic acid is separated quantitatively from mannonic acid in 0.075 *M* sodium acetate solution. At this eluant concentration the mutual separation of various acids within each group is far from complete.

Although the separation factor, *i.e.*, the ratio between the distribution coefficients for the various aldobionic acids depends only slightly upon the eluant concentration (Fig. 1), their mutual separation is improved to a large extent when the concentration is lowered. This improvement, according to Glueckauf's theory (*cf.* ref. 9), can be explained by the increased number of theoretical plates at a lower concentration. Similar results have been reported in an earlier paper for the separation of lactic and glycolic acids[10] in acetate medium. A chromatogram of a mixture

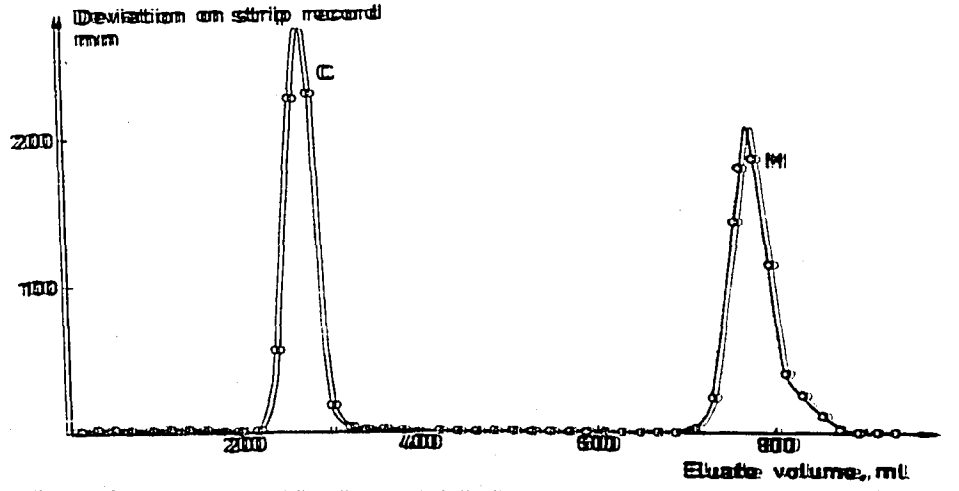

Fig. 2. Separation of 20 mg cellobionic acid (C) from 19 mg mannonic acid (M). Eluant: 0.075 *M* sodium acetate.

of melibionic, lactobionic, and cellobionic acids together with gluconic and mannonic acids run in 0.015 *M* sodium acetate is reproduced in Fig. 3, where it can be seen that the separation of the aldobionic acids from each other and from the aldonic acids is satisfactory for analytical purposes. The two aldonic acids are also separated, but at

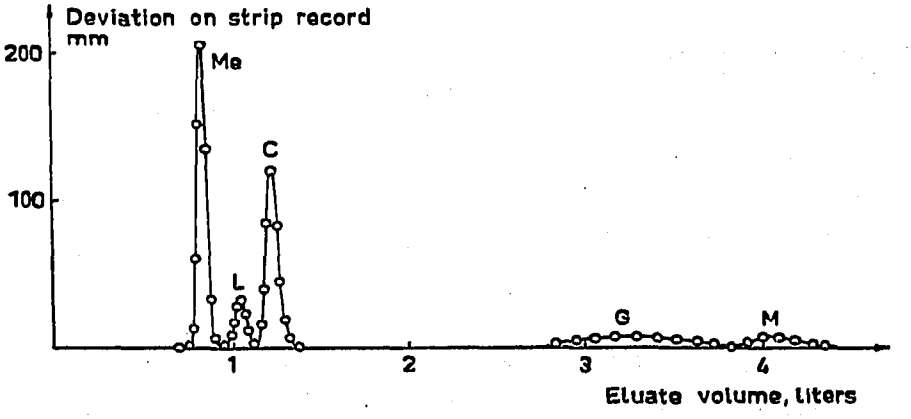

Fig. 3. Separation of various aldobionic and aldonic acids. Eluant: 0.015 *M* sodium acetate. Me: melibionic acid (17 mg); L: lactobionic acid (8 mg); C: cellobionic acid (19 mg); G: gluconic acid (17 mg); M: mannonic acid (10 mg).

this low eluant concentration the elution curves are wide and less suitable for quantitative calculations. In systems where several aldobionic acids are present together with only one simple aldonic acid it is preferable to use a stepwise elution, *i.e.*, to increase the eluant concentration after the aldobionic acids have been eluted.

