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The alkaline anthraquinone-2-sulfonate–$\text{H}_2\text{O}_2$-catalyzed oxidative degradation of lactose: An improved Spengler–Pfannenstiel oxidation*

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ABSTRACT

The alkali-catalyzed oxidative degradation of lactose (1) to potassium $\alpha$-/$\beta$-D-galactopyranosyl-(1→3)-D-arabinonate (2) has been studied and compared with that of $\alpha$-glucose to $\alpha$-arabinonate and $\alpha$-galactose to $\alpha$-lyxonate. A mechanism for the degradation of 1 catalyzed by alkali only is presented and discussed, taking into consideration the main reaction products. Increasing the reaction temperature from 293 to 318 K resulted in a drastic decrease of the selectivity for 2. Increasing the oxygen pressure from 1 to 5 bar did not significantly influence the selectivity. The overall reaction kinetics followed first-order behavior with respect to lactose, $\alpha$-glucose, or $\alpha$-galactose. The simultaneous addition of catalytic, equimolar amounts of sodium 2-anthraquinonemonosulfonate and $\text{H}_2\text{O}_2$ showed a pronounced effect on the selectivity. A reaction mechanism for this type of alkali-catalyzed oxidative degradation of carbohydrates is presented and discussed. Lactose could be oxidized up to almost complete conversion with a selectivity of 90–95% (mol/mol), whereas $\alpha$-glucose was oxidized to $\alpha$-arabinonate with a selectivity of 98%. This increased selectivity was maintained at temperatures from 293 up to 323 K, allowing a reduction of the batch time necessary for almost complete conversion from 50 to 1.5 h. The overall reaction kinetics still followed first-order behavior with respect to lactose, $\alpha$-glucose or $\alpha$-galactose. The apparent activation energy amounted to 114 ± 2 kJ mol$^{-1}$ for lactose, to 109 ± 2 kJ mol$^{-1}$ for $\alpha$-glucose, and to 104 ± 9 kJ mol$^{-1}$ for $\alpha$-galactose.

INTRODUCTION

Lactose (1, milk sugar) is obtained from whey, the liquid residue of cheese production. Upgrading of this residue can be performed by the heterogeneous catalytic oxidation of lactose to lactobionate$^1$ or by the homogeneous alkaline-oxidative degradation of lactose to $\alpha$-D-galactopyranosyl-(1→3)-D-arabinonate (potassium salt, 2). Conversion of the aldobionates produced into the corresponding aldobionolactones, eventually followed by combination with lipophilic long-chain amines$^2$ or alcohols$^3$, allows their application as surfactants, polymers, or liquid crystals. However, a commercial breakthrough of such technology depends strongly on the selectivity at which the oxidation of the carbohydrates can be performed. This paper reports on the

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alkali-catalyzed oxidative degradation of lactose, the so-called Spengler-Pfannenstiel reaction, shown in Fig. 1.

In alkaline media, monosaccharides equilibrate with their cyclic anions, from which enediol anions are formed. Isomerisation can occur by means of the well known Lobry de Bruyn-Alberda van Ekenstein reaction. The reactions starting from these enediol anions are potentially faster than their formation. Early investigations on the alkaline degradation reactions of monosaccharides by Nef and Spengler-Pfannenstiel showed a difference in product distribution between the oxidative- and the non-oxidative alkaline degradation of the monosaccharides.

Under non-oxidative conditions, the enediol anion reacts mainly according to two reaction-pathways, namely β-elimination leading to saccharinic acids, or retro-aldol condensation leading to lactic acid. Depending on alkalinity and reactant concentration, aldol condensation may also occur. Under oxidative conditions, glucose and fructose are oxidized into arabinonic acid and formic acid through cleavage of the enediol anion. The molar yield of arabinonic acid from glucose is generally believed to be 75%, although Scholtz and Gotsmann claimed a molar yield of 98% at higher oxygen pressures.

