Separation of Uronic Acids on Anion Exchange Columns

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Chromatography on anion exchange columns has been used for the separation of oligouronides of different molecular weight. The method has also been used for the separation of monomeric uronic acids. Khym and Doherty separated galacturonic and glucuronic acid on columns of Dowex 1 by elution with 0.15 M acetic acid. Mannuronic and glucuronic acid were, however, not separated. Hallén separated galacturonic and glucuronic acid by chromatography on Dowex 2 columns with 0.03 M borax in 0.6 M sodium hydrogen carbonate as eluant.

The absorption of organic acids on an anionic exchange column is partly due to ionic absorption and partly to molecular absorption. The ionic absorption depends on the dissociation of the acid, and if this type of absorption predominates, it is possible to separate organic acids provided that the dissociation constants are sufficiently different. A difference of 0.2 pH units will often permit a separation of the acids.

The dissociation constants of galacturonic, glucuronic, guluronic and mannanuronic acid are given in a previous note in this journal. The pH values indicate that it should be possible to separate guluronic and glucuronic acid from each other and from mannanuronic and galacturonic acid, while the two latter should be inseparable on a laboratory column of convenient size.

Materials and methods. The samples of uronic acids used have been described earlier. Dowex 1 X 8, 200—400 mesh, was used as ion exchange resin and the dimension of the column was 2 × 45 cm. Approximately 0.75 mequiv of uronic acids were added to the column in each run and the flow of the liquid was 0.3—0.5 ml/min. The resin was activated with 1 N sodium hydroxide and washed with 2 N acetic acid followed by 0.2 N acetic acid. The uronic acids were applied to the column in slightly alkaline solution. The elution was carried out with acetic acid varying from 0.5 N to 2 N with a linear gradient. The elution was followed by the colour reaction with phenolsulphuric acid. The figures given for optical density refer to 2 ml eluate, and have been corrected for dilution when necessary. The elution products were identified by paper electrophoresis in 0.01 borate buffer containing 0.005 M CaCl₂.

Results. The elution curve from a typical experiment is shown in Fig. 1. Table 1 gives the identity of the elution product in the different fractions. The results show that glucuronic and mannanuronic acids are separated from each other and from guluronic and galacturonic acid while the separation of the two latter acids was not satisfactory.

The order of elution of guluronic, mannanuronic and glucuronic acids is in good agreement with the pH₅ values of the uronic acids. Galacturonic acid forms an exception, thus indicating that the order of elution is not controlled solely by ionic absorption.

![Graph](image-url)
Table 1. Uronic acids eluted from anion exchange columns.

<table>
<thead>
<tr>
<th>Tube</th>
<th>M HAE</th>
<th>Galacturonic acid</th>
<th>Gluronic acid</th>
<th>Mannuronic acid</th>
<th>Glucuronic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>89—96</td>
<td>0.90—0.95</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>97—103</td>
<td>0.95—1.00</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>104—111</td>
<td>1.00—1.05</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>139—150</td>
<td>1.20—1.25</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>151—175</td>
<td>1.25—1.40</td>
<td>+</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Hydrogen chloride is evolved during the reaction and the oxides are dissolved. After cooling a crystalline product appears, e.g. \( \text{MnO}_2\text{P}_2\text{O}_5\text{Cl}_4\cdot(\text{CH}_2\text{COOC}_2\text{H}_5)_2 \).

All the compounds mentioned are rather hygroscopic, and we selected the manganous compound for an X-ray investigation as it was apparently less hygroscopic than the others. The chemical analysis confirmed the results of Bassett and Taylor: (Found: Cl 28.84; P 12.38. Calc. for \( \text{MnO}_2\text{P}_2\text{O}_5\text{Cl}_4\cdot(\text{CH}_2\text{COOC}_2\text{H}_5)_2 \): Cl 28.45; P 12.42).

A crystal of the dimensions: 1.7 mm × 0.8 mm × 0.4 mm was sealed in a Lindemann tube. Weissenberg and precession photographs have been taken using both Cu and Mo radiation.

The crystal proved to be monoclinic. The unit cell has the following dimensions:

\[
\begin{align*}
\alpha &= 14.27 \text{ Å} \\
\beta &= 13.87 \text{ Å} \\
\gamma &= 10.04 \text{ Å} \\
\beta &= 95.58^\circ
\end{align*}
\]

The following spectra were absent: \( h0l \) for \( h = 2n + 1 \), 060 for \( k = 2n + 1 \). Thus the space group is \( P2_1\alpha \). The density of the crystal is found as 1.671. The general point in \( P2_1\alpha \) is fourfold so the molecular weight of the asymmetric unit is 900. The calculated molecular weight for \( \text{MnO}_2\text{P}_2\text{O}_5\text{Cl}_4\cdot(\text{C}_4\text{H}_8\text{O})_2 \) is 499.

A full structure determination of this compound has been started.


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Crystal Data of \( \text{MnO}_2\text{P}_2\text{O}_5\text{Cl}_4\cdot(\text{CH}_2\text{COOC}_2\text{H}_5)_2 \)

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Bassett and Taylor\(^{13}\) investigated 40 years ago the reactions between metal oxides and phosphorus oxychloride. Their aim was to establish the molecular structure of apatite and related compounds. They discovered that the oxides of divalent manganese, zinc, magnesium and calcium react with phosphorus oxychloride to yield crystalline products. To a crystal chemist of to day this is somewhat surprising and these compounds deserve a structural investigation, as they certainly are not related to apatite.

Bassett and Taylor discovered also that \( \text{POCl}_3 \) dissolved in acetone or ethylacetate or other related compounds reacts violently with the metal oxides mentioned above.