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Polymer-Analogous Preparation of Cellulose Tricarbanilates: Mechanisms of Degradation in Dimethylsulfoxide

Martin Fischer,[1] *Klaus Fischer**[2]

[1] Christian Doppler Laboratory for Pulp Reactivity, Institute of Chemistry, University of Agricultural Sciences Vienna, Muthgasse 18, 1190 Vienna, Austria
Fax: (+43) 1 36006 6059; E-mail: mfischer@edv2.boku.ac.at
[2] Institute of Plant and Wood Chemistry, Technical University of Dresden, Pienner Straße 19, 01737 Tharandt, Germany

Summary: Two cellulose samples were subject to treatment with phenylisocyanate (PhNCO), both in pyridine and in dimethylsulfoxide (DMSO). In each case, DMSO degraded cellulose in an extent depending on the derivatized cellulose. The formation of dimethylsulfonium ions was recognized as the cause leading to an oxidation of hydroxyl groups. This *Pfitzner-Moffatt* type oxidation is dependent on carboxyl groups and therefore occurs most probably in their neighbourhood. It was deduced that formation of oxidised sections along the cellulose chains occurs containing either carbonyl and carboxyl groups in the same anhydroglucose unit (AGU) or singly oxidised AGUs in direct neighbourhood to each other. This leads to much facilitated eliminative degradation.

Keywords: carbanilation; cellulose; degradation; oxidation; polysaccharides

Introduction

For the determination of molecular weights and molecular weight distributions (MWD) of cellulose samples derivatives of cellulose or directly dissolved cellulose are used. Common derivatives employed are cellulose-*O*-trinitrates (CTN)[1-6] and cellulose-*O*-tricarbanilates (CTC).[6-9] CTC has favourable properties for the characterisation of cellulose. The mass increase by factor 3.204 is especially useful when the focus lies on the lower molecular weight fractions. A high dn/dc (e.g. 0.165 in tetrahydrofuran - THF)[10] and the UV-activity allow good detector responses. Furthermore, CTC is of sufficient chemical stability and therefore easy to handle and to store, particularly when compared with CTN.[11]

Although derivatization methods have been known for a long time, big differences between results of the participants of a round robin were noticed, for instance.[12] The

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results obtained by the measurement of CTC samples were differing, too. Probably, this can be ascribed to different preparation protocols, because it is known that the applied solvent is of great influence on the molecular weight of the resulting carbanilates. Especially, preparation in DMSO- or DMSO-containing media can lead to polymer degradation in comparison with CTC prepared in pyridine.[7-9]

The present work confirms this solvent-specific degradation and reports a clear correlation of its extent with the starting cellulose. A mechanism is proposed consisting of a *Pfitzner-Moffatt* type oxidation and a subsequent β-elimination. This proposal was confirmed by means of NMR and reaction studies on model systems and also by the effects of certain additives on carbanilation of cellulose in DMSO. The results led to the formulation of specific reaction schemes most probably being responsible for the partly large losses in degree of polymerization (DP) if carbanilation is carried out in DMSO.

Experimental

Two fully bleached acidic sulphite chemical pulps were chosen for carbanilation experiments. Sample 1 originated from eucalypt, sample 2 from spruce. Drying of the two cellulose samples was achieved under vacuum at room temperature. Carbanilation was carried out in "Schlenk" flasks at completely anaerobic conditions at 70 °C under slight stirring. 100 mg samples reacted with 5 ml PhNCO (25-fold molar excess) in approximately 50 ml solvent. Derivatization times were chosen from 12 hours to 2 days at maximum. Recovery of CTC was accomplished by precipitation in a methanol-water mixture (70:30, v/v) as was described in the literature.[9] The crude CTCs were dried under vacuum, redissolved in acetone and precipitated again. This procedure was repeated yielding practically pure CTC samples as proven by ^{1}H-NMR.

Conversion of methyl-4-*O*-methyl-β-D-*ribo*-hex-3-ulopyranoside with PhNCO in pyridine was carried out in a NMR tube at 4 °C over 14 days followed by 6 hours at room temperature. During the latter time the pyridine was removed by a slow stream of nitrogen. Thereafter the residue was dissolved in $CDCl_3$ and measured by NMR.

