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Isomerisation of D-Glucuronic Acid in Neutral Aqueous Solution

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The isomerisation products from the treatment of D-glucuronic acid in an aqueous solution of pH 7 at 100° and 110° have been separated by anion exchange chromatography and identified. The predominant product was D-*lyxo*-5-hexulosonic acid and the others were L-*ribo*-5-hexulosonic, D-mannuronic, D-altruronic, and D-alluronic acid (the amounts of which decreased in the order given). L-Iduronic acid, previously stated as the main product by these conditions, was not detected.

The isomerisation of reducing sugars under the influence of alkali has been extensively studied and the Lobry de Bruyn-Alberda van Ekenstein mechanism,[1] postulating enediol intermediates in the aldose-ketose conversions, is generally accepted. The corresponding reactions of uronic acids, however, have been very little studied.

Siddiqui and Purves [2] found that uronic acids were isomerised when treated with aqueous sodium hydroxide at room temperature. They thus presented evidence for the formation of D-mannuronic acid and a keto acid from D-glucuronic acid, and taluronic acid from D-galacturonic acid.

Fischer and Schmidt [3] found that uronic acids were readily isomerised even at pH 7 (at 100°) and reported that D-glucuronic, D-galacturonic, and D-mannuronic acid, respectively, formed mainly (by C-5 epimerisation) L-iduronic, L-altruronic, and L-guluronic acid.

In connection with studies of uronic acids in sulphate pulps [4] and of the influence of uronic acids upon the colour reversion of cellulose products,[5] it was observed that the isomerisation of D-glucuronic acid at pH 7 resulted

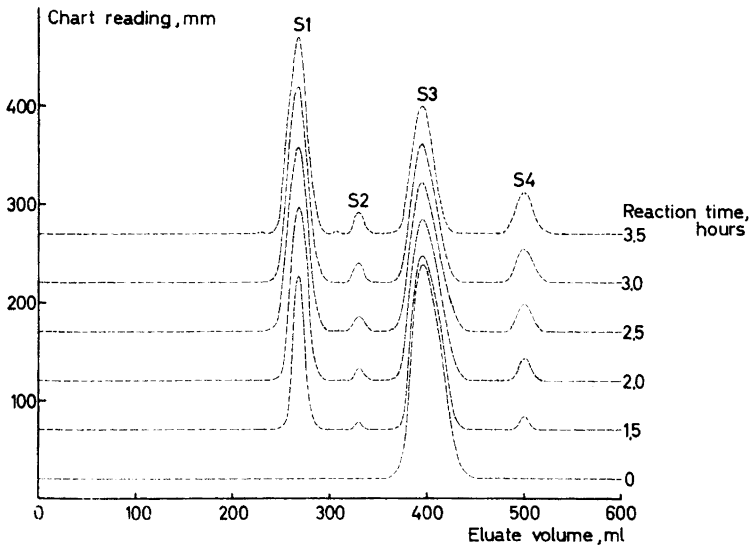


Fig. 1. Separation of isomerisation products formed at 100° after various reaction times. The curves refer to the reaction with carbazole. Resin bed: 6 × 670 mm; Dowex 1−X8; 13−18 μm; Eluent: 1 M acetic acid; Flow rate: 5.0 ml min^{-1} cm^{-2}.

in a complex mixture of acids with D-*lyxo*-5-hexulosonic acid (5-keto-D-mannonic or 5-keto-L-gulonic acid) as the main reaction product, in analogy with the formation of fructose as the main product from glucose.

The present investigation deals with the separation by anion exchange chromatography of the acids obtained by the treatment of D-glucuronic acid

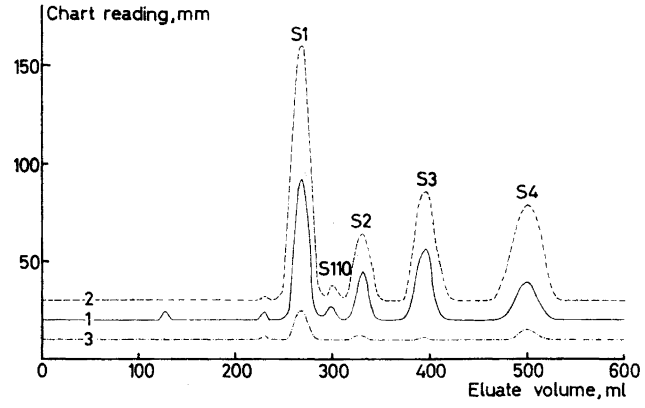


Fig. 2. Separation of isomerisation products formed at 110° after 3 h. Chromic acid oxidation: 1; Carbazole method: 2; Periodate oxidation: 3. Conditions: see Fig. 1.

in neutral aqueous solution and their characterisation and identification by various methods.