Fig. 4 shows that xylonic and mannonic acids can be almost completely separated even at a higher sodium acetate concentration. However, the separation factors for the simple aldonic acids are, in general, less favorable in acetate medium than in borate medium.

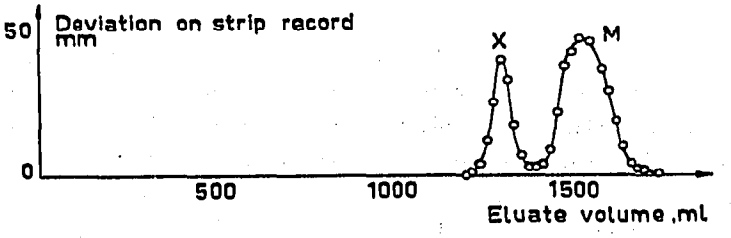

Fig. 4. Separation of 10 mg xylonic acid (X) from 20 mg mannonic acid (*M*). Eluant: 0.0375 *M* sodium acetate.

Separations in borate medium

In borate medium the aldonic acids appear in the following order: xylonic, arabonic, mannonic, gluconic, galactonic acid[1]. The pentonic acids appear ahead of the hexonic acids. The uptake can be ascribed to the combination of ion exchange due to the presence of a carboxyl group and the formation of borate complexes in which the hydroxy groups are involved. Rhamnonic acid (6-deoxy-mannonic acid), which contains one hydroxy group less than the hexonic acids, would be expected to appear before the hexonic acids. As can be seen in Fig. 5, rhamnonic acid appears even before xylonic acid, *i.e.*, it is first among all the aldonic acids studied. A qualitative separation can thus be obtained of all aldonic acids hitherto investigated. A comparison

between the results obtained in borate medium with those in acetate medium shows that borate solutions are preferable when a complicated mixture of aldonic acids is to be separated.

Certain systems containing a mixture of aldonic and aldobionic acids can be satisfactorily analyzed by the borate method. The separation of rhamnonic, xylonic,

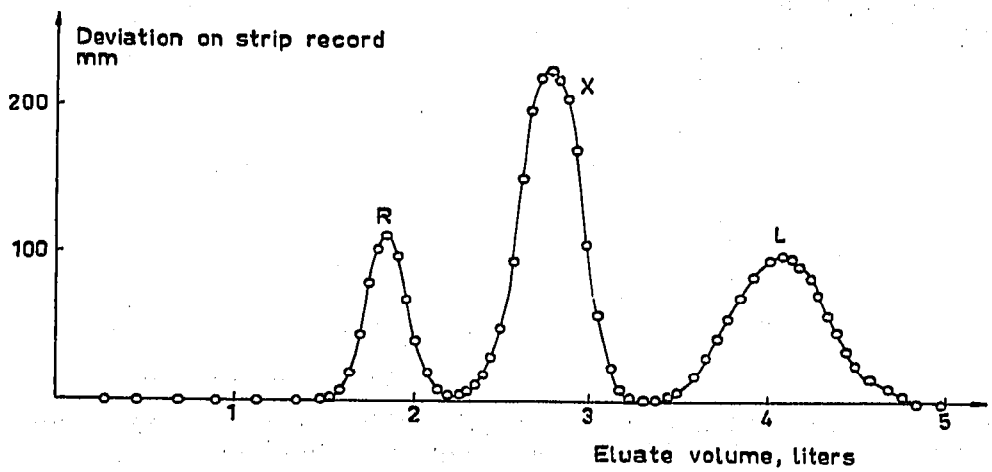

Fig. 5. Separation of 70 mg rhamnonic acid (R), 140 mg xylonic acid (X), and 150 mg lactobionic acid (L). Eluant: 0.1 *M* sodium tetraborate.

and lactobionic acids is indicated in Fig. 5. Similarly, rhamnonic acid can be separated from melibionic acid (Fig. 6). Cellobionic acid is also eluted as a well separated band after rhamnonic acid, but the peak elution volume differs too little from that of melibionic acid to permit a quantitative separation at the eluant concentration used in these experiments (0.1 *M*). Working at a lower concentration is known to give an improved separation of other acids, but the elution is tedious and the elution bands are broadened to such an extent that the accuracy is decreased in quantitative determinations.