SEC-MALLS on solutions of CTC samples was performed either in THF or in *N,N*-dimethylacetamide / lithium chloride (DMAc-LiCl). Results are given as degrees of polymerisation (P) instead of molecular weights (M) because of better comparability.

SEC system 1 (THF): Waters 510 pump; 3 PL gel columns (300 x 8 mm, 10 µm, 10^4 Å, 500 Å and 50 Å (Knauer); detectors: Knauer RI or Optilab DSP (Wyatt Technology, Santa

Barbara, USA) and MALLS DAWN DSP laser photometer (Ar ion laser, λ = 488nm, Wyatt Technology).

SEC system 2 (DMAc-LiCl): Kontron 420 pump; PL gel mixedA ALS, 20 µm, 300 x 7.5 mm; autosampler: HP 1100 series; detectors: Shodex RI-71 and a DAWN DSP laser photometer (λ = 488nm).

CTC samples were labelled with *N*9-(7-Amino-1,4,7-trioxaheptyl)-9*H*-9-carbazolcarboxamid (CCOA) in THF solutions. 2 mg CCOA · HCl and 1 ml triethylamine were added to each solution of 100 mg CTC in 5 ml THF, followed by slight stirring at room temperature and in the dark. After 1 day the CTC were precipitated twice in methanol-water (70:30, v/v) with intermediate vacuum-drying and dissolution in THF. After drying, the labelled samples were dissolved in THF and samples of progressive dilution were prepared. Fluorescence of these solutions was measured using a HP LS 50 spectrometer affording intensity-to-concentration plots with a linear range at the lowest concentrations intersecting the zero point. The quantity of bound CCOA was calculated from the slope of the linear curve range (Figure 1).

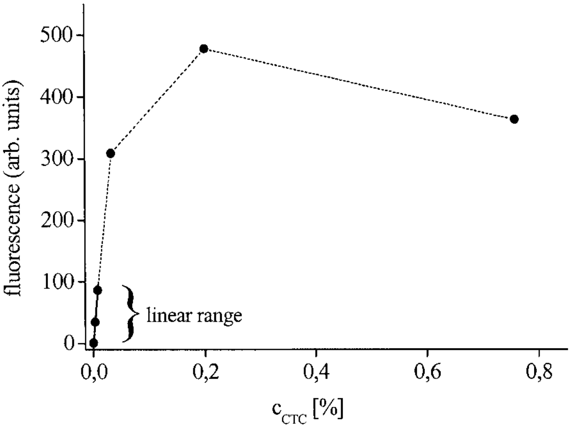


Figure 1. Fluorescence of a CCOA-labelled sample of cellulose-*O*-tricarbanilates at different concentrations in THF.

^{1}H-NMR spectroscopy of a reaction mixture of DMSO, PhNCO and monochloracetic acid (MCA) was performed with a Bruker DRX 500 MHz spectrometer at room temperature. A measurement time of a few minutes could be realised only because of the evolution of CO_2. For this reason, ^{13}C and two-dimensional NMR spectra were not accessible.

Model oxidations were carried out in DMSO as the solvent (25 mmol) employing 2 mmol 3-methoxyphenylisocyanate, 0.7 mmol methanol and 0.35 mmol of the respective additive (trifluoroacetic acid (TFA), pyridinium trifluoroacetate (Py·TFA), MCA or pyridine). The conversions were carried out at room temperature over 4 hours until quantitative conversion. After dilution with methanol and water the formed HCHO was derivatized with *O*-benzylhydroxylamine and determined as formaldehyde-*O*-benzyloxime. Analysis was performed on a HP LC-MS 1100. Chromatographic separation was carried out on an Eclipse XDB C-8 (4.6 x 150 mm, Agilent) column using a gradient from 20:80 methanol-water to 100 % methanol during 10 minutes. All reagents were confirmed to be HCHO-free with the same method.

Results and Discussion

The carbanilation of the eucalypt chemical pulp was carried out two times in pyridine and two times in DMSO under completely identical conditions (12 hours reaction time). The results were almost identical for each solvent ($\Delta P_w < 1$ %) but differed considerably between the two sample sets (Table 1, Figure 2).

