

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 weight¹. The method has also been used for the separation of monomeric uronic acids. Khym and Doherty² 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. Hallén³ separated galacturonic and glucuronic acids 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 absorption⁴. 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 acids⁴.

The dissociation constants of galacturonic, glucuronic, guluronic and mannuronic acid are given in a previous note in this journal⁵. The pK_S values indicate that it should be possible to separate guluronic and glucuronic acid from each other and from mannuronic 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 earlier⁵. 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 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 acid⁶. 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 CaCl_2 ⁵.

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

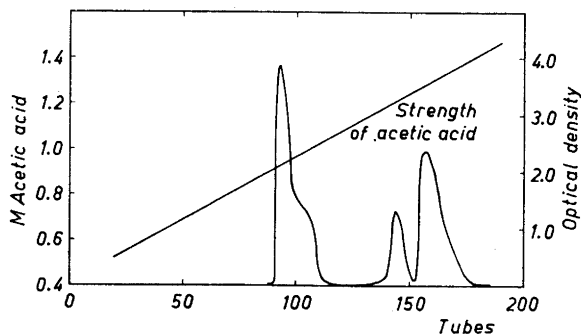


Fig. 1.

Table 1. Uronic acids eluted from anion exchange columns.

Tube	M HAc	Galacturonic acid	Guluronic acid	Mannuronic acid	Glucuronic acid
89-96	0.90-0.95	+			
97-103	0.95-1.00	+	+		
104-111	1.00-1.05		+		
139-150	1.20-1.25			+	
151-175	1.25-1.40				+

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Crystal Data of $\text{MnO}_2 \cdot \text{P}_2\text{O}_5 \cdot \text{Cl}_4 \cdot (\text{CH}_3\text{COOC}_2\text{H}_5)_2$

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Bassett and Taylor^{1,2} 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 POCl_3 dissolved in acetone or ethylacetate or other related compounds reacts violently with the metal oxides mentioned above.

Hydrogen chloride is evolved during the reaction and the oxides are dissolved. After cooling a crystalline product appears, e.g. $\text{MnO}_2 \cdot \text{P}_2\text{O}_5 \cdot \text{Cl}_4 \cdot (\text{CH}_3\text{COOC}_2\text{H}_5)_2$.

All the compounds mentioned are rather hygroscopic, and we selected the manganese 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 $\text{MnO}_2 \cdot \text{P}_2\text{O}_5 \cdot \text{Cl}_4 \cdot (\text{C}_4\text{H}_8\text{O}_2)_2$: Cl 28.45; P 12.42).

A crystal of the dimensions: 1.7 mm \times 0.8 mm \times 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:

$$\begin{aligned} a &= 14.27 \text{ \AA} \\ b &= 13.87 \text{ \AA} \\ c &= 10.04 \text{ \AA} \\ \beta &= 95^\circ 56' \end{aligned}$$

The following spectra were absent: $h0l$ for $h = 2n + 1$, $0k0$ for $k = 2n + 1$. Thus the space group is $P2_1/a$. The density of the crystal is found as 1.671. The general point in $P2_1/a$ is fourfold so the molecular weight of the asymmetric unit is 500. The calculated molecular weight for $\text{MnO}_2 \cdot \text{P}_2\text{O}_5 \cdot \text{Cl}_4 \cdot (\text{C}_4\text{H}_8\text{O}_2)_2$ is 499.

A full structure determination of this compound has been started.

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