Bamford and Collins as well as Dubourg and Naffa explained their results by means of an enediol anion peroxide mechanism. More recently, Isbell proposed a diradical mechanism for the oxidative degradation of reducing sugars in alkaline media. This diradical mechanism does not require the addition of triplet oxygen to the enediol anion, which is spin-forbidden. Moreover, Vuorinen clearly demonstrated that the reaction does not proceed via the 1,2-dioxetane structure postulated in the enediol anion-peroxide mechanism, but that the cleavage proceeds via the C-1 and C-2 hydroperoxides in a ratio of 1:2. Fig. 2 summarizes the present insights in the oxidative cleavage of enediol anions obtained from carbohydrates.

Less is known about the alkaline degradation reactions of disaccharides, especially lactose. Corbett and Kenner studied the non-oxidative degradation of lactose and found α- and β-isosaccharinic acids and galactose as the main products. Hardegger et al. studied the alkali-catalyzed oxidative degradation of cellobiose, maltose, and lactose, but obtained only low yields of the expected products. Recently Röger et al. reported on the oxidative cleavage of such disaccharides as maltose, isomaltulose, lactose, lactulose, and cellobiose, and claimed selectivities up to 90-95% based on measured quantities of formic acid and not on that of the main products formed.
Nowadays, alkaline pulping processes are extensively used in the paper industry for the separation of (wood) polysaccharides, the kraft pulping process being the most important today. The most serious drawbacks in this process are the non-selective delignification and the extensive degradation of polysaccharides by the so-called peeling reaction, resulting in a low pulp (namely carbohydrate) yield. Treatment of the alkaline pulp with oxygen oxidizes most of the functional end-groups of the polysaccharides to carboxylic acids, which stabilizes the polysaccharides against further degradation. Cellobiose and hydrocellulose were extensively studied as model component for cellulose, the most abundant polysaccharide in wood. It was found by Bach and Fiehn that the alkali stability of hydrocellulose could be improved by treatment with an excess of anthraquinone-2-sulfonate (AMS), or other sulfonated anthraquinones. Ruoho and Sjöström observed that the amount of AMS could be drastically decreased in the presence of oxygen because the hydroquinone formed was reoxidized by oxygen. It is known that, in alkaline medium, the quinone and corresponding hydroquinone form a redox couple, and that the hydroquinone may be readily reoxidized by almost any mild oxidant.

The primary products of the reaction of hexoses and AMS in alkali are the hexosuloses, obtained by hydride transfer from the 1,2-enediol to AMS. For D-glucose, Vuorinen demonstrated that the reaction product, D-arabino-hexosulose, is selectively cleaved by H₂O₂ to D-arabinonic acid and formic acid. Vuorinen and Sjöström mentioned that the improved alkali stability of polysaccharides was the result of a more-selective oxidation of the functional end-groups.

In this paper, a degradation mechanism for lactose is presented and discussed, that accounts for the main reaction-products observed. More importantly, the advantages of performing the reaction in the simultaneous presence of anthraquinone-2-
sulfonate and H₂O₂ are demonstrated. Addition of a catalytic amount of both of these components leads to a significant increase of the selectivity when compared to the "classical" alkali-catalyzed oxidative degradation of carbohydrates, and allows a pronounced reduction of the batch time required for almost complete conversion.

EXPERIMENTAL

Materials. — Lactose, D-glucose, and D-galactose were all commercial high-purity samples obtained from D.M.V. Campina B.V. (Veghel, Holland) and Gist-Brocades N.V. (Maarssen, Holland). Potassium hydroxide (very pure) was obtained from Broom B.V. (Meppel, Holland). Sodium anthraquinone-2-sulfonate monohydrate was obtained from Janssen Chimica (Beerse, Belgium) and H₂O₂ was obtained as a 27% (m/m) solution from Gist-Brocades N.V. (Maarssen, Holland).

Equipment. — All experiments were performed in a stirred tank batch-reactor as shown in Fig. 3; the reactor volume being 1.0 dm³. The temperature of the reactor was kept constant within 1.0 K by means of a thermostat [4]. The pressure in the reactor system was kept at 0.1 MPa by admitting water from the water burette [7] to the oxygen-supply vessel [6] directed by a contact manometer [8]. The consumption of oxygen was recorded as a function of time.