Table 1. Values of the mass average of the degree of polymerisation (P_w) of cellulose-*O*-tricarbanilates prepared from pulp 1 (eucalypt sulphite pulp).

solvent	P_w
pyridine	1.653
	1.628
DMSO	799
	792

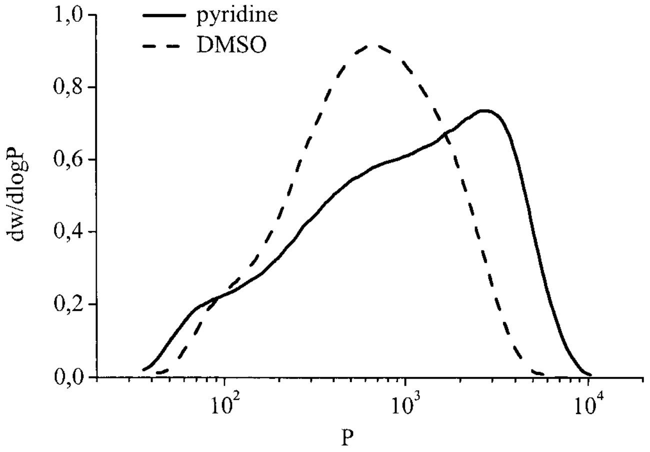


Figure 2. Molecular weight distributions of cellulose-*O*-tricarbanilates prepared from sample 1 (eucalypt sulphite pulp). THF was used as solvent for SEC. Each curve is an average of the measurements of 2 samples.

Upon carbanilation in DMSO, less degradation of sample 2 (spruce pulp) occurred. Because of variations in preactivation and some slight variations of the reaction conditions, the differences in P_w between the respective CTC samples are bigger than for sample 1 (Table 2, Figure 3).

Nevertheless, it can be seen that the difference between P_w of CTC prepared in pyridine and CTC prepared in DMSO are significant. This difference amounts to 51.5 % for pulp 1 and to 9.5 % for pulp 2. Therefore the extent of degradation must be dependent on a certain property of the respective pulp, which was still unknown up to now. Considering the joint presence of DMSO and PhNCO it was logical to suggest a degradation mechanism with an oxidative step.

Table 2. Values of the mass average of the degree of polymerisation (P_w) of cellulose-*O*-tricarbanilates prepared from pulp 2 (spruce sulphite pulp).

solvent	P_w
pyridine	2.340
	2.283
DMSO	2.010
	2.104
	2.159

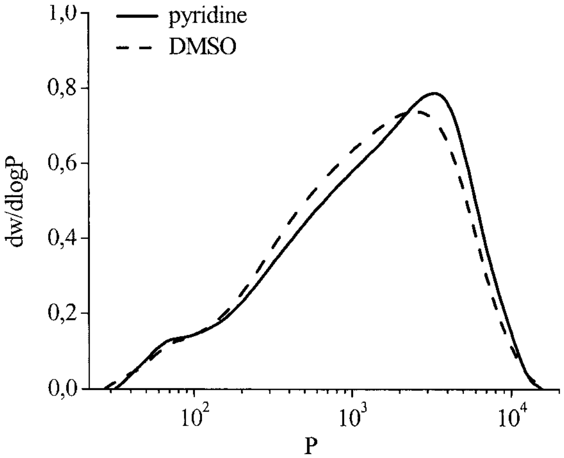


Figure 3. Molecular weight distributions of cellulose-*O*-tricarbanilates (CTC) prepared from sample 2 (spruce sulphite pulp). The curves are averages of two measurements of two CTC samples in the case of pyridine and three in the case of DMSO. THF was used as solvent for SEC.

