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ISOMERIZATION OF 4-*O*-METHYL-D-GLUCURONIC ACID IN NEUTRAL, AQUEOUS SOLUTION

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ABSTRACT

Aqueous solutions of 4-*O*-methyl-D-glucuronic acid at pH 7 were heated at 100°, and the monocarboxylic acids formed by isomerization were separated by anion-exchange chromatography and further identified by gas–liquid chromatography–mass spectrometry After 6 h, the following yields of acids were obtained 3-*O*-methyl-D-*lyxo*-5-hexulosonic (47%), 3-*O*-methyl-L-*ribo*-5-hexulosonic (12%), 4-*O*-methyl-D-mannuronic (4%), and 3-*O*-methyl-L-*ribo*-4-hexulosonic (1%)

INTRODUCTION

Earlier investigations showed that D-glucuronic and D-galacturonic acids, when heated in a neutral, aqueous solution, gave rise to several hexuronic and hexulosonic acids[1–3]. Their formation could be explained by Lobry de Bruyn–Alberda van Ekenstein transformations[4]. Large proportions of 4-*O*-methyl-D-glucuronic acid moieties are present in wood hemicellulose As a rule, hydrolysates, as well as acid and neutral spent-liquors obtained from wood, which contain this uronic acid also contain a number of unknown acids; from their chromatographic behaviour and color responses, it is suspected that these acids are formed by isomerization We now report on the monocarboxylic acids formed by isomerization of 4-*O*-methyl-D-glucuronic acid in neutral, aqueous solution

EXPERIMENTAL

4-*O*-Methyl-D-glucuronic acid was prepared from extracted birch powder (*Betula verrucosa*) by partial hydrolysis with 0 25M sulphuric acid at 100° for 15 h, and isolated as a syrup after anion-exchange chromatography in acetic acid and sodium acetate[5] An aqueous solution containing 1 g of the syrup was neutralized with M sodium hydroxide and kept at room temperature at pH 8 0 for 5 h with the help of an autotitrator Acetic acid was added to pH 7 0, followed by water to obtain a final concentration of 12 mg of uronic acid per ml The solution was then heated in round-bottomed flasks under reflux for 4 and 6 h, respectively, the temperature of the polyglycol heating-bath was 100° In the 6-h experiment, a stream of nitrogen was passed over the solution The pH dropped to ~6 in both experiments

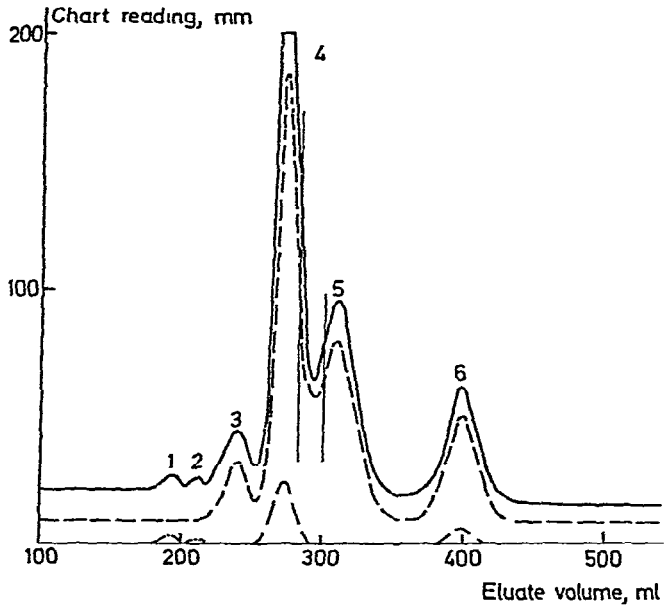

Fig 1. Separation of isomerization products formed at 100° after 6 h Resin bed 6×620 mm, Dowex-1 X8, 23–27 μm, eluent M acetic acid, nominal flow 4 3 cm/min Chromic acid (———), carbazole (– – –), and periodate–formaldehyde (— — —) channels.

After the heating period, sodium hydroxide was added to pH 8 0 and the acids were separated on a column (87×2 cm) of Dowex-1 X8 resin (25–39 μm) with M acetic acid as eluent. Fractions were collected as indicated in Fig 1, which refers to a parallel run on an analytical column coupled with a three-channel analyzer[6] In addition to the numbered fractions, the overlapping zone between peaks 4 and 5 was analyzed separately and found to contain no additional compounds The amounts of acids present in this zone were added to those found in fractions 4 and 5 The acids contained in fractions 2 and 6 were re-chromatographed on a preparative scale in 0 08M sodium acetate (with acetic acid added to pH 5 9) This medium, as well as M acetic acid and 0 15M potassium tetraborate, was used to determine the volume distribution coefficients of the acids contained in the fractions isolated by anion-exchange chromatography on Dowex-1 X8

All acids isolated were reduced with sodium borohydride, as described by Perry and Hulyalkar[7], and the reduction products were studied by anion-exchange chromatography in sodium acetate and tetraborate, using the eluent concentrations given above, and also in 0 5M acetic acid, which gives a better separation of aldonic acids than the higher concentration used above The three-channel analyzer was used in all experiments to facilitate the identification

