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Note

Preparation of aldononitrile acetates using *N*-methylimidazole as catalyst and solvent

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Gas-chromatographic analysis of carbohydrates has been performed by a number of methods, the most widely used[1] being that involving trimethylsilyl ethers, whose major advantage is their rapid formation. Nevertheless, water reacts with the silylating reagents and hydrolyzes the silylated products, and thus the sample to be derivatized needs to be reasonably dry. Moreover, each individual sugar gives at least two peaks because of the various anomeric forms of each sugar in solution. Quantitative analysis of simple mixtures is very good, but accurate analysis of complex mixtures is much more difficult as the concentration of sugar depends on the reproducibility of the ratio of isomers in solution at equilibrium and the ability of the column to separate the individual isomers at least partially. Some investigators[2,3] have converted the sugars into oximes before trimethylsilylation. When the *syn* and *anti* isomers of the trimethylsilylated oximes are not resolved, each sugar shows only one peak. Nevertheless, this derivative still inherits the other disadvantages of trimethylsilyl ethers.

As alternatives to the aforementioned derivatives, the alditol acetates[4] and aldononitrile acetates[5–7] are widely used. Each sugar derivative gives only one peak and has good chromatographic properties. However, the sample to be derivatized has to be dry, and preparation of these derivatives is generally too elaborate to be convenient for routine carbohydrate analysis.

In a previous report[8] the aldononitrile acetates of some neutral sugars were prepared by using *N*-methylimidazole as solvent and catalyst. Their preparation was found to be both rapid and simple. The present study reports a more-detailed examination of the formation of aldononitrile acetates of both neutral and amino sugars. The effects of water and aqueous acid on the reaction were also investigated.

EXPERIMENTAL

Preparation of aldononitrile acetates of neutral sugars in aqueous solution. — A stock solution of hydroxylamine · HCl was prepared by dissolving hydroxylamine ·

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HCl (0.5 g) and methyl α-D-glucopyranoside (0.1 g, internal standard) in 20 mL of *N*-methylimidazole (Sigma Chemical Company). A sugar stock was prepared by dissolving 0.5 g of the sugars (L-arabinose, D-glucose, and D-galactose; 100 mg of each sugar in 10 mL of water). Six samples were prepared to check the reproducibility of the method. Each was prepared by adding 0.4 mL of hydroxylamine · HCl stock solution to 0.2 mL of sugar solution in a vial having a vinyl-lined screw cap. The vial was placed in a heating block for 10 min at 80°, removed, cooled, and 1 mL of acetic anhydride added. After 5 min, chloroform (1 mL) was added and the solution was washed twice with 1 mL of water. The aqueous fraction was discarded and ~0.39 g of anhydrous sodium sulfate was added to the chloroform fraction. The samples were analyzed by g.l.c. with a nickel alloy column (3.05 m × 3.2 mm) packed with 1% diethylene glycol adipate on Chromosorb WHP (100–120 mesh) (Supelco, Inc., Bellefonte, PA). Aldononitrile acetates were eluted isothermally at 195° with helium as the carrier gas at 25 mL per min.

Preparation of the aldononitrile acetate of 2-amino-2-deoxyglucose in aqueous solution. — A stock solution of hydroxylamine · HCl was prepared by dissolving 1 g of hydroxylamine · HCl and 0.1 g xylitol (internal standard) in 20 mL of *N*-methylimidazole. A sugar stock-solution was prepared by dissolving 1 g of 2-amino-2-deoxy-D-glucose hydrochloride in 10 mL of water. Six samples were prepared to check the reproducibility. Each sample was prepared by adding 0.4 mL of hydroxylamine · HCl stock solution to 0.2 mL of sugar stock-solution in a vial. The sample was heated for 5 min at 80° and then treated as for neutral sugars. The samples were analyzed with a stainless-steel column (1.83 m × 3.2 mm) packed with 5% OV-17 on Chromosorb W, AW (60–80 mesh). The derivative was eluted isothermally at 240°.