^{1}H-NMR measurements of model systems were carried out in order to confirm the occurrence of dimethylsulfonium ions as a possible cause of an oxidation. As model systems mixtures of DMSO and PhNCO with and without MCA as the carboxylic acid were used. MCA was chosen because of almost identical acidity values of MCA ($pK_a = 2.87$) and carboxyl groups in cellulose ($pK_a = 2.80 \pm 0.1$).[13] While only minute amounts of a zwitterionic adduct could be detected in a mixture of DMSO and PhNCO, the addition of MCA led to significantly higher amounts of one out of two possible types of dimethylsulfonium ions. These were observed ten minutes after starting the reaction by addition of MCA (2.90 – 2.93 ppm, Figure 4). These dimethylsulfonium ions were identified as *N*-phenylcarbamoyloxydimethylsulfonium ions, because none of the signals of methylene hydrogens could be assigned to the alternative chloroacetoxydimethylsulfonium ions. There, the appropriate signal would have been diminished in a proportion of 3:1 relative to the decrease of the signal of the observed dimethylsulfonium ions after 3 hours. As this was not the case, the presence of chloroacetoxydimethylsulfonium ions could be ruled out within the precision of the measurement.

These measurements suggested the formation of *N*-phenylcarbamoyloxy-dimethylsulfonium ions upon carbanilation of cellulose in DMSO containing carboxyl groups. Consequently, the oxidation capabilities of several model systems were investigated.

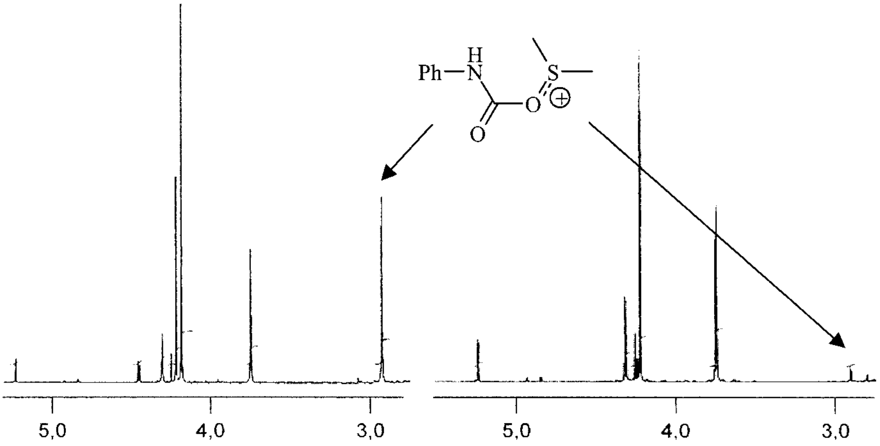


Figure 4. ^{1}H-NMR spectra of a system of DMSO, phenylisocyanate and chloroacetic acid after 10 minutes (left) and after 3 hours (right), illustrating the formation and the decomposition of the labile *N*-phenylcarbamoyloxydimethylsulfonium ions. Chemical shifts are given in ppm.

These systems consisted of DMSO, an isocyanate and different additives. The latter were compounds with varying acidity / basicity, as it is known that this is of great influence on the yield of *Pfitzner-Moffatt* oxidations. This mechanism was suggested to be paralleled by the reactions of sulfoxides and isocyanates.

Methanol was used as the alcohol to be oxidised. PhNCO, 3-methoxyphenylisocyanate and cyclohexylisocyanate were employed as the acylating agents, mainly leading to the formation of the respective carbamoylation products of methanol. The formation of low amounts of HCHO was expected as the result of a *Pfitzner-Moffatt* type oxidation.

3-Methoxyphenylisocyanate was chosen for systematic investigations because of its most suitable reactivity. Conversions with PhNCO proceeded too vigorously, so that temperature control was more difficult, whereas cyclohexylisocyanate had a much too low reactivity.

The results of the experiments, as given in Figure 5, support the assumption of a *Pfitzner-Moffatt* reaction mechanism because of the same dependence of the yield of the oxidation product HCHO on the acidity / basicity of the respective reagents employed as additive and on temperature.[14] Considering the especially good suitability of Py·TFA as catalyst in the *Pfitzner-Moffatt* oxidation of alcohols with DMSO and *N,N'*-dicyclohexylcarbodiimide,[14] MCA must be considered as an appropriate catalyst, too. Because of similar acidities of MCA and carboxyl groups in cellulose the presumed oxidation of OH-groups in reactions with PhNCO and DMSO is strongly supported.

