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ALKALI-CATALYZED OXIDATION OF D-GLUCOSE WITH SODIUM 2-ANTHRAQUINONESULFONATE IN ETHANOL–WATER SOLUTIONS

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ABSTRACT

The kinetics of oxidation of D-glucose with 0.01–10mM 2-anthraquinonesulfonic acid in 0.001–1M sodium hydroxide in ethanol–water of up to 61% (w/w) concentration of ethanol were studied over a temperature range of 25–45°. At high concentrations of the quinone, the rate of oxidation of D-glucose was determined by its rate of enolization, which was higher in ethanol–water solutions than in water. The oxidation of the enolized D-glucose was first-order with respect to the quinone, and it competed with isomerization to D-fructose. The ratio of the rates of oxidation and isomerization was increased with decreasing ethanol concentration and increasing hydroxyl-ion concentration. D-Glucosone was proved to be the only primary oxidation product of D-glucose.

INTRODUCTION

From the early 1970's, when the beneficial effect of quinonoid compounds in the alkaline pulping of wood was first reported[1], studies on the oxidation of mono-[2], di-[2–4], and poly-saccharides[4,5] with anthraquinone and its derivatives have been conducted. Most of these experiments have been concentrated on analysis of the reaction products, and search for possible reaction-mechanisms, whereas less attention has been paid to the reaction kinetics. The oxidation of D-glucose with sodium 2-anthraquinonesulfonate (AMS) is here discussed from the point of view of the kinetics, with special emphasis on the primary oxidation-step. The effect of solvent has been included, as it has been discussed in earlier publications dealing with other alkali-catalyzed reactions of reducing carbohydrates[6–8].

RESULTS AND DISCUSSION

Rate of disappearance of D-glucose. — It would be expected that the rate of oxidation of D-glucose would be determined by its rate of enolization. To verify this assumption, the effects of hydroxyl-ion concentration, ethanol concentration, and temperature on the rate of disappearance of D-glucose was studied. The oxidations

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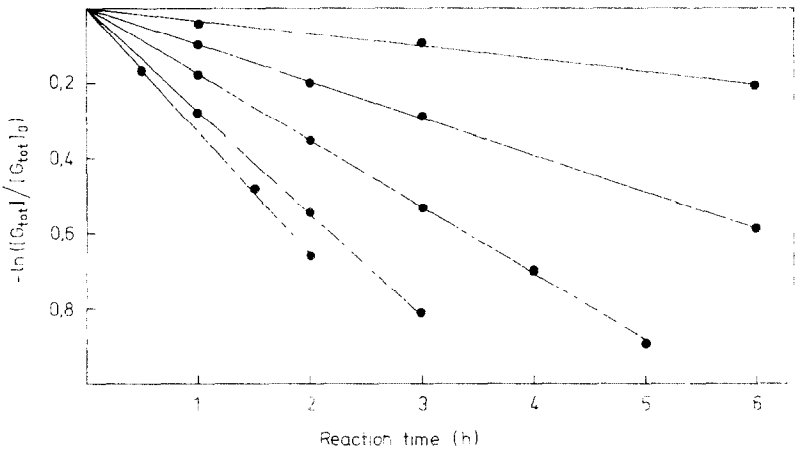

Fig. 1 Disappearance of D-glucose in 10mM AMS solution at 35°. [The hydroxyl-ion concentrations corresponding to the lines are 0.003, 0.01, 0.03, 0.1, and 0.3M, reading down.]

