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Kinetic and chemical studies on the isomerization of monosaccharides in *N*-methylmorpholine-*N*-oxide (NMMO) under Lyocell conditions

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Abstract—The Lyocell process is a modern and environmentally fully compatible industrial fiber-making technology. Cellulosic pulp is dissolved without chemical derivatization in a melt of *N*-methylmorpholine-*N*-oxide monohydrate (NMMO). In the present work, the reactions of monosaccharides under Lyocell conditions were investigated in detail, using capillary zone electrophoresis as the analytical technique to clarify the composition of reaction mixtures and to follow the kinetics. Under Lyocell conditions, xylose and glucose undergo two competitive reactions: rapid conversion to nonreducing products, and complete isomerization involving the whole carbohydrate backbone, via ketose intermediates. Sugar acids are present in minor amounts only, as demonstrated by employing isotopically labeled material for NMR techniques.

Keywords: Capillary electrophoresis; Isomerization; Degradation; NMMO; Monosaccharides; Aldoses

1. Introduction

Within the past decade, a new, environmentally compatible cellulose-fiber technology has emerged, the Lyocell process, which employs a melt of *N*-methylmorpholine-*N*-oxide monohydrate (NMMO) for direct dissolution of cellulosic pulp without chemical derivatization. Other benefits with regard to environmental compatibility are the use of air and water as the only spinning bath 'chemicals', and the nearly complete recycling of the amine oxide solvent. Although the dissolution process is supposed to be entirely physical according to theory, manifold chemical side reactions occur in reality due to the elevated process temperatures and the oxidative nature of the solvent.[1] These chemical processes are reflected for instance in a pronounced chromophore formation in the Lyocell spinning dope, which might even cause discoloration of the fiber products. Resulting from multiple pathways, a large number of chromophores is formed, of which all contribute to overall discoloration or yellowing. In a recent work, most of the identified chromophores have been shown to be degradation and condensation products of monosaccharides, which in turn must have originated from degradation reactions of the pulp polymers.[2] The rates of chromophore formation in NMMO solutions from different carbohydrates and pulps have also been studied, finding pentoses to react faster than hexoses.[3] Carbonyl groups were determined as the main influential factor regarding chromophore formation.[3] In the present study, the behavior of monosaccharides under Lyocell conditions will be addressed, and kinetic and chemical studies will be presented.

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2. Results and discussion

2.1. Isomerization and degradation kinetics of monosaccharides under Lyocell conditions

While the reactions of carbohydrates and cellulose under aqueous alkaline conditions have been intensively investigated in the past,[4–6] as well as recently,[7–10] the behavior of low-molecular-weight carbohydrates under Lyocell conditions—that is in an NMMO melt at about 110 °C—remained unclear so far. In the present study, we used an optimized CZE method,[11] based on reductive amination of aldoses with aminobenzoic acid ethyl ester (ABEE) carried out as pre-column derivatization. In the case of carbohydrates dissolved in the cellulose solvent *N*-methylmorpholine-*N*-oxide monohydrate (NMMO), no negative influence of residual NMMO present in the aqueous saccharide solutions on the reductive amination reaction was observed.

Monosaccharides under Lyocell conditions undergo isomerization reactions similar to the reactions under aqueous alkaline conditions, even though the pH value of the NMMO/carbohydrate reaction mixtures, which is about 6–7 after dissolution in excess water, is much lower than that of the alkaline media used. The isomerization was largely complete and proceeded along the whole carbon chain of the sugar. Starting for instance with galactose, isomerization proceeded to give glucose, and all eight isomeric aldohexoses were found (Fig. 1).

The isomerization processes are only a relatively small part of the overall chemical conversions in the system. The major part are degradations and reactions to non-reducing products (including ketoses), which are thus not detectable by the capillary electrophoresis method used,[11] as the presence of a reducing end is required for attaching the UV label by reductive amination. In the case of glucose, after 4 h reaction time only 20% of the starting material was still present, along with about 10%

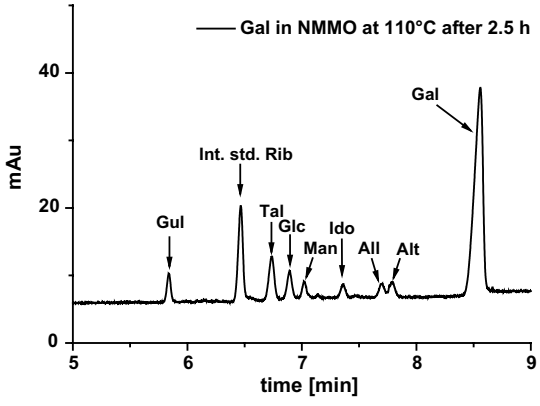


Figure 1. Electropherogram of the reaction mixture of galactose after 2.5 h in NMMO at 110 °C.