It can be concluded from the experimental results that the acetate method is preferable when melibionic, lactobionic, and cellobionic (or maltobionic) acids are to be separated from each other, but the acetate method fails to separate cellobionic and maltobionic acids. The separation of cellobionic acid from maltobionic acid is

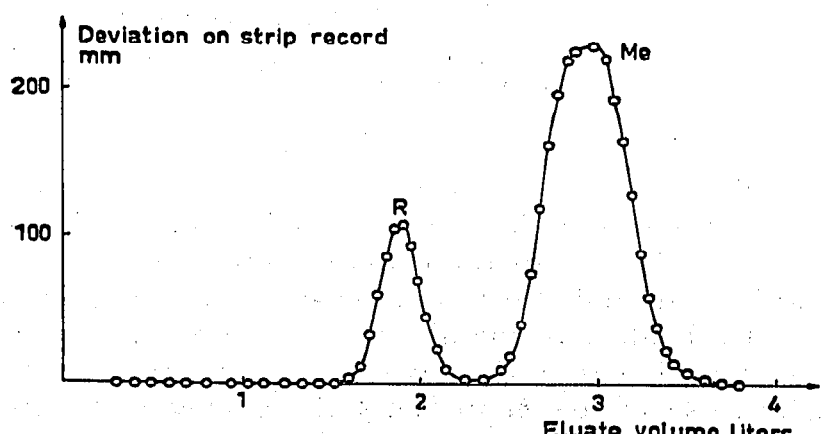

Fig. 6. Separation of 70 mg rhamnonic acid (R) from 160 mg melibionic acid (Me). Eluant: 0.1 *M* sodium tetraborate.

very difficult in borate medium. Using two columns coupled in series it is possible to achieve a partial separation in 0.1 *M* sodium tetraborate solution (Fig. 7). The curves overlap to such an extent that the method can be used only for semi-quantitative determinations of these acids.

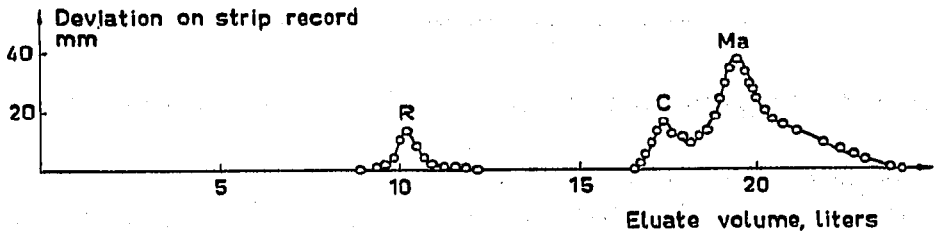

Fig. 7. Separation of 70 mg rhamnonic acid (R), 70 mg cellobionic acid (C) and 180 mg maltobionic acid (Ma). First column: 20 × 1480 mm. Second column: 10 × 920 mm. Flow rate: 2 ml/min. Eluant: 0.1 *M* sodium tetraborate.

In borate medium the aldobionic acids studied have peak elution volumes which differ only slightly from those of the aldonic acids which appear after rhamnonic acid. For this reason the borate method is unsuitable when complicated mixtures of aldonic acids are to be separated from aldobionic acids. When such mixtures are to be analyzed, a group separation is first carried out in acetate medium. If desired, melibionic, lactobionic, and cellobionic acids (together with maltobionic acid), which appear first in the eluate (Fig. 3), can be separated as three elution bands. After cellobionic acid has been eluted the acetate concentration is increased to 0.1 *M* and the aldonic acids are eluted as a group. Subsequently, the borate method is employed to separate all aldonic acids as individual bands.

ACKNOWLEDGEMENT

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SUMMARY

Several aldobionic acids can be separated from each other by chromatography on anion exchange resins. These separations as well as mutual separations of some simple aldonic acids can be carried out by elution with sodium acetate. When complicated mixtures of aldonic acids are to be separated from aldobionic acids, the aldobionic acids are separated in acetate medium. The aldonic acids are eluted as a group and, subsequently, separated in borate medium.

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