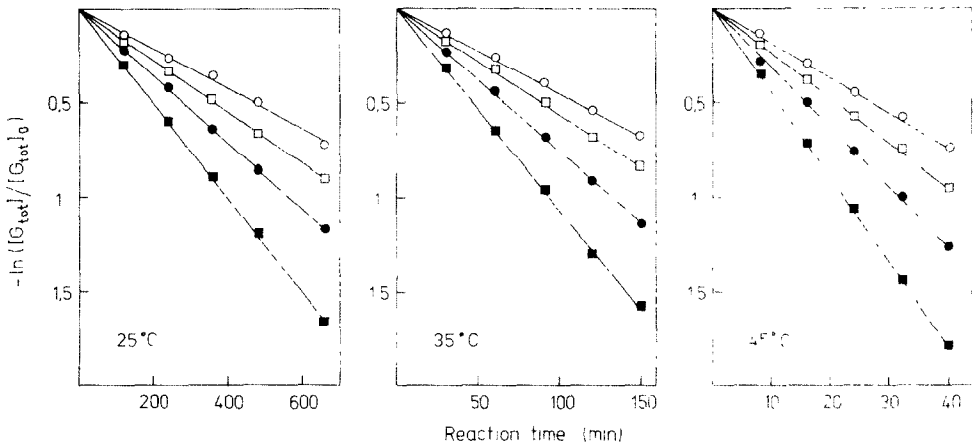

Fig. 2. Disappearance of D-glucose in 0.1M sodium hydroxide in the presence of AMS (10mM) in 0 (○), 16 (□), 33 (●), and 61% (■) (w/w) ethanol at 25, 35, and 45°.

were conducted under a nitrogen atmosphere, and an excess of AMS was used (to prevent isomerization).

The disappearance of D-glucose (G) followed *pseudo*-first-order kinetics, independent of the reaction conditions, according to Eq. *1*.

$$ln([G_{tot}]/[G_{tot}]_0) = -k_{app} \cdot t, \qquad (1)$$

where $[G_{tot}]$ denotes the overall (ionized and un-ionized) D-glucose concentration. Experiments conducted at different hydroxyl-ion concentrations (see Fig. 1) indicated existence of a rate-determining intermediate having an ionization constant of

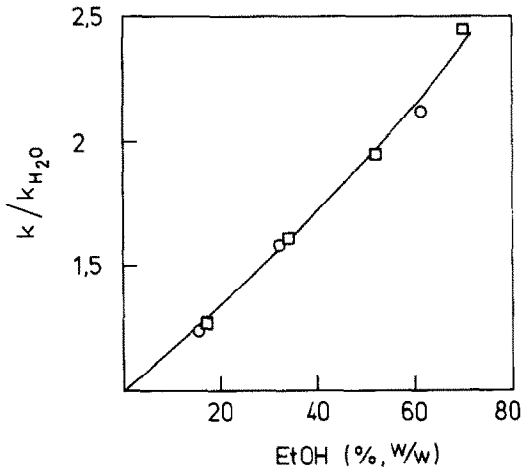

Fig. 3. The ratio of the hydroxyl-ion-independent isomerization[7] (□) and oxidation (○) rate-constants of D-glucose in ethanol–water solutions to the corresponding values in water.

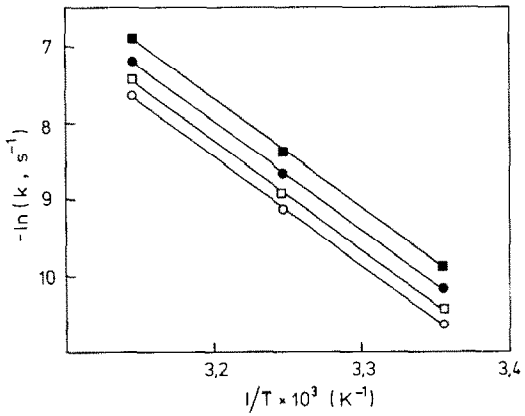

Fig. 4. The hydroxyl-ion-independent disappearance rate-constant of D-glucose in 0 (○), 16 (□), 33 (●), and 61% (■) (w/w) ethanol between 25 and 45°.

~30. This value corresponds reasonably well to the ionization constant of D-glucose[7]. At high concentrations of sodium hydroxide (>0.5M), part of the AMS and, possibly, also of the D-glucose, was precipitated; this resulted in erroneously low reaction-rates (lower than in 0.3M sodium hydroxide).