Rate studies on the formation of oximes and aldononitrile acetates of glucose and 2-amino-2-deoxy-D-glucose hydrochloride. — A stock solution of hydroxylamine · HCl was prepared by dissolving 1 g of hydroxylamine · HCl in 20 mL of *N*-methylimidazole, and a sugar stock-solution by dissolving 1 g of glucose or 2-amino-2-deoxy-D-glucose · HCl in 10 mL of water. The sample was prepared by adding 0.4 mL of hydroxylamine · HCl solution to 0.2 mL of sugar solution.

The rate of formation of glucose oximes was determined by heating each sample at 80° for various lengths of time. The sample was analyzed by high-performance liquid chromatography (l.c.). The separations were accomplished in a column (4.6 mm × 30 cm) of "Chromegabond NH_2" (ES Industries, Marlton, NJ) connected to a differential refractometer. The mobile phase consisted of 4:1 (v/v) acetonitrile–0.1% acetic acid in water. Flow rate was 2 mL per min.

The rate of formation of the 2-amino-2-deoxy-D-glucose oxime was determined by heating each sample at 80° for various lengths of time. After the samples had been removed from the heating block, 1 mL of acetic anhydride was added. The reaction was allowed to proceed for 10 min to allow sufficient time for complete dehydration and acetylation, and then 1 mL of chloroform was added. The solution was washed twice with 1 mL of water and analyzed as for the amino sugar as already described.

The rate of formation of aldononitrile acetates was determined by heating

each sample for 10 min at 80°. The vials were removed from the heating block and 1 mL of acetic anhydride was added. The reaction in each sample was allowed to proceed for various lengths of time, and then 1 mL of chloroform was added. The solution was washed twice with 1 mL of water, and analyzed by g.l.c. under the conditions already described.

Effect of N-*methylimidazole–water ratio on the formation of* D-*glucose oxime.* — Six samples were prepared for this study, each by dissolving 100 mg of glucose and 100 mg of hydroxylamine · HCl in 3 mL of *N*-methylimidazole and water solution. The ratio of *N*-methylimidazole to water for each sample was varied. The samples were heated for 5 min at 80° and analyzed by l.c. under the same conditions already given.

Application of the method for analysis of sugars in a wood hydrolyzate. — A sample of wood residue was hydrolyzed with sulfuric acid by the procedure of Laver *et al.*[9]. After hydrolysis, glucitol was added as the internal standard. One-half of the solution was made neutral with lead carbonate to pH 6 and filtered. Five aliquots of each solution (hydrolyzed and neutralized) were derivatized, and analyzed by the method used for neutral sugars.

Preparation of alditol acetates of neutral sugars in aqueous solution. — A sugar solution was prepared by dissolving 0.1 g of the individual sugars (L-arabinose, D-xylose, D-mannose, D-glucose, and D-galactose) in 10 mL of water. The sugar solution (0.1 mL) was reduced by the addition of 0.1 mL of 5% sodium borohydride. After 1 h, *N*-methylimidazole (0.4 mL) and acetic anhydride (1 mL) were added, followed 5 min later by chloroform (1 mL). The solution was washed twice with 1 mL of water, and analyzed by g.l.c. with a stainless-steel column (1.83 m × 3.2 mm) packed with 3% OV-225 plus 2.5% high efficiency 9 BP (80–100 mesh) (Supelco, Inc., Bellefonte, PA) at 215°.

RESULTS AND DISCUSSION

Aldononitrile acetates of carbohydrates are prepared by initial condensation with hydroxylamine hydrochloride to form the oxime, followed by dehydration and acetylation to produce the corresponding aldononitrile acetate. *N*-Methylimidazole catalyzes the conversion of carbohydrates into the corresponding aldononitrile acetates (Fig. 1). This reaction may be performed in both aqueous and non aqueous solution. The reproducibility of this method with a series of standard solutions of sugars is shown in Table I.

The rate of the reactions (oximation, dehydration, and acetylation) was studied with D-glucose and 2-amino-2-deoxy-D-glucose as model compounds in 1:2 water–*N*-methylimidazole.