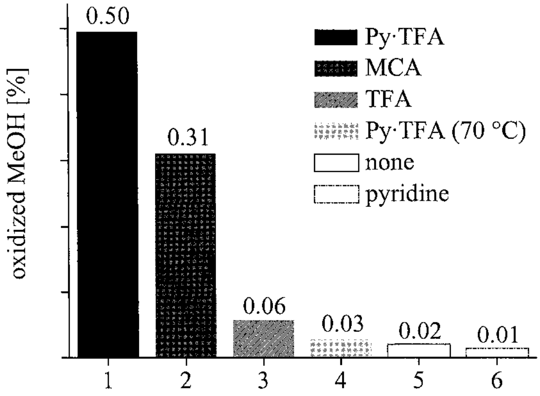


Figure 5. Proportions of methanol which were oxidised in different model systems.

In order to confirm these findings the effects of several additives on the carbanilation in DMSO was elucidated. Pulp 1 was carbanilated in pure pyridine and DMSO and in DMSO either with Py·TFA or with 1,8-bis(dimethylamino)naphthalene (proton sponge®, pK_a in water = 12.1).[15] The addition of Py·TFA led to the expected increase of the degradation, whereas the addition of proton sponge® caused a distinct decrease of the degradation compared with the use of pure DMSO (Figure 6). Although the amount of proton sponge® was about hundredfold the amount of carboxyl groups of the cellulose reacted, the degradation was not avoided completely. Most probably this has to be ascribed to the presence of carboxyl groups in portions of the cellulose with restricted accessibility.

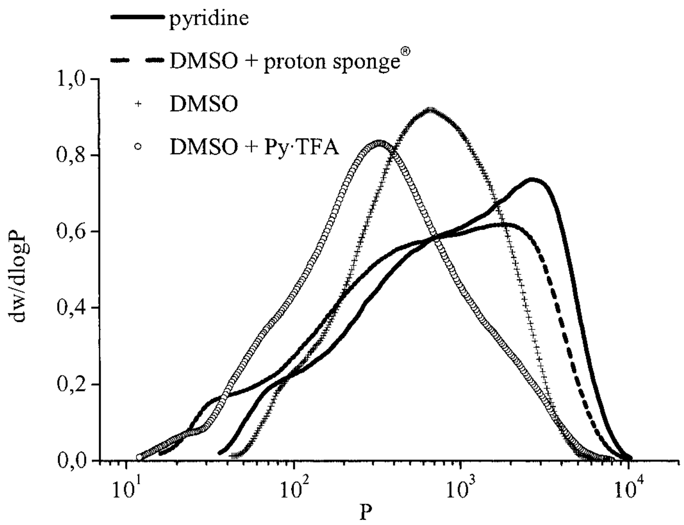


Figure 6. Effects of several carbanilation media on the molecular weight distribution of the resulting cellulose-*O*-tricarbanilates prepared from sample 1 (eucalypt sulphite pulp). SEC was carried out in THF.

The amount of Py·TFA was about 130-fold of the amount of carboxyl groups of the cellulose reacted, but the degradation was lower than expected. In part, this can be ascribed to a crosslinking of the macromolecules as revealed in handling the sample solutions and by SEC-MALLS (Figure 7). Solutions of the respective CTC sample were hardly to filter through 0.45 µm PTFE filters as it is carried out prior to injections into SEC systems. The SEC measurement revealed that the relation between radii of gyration (R_g) and P are that of crosslinked macromolecules (equation 1, Figure 7, Table 3).[16]