Procedure. — A solution of lactose, D-glucose, or D-galactose, eventually containing a catalytic amount of AMS, was saturated with O₂ gas in the reactor [1] by vigorously stirring at the temperature of reaction, while the alkali solution was sat-

Fig. 3. Experimental reactor set-up. [1, reactor; 2, reactant supply vessel; 3, turbine stirrer; 4, thermostat bath; 5, temperature sensor; 6, oxygen supply vessel; 7, water burette; 8, contact manometer for pressure control; 9, condenser; 10, gas circulation pump; 11, pressure relief; 12, pH-electrode; 13, pH-meter; 14, titrator; 15, automatic burette; 16, drain].
urated with oxygen by bubbling O₂ gas through it at the reaction temperature in the reactant supply vessel [2]. Both solutions were kept under oxygen for ~900 s. The reaction was started by adjusting the gas-circulation system to the contact manometer [8], opening the valve of the reactant-supply vessel [2], and starting the gas-circulation pump [10]. Solutions containing a catalytic amount of AMS had an equimolar amount of H₂O₂ injected at the start of the experiment.

Samples (6 mL) taken with a syringe were protected from non-oxidative degradation and polymerization reactions by the following procedure: 5.00 mL of the mixture was quickly pipetted and acidified with 2n HCl to pH 8.5–9.5. The acidified solution was diluted with distilled water to an end volume of 25.00 mL and stored at ~278 K. The composition of the reaction mixtures was determined by h.p.l.c.-analysis as a function of time. Neutral carbohydrates were analyzed by use of an anion-exchange column in the Ac form in series with a cation-exchange column in the H⁺ form and with water as the eluent. The anionic oxidation and/or degradation products were analyzed by use of an anion-exchange column in the Cl⁻ form or in the SO₄²⁻ form with respectively an aqueous NaCl–MgCl₂ solution and (NH₄)₂SO₄ solution as the eluent.

RESULTS AND DISCUSSION

"Classical" alkali-catalyzed oxidative degradation of lactose. — Lactose was oxidized under the standard reaction conditions used by Lichtenthaler and Klimesch, Kunz and Röger, and Röger et al. to investigate the alkaline oxidative degradation of isomaltulose and other carbohydrates. Fig. 4 shows the typical results of an experiment in which the concentration of lactose [1], the galactopyranosyl-arabinonate [2], formate [3], the carbon balance and the O₂-consumption were recorded as a function of time.

To obtain high selectivity for 2, the reaction was performed at 293 K, resulting in a batch time of about 50 h. The decrease in concentration of lactose can be adequately described by first-order kinetics in lactose, the pseudo-first-order rate constant amounting to 1.19 10⁻⁵ s⁻¹. This result is in good agreement with the value obtained by Kunz and Klimesch who found a value of 1.14 10⁻⁵ s⁻¹ for lactose. The oxygen consumption
expected from the stoichiometry given in Fig. 1, as well as the production of 3 exceeds the production of 2, indicating that the selectivity is < 100%. Also some D-galactose (4), D-lyxionate (5), 2-deoxytetronate (6), and glycolate (7) were found by h.p.l.c. analysis.

As not all oxidation products detected by h.p.l.c. analysis could be identified and/or quantified, the carbon balance decreases slightly during the reaction. However, the carbon balance of the reaction was satisfied within 95% at almost complete conversion.

To account for the main reaction products formed, a reaction path is presented in Fig. 5 for the alkali-catalyzed oxidative degradation of lactose. The main reaction is the oxidative cleavage of the lactose 1,2-enediol anion into D-β-D-galactopyranosyl (1 → 3)-D-arabinonate (2) and formate (3). However, because of intramolecular proton transfers in the alkaline medium, the 2,3-enediol anion may also be formed. Because of the presence at C-4 of the galactopyranosylxy group, a better leaving group than a hydroxyl group, this 2,3-enediol anion can easily undergo a β-elimination to yield D-galactose (4) and 4-deoxy-D-glycerol-2,3-hexodiolose. The latter can readily be oxidized to glycolate (7) and 2-deoxy-D-tetronate (6); D-galactose (4) can be oxidized to D-lyxionate (5) and formate, in a manner analogous to the oxidative cleavage of lactose. Clearly, the selectivity for 2 depends strongly on the reaction conditions. Non-oxidative conditions enhance the formation of side products, especially saccharinic acids (including lactic acid), whereas oxidative conditions lead to high selectivities.