The chromatograms reproduced in Fig. 1 show that at least three acids were rapidly produced when a solution of glucuronic acid of pH 7 was kept at 100°. The acid fractions were isolated from a run on a larger scale and rechromatographed in 0.08 M sodium acetate at pH 5.9. Band S2 was resolved into two bands denoted S2:A1 and S2:A2 whereas the other fractions gave rise to single bands.

Two additional bands were recorded in the chromatogram obtained when the temperature was increased to 110° (Fig. 2). None of the bands showed elution properties identical with those of iduronic acid. The small band which appeared before S1 in the run at 110° was not studied further whereas the other six acids were isolated in larger amounts and identified by various methods.

All acids gave a strong response with carbazole indicating that the acids were hexuronic or 5-hexulosonic acids. The periodate response index [6] and the colour of the spots obtained by paper chromatography gave information about the group of acids to which each compound belonged. Gas chromatography-mass spectrometry of the trimethylsilyl derivatives showed that all acids, except S1 and S4, were hexuronic acids.

A valuable tool for providing further confirmation of the structures of previously known acids and conclusive evidence for the structures of unknown species was reduction with sodium borohydride to aldonic acids which were then identified by well established methods using authentic compounds for comparison. Model experiments confirmed that hexuronic acids gave rise to a single aldonic acid and showed that 5-hexulosonic acids yielded similar amounts of two epimeric aldonic acids.

Three of the acids exhibited D_v values which in both media were identical with those of available reference compounds (Table 1). Paper chromatographic and electrophoretic studies (Table 2) as well as gas chromatography-mass spectrometry confirmed the identity of these compounds.

The acid contained in band S3 was identified as glucuronic acid by all these methods. After reduction gulonic acid was obtained. As can be seen

Table 1. Characteristics of isolated chromatographic bands(s) compared with those of reference acids(r).

Band number	Compound	Volume distribution coefficient, D_v 1 M HAc		0.08 M NaAc		Periodate [6] response index
		s	r	s	r	
S1	D-*lyxo*-5-Hexulosonic	15.2	15.2	14.1	14.2	strong
S110	D-Alluronic	16.9		12.6		weak
S2:A1	D-Altruronic	19.0		13.1		weak
S2:A2	D-Mannuronic	19.0	18.9	14.3	14.3	medium
S3	D-Glucuronic	22.7	22.7	13.0	13.0	weak
S4	L-*ribo*-5-Hexulosonic	28.8		16.5		strong

Table 2. Paper chromatography and electrophoresis of uronic and hexulosonic acids. (1)=spot, giving positive lactone reaction

Compound	Paper chromatography $R_{glucurono\text{-}6,3\text{-}lactone}$ in solvent A	Paper electrophoresis $M_{vanillin}$ buffer B	Paper electrophoresis $M_{glucose}$ buffer C	Colour with *p*-anisidine-HCl
Acids isolated in the present investigation:				
D-Glucuronic	0.42; 1.00 (1)	1.24; 0.91 (1)	2.88; 2.10 (1)	Brick-brown
D-Mannuronic	0.50; 0.85 (1)	1.45; 1.06 (1)	2.71	»
D-Altruronic	0.50	1.49; 1.37 (1)	2.91	»
D-Alluronic	0.51	1.35	3.22	»
D-*lyxo*-5-Hexulosonic;	0.58; 0.97 and 1.35 (1)	1.25	3.11	Yellowish-brown
L-*ribo*-5-Hexulosonic	0.79; 0.83 and 1.52 (1)	1.32	3.33	»
Acids given as comparison:				
D-Galacturonic	0.38	1.33	2.50	Brown
L-Iduronic	0.58; 1.33 (1)	1.59; 1.05 (1)	3.07	»
L-Guluronic	0.45; 1.24 (1)	1.41	2.70	Brick-brown
D-*xylo*-5-Hexulosonic (5-keto-D-gluconic)	0.77	1.29	3.22	Yellowish-brown
D-*arabino*-Hexuloson-ic (2-keto-D-gluconic)	0.45	1.65	3.04	Reddish-brown
L-*xylo*-Hexulosonic (2-keto-L-gulonic)	0.47	1.60	2.96	»

from Fig. 1 the area of the glucuronic acid band decreased continuously upon prolonged reaction time.