$$R_g = k \cdot P^{a(R_g)} \tag{1}$$

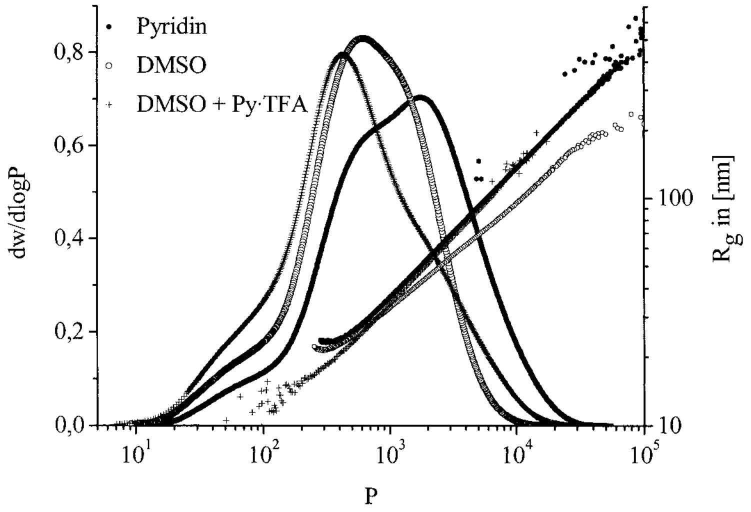


Figure 7. Molecular weight distribution and R_g(P)-relation of cellulose-*O*-tricarbanilates prepared from pulp 1 (eucalypt) as revealed by SEC-MALLS analysis, carried out in DMAc-LiCl.

Table 3. Exponents a(R_g) of the R_g(P)-relation (1) of CTC prepared from pulp 1 (Fig. 7).

solvent	a(R_g)
pyridine	0.57
DMSO	0.59
DMSO + Py· TFA	0.46

The crosslinking is explained by the oxidation of hydroxyl to aldehyde groups and subsequent formation of semiacetal bonds. Free semiacetal hydroxyls react with PhNCO to stable products. These links can be formed intra- and intermolecularly leading to solvent exclusion and therefore to a contraction of the macromolecules.[16, 17] This effect was not observed for the CTC samples prepared in pure DMSO indicating that formation of aldehyde groups or oxidation in general must have been negligible in comparison to the conversion in DMSO + Py· TFA. This is easily understandable regarding the high amount of Py·TFA used.

The degree of oxidation has further been proved by labelling with CCOA, a reagent specific for the conversion of carbonyl groups into the corresponding oximes.[18-20] The fluorescence of the purified CTC prepared in DMSO + Py·TFA revealed an amount of approximately 50-60 μmol carbonyl groups (calculated for cellulose) which is low considering the amount of added Py·TFA. In contrast, CTC prepared in pure pyridine or DMSO had no content of carbonyl groups which could be derivatized with CCOA. Obviously carbonyl groups of the starting cellulose (30.3 μmol / g) were masked almost completely during the carbanilation reaction. While aldehyde groups can be blocked by crosslinking, keton groups are in equilibrium with their respective enoles. Resulting enolic groups will be blocked by the reaction with isocyanate, forming enediol dicarbanilates. This was proven with methyl-4-*O*-methyl-β-D-*ribo*-hex-3-ulopyranoside as a model compound for keto groups in cellulose.[21] The compound was converted into two enediol carbanilates quantitatively (Figure 8).