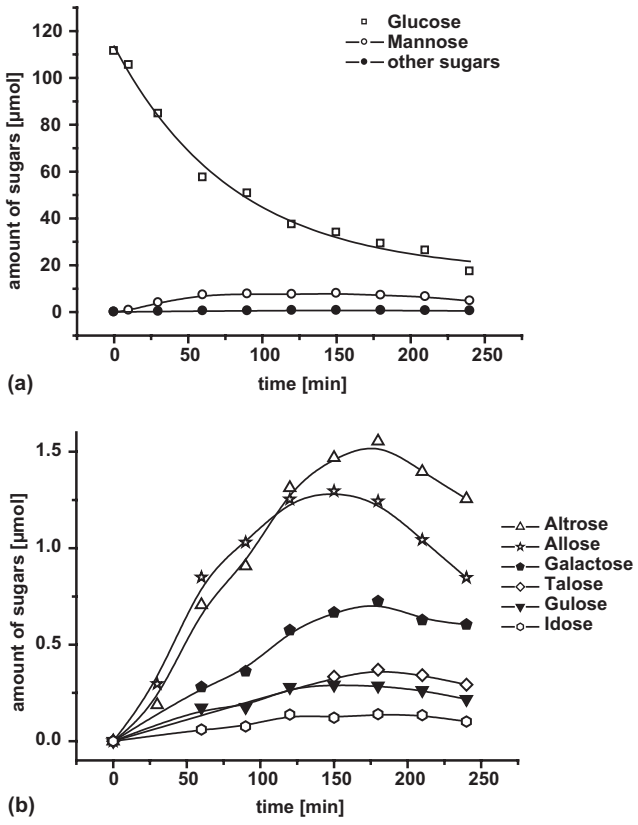


Figure 2. Isomerization of glucose in NMMO at 110 °C: (a) formation of the C-2 epimer mannose; (b) build-up and degradation of the C-3, C4, and C-5 epimers of glucose. Note the different scales of the *y*-axes.

of isomerized aldoses (Fig. 2a). Since each isomerization step involves the formation of an enediol structure as intermediate and these strongly reducing structures ('reductones') are supposed to be the starting points of the degradation reactions in case of alkaline treatment,[4–9] similar behavior can be expected in the presence of the reactive NMMO especially at the elevated reaction temperature used.

Figure 2 presents the kinetics of D-glucose isomerization/degradation under Lyocell conditions. An exponential decay according to pseudo first-order kinetics was found for the starting material (Fig. 2a); formation of the isomeric sugars showed the typical course of intermediates, their generation being superimposed by consumption in degradation/isomerization processes. From all reducing intermediates, the C-2 epimer mannose was found in highest concentration, with a maximum at approx. 150 min reaction time, followed by allose and altrose (Fig. 2a and b). The isomers galactose, talose, gulose, and idose were formed only in rather small amounts, as plotted in Figure 2b.

Also in the case of D-galactose an exponential decay was observed, but the reaction rate was significantly higher as compared to glucose. The concentration of the C-2 epimer talose reached its maximum after 60 min

already, whereas glucose was still in the build-up phase after 3 h reaction time.

In the course of the isomerization steps, keto-sugars must have formed in accordance with the isomerization schemes in Figures 6 and 7. Even though these species cannot be detected and quantified by the CZE method used,[11] their *aldose* isomerization products should be detectable, which would provide strong support for the suggested mechanism. Therefore, the ketohexoses D-fructose and D-sorbose were subjected to the NMMO treatment. Further direct evidence for the intermediacy of ketohexoses in the isomerization reactions was provided by ^{13}C NMR spectroscopy, see Chapter 2.2.

In Figure 3a and b, the D-fructose isomerization kinetics are presented. In contrast to the aldoses glucose and galactose, where epimerization could only progress 'in one direction', the keto group in fructose can isomerize 'in two directions'. The main products are glucose followed by mannose (Fig. 3a), in accordance with the expectations. Further products in the order of decreasing intermediate concentration were allose, altrose, galactose, talose, gulose, and idose (Fig. 3b). After approx. 60 min, the sum of all aldoses reached its maximum corresponding to 34% of the consumed starting material.