The rate of the first reaction was determined by monitoring, by l.c., the disappearance of glucose and the formation of glucose oxime (Fig. 2). The peak heights of the highest peaks for glucose and glucose oxime are designated as 100 and the

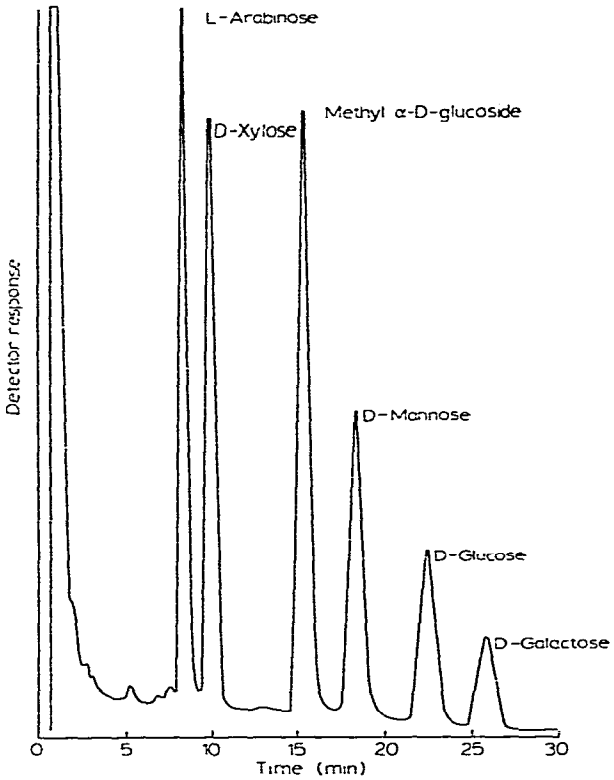

Fig. 1. Gas–liquid chromatogram of aldononitrile acetates at 195°.

TABLE I

ACCURACY OF THE ALDONONITRILE ACETATE PROCEDURE USING *N*-METHYLIMIDAZOLE

Sugar	*Retention time (min)*[a]	*Actual amount of sugar (mg)*	*Calculated mean ±standard deviation (mg)*[b]
L-Arabinose	8.3	2	2.00 ±0.02
D-Xylose	9.9	2	2.03 ±0.07
D-Mannose	18.3	2	1.99 ±0.02
D-Glucose	22.4	2	1.97 ±0.08
D-Galactose	25.8	2	2.02 ±0.04
2-Amino-2-deoxy-D-glucose · HCl	9.5	20	19.68 ±0.54

[a]Methyl α-D-glucoside (15.3 min) was used as the internal standard for neutral sugars. Xylitol (4 min) was used as the internal standard for the amino sugar. [b]Six replications. The amount of sugar was calculated based on the peak height and internal standard.

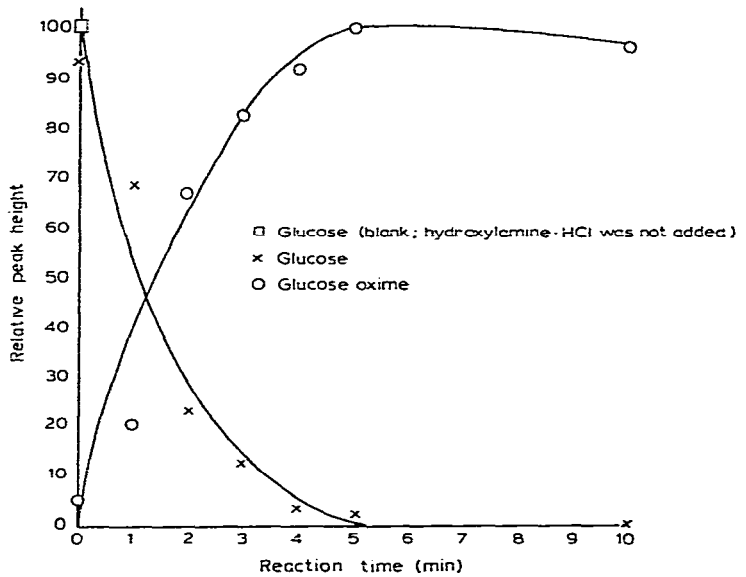

Fig. 2. Disappearance of glucose and formation of glucose oxime versus reaction time.