In ethanol–water solutions, the rate of disappearance of D-glucose was increased significantly (see Fig. 2). The relative increase in the hydroxyl-ion-independent rate-constant (Eq. 2) was equal to that reported earlier for the isomerization of D-glucose[7] (see Fig. 3).

$$k = k_{app}(1 + K_G[HO^-])/K_G[HO^-], \quad (2)$$

where K_G is the ionization constant of D-glucose.

The activation energy for the hydroxyl-ion-independent rate-constant was 118 kJ.mol^{-1}, and ethanol concentration had no influence on it (see Fig. 4). In earlier work[7], almost the same activation energy (122 kJ.mol^{-1}) was obtained for the isomerization of D-glucose.

As the effect of hydroxyl-ion concentration, ethanol concentration, and temperature is similar for both the isomerization and the oxidation of D-glucose, it seems plausible that enolization is also the rate-determining step in the latter reaction. The absolute values of oxidation rate-constant are somewhat higher than the corresponding values of isomerization rate-constant, because, in the absence of oxidant, part of the enolate ion is reconverted into D-glucose.

Oxidation and isomerization as competing reactions. — The overall reaction-rate of D-glucose is determined by its rate of enolization. The enolate ion yields D-fructose (F) and D-glucose in a certain ratio at the following rate:

$$d([F_{tot}] + [G_{tot}])/dt = a \times d[F_{tot}]/dt = k_{is}[E^-]dt, \quad (3)$$

where a is a constant.

The rate of oxidation is determined by Eq. *4* if the oxidation reaction is first-order with respect to AMS.

$$d[A]/dt = k_{ox}[E^-][AMS]dt, \quad (4)$$

where $[A]$ denotes the concentration of oxidation products. On the other hand, the oxidation rate is

$$d[A]/dt = a(d[F_{tot}]/dt)_o - a\, d[F_{tot}]/dt, \quad (5)$$

where the subscript is used for conditions without AMS. By combining Eqs. *3*, *4*, and *5*, the following expressions are obtained for the relationship between the rates of oxidation and isomerization.

$$\frac{(d[F_{tot}]/dt)_o - d[F_{tot}]/dt}{d[F_{tot}]/dt} = \frac{x_{ox}}{1 - x_{ox}} = \frac{k_{ox}}{k_{is}}[AMS], \quad (6)$$

where x_{ox} denotes the molar proportion of oxidized D-glucose.

The initial rates of formation of D-fructose obey Eq. *6* very well (see Fig. 5), which means that the oxidation reaction is first-order with respect to AMS. The oxidation efficiency of AMS is lower in ethanol–water solutions than in water solution. The relative rate of oxidation is increased constantly with increasing hydroxyl-ion concentration (see Fig. 6). When the hydroxyl-ion concentration is <0.1M, the increase is moderate, whereas, above 0.1M hydroxyl-ion concentration, the relative oxidation-rate increases radically, being roughly proportional to the square of the hydroxyl-ion concentration.

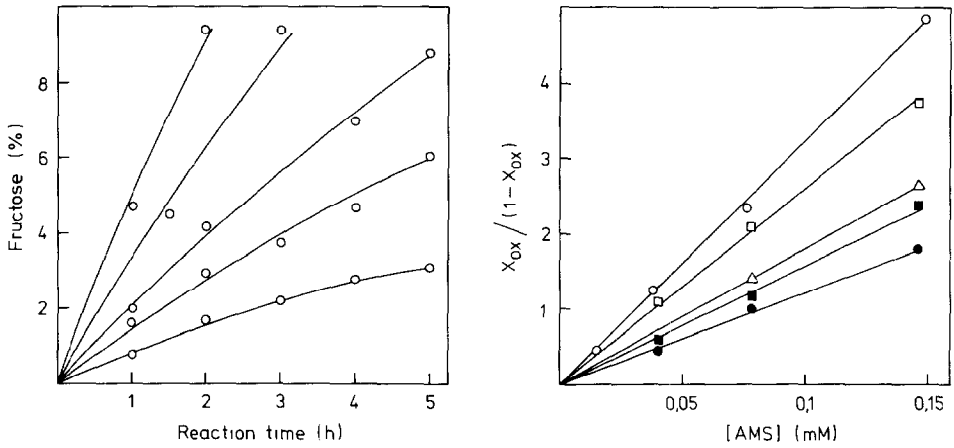

Fig. 5. Effects of AMS concentration on the relative oxidation rate of D-glucose in 0 (○), 16 (□), 24 (△), 33 (●), and 61% (■) (w/w) ethanol at 25° On the left, the concentrations of AMS corresponding to the curves are 0, 0.015, 0.038, 0.076, and 0.15mM, reading clockwise.