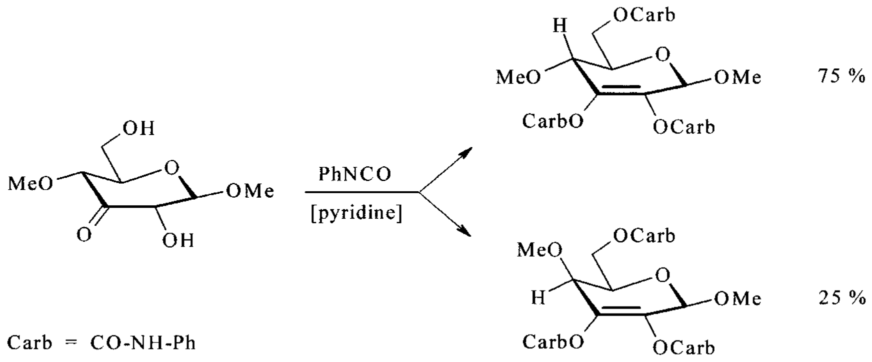
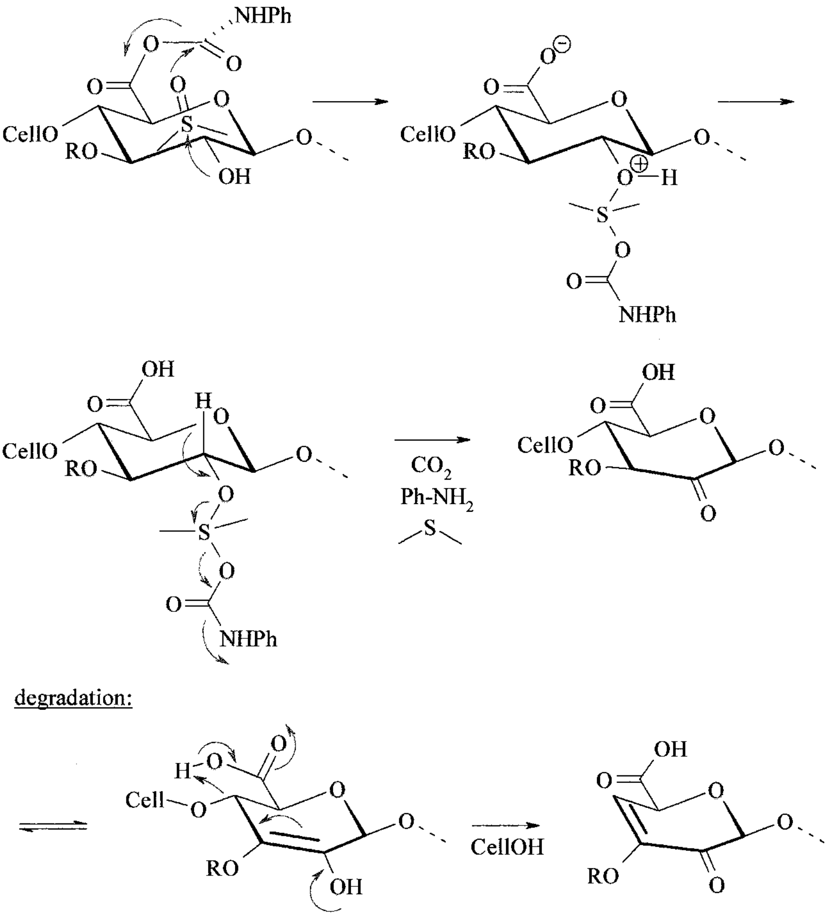


Figure 8. Reaction of methyl-4-*O*-methyl-β-D-*ribo*-hex-3-ulopyranoside with PhNCO in pyridine at 4 °C (14 days) and room temperature (6 hours). Conversion of the ketosugar into the two epimeric enedioldicarbanilates was quantitative.

Comparing the degradation of the cellulose molecules in DMSO or in DMSO + Py·TFA, the efficiency of the added Py·TFA was assessed as low. Thus, the degradation occurring when carbanilation is carried out in pure DMSO must be the result of a rather low degree of oxidation. Then, the so formed carbonyl groups must lead to much more fissions of the polymer chains than arbitrarily formed or already present carbonyls. This is especially true regarding the fact that the much higher basicity of the solvent pyridine did not effect a degradation as high as DMSO did. The degradation in pyridine proceeded very slowly and was negligible if the conversion times were about 12 hours up to two days, as will be published elsewhere.

The first step in the mechanism of the *Pfitzner-Moffatt*-analogous oxidation as a side reaction of carbanilation of cellulose must consist in the formation of a mixed anhydride between a carboxyl group of cellulose and *N*-phenylcarbamidic acid. Thus, these mixed anhydrides are formed along the cellulose chains (Figure 9). The second step consists in an *O*-acylation of DMSO leading to the formation of dimethylsulfonium ions. These can be *N*-phenylcarbamoyloxydimethylsulfonium ions or dimethylsulfonium ions situated along the cellulose chains (Figure 9 and 10).



R = H, CO-NHPh
Cell = cellulosyl

Figure 9. Possible oxidation and degradation mechanisms for the carbanilation of cellulose in DMSO at anhydroglucose units containing carboxyl groups.

