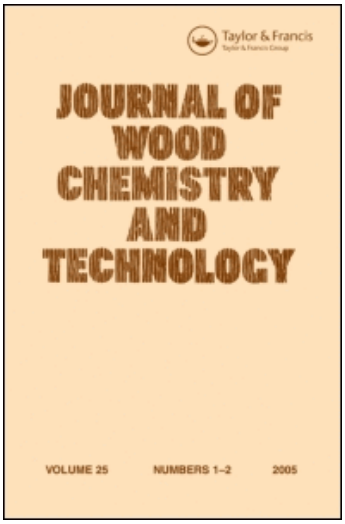

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Confirmation of the Presence of Hydroxyl Radicals During Pre-Ripening of Alkali Cellulose

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Confirmation of the Presence of Hydroxyl Radicals During Pre-Ripening of Alkali Cellulose

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Abstract: The presence of hydroxyl radicals during aging ("ripening") of alkali cellulose has been confirmed by selective trapping. As none of the spin traps available to date was working under such strongly alkaline conditions, a novel, alkali-stable trapping agent was developed, γ-tocopheryl-*iso*-propyl ether. This spin trap reacts selectively with hydroxyl radicals, but not with other reactive oxygen species or radicals present. The presence of hydroxyl radicals was confirmed additionally by the inhibition of the chain degradation in the presence of the OH-trap, which allowed estimating the contribution of OH radicals to the overall degradation of alkali cellulose during aging.

Keywords: Alkali cellulose, aging, ripening, hydroxyl radical, spin trapping, γ-tocopheryl-*iso*-propyl ether

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INTRODUCTION

Alkalization of cellulose, also called steeping, is a central process step in the production of many cellulose derivatives, most notably xanthation[1] in the viscose process or etherification in the production of carboxymethyl cellulose,[2] which is employed prior to the actual derivatization reactions. In viscose rayon production, alkalization is not only used for activating the hydroxyl groups toward xanthation, but also to free the pulp from impurities, such as hemicelluloses. The alkalization step involves treatment of dissolving pulp with strong alkali hydroxides, mostly 18% NaOH, which converts cellulose into sodium cellulose I. The alkalization step is followed by the (pre-)ripening procedure: excess sodium hydroxide is pressed off and the press cake, consisting of 34–35% cellulose and 15–16% NaOH, is left in the presence of air for several hours. In this stage, the appropriate pulp viscosity, that is, the cellulose DP, is adjusted for further processing to viscose. It is known that the changes in the molecular weight distribution are brought about by oxidative processes,[3,4] which involve introduction and conversion of oxidized functions, such as keto and aldehyde groups,[5] in addition to chain shortening.

Mechanistic aspects of the alkaline degradation of cellulosic materials have mainly been addressed by research groups in the 1950s and 1960s.[6–8] Chain cleavage was shown to be predominantly caused by introduction of keto groups followed by β-elimination. Already in 1949 Entwistle et al. proposed a radical chain mechanism for the aging of alkali cellulose.[9,10] The formation of hydrogen peroxide during aging of alkali cellulose under oxygen was demonstrated,[11] as well as the formation of a number of low-molecular weight acids. Thus, reactive oxygen species, namely hydroperoxyl, alkylperoxyl, and hydrogen peroxide along with the respective anions, play a key role in the aging of alkali cellulose. So far, the question of whether extremely reactive hydroxyl radicals are also involved is controversially discussed. On one hand, hydroxyl radicals, if present in reasonably high concentrations, should cause much more pronounced chain degradation than usually observed. On the other hand, formation of hydroxyl radicals from peroxides and other reactive oxygen species should proceed relatively easily.

Questions concerning the presence of a distinct radical species are usually addressed by specific trapping,[12] employing spin traps that react with transient single-electron species to form stable radicals, which are subsequently analyzed by EPR or UV spectrometry. Such approaches have been widely and successfully used for a large variety of structurally different radicals, employing numerous special spin traps. Almost all of these applications have been concerned with the detection of radicals under physiological conditions or conditions coming close to those in living systems. The number of spin traps remaining operational under extreme conditions, that is, at high temperatures or in the presence of concentrated acids,

bases, or other aggressive chemicals, is extremely limited. There have been reports on spin traps working under alkaline bleaching conditions,[15] but it was not clear whether these traps would remain operative under the far more drastic conditions of alkalization, meaning a reaction essentially carried out in concentrated aqueous NaOH. In addition, the recovery of the spin traps and the separation from the alkalization byproducts would remain as an obstacle, whereas the lipophilic trap and its products we describe herein are very easy to recover and to separate.