The acid corresponding to band S1, the main reaction product, showed properties identical with those of D-*lyxo*-5-hexulosonic acid. On reduction mannonic and gulonic acids were identified as the only aldonic acids formed.

Band S110 gave only one peak when rechromatographed in sodium acetate. Mass spectrometry, periodate index, as well as colour reaction with the anisidine reagent, indicated that the acid was a uronic acid. On reduction only one aldonic acid, indistinguishable from allonic acid, was formed. Thus band S110 must correspond to D-alluronic acid (not previously reported).

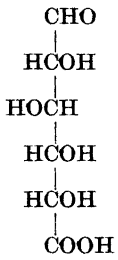
The acid corresponding to band S2:A1 also had properties of a uronic acid and it was proved to be D-altruronic acid (not previously reported) as it gave on reduction only one aldonic acid, indistinguishable from talonic acid.

The acid corresponding to band S2:A2 was indistinguishable, by all methods used, from D-mannuronic acid. On reduction it gave only one aldonic acid, corresponding to the expected acid, namely mannonic acid.

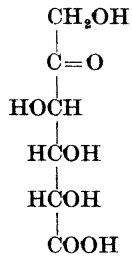
Finally the acid corresponding to band S4 had properties similar to those of the acid, contained in band S1, indicating a 5-hexulosonic acid. On reduction two aldonic acids, indistinguishable from talonic and allonic acids, respectively, were formed in about equal amounts, which proved the acid to be

L-*ribo*-5-hexulosonic acid (5-keto-D-talonic or 5-keto-L-allonic acid), not previously reported.

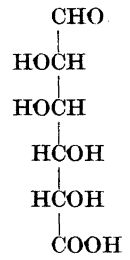
This investigation shows that D-glucuronic acid isomerises more readily than the corresponding monosaccharide, D-glucose, and yields a higher amount of isomerisation products (also including considerable amounts of C-3 epimers). The relative proportions of acids formed by the treatment at pH 7 and 110° for 3 h are given below. As no appreciable amounts of products originating from enolisation further down the carbon chain were detected among the reaction products, it is likely that the acids isolated have retained the original configuration at C-4 and C-5 and thus exist in the forms as shown here.



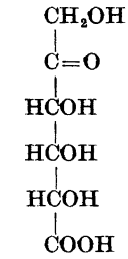
D-Glucuronic acid (36 %)



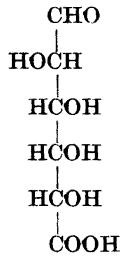
D-*lyxo*-5-Hexulosonic acid (35 %)



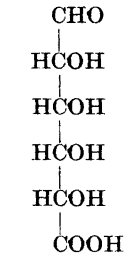
D-Mannuronic acid (8 %)



L-*ribo*-5-Hexulosonic acid (15 %)



D-Altruronic acid (5 %)



D-Alluronic acid (1 %)

EXPERIMENTAL

The acids used as reference substances were either obtained commercially or prepared and identified by previously reported methods. D-Allonic acid [isolated as 1,4-lactone; m.p. 127–128° and $[\alpha]_D$ −4.4 (*c* 2; water)] was prepared from the now readily available D-allose,[7] using the usual bromine oxidation procedure.

The optical rotations given below of chromatographically pure but amorphous compounds are equilibrium values (of the acid and possible lactone forms) in water.