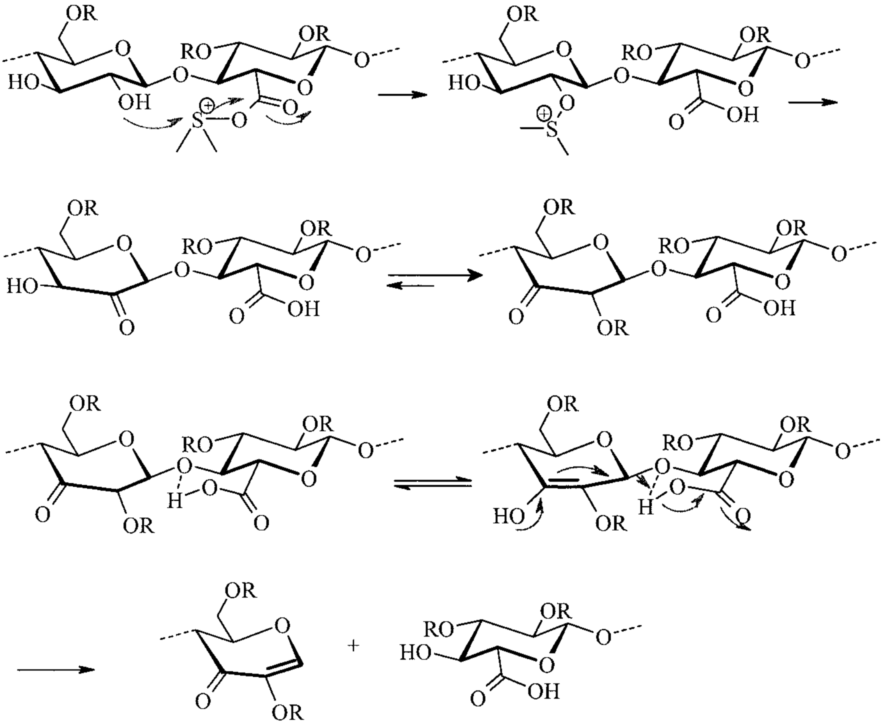
In Figure 9 a hypothetical intermolecular acylation step is drawn considering the presence of excess DMSO in the reaction mixtures. The third step of the oxidation consists of an exchange of the acyloxy- against alkoxy-moieties. This is the prerequisite to the following fragmentation as the fourth step of the reaction and the actual oxidation step. The third step will occur most probably in the neighbourhood of the acyloxy-moieties. Thus, formation of carbonyl groups is expected to occur at AGUs containing carboxyl groups themselves or in neighbouring AGU. This leads to a special constellation of functional groups, where polymer degradation can occur much more readily than at isolated carbonyl or carboxyl groups.

The most common degradation mechanism at carbonyl groups in carbohydrates is β-elimination, particularly in the presence of basic reagents. Moreover, it is known that carboxyl groups in cellulose effect an acceleration of acidic hydrolysis, most probably by intermolecular protonation.[22, 23] If the formation of the highly localized charges in intermediates (carbenium ions) of both reactions is avoided, the reaction rate will increase. This is fulfilled when β-elimination and protonation of the respective leaving groups occurs according to a concerted mechanism (Figure 9 and 10).

Conclusion

Carbanilation of cellulose in DMSO causes degradation to the polymer. This DMSO-specific degradation occurs at oxidised positions, which are generated during the carbanilation exclusively. The oxidation and thereby the degradation are dependent on the presence of carboxyl groups in the starting cellulose material, as proven by the significant decrease of depolymerization by addition of proton sponge.

It is highly likely that the oxidation follows the known *Pfitzner-Moffatt* mechanism, leading to oxidised positions containing carboxyl and carbonyl groups together in the AGU. These oxidised units degrade much easier than singly oxidised AGU. Therefore, the extent of degradation in carbanilation in DMSO must be dependent on the sample-specific distribution of accessible carboxyl groups along and between the cellulose chains. Up to now, no possibility for a complete prevention of this degradation is known. Therefore, carbanilation of cellulose in DMSO is not suitable if a polymer-analogous derivatization is desired.



R = H, CO-NHPh

Figure 10. Possible degradation mechanism for cellulose upon carbanilation in DMSO. An intracatenar oxidation occurs followed by a keto shift into the more favoured 3-position,[24] resulting in a concerted β-elimination / protonation. Thus, the chain is split much more readily than through β-elimination at isolated keto groups.

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