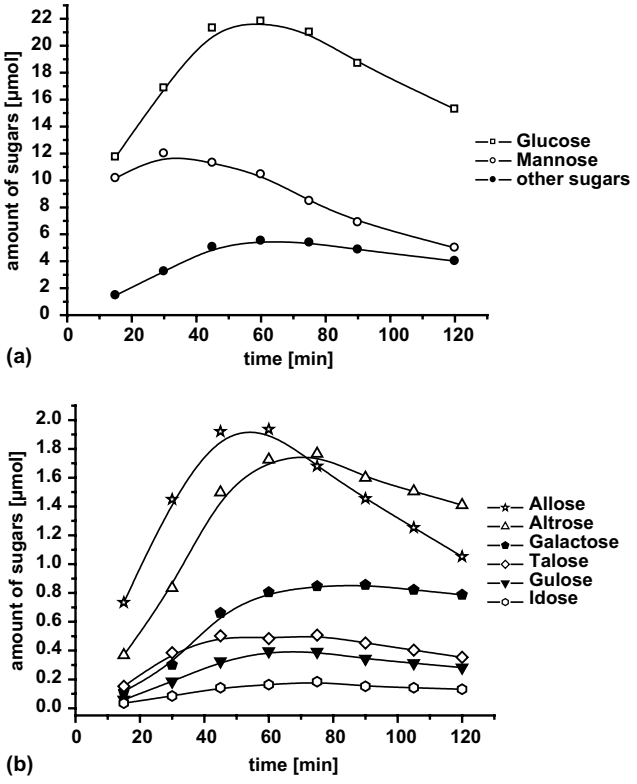


Figure 3. Isomerization of fructose in NMMO at 110 °C: (a) course of the main intermediates glucose and mannose; (b) profile of the minor isomerization products allose, altrose, galactose, talose, gulose, and idose. Note the different scales of the *y*-axes.

It is interesting to note that the isomerization of D-sorbose proceeded faster, and that sorbose as well as the main products gulose and idose were less stable under the prevailing conditions. The sum of all aldoses reached its maximum already after 40 min, with 18% of the starting material having been converted into aldoses at this time. Due to the instability of the keto sugar and its quick conversion, the build-up phase for the two main products gulose and idose was so fast, that only a decline was monitored. On the other hand, the more stable glucose was still in the build-up phase after a reaction time of 2 h.

In the next step, the isomerization of pentoses in NMMO was investigated, since the chromophore formation rate under Lyocell conditions had been shown to be much higher for monomeric pentoses as compared to hexoses, and also for polymeric pentoses (xylan) in comparison to their hexose counterpart (cellulose).[3]

D-Xylose, as the starting material, was consumed according to pseudo-first-order kinetics (Fig. 4a). The

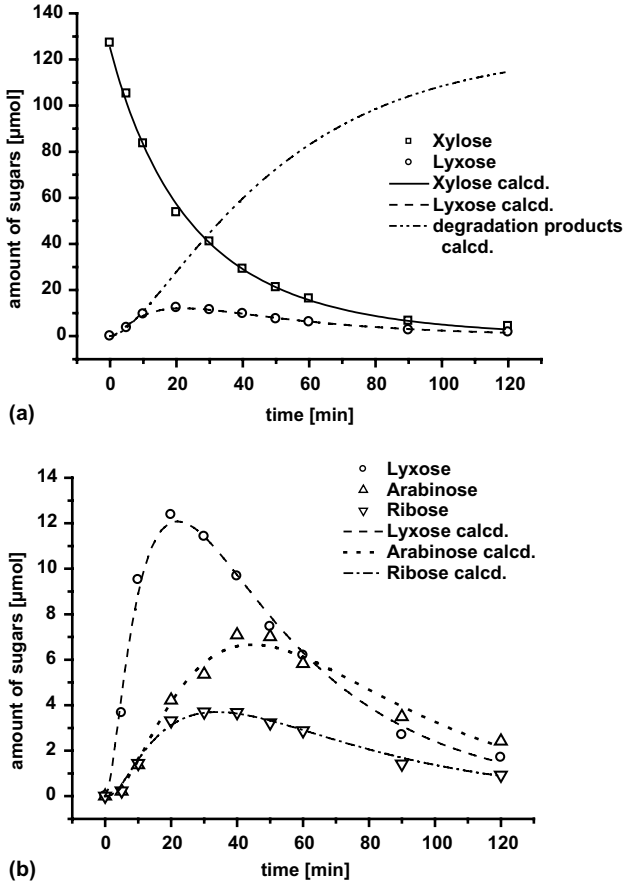
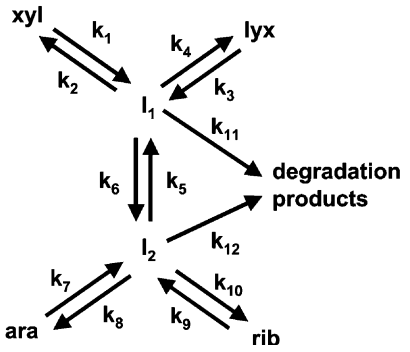