Both the isomerization acids and their reduction products were studied by g l c–m s after transformation of their sodium salts into fully trimethylsilylated esters[8] The silicone fluid, QF-1, was used as the stationary phase

The distribution coefficients listed in Tables I and II refer to the experiment carried out for 6 h The values relative to gluconic and galacturonic acids (applied as standards) differed by less than 2% from those obtained in the 4-h experiment

TABLE I

YIELDS AND CHROMATOGRAPHIC DATA OF ACIDS[a]

Identified acid	*Yield* (%)		*Distribution coefficient* (D_v)			*Acids produced by reduction*
	4 h	*6 h*	*HOAc*	*NaOAc*	$K_2B_4O_7$	
4-*O*-Methyl-D-glucuronic	32	22	16 8	7 07	3 26	3-*O*-Methyl-L-gulonic
3-*O*-Methyl-D-*lyxo*-5-hexulosonic	34	47	15 0	10 7	11 5	3-*O*-Methyl-L-gulonic 3-*O*-Methyl-D-mannonic
4-*O*-Methyl-D-mannuronic	3 5	4 2	13 0	7 71	5 11	3-*O*-Methyl-D-mannonic
3-*O*-Methyl-L-*ribo*-4-hexulosonic	0 9	1 0	10 2	10 0	11 0	3-*O*-Methyl-L-gulonic 3-*O*-Methyl-L-allonic
3-*O*-Methyl-L-*ribo*-5-hexulosonic	5 6	12	22 0	9 56	6 41	3-*O*-Methyl-L-allonic 3-*O*-Metnyl-D-talonic
3-Deoxy-4-pentulosonic	0 6	0 4	11 3	11 3		3-Deoxy-*erythro*-pentonic 3-Deoxy-*threo*-pentonic

[a]Present after heating an aqueous solution of 4-*O*-methyl-D-glucuronic acid at 100° for 4 and 6 h

TABLE II

CHROMATOGRAPHIC DATA[a] OF REDUCTION PRODUCTS

Identified acid	*Distribution coefficient* (D_v)		
	HOAc	*NaOAc*	$K_2B_4O_7$
3-*O*-Methyl-L-gulonic	10 9	4 87	5 54
3-*O*-Methyl-D-mannonic	14 5	6 32	7.51
3-*O*-Methyl-L-allonic	8 76	6 87	6 81
3-*O*-Methyl-D-talonic	4 95	5 23	6 35

[a]In 0 5M acetic acid, 0 08M sodium acetate (pH 5 9), 0 15M potassium tetraborate

The yields of acids were determined by weighing the isolated, syrupy fractions A correction for minor impurities was obtained by planimetration of the chromatograms The results from the isomerization experiments carried out for 4 and 6 h are included in Table I.

SEPARATION AND IDENTIFICATION

As can be seen in Fig 1, after heating a neutral solution of 4-*O*-methyl-D-glucuronic acid at 100° for 6 h, six peaks were recorded on the chromatogram obtained

by anion-exchange chromatography in acetic acid Peak 5, which was recorded only in the chromic acid and carbazole channels, had a position corresponding to that of the starting material The identity was confirmed by g l c.–m s (all mentions of this technique imply analysis of the trimethylsilylated esters), and by identification of the acid formed after reduction with sodium borohydride (3-*O*-methylgulonic acid, Table II)

The most prominent peak, 4, gave a response in the periodate–formaldehyde channel also The formation of formaldehyde, together with the strong response with carbazole, suggested that the solute was a ketoaldonic acid G l c –m s showed the presence of a 3-*O*-methyl-5-hexulosonic acid derivatised in the acyclic and one furanoid form As expected, ion-exchange chromatography of the reduction products gave two peaks with strong responses in the chromic acid and periodate–formaldehyde channels, typical of aldonic acids One of the acids exhibited distribution coefficients identical with those of 3-*O*-methylgulonic acid in acetate and acetic acid G l c –m s confirmed its identity and showed that, as expected, the other reduction product was a 3-*O*-methylhexonic acid The results permit the conclusion that the keto-acid formed by isomerization was 3-*O*-methyl-*lyxo*-5-hexulosonic acid, and that the other aldonic acid formed by its reduction was 3-*O*-methylmannonic acid.

The third peak in Fig 1 was recorded in the carbazole channel, but not in the periodate–formaldehyde channel, suggesting the presence of a uronic acid G l c –m s showed that the acid was a 4-*O*-methylhexuronic acid; both derivatised pyranoid forms were recorded Reduction with sodium borohydride and ion-exchange chromatography showed the presence of one aldonic acid as the only reduction product Its behaviour during ion-exchange chromatography in acetate and acetic acid was identical with that of the 3-*O*-methylmannonic acid obtained from fraction 4 in Fig 1 The identity of the aldonic acid was confirmed by g l c –m s The results show that the original isomerization product was 4-*O*-methylmannuronic acid.