Similar results were obtained with glucose (Glc, 8). Arabinonate (9) and formate were produced with 90-95% selectivity, the pseudo-first-order rate constant of glucose
Fig. 5. Alkali-catalyzed oxidative degradation of lactose. [2 = α-galactopyranosyl-α-arabinonate, 3 = formate, 4 = α-galactose, 7 = glycolate, 6 = 2-deoxy-α-tetronic acid, and 5 = α-lyxonate].

Consumption amounting to $1.36 \times 10^{-3} \text{ s}^{-1}$. This agrees with the value of $1.33 \times 10^{-3} \text{ s}^{-1}$ reported by Kunz and Röger.34

**Influence of the reaction temperature.** Increasing the reaction temperature to decrease the batch time caused a sharp decrease in the selectivity for 2, as could be seen by a drastic increase in $O_2$ consumption, an increased production of 4 and 7 and a decreased production of 2. Similar results were obtained by Röger et al.17 for the alkaline oxidative degradation of isomaltulose.

**Influence of the oxygen pressure.** To study the influence of the oxygen pressure on the alkali-catalyzed oxidative degradation of lactose, reactions were performed with air at 1 bar and with pure oxygen at 1 bar and at 5 bar. In the reaction with air, a browning of the mixture was observed, indicating an increasing importance of the non-oxidative degradation pathway of lactose, resulting in a decreased selectivity for 2. In accordance with Fig. 5, an increased production of 4 and 7 was observed, along with unidentified components.
No significant differences were found between the alkali-catalyzed oxidative degradation of lactose with pure oxygen at either 1 bar or 5 bar. These findings are in agreement with the results of the alkali-catalyzed oxidative degradation of isomaltulose found by Kunz and Röger, Lichtenthaler and Klimesch, and Röger et al. However, Scholtz and Gotsmann, who studied the alkaline oxidative degradation of d-glucose at oxygen pressures up to 40 bar, found improved selectivities at higher oxygen pressures.

The alkaline AMS–H$_2$O$_2$-catalyzed oxidative degradation of lactose. To study the effect of AMS on the alkali-catalyzed oxidative degradation of lactose, portions of 1–10% AMS (w/w, based on lactose) were added to the mixture under the reaction conditions described for the “classical” alkali-catalyzed oxidative degradation of lactose. No significant influence on the selectivity for 2 nor on the pseudo-first-order rate constant was observed.

The effect of adding small amounts of H$_2$O$_2$ to the reaction mixture was also studied, and no significant influence was observed. The simultaneous addition of catalytic amounts of AMS and H$_2$O$_2$ led to a drastic improved selectivity for 2, however. Fig. 6 shows the influence of the addition of 1% (w/w, based on lactose) of AMS and an equimolar amount of H$_2$O$_2$ to the reaction mixture as described for the “classical” alkali-catalyzed oxidative degradation of lactose.

As compared with this “classical” oxidative degradation of lactose as shown in Fig. 4, Fig. 6 clearly shows the beneficial effects of the addition of AMS–H$_2$O$_2$. The production of 2 was increased while the production curve for 3 almost matches the O$_2$.
Fig. 7. Selectivities and yields versus conversion for the "classical" alkali-catalyzed oxidative degradation and the alkaline AMS-H₂O₂-catalyzed oxidative degradation of lactose. Selectivity with (□) and without (■) AMS-H₂O₂. Yield with (+) and without (×) AMS-H₂O₂. Reaction conditions as given for Figs. 4 and 6.

consumption curve. The decrease in [ l ] still follows first order behavior, the pseudo-first-order rate constant amounting to 1.23 10⁻⁵ s⁻¹. Hence, the simultaneous addition of AMS and H₂O₂ only improved the selectivity for 2 but does not increase the rate of consumption of lactose.