Figure 4. (a) Isomerization of xylose in NMMO at 110 °C; (b) build-up and degradation of the C-2 epimer lyxose and the C-3 isomers arabinose and ribose. Note the different scales of the *y*-axes. The symbols represent the experimental values, the full lines the mathematically modeled fits.



$$[xyl]' = -k_1 \cdot [xyl] + k_2 \cdot [I_1]$$
$$[lyx]' = -k_3 \cdot [lyx] + k_4 \cdot [I_1]$$
$$[ara]' = -k_7 \cdot [ara] + k_8 \cdot [I_2]$$
$$[rib]' = -k_9 \cdot [rib] + k_{10} \cdot [I_2]$$
$$[I_1]' = k_1 \cdot [xyl] + k_3 \cdot [xyl] + k_5 \cdot [I_2] - (k_2 + k_4 + k_6 + k_{11}) \cdot [I_1]$$
$$[I_2]' = k_6 \cdot [I_1] + k_7 \cdot [ara] + k_9 \cdot [rib] - (k_5 + k_8 + k_{10} + k_{12}) \cdot [I_2]$$

Figure 5. Simplified model of the isomerization of pentoses and corresponding differential rate laws.

Table 1. Calculated reaction constants for pentose isomerization in NMMO at 110 °C using D-xylose as the starting material

Reaction constants k [s^{-1}]	
k_1	4.41E−02
k_2	3.45E−02
k_3	2.57E−01
k_4	1.28E−01
k_5	2.75E−01
k_6	5.68E−02
k_7	8.05E−02
k_8	2.03E−01
k_9	3.36E−01
k_{10}	4.26E−01
k_{11}	6.58E−02
k_{12}	1.02E−01

formed amount of 'degradation products', calculated as the difference between starting material and detectable aldohexoses, gave the expected delayed exponential increase. As shown in the zoomed plot in Figure 4b, the main intermediate lyxose reached its concentration maximum already after 20 min, ribose after about 30 min, and arabinose after approx. 40 min.

The experimental kinetic data were fitted by a mathematical model. For that purpose, the quite complex overall isomerization processes (cf. Fig. 7) were simplified using the chemical model in Figure 5. The keto and enediol structures were combined into two virtual intermediates I_1 and I_2 as indicated in Figure 7. Furthermore, the model was based on the assumptions that all reactions are of first order, all equilibria are established, and degradation reactions start exclusively from the intermediates I_1 and I_2. The resulting set of differential equations was solved, and the kinetic rate constants were determined by iterative fit optimization (Table 1).

Considering that the data points in Figure 4 show the experimental values and the full lines represent the solutions of the differential equations in Figure 5, the match between experimental and calculated values is excellent. This outcome proved the validity of the assumptions used in the model. In particular, it confirmed the first-order kinetics[†] for all individual reaction steps in the isomerization scheme.

As expected, isomerization occurred also in the case of D-arabinose, and the reaction proceeded much faster than in the case of hexoses. Consumption of the starting sugar followed a pseudo-first-order exponential decay, and the isomerization products showed the typical course of intermediates.

The complete set of isomerization reactions is summarized in Figure 6 for hexoses and Figure 7 for pentoses.

2.2. ^{13}C NMR investigations of [1-^{13}C]glucose under Lyocell conditions

Another issue in reactions of monosaccharides under Lyocell conditions is the oxidation of aldoses to the corresponding aldonic acids, especially as NMMO is considered a rather strong oxidant. Preliminary CZE runs indicated aldonic acids to be present in very small amounts only. The drawback of the CZE method was its low sensitivity, since direct UV detection at 200 nm was used. Ion chromatography further supported that the amount of aldonic acids in the monosaccharide reaction mixtures was well below 5% (data not shown). To clarify this issue and to finally confirm the intermediacy of keto-sugars (which were not detectable by the used CZE method,[11] [1-^{13}C]-glucose was subjected to the usual isomerization conditions. A reaction time of 1.5 h was chosen, because CZE experiments had shown that after this time approx. half of the glucose starting material had been consumed. After cooling, the reaction mixture was dissolved in D_2O and analyzed by quantitative ^{13}C NMR spectroscopy.[12] Due to the selective ^{13}C enrichment, C-1 produces a signal about 100 times stronger than the other carbon atoms present, which are thus

[†] At present, it can be stated that the kinetics are actually of pseudo-first-order. Assuming both the sugar and NMMO to participate in the rate-determining step, the NMMO concentration would be much larger and would thus remain nearly constant over time.