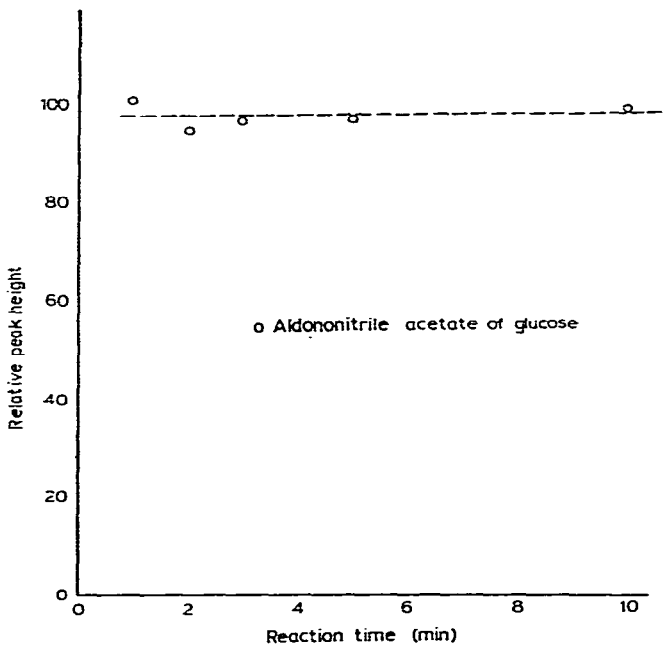

Fig. 3. Formation of aldononitrile acetate from glucose (dehydration and acetylation of glucose oxime) versus reaction time.

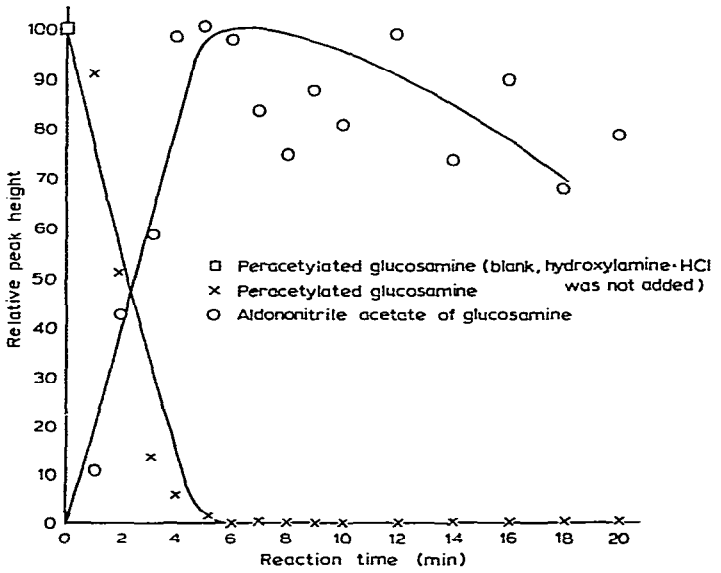

Fig. 4. Disappearance of peracetylated 2-amino-2-deoxy-D-glucose · HCl and formation of aldononitrile acetate of 2-amino-2-deoxy-D-glucose (2-amino-2-deoxy-D-glucose oxime) versus reaction time.