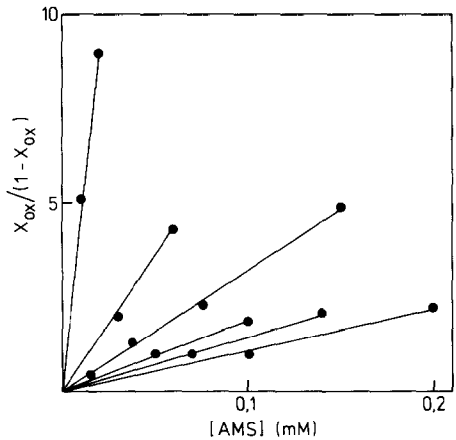

Fig. 6. Effect of AMS concentration on the relative oxidation rate of D-glucose at 25°. The hydroxyl ion concentrations corresponding to the lines are 1, 0.3, 0.1, 0.01, 0.003, and 0.001M, reading clockwise.

In preliminary experiments, it has also been shown that the oxidation efficiency of AMS is significantly decreased, especially in ethanol–water solutions, if 9,10-anthradiol-2-sulfonate is added to the reaction mixture. In theory, this phenomenon can be explained by deactivation of AMS through formation of a semiquinone anion-radical[9].

Primary oxidation product. — It had earlier been supposed that the primary oxidation product of an aldohexose with a quinone would be the corresponding hexulose[2,4,5,10]. This assumption was based mainly on the fact that an epimeric pair of hexonic acids is obtained from an aldohexose, but it is difficult to draw conclusions from the quantity of the primary reaction products, as hexonic acids form only a minor proportion of the reaction products.

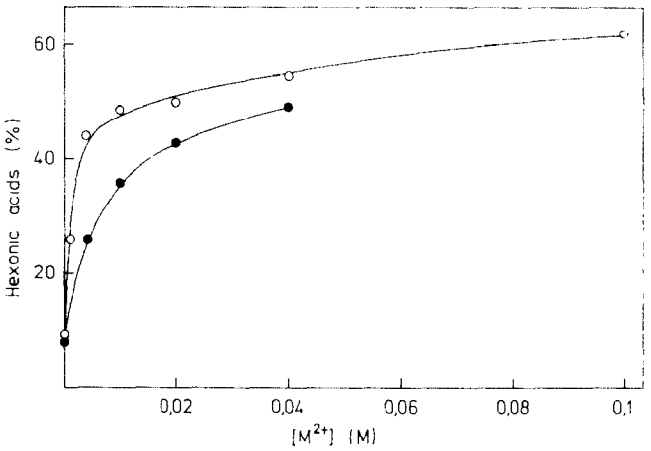

Fig 7 Effect of calcium (○)and barium (●) ion concentrations on the formation of hexonic acids (expressed as % of nonvolatile acids) from D-glucose at 50° The hydroxyl-ion concentrations corresponding to the curves are 0.01 (○) and 0 1M (●), respectively