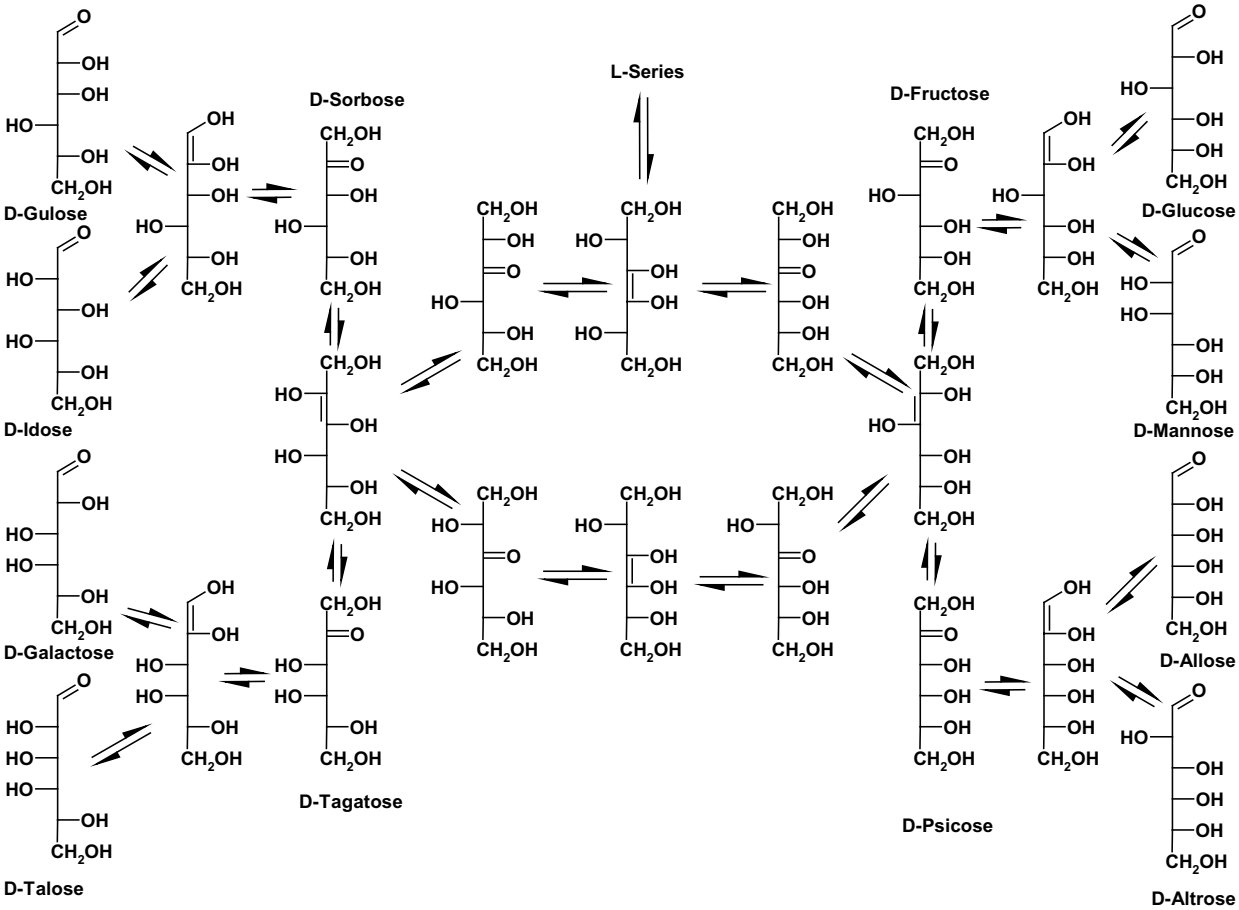


Figure 6. Isomerization of hexoses.

'blinded out'. The amount of carboxylic acid oxidation products, seen in the region of 170–180 ppm for C-1 signals, was found to be very low as compared to the α- and β-pyranose forms of glucose (Fig. 8). The extremely small signal at 179.99 ppm (see the inset in Fig. 8) is likely to originate from an aldonic acid, the signal at 171.59 ppm from another, hitherto unidentified C-1 carboxylic degradation product. In addition, a CH_3 signal at 24.07 ppm was observed.