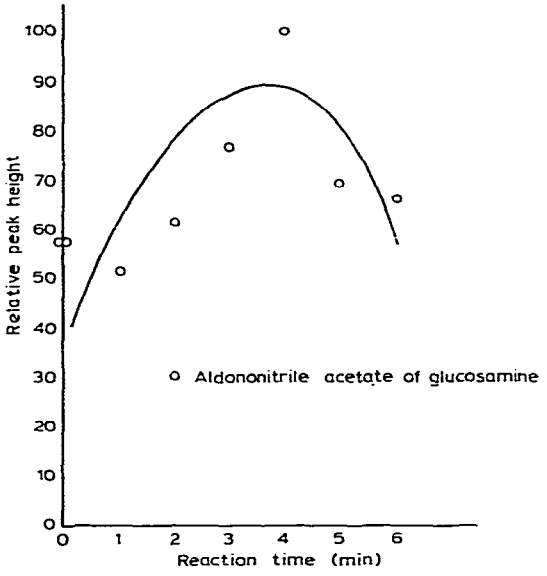

Fig. 5. Formation of aldononitrile acetate of 2-amino-2-deoxy-D-glucose (dehydration and acetylation of 2-amino-2-deoxy-D-glucose oxime) versus reaction time.

heights of other peaks are relative to this standard. All glucose was converted into its oxime in <10 min.

The second step of the reaction (formation of aldononitrile acetate from the glucose oxime) was monitored by g.l.c. (Fig. 3). Analysis of the first samples, taken

1 min. after addition of acetic anhydride, indicated that the reaction was complete. It appears from these data that the complete reaction, conversion of glucose into the aldononitrile acetate, occurs in <7 min.

The rate of oxime formation with 2-amino-2-deoxy-D-glucose was determined by treating the sugar for various lengths of time with hydroxylamine hydrochloride, followed by addition of acetic anhydride, which converted the unreacted amino sugar into the corresponding peracetate and the reacted 2-amino-2-deoxy-D-glucose into the aldononitrile acetate. The relative amounts of both products were determined by g.l.c. (Fig. 4) and indicate that, after ~ 6 min, the initial reaction between 2-amino-2-deoxy-D-glucose and hydroxylamine was complete, as all of the unreacted amino sugar had disappeared. However, the yield of the aldononitrile acetate decreased at prolonged reaction-times, indicating some degradation of product.

The rate of formation of the aldononitrile acetate from 2-amino-2-deoxy-D-glucose oxime was determined by treating the amino sugar with hydroxylamine for 10 min and by adding acetic anhydride for various lengths of time. The results (Fig. 5) indicate that the dehydration and/or acetylation of 2-amino-2-deoxy-D-glucose oxime are slower than for glucose oxime. The reactions were complete in ~ 4 min. The yield decreased after longer times, indicating degradation of product. Although product degradation may occur in the reaction medium, it is surprising that reasonably good reproducibility was obtained for 2-amino-2-deoxy-D-glucose hydrochloride, as shown in Table I. It appears that the reaction time for amino sugars is very critical for obtaining maximal yield and good reproducibility. Four or 5 min for each step could be a proper choice for the derivatization of 2-amino-2-deoxy-D-glucose hydrochloride.

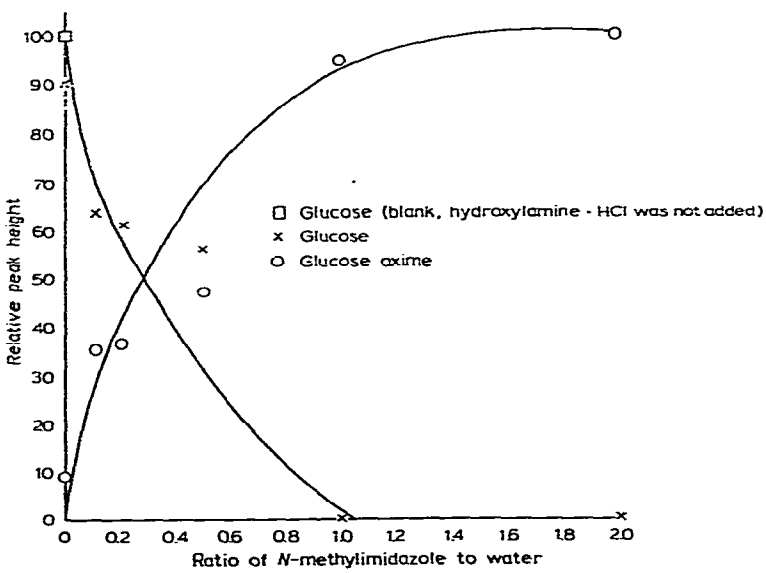

Fig. 6. The effect of *N*-methylimidazole–water ratio on the rate of formation of glucose oxime.