Whatman No. 1 papers were used for paper chromatography and electrophoresis and the following solvents and buffers (see Table 2) were used:

A. Ethyl acetate, acetic acid, water, 3:1:1.
B. 0.1 M Hydrogen sulphite-acetate buffer, pH 4.7 (used at 40°).[8]
C. 0.4 M Boric acid-1.0 M glycerol, adjusted with NaOH to pH 6.8 (used at 40°).[9]

Conventional spray reagents for carbonyl compounds (*p*-anisidine hydrogen chloride and silver nitrate-sodium hydroxide), acids (bromophenol blue) and lactones (hydroxylamine-ferric chloride) were used. The sharpest spots for the free acids were obtained using the indicator reagent.

After trimethylsilylation the acids were investigated by gas chromatography. Each hexuronic acid gave rise to several peaks (see also Ref. 10) and the chromatogram (obtained on a QF-1 column) could be used as additional evidence for the identification of those acids of which authentic reference samples were available. Mass spectra were obtained from a gas chromatograph-mass spectrometer. The TMS-derivatives of the hexuronic acids and the lactones of the hexuronic acids gave characteristic mass spectra. Only small differences existed between diastereomers. Investigation of trimethylsilyl derivatives of the acids S1 and S4 revealed different structures. This part of the investigation was carried out by Göran Petersson and a complete report will be given in a forthcoming paper.

The reduction of uronic and hexulosonic acids by sodium borohydride to aldonic acids was carried out according to the procedure of Perry and Hulyalkar.[11]

The identification of aldonic acids by anion exchange chromatography was made as previously described [12] using acetic acid and sodium acetate as eluents. The allonic and talonic acids were also identified using 0.12 M sodium tetraborate.

The gas-liquid chromatography of the aldonic acids as their fully trimethylsilyl substituted 1,4-lactones was carried out using a QF-1 column.[13]

Isomerisation of D-*glucuronic acid and separation of the isomerisation products.* An aqueous solution of D-glucurono-6,3-lactone (1.30 g) was treated with sodium hydroxide (2 M) at pH 8 to saponify the lactone. The reaction was carried out at room temperature for 6 h the pH being kept constant with the help of an autotitrator after which the pH was adjusted to 7 with acetic acid. The neutralised solution (15 ml) was divided into six equal parts, five of which were placed in glass tubes and after deaeration with nitrogen sealed by melting. The tubes were treated in a polyglycol bath at 100° for various times. The isomerised solutions were then chromatographed on an analytical anion exchange column using a three channel analyser,[8] which permitted simultaneous registration of the chromic acid consumption, the colour reaction with carbazole and the formation of formaldehyde after periodate oxidation. From the peak elution volumes the distribution coefficients (D_v) were calculated as usual [14] and compared with those of available substances (see Fig. 1 and Table 1).

In the preparative scale experiments the neutralised solutions (1.30 g glucuronolactone in 15 ml and 3.00 g in 30 ml, respectively) were placed in glass tubes and treated as described above for 3 h at 100° and 110°, respectively. A part of each of the solutions (5 mg of starting substance) was chromatographed on the analytical column, the main parts being separated on a preparative column (Dowex 1-X8; 25—32 μm; 20×870 mm) with 1 M acetic acid as eluent (see Fig. 2, Table 1 and the formulae above).

Band S2, the only band which when rechromatographed in sodium acetate gave rise to two bands, was separated on a preparative column (Dowex 1-X8; 27—32 μm; 10×920 mm) with 0.08 M sodium acetate at pH 5.9 as eluent.

The $[\alpha]_D^{25}$-values of D-alluronic and D-altruronic acids were +10° and −10°, respectively (*c* 0.6; water).

Two larger scale preparative separations were made starting from (A) 4.00 g and (B) 3.00 g glucurono lactone, treated in a 10 % aqueous solution kept at pH 7.0 at 96° for 2 h and carrying out the separation on a column of Dowex 1—X8, 200—400 mesh (30×1100 mm) and eluting with 1 M acetic acid. The following chromatographically pure fractions (except fraction 2 which as indicated below was a mixture) were collected.

	Acid	A g	B g	$[\alpha]_D^{22}$ (*c* 2)
1.	D-*lyxo*-5-Hexulosonic	0.61	0.53	−4°
2.	D-Mannuronic / D-Altruronic	0.12	0.10	
3.	D-Glucuronic	2.57	2.07	
4.	L-*ribo*-5-Hexulosonic	0.15	0.08	+12°

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