The chemical shifts and coupling constants ($J_{C1,C2}$) of the α- and β-anomers of glucose as well as the ratio between these anomers were in complete agreement with published values.[13–15] Anomeric signals of mannose (α- and β-pyranose form) were also detected. Three forms of fructose (β-pyranose and α,β-furanose; the α-furanose form being very small) were found, thus directly confirming the presence of keto-sugars. Also here, chemical shifts and the ratios of the different hemiacetal/hemiketal isomers fully corresponded with literature data.[13,16,17] Compared to glucose, a rather high amount of fructose (33%) was present. The content of mannose was 9%, which corresponded roughly to the CZE results. Thus, the ^{13}C NMR study supported not only the outcome of the CZE experiments, it moreover provided additional information about the formation of fructose, which was not accessible with the CZE method used. Nevertheless no significant amounts of degradation products were found, though—according to CZE experiments—about half of the glucose had been consumed, that is, the integrals of the 1-^{13}C signals of glucose should be roughly the same as the integrals over all other ^{13}C-signals. In the present case, considerable ^{13}C intensity (about 40%) was missing. This could be due to loss of C-1 via decarboxylation reactions. Further investigations using [2-^{13}C] and [6-^{13}C]glucose are underway.

2.3. Isomerization of carbohydrates in other *N*-oxides

Finally the question was addressed whether the induction of isomerization in monosaccharides is a unique ability of NMMO, or whether it is a property of tertiary amine *N*-oxides in general. D-Xylose was chosen as the test carbohydrate because of its quite high reactivity, and *N*,*N*-dimethyloctylamine-*N*-oxide as well as *N*,*N*-dimethyldodecylamine-*N*-oxide were used as the reaction media. A reaction time of 1.5 h was scheduled.