When re-chromatographed in 0 08M sodium acetate, the first peak gave rise to one major acid, recorded in all channels, indicating a ketoaldonic acid In addition, trace amounts of at least four other acids were recorded (D_v in sodium acetate 3 03, 3 60, 5 42, and 7 60) G l c –m s showed that the major acid was a 3-*O*-methyl-4-hexulosonic acid Ion-exchange chromatography of the reduction products in acetate, as well as in acetic acid, showed that two acids with color responses typical of aldonic acids were obtained G l c –m s showed the presence of two 3-*O*-methylhexonic acids One of these exhibited chromatographic data identical with those of 3-*O*-methylgulonic acid Since the keto group was located at C-4, it can be concluded that the isomerization product was 3-*O*-methyl-*ribo*-4-hexulosonic acid and that the other reduction product was 3-*O*-methylallonic acid

Re-chromatography of peak 6 in sodium acetate revealed that the main acid, which was recorded in all three channels, was contaminated with a trace amount of another acid (D_v in sodium acetate, 11 3) The color responses suggested that the main acid was a hexulosonic acid G l c –m s showed the presence of a 3-*O*-methyl-5-hexulosonic acid (derivatised as its acyclic and furanoid forms) Ion-exchange

chromatography (in acetate and acetic acid media) of its reduction products gave responses typical of two aldonic acids The peak positions of one of them were the same as those of 3-*O*-methylallonic acid prepared from fraction 1 in Fig 1, whereas the elution properties of the other acid, obtained after reduction, differed from those of the other 3-*O*-methylaldonic acids G l c –m s confirmed that both acids were 3-*O*-methylhexonic acids This means that the acid contained in peak 6 was 3-*O*-methyl-*ribo*-5-hexulosonic acid, and that the other reduction product was 3-*O*-methyltalonic acid. To obtain futher evidence, the reduction products were separated on a preparative scale The 3-*O*-methyltalonic acid thus isolated was demethylated[9] Ion-exchange chromatography in acetate and acetic acid media showed that the only acid produced by demethylation was talonic acid

The second peak, which contained only minor amounts of acid, gave three peaks when re-chromatographed in acetate Two of these, which contained only trace amounts, gave responses in the chromic acid and carbazole channels (D_v in sodium acetate, 3 77 and 5 51) The major acid was recorded in all channels The isolated acid was studied by g l c –m s and shown to be a 3-deoxy-4-pentulosonic acid Ion-exchange chromatography (in acetic acid) of its reduction products gave responses typical of two aldonic acids The D_v values were the same as those obtained for 3-deoxy-*erythro*-pentonic and 3-deoxy-*threo*-pentonic acids G l c –m s confirmed the identity of these acids These results confirmed that the acid, which was a minor constituent, was 3-deoxy-4-pentulosonic acid. The amount was too small for determination of the absolute configuration

DISCUSSION

The results presented above show that 4-*O*-methyl-D-glucuronic acid is isomerized readily in neutral, aqueous solution The major products were three hexulosonic acids and the C-2-epimeric uronic acid. Evidently, other reactions, such as the formation of aromatic compounds[10] or reactions analogous to saccharinic acid formation, are less important

The appearance of the hexulosonic acids and the epimeric uronic acid is explained by Lobry de Bruyn–Alberda van Ekenstein transformations, in which the substituents at C-1, C-2, and C-3 are involved As in the previous studies of glucuronic[1,2] and galacturonic[3] acids, no isomerization products were found having an inverted configuration at C-4 and C-5 relative to the starting material It can therefore be concluded that the absolute configurations of the acids are those given in the Tables Like D-glucuronic acid, the methylated acid gave rise to the corresponding D-*lyxo*-5-hexulosonic acid as the main isomerization product With both starting materials, the second most-abundant products were the L-*ribo*-5-hexulosonic acids The third most-abundant products were D-mannuronic and 4-*O*-methyl-D-mannuronic acids, respectively D-Altruronic and D-alluronic acids were obtained in smaller amounts from D-glucuronic acid[1], whereas no detectable amounts of the corresponding methylated species were formed from 4-*O*-methyl-D-glucuronic acid. Likewise, not

only was the C-2-epimeric uronic (taluronic) acid obtained from D-galacturonic acid[3], but minor amounts of D-guluronic and D-iduronic acids were also found A possible explanation of the absence of methylated altruronic and alluronic acids is that HO-4 is substituted and that, for this reason, the furanoid form cannot be formed

Very small proportions of L-*ribo*-4-hexulosonic acid were produced from D-glucuronic acid[2] and, as can be seen from Table I, a small proportion of the corresponding methylated acid was obtained in the present work The corresponding 5-hexulosonic acids and the C-2-epimeric uronic acid were the preponderant acids formed from galacturonic acid as well, but the formation of 4-hexulosonic acids was also established here The results show that, like D-glucuronic and D-galacturonic acids, 4-*O*-methyl-D-glucuronic acid isomerizes very easily and gives very good yields of several isomeric acids The isomerization occurs more rapidly with the methylated acid than with the non-methylated species The results explain the appearance of the isomerization products in several types of waste water from the wood-pulp and wallboard industries

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