Fig. 7 clearly demonstrates the effect of the addition of AMS-H₂O₂ on the selectivity of the alkali-catalyzed, oxidative degradation of lactose; the selectivity for 2 increased from 75–80% for the "classical" oxidative degradation of lactose up to 90–95% for the alkaline AMS-H₂O₂-catalyzed oxidation of lactose. However, the reaction product, potassium O-β-D-galactopyranosyl-(1→3)-D-arabinonate (2) could not be isolated from the reaction mixture because of its very hygroscopic character.

Fig. 8 presents a reaction scheme that accounts for the observations reported. The 1,2-enediol anion is selectively oxidized by AMS to an aldulosulose. The hydroquinone anion formed, AMSh⁻, is reoxidized in situ by oxygen to AMS, which can oxidize an new 1,2-enediol anion molecule. In the reoxidation of AMSh⁻, oxygen is reduced to a H₂O₂ anion. The aldulosulose formed in the oxidation of the 1,2-enediol anion with AMS, reacts selectively with the H₂O₂ anion leading to the corresponding peroxy compounds, which can undergo hydroxylation, leading to the cleavage of the C-1–C-2 bond.

Adding catalytic amounts of AMS alone does not provide sufficient H₂O₂ anion for the selective peroxidation of the aldulosulose formed and, hence, does not lead to improved selectivities. On the other hand, addition of a small amount of H₂O₂ alone does not lead to the selective oxidation of the 1,2-enediol anion to the aldulosulose. The
Fig. 8. Reaction scheme for the AMS $H_2O_2$-catalyzed oxidative degradation of carbohydrate enediol anions.
Fig. 9. Alkaline AMS\(\cdot\)H\(_2\)O\(_2\)-catalyzed oxidative degradation of d-glucose (8). Concentrations of 8 (□), 9 (○), 3 (▲), \(O_2\)-consumption [meq.] (△) and carbon balance (▽) versus time. Reaction conditions: \([8]_0 = 0.25\) kmol m\(^{-3}\), \([\text{KOH}]_0 = 0.75\) kmol m\(^{-3}\), pH > 13.5, \([\text{AMS}] = 2.75\) mol m\(^{-3}\), \([\text{H}_2\text{O}_2] = 2.75\) mol m\(^{-3}\), T = 303 K, and \(T = 100\%\).

Fig. 10. Selectivities and yields versus conversion for the “classical” alkali-catalyzed oxidative degradation and the alkaline AMS\(\cdot\)H\(_2\)O\(_2\)-catalyzed oxidative degradation of d-glucose. Selectivity with (□) and without (■) AMS\(\cdot\)H\(_2\)O\(_2\). Yield with (+) and without (+) AMS\(\cdot\)H\(_2\)O\(_2\). Reaction conditions as given for Fig. 9.
simultaneous addition of catalytic amounts of AMS and H₂O₂ was essential for the improved selectivity of the alkali-catalyzed oxidative degradation. The similar results of the pseudo-first-order rate constant of the “classical” alkaline catalyzed oxidative degradation of lactose \( (k = 1.19 \times 10^{-5} \text{ s}^{-1}) \) and of the alkaline AMS–H₂O₂-catalyzed oxidative degradation of lactose \( (k = 1.23 \times 10^{-5} \text{ s}^{-1}) \) leads to the conclusion that the essential difference between both routes lies beyond the formation of the 1,2-enediol anion.

Figs. 9 and 10 show similar results as obtained for the alkaline AMS–H₂O₂-catalyzed oxidative degradation of glucose (8) to arabinonate (9). Fig. 9 shows almost complete conversion of 8 into 9, and a production curve for 3 and an O₂ consumption curve matching the production curve for 9. Fig. 10 shows the effect of the addition of AMS–H₂O₂ on the selectivity of the alkali-catalyzed oxidative degradation of glucose. The selectivity for 9 increased from 90–95% for the “classical” alkali-catalyzed oxidative degradation of glucose up to 95–98% for the alkaline AMS–H₂O₂-catalyzed oxidative degradation of glucose. The reaction product, potassium D-arabinonate (9) could be isolated readily from the reaction mixture by adding EtOH to the mixture after neutralization to pH ~ 8.