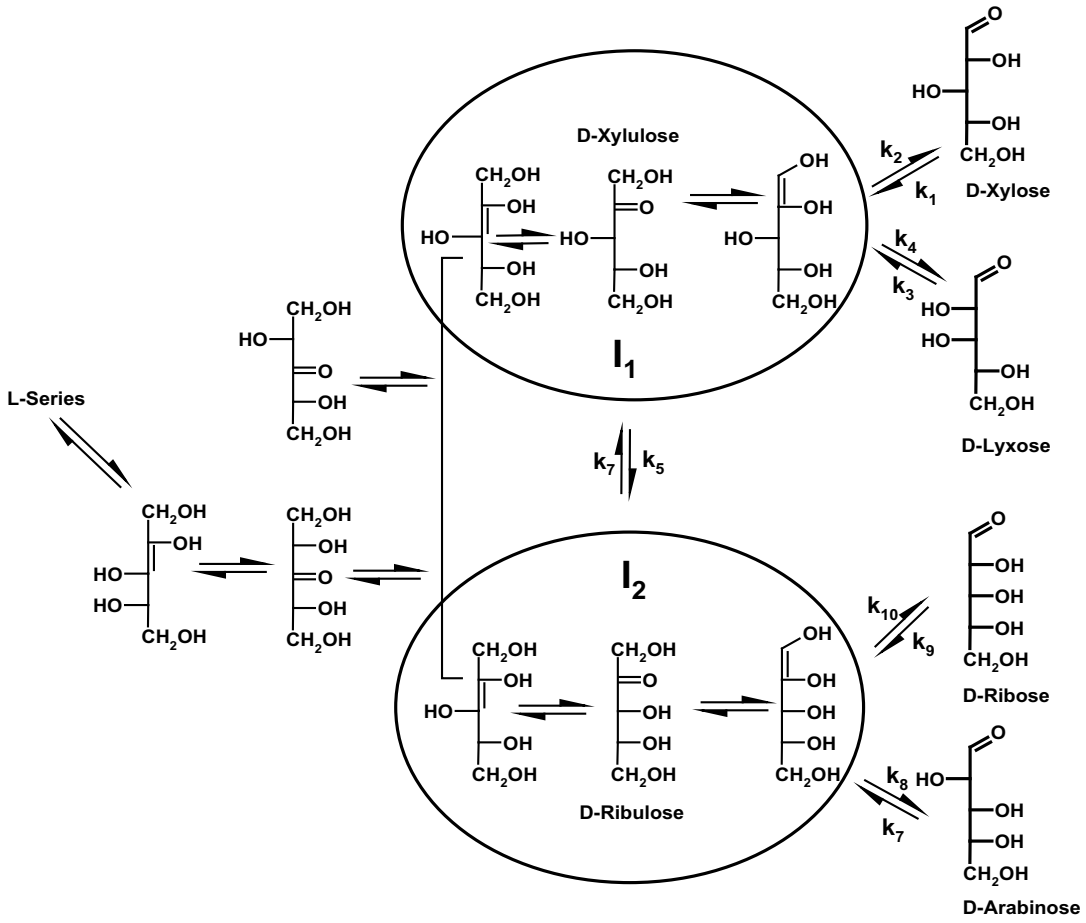


Figure 7. Isomerization of pentoses. The simplifications of intermediates I_1 and I_2 chosen for mathematical modeling of the rate constants are indicated by circles.

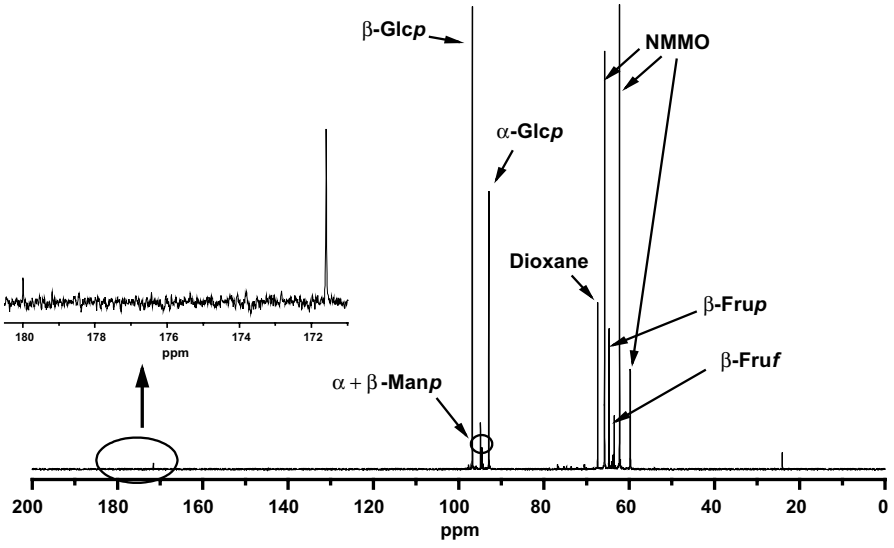


Figure 8. ^{13}C NMR spectrum of the reaction mixture of [1-^{13}C]glucose in NMMO at 110 °C after 1.5 h. Dioxane was used as the internal reference (67.40 ppm).

Isomerization occurred with all amine oxides tested, even though the reactions were slower than in the reference case of NMMO. Thus, the results confirm that other tertiary amine-*N*-oxides besides NMMO possess

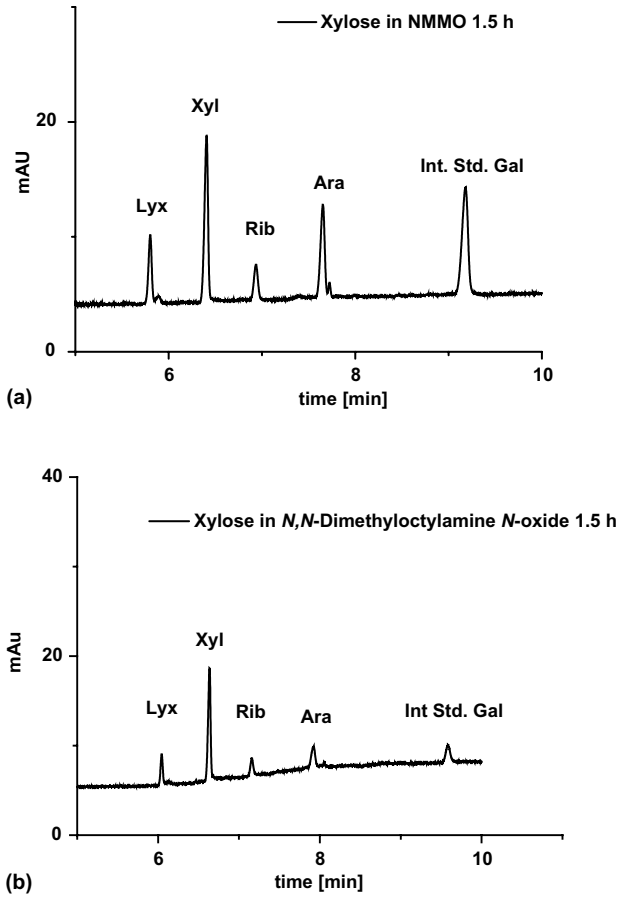


Figure 9. Electropherograms of xylose heated for 1.5 h at 110 °C in (a) *N*-methylmorpholine-*N*-oxide monohydrate (NMMO) and (b) *N*,*N*-dimethyloctylamine-*N*-oxide.

isomerizing capabilities as well. In Figure 9, a comparison of electropherograms of the reaction mixtures is presented.