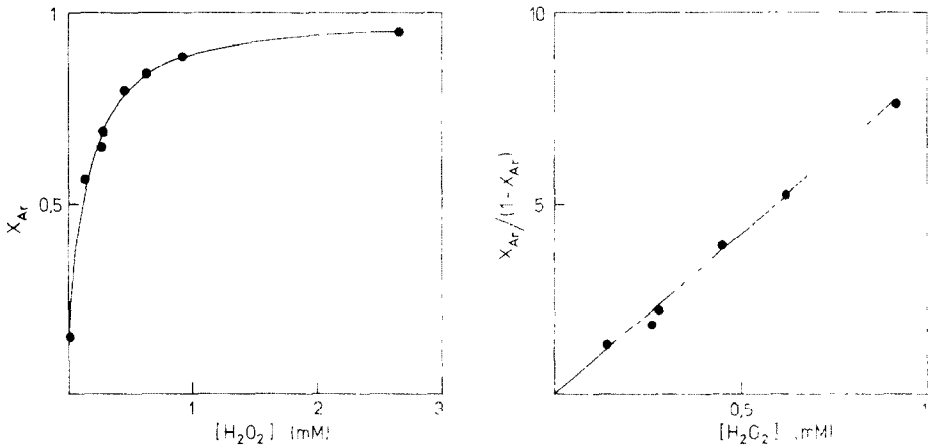

Fig. 8. Effect of hydrogen peroxide on the formation of arabinonic acid from oxidized D-glucose in 0 1M sodium hydroxide at 35°

In the present experiments in sodium hydroxide, ~5 and 3% of D-mannonic and D-gluconic acid, respectively, were formed from D-glucose. When the hydroxyl-ion concentration was maintained constant, and calcium chloride was added to the reaction mixture, up to 60% of hexonic acids was formed (see Fig. 7). The hexonic acids consisted mainly, or to >90%, of D-mannonic acid According to Lindberg and Theander[11], D-glucosone yields hexonic acids in almost the same proportion and yield. With addition of barium chloride, a somewhat less-pronounced effect was observed: this might partly result from the low solubility of the barium salt of AMS.

Both arabinonic and ribonic acid were formed to the extent of 10 to 20% of the D-glucose, depending on the reaction conditions. The yield of arabinonic acid

was increased up to 95% when a small proportion of hydrogen peroxide was included in the reaction mixture (see Fig. 8). Hydrogen peroxide alone caused no appreciable oxidation under similar conditions. Because formation of arabinonic acid is a well known result of the reaction of D-glucosone with hydrogen peroxide, it seems plausible that D-glucosone is practically the only primary oxidation product of D-glucose[12].

Formation of arabinonic acid from D-glucosone competes with other reactions, and the corresponding reaction-rates are determined by Eqs. *7* and *8*, provided that the AMS and hydroxyl-ion concentrations are constants.

$$d[Ar]/dt = k_{Ar}[GO][H_2O_2] \quad (7)$$

$$d[A]/dt = k_A[GO], \quad (8)$$

where GO denotes D-glucosone; Ar, arabinonic acid; and A, other reaction-products. As the sum of concentrations of arabinonic acid and other reaction products is equal to the concentration of reacted D-glucosone (or D-glucose), the following expression (*9*) is obtained for the dependence of the yield of arabinonic acid on the concentration of hydrogen peroxide.

$$x_{Ar}/(1 - x_{Ar}) = k_{Ar}/k_A \times [H_2O_2], \quad (9)$$

where x_{Ar} is the molar yield of arabinonic acid from one mole of reacted D-glucose. The experimental data fit this equation very well (*cf.*, Fig. 8).

In separate experiments, it was shown that the oxidation rate of 2-deoxy-D-*arabino*-hexose is only one thousandth to one hundredth of the oxidation rate of D-glucose, as determined by appearance of the brownish-red color of the reduced species of AMS. This observation confirms the conclusion that D-glucosone is practically the only primary oxidation product of D-glucose.

Concurrent with the formation of D-glucosone, AMS is reduced, probably to 9,10-anthradiol-2-sulfonate (the reaction is first-order with respect to AMS, and it involves transfer of two electrons), although the semiquinone anion-radical is also formed as an equilibrium product[9,13]. When AMS was first reduced with a large excess of D-xylose and then, under vigorous stirring, D-glucosone was added dropwise, neither D-glucose, nor D-mannose, nor D-fructose was formed. Accordingly, the oxidation of D-glucose may be regarded as an irreversible reaction.