TABLE II

ANALYSIS OF SUGARS FROM WOOD HYDROLYZATES BEFORE AND AFTER NEUTRALIZATION[a]

Sugar	*Acidic sample mean ±standard deviation (mg)*	*Neutralized sample mean ±standard deviation (mg)*
L-Arabinose	2.7 ±0.3	3.7 ±0.4
D-Xylose	10.8 ±1.9	10.3 ±0.5
D-Mannose	9.3 ±0.7	8.9 ±0.5
D-Glucose	80.0 ±4.5	79.3 ±3.8
D-Galactose	6.1 ±0.9	6.6 ±0.7

[a]Five replications.

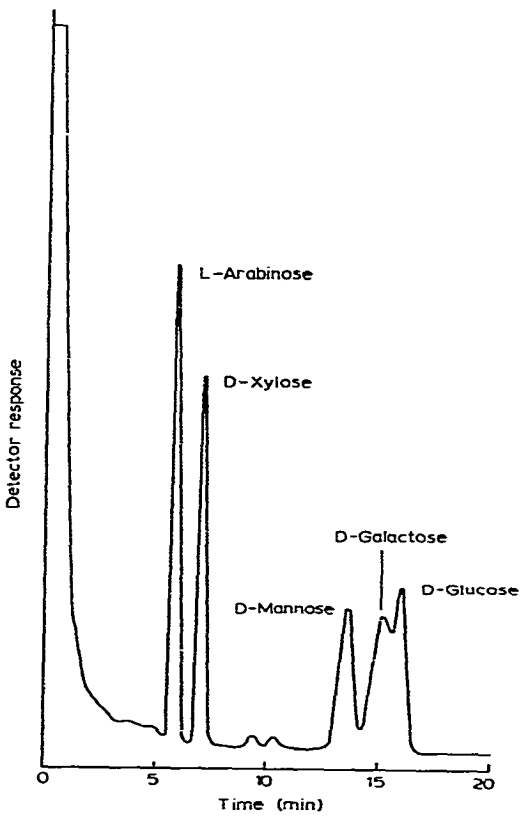

Fig. 7. Gas-liquid chromatogram of alditol acetates. Stainless-steel column (1.83 m × 3.2 mm) with 3% OV-225 plus 2.5% high efficiency 9BP on Supelcoport (80–100 mesh) at 215°.

The ratio of *N*-methylimidazole to water in the medium affects the rate of derivatization. As shown in Fig. 6, glucose oxime may be prepared in a medium consisting of any proportion of *N*-methylimidazole to water. The reaction is complete in 5 min. when the ratio is one or greater. The presence of water in the medium does not seem to affect the dehydration and acetylation of sugar oximes. Nevertheless, an excess of acetic anhydride should be used to compensate for its possible loss from hydrolysis.

The presence of mineral acid in the medium does not seem to interfere with the reaction, provided that there is an excess of acetic anhydride and *N*-methylimidazole. Acid hydrolyzates of polysaccharides may be analyzed directly without removing the water or neutralizing the acid (Table II).

Alditol acetates are also useful derivatives for g.l.c. analysis of aldoses. They may be prepared conveniently in aqueous medium by using *N*-methylimidazole. A gas–liquid chromatogram of the alditol acetates of 5 neutral sugars is shown in Fig. 7. They were prepared by treating the reduced sugars with acetic anhydride in *N*-methylimidazole for 5 min. No particular effort was made to optimize the reaction conditions or to separate these derivatives.

This procedure for preparing aldononitrile acetates of aldoses is relatively fast and may be used in the presence of water and acids, and therefore it should be useful for the routine analysis of a wide range of aldoses.

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