The electropherogram of the reaction mixture in *N*,*N*-dimethyldodecylamine-*N*-oxide was similar to Figure 9b with the amount of isomerized pentoses being even lower than in *N*,*N*-dimethyloctylamine-*N*-oxide.

3. Experimental

Chemicals: NMMO monohydrate was recrystallized from acetone and chloroform (mp 76–78 °C). 4-Aminobenzoic acid ethyl ester (ABEE) 98%, sodium cyanoborohydride 95%, boric acid (99.5+%), D-arabinose (99%), D-xylose (99+%), and D-glucose (A.S.C. grade) were obtained from Sigma-Aldrich (Milwaukee, WI, USA). D-Lyxose (99+%), D-galactose (99+%), D-allose (99+%), D-altrose (99+%), D-talose (99+%) were obtained from Fluka (Buchs, Switzerland). D-Mannose (98+%) was obtained from Merck E. (Darmstadt, Germany). D-Ribose (99+%), D-gulose (95%), and L-idose (95%) were obtained from Sigma (St. Louis, MO, USA). The water used was purified (>17 MΩ/cm) with an HQ5 filter apparatus (REWA, Gladbeck, Germany).

Ion chromatography: For the determination of aldonic acids, a Dionex CarboPac PA10 4×250 mm column fitted with a Dionex CarboPac PA10 Guard 4×50 mm, 0.6 M sodium hydroxide solution as eluant, a flow of 0.5 mL/min and pulsed amperometry (Dionex ED40) for detection was used.

^{13}C NMR spectroscopy: ^{13}C NMR spectra were recorded on a Bruker Avance DPX instrument operating at 75.47 MHz for ^{13}C using D_2O as the solvent. ^{13}C NMR spectra were measured at 293 K and referenced to 1,4-dioxane (δ 67.40). Quantitative ^{13}C NMR spectroscopy was performed with Bruker standard software using inverse gated decoupling with 4.0 s delay.[12]

Kinetic studies: Twenty milligrams of the respective sugar was mixed with 200 mg NMMO·H_2O in a 4 mL vial and immersed into an oil bath at 110 °C with magnetic stirring. After a certain reaction time, the vessel was quickly cooled in an ice/water bath and the reaction mixture was dissolved in water (2 mL). A 200 μL aliquot was taken and analyzed by capillary zone electrophoresis (CZE) after pre-column derivatization by reductive amination with ABEE and $NaCNBH_3$. Pre-column derivatization and analytical procedure have been described in detail previously.[11]

For modeling and experimental data fitting the software Scientist® for Windows Version 2.0 by MICROMATH Scientific Software was used.

4. Conclusions

Isomerization of monosaccharides in NMMO and other amine *N*-oxides under Lyocell conditions proceeds quite fast and affects the whole carbon chain, producing all four isomeric aldopentoses from xylose and all eight isomeric aldohexoses from glucose. Similar isomerization processes start also from ketosugars. The isomerization reactions involve the formation of enediol intermediates, which are likely to be the starting points for degradation/oxidation reactions finally leading to chromophores. Oxidation of aldoses to the corresponding aldonic acids is of minor importance only. The degradation or isomerization to ketoses seem to dominate over the isomerization to CZE-detectable aldoses. The isomerization kinetics of glucose, galactose, fructose, sorbose, arabinose and xylose were fully recorded, that of xylose was mathematically modeled in addition.

Besides fructose and mannose as isomerization products no significant amounts of degradation products were observed in the ^{13}C NMR spectrum of [1-^{13}C]glucose after NMMO treatment. Thus, in contrast to the clarified isomerization, further investigations

using [2-^{13}C] and [6-^{13}C]glucose are needed to study the degradation reactions under Lyocell conditions.

The results of this study help to improve the understanding of the very complex topic of color generation under Lyocell conditions, especially by narrowing the gap between the polymer cellulose and the monosaccharide-derived chromophores. The missing link—the mechanism of monosaccharide release from the polymeric starting materials (cellulose/xylan)—will be addressed in further studies.

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