EXPERIMENTAL

Materials. — All materials used were commercial products, except for D-glucosone, which was prepared from D-*arabino*-hexose phenylosazone[14] according to Bayne[15]. The purity of the D-glucosone was checked by allowing it to react with hydrogen peroxide in alkaline solution under vigorous stirring. The yield of D-

arabinonic acid was 95% (corresponding to the minimum purity of the D-glucosone).

Rate of disappearance of D-glucose. — Reaction solutions lacking D-glucose were prepared in vials sealed with septa. Air was removed by evacuation, and replaced by nitrogen. After thermostating, a solution of D-glucose in a small amount of water was added. The initial concentrations of AMS and D-glucose were 10 and 1mM, respectively. The concentration of D-glucose was monitored by colorimetry. The rate of disappearance of D-glucose was calculated from Eq. 2. Ionization constants of D-glucose were obtained from the results of earlier work[7].

Relative oxidation rate of D-glucose. — The reaction was conducted as before. The initial concentrations of AMS, D-glucose, and xylitol (internal standard) were equal. The sugars were separated from the reaction mixture by ion-exchange chromatography, and analyzed as their per(trimethylsilyl)ated oximes by g.l.c.[7]. The relative oxidation-rates were calculated from the initial rates of formation of D-fructose according to Eq. 6.

Oxidation of D-glucose in the presence of calcium and barium ions. — The reactions were conducted in sealed vials under a nitrogen atmosphere. The initial concentrations of AMS and D-glucose were 2 and 0.5M, respectively. The concentration of sodium hydroxide was 0.01M (experiments with calcium) or 0.1M (experiments with barium). The calcium and barium ions were added as their chlorides. Part of the AMS was precipitated, even at 4mM concentration of barium chloride. After 3 h at 50°, the carboxylic acids were separated from the reaction mixture by ion-exchange chromatography, and analyzed by g.l.c.[16]

Oxidation of D-glucose in the presence of hydrogen peroxide. — The reactions were conducted in a polyethylene reactor under a nitrogen atmosphere. A peristaltic pump fitted with Tygon tubing was used to circulate the liquor, as well as to add and remove samples. The initial concentrations of AMS and D-glucose were 2 and 1mM, respectively. The concentration of hydrogen peroxide was monitored by iodometric titration[17], and maintained roughly constant throughout the reaction. After 3 h at 35°, the amounts of D-arabinonic acid and unreacted D-glucose were respectively determined by g.l.c.[16] and colorimetry.

Colorimetric determination of D-glucose. — The concentration of D-glucose was determined by a slightly modified, anthrone method[18]. A sample (400 μL) of reaction solution containing D-glucose (<1mM) was injected into a test tube containing 5 mL of cold, 0.1% solution of anthrone in 80% sulfuric acid. The stoppered tube was shaken, and kept in a refrigerator until all of the samples within the series had been taken. The tubes were thermostated for 17.5 min at 90° (under these conditions, the absorptivities of D-glucose and D-fructose were equal), cooled in a water bath at ambient temperature, and reshaken. The absorbances of the solutions in a 1-cm cuvet were measured at 625 nm with a Zeiss (Opton) PMQ II spectrophotometer. The Lambert–Beer law was obeyed throughout the measuring region ($A < 0.7$), and the measurements were not interfered with by AMS.

Separation of reaction products. — Samples of reaction mixture were made

neutral in a column of Dowex 50-W (H^+) cation-exchange resin, and the eluate was passed directly into another column containing Dowex-1 X-8 (Ac^-)anion-exchange resin, to remove carboxylic acids and AMS. The sugars were eluted with water. The carboxylic acids were eluted from the lower column with M acetic acid. The eluate was evaporated to dryness, and the residue was dissolved in a small amount of 0.1M sodium hydroxide to open up lactone rings. Sodium ions were again removed with Dowex 50-W (H^+) cation-exchange resin, the resin was washed with water, and the eluate was immediately made neutral with 0.1M ammonia to pH 7, to prevent lactonization.

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