**Influence of the reaction temperature.** Compared with the “classical” alkali-catalyzed oxidative degradation of lactose, an increase of the reaction temperature of the alkaline AMS–H₂O₂-catalyzed oxidation of lactose did not result in a decrease of selectivity for 2. No significant influence on selectivity for 2 was noticed in the temperature range 293–323 K; the batch time of the process could be decreased from 50 to 1.5 h.

**Influence of the oxygen pressure.** To study the influence of the O₂ pressure on the alkaline AMS–H₂O₂-catalyzed oxidative degradation of lactose, reactions were performed with pure oxygen at 1 and 7 bar, but, no significant differences were observed.

Because the reduced anion of AMS, AMSH⁻, has a deep red color, the AMS added also acts as an indicator for the oxygen saturation in the mixture. Under reaction conditions where the mixture turns red, non-oxidative alkaline degradation reactions become more important and the selectivity to 2 decreases. Under the conditions investigated no coloration of the mixture was observed, indicating a reoxidation of AMSH⁻ that is potentially faster than the reduction of AMS.

**Determination of the overall activation energy.** Because the selectivity for O-β-D-galactopyranosyl-(1→3)-D-arabinonate (2) was not affected by increasing the reaction temperature, the activation energy of the reaction shown in Fig. 1 could be determined. In Fig. 11, ln(k) of the alkaline AMS–H₂O₂-catalyzed oxidation is plotted against T⁻¹ for the reactants D-glucose, D-galactose, and lactose. The apparent activation energies amounted for lactose to 114 ± 2 kJ mol⁻¹, for D-glucose to 109 ± 2 kJ mol⁻¹, and for D-galactose to 104 ± 9 kJ mol⁻¹. The activation energy for D-glucose is in reasonable agreement with the values reported by de Wit et al.⁷, who estimated an activation energy of 111 kJ mol⁻¹, based on u.v.-absorbance measurements, and by Vuorinen⁸, who estimated an activation energy of 118 kJ mol⁻¹ based on colorimetric measurements. Estimates of the apparent activation energies for D-galactose and lactose have not yet been reported.
OXIDATIVE DEGRADATION OF LACTOSE

CONCLUSIONS

A degradation mechanism for lactose is presented that accounts for the main reaction products of the alkali-catalyzed oxidative degradation of lactose. The selectivity for $O$-$\beta$-$D$-galactopyranosyl-(1$\rightarrow$3)-$D$-arabinonate (2) may be drastically improved by the simultaneous addition of catalytic amounts of sodium anthraquinone-2-sulfonate and $H_2O_2$. The increase in selectivity may be attributed to selective oxidation by AMS of the 1,2-enediol anion into an aldosulose, which is selectively cleaved by $H_2O_2$ to $O$-$\beta$-$D$-galactopyranosyl-(1$\rightarrow$3)-$D$-arabinonate (2) and formate (3).

This increased selectivity was maintained at temperatures from 293 up to 323 K, allowing a decrease of the batch time necessary for almost complete conversion from 50 to 1.5 h. The procedure developed is believed to be applicable to carbohydrates in general.

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LIST OF SYMBOLS

\([I]_{i=0}\): initial concentration of lactose, kmol m\(^{-3}\).
\([\text{KOH}]_{i=0}\): initial concentration of KOH, kmol m\(^{-3}\).
\([I]\): concentration of lactose (as determined by h.p.l.c. and corrected for dilution), kmol m\(^{-3}\).
\([2]\): concentration of potassium \(\alpha\)-galactopyranosylarabinonate, kmol m\(^{-3}\).
\([3]\): concentration of potassium formate, kmol m\(^{-3}\).
C-balance: carbon balance, kmol m\(^{-3}\).
\(k\): pseudo-first-order rate constant, s\(^{-1}\).
\([\text{AMS}]\): concentration of sodium anthraquinone-2-sulfonate, mol m\(^{-3}\).
\([\text{H}_2\text{O}_2]\): concentration of hydrogen peroxide, mol m\(^{-3}\).
\(I\): oxygen concentration with respect to equilibrium oxygen concentration, \(\text{atm}\)
\(X\): conversion of lactose
\(S\): selectivity to potassium galactopyranosylarabinonate (2)
\(Y\): yield of potassium galactopyranosylarabinonate (2)

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