RESULTS AND DISCUSSION

Development of a Hydroxyl Radical Trap Capable of Working under Conditions of Alkali Cellulose Aging

Spin traps scavenge reactive radical species by converting them into more stable radicals. Stable nitroxyl radicals formed from nitrones and stabilized phenoxyl radicals formed from sterically hindered phenols are the two most prominent examples. Despite the large number of spin traps available, none of them is applicable under extremely high alkalinities present during the aging of alkali cellulose. In most cases, both the starting trap and the trapping product cannot withstand the concentrated alkaline medium. Therefore, we turned our attention to aromatic hydroxylation as another means to detect the presence of hydroxyl radicals. Aromatic systems are converted into hydroxyaromatics (phenols) in the presence of hydroxyl radicals. Usually, salicylic acid or phenylalanine are used as aromatic traps in hydroxylation assays[16] However, application to alkali cellulose poses the problem of retrieving the spin trap and its trapping products, as the mixture is rather complex and contains a complex, inseparable blend of aromatic degradation products from low-molecular weight celluloses and hemicelluloses.

In response to these problems, we used γ-tocopheryl-*iso*-propyl ether (**2**) as a new, selective hydroxyl radical spin trap. The reagent was synthesized according to a Williamson etherification procedure starting from γ-tocopherol (**1**) and 2-bromo-propane (Figure 1). The reagent is stable under ambient conditions, but should be kept in the dark under inert gas for long-term storage. The selectivity of the trap toward hydroxyl radicals was demonstrated by several experiments, such as treatment with enzyme systems, hydrogen peroxide, and potassium superoxide (KO_2) under different conditions, showing that the formation of the respective hydroxylation product, 5-hydroxy-6-*iso*-propoxy-γ-tocopherol (**3**), is only caused by hydroxyl radicals, but not by hydroperoxyl, alkylperoxyl, alkoxyl, nitroxyl, or superoxide anion radicals.[17]

For testing the trapping selectivity and evaluating the performance of the spin trap **2**, different radical generating systems summarized in previous work were used for comparison. These included the well-established nitrone

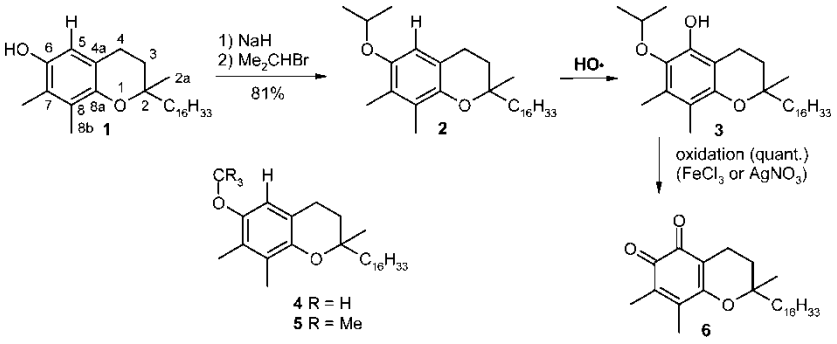

Figure 1. Synthesis of the hydroxyl radical trap γ-tocopheryl-*iso*-propyl ether, and its trapping reaction and detection as the red *ortho*-quinone **6**.

based[13,14,18,19] and pyrroline-*N*-oxide based[13,20,21] spin traps. The reaction system for the generation of alkoxyl and alkylperoxyl radicals consisted of peroxidized linoleic acid and the respective spin trap dissolved in 20 mM phosphate buffer, pH 7.4, containing 1% acetonitrile. To this solution Fe^{2+}, dissolved in nitrogen-purged water, was added in order to start the formation of free radicals in a Fenton-type reaction, as recently tested with different DEPMPO[20] or EPPN[13] spin trap derivatives. The starting linoleic acid hydroperoxide was synthesized according to O'Brien,[22] and its concentration was determined by UV spectroscopy based on an extinction coefficient of $\varepsilon_{233\,nm} = 25250\ M^{-1}\ cm^{-1}$ in ethanol.

Superoxide radical adducts were generated in the xanthine/xanthine oxidase system at pH 7.4. Used as a blank, no adducts were obtained in the additional presence of superoxide dismutase (SOD). Furthermore, an incubation system with solid KO_2 was used as the source of superoxide anion radicals. A Fenton system was used for the generation of hydroxyl radicals, and a Fenton system in the presence of 5% methanol according to Roubaud et al.[23] for the formation of the respective hydroxymethyl radical. Methoxyl radicals were generated according to Dikalov and Mason.[24]

With the exception of the hydroxyl radical, the presence of all radicals tested caused the formation of spin adducts from the nitrone- and pyrroline-derived spin traps used, but in no case any chemical changes of spin trap **2**. This result was independent of the use of monophasic, biphasic, or micellar reaction systems. In OH radical generating systems, the trapping product of **2** was found, and the respective OH radical adducts were formed from the nitrone- and pyrroline-derived spin traps. This confirmed the trapping selectivity of **2** for hydroxyl radicals.

It is interesting to note that the trapping selectivity of **2** is obviously due to a combination of both electronic and steric effects. The aromatic part in tocopherols is electron-rich, which is the prerequisite for a high trapping efficiency toward electrophilic radicals. The electron density is increased by the

mesomeric effects of the two oxygens at C-6 and C-8a and the inductive influence of the alkyl groups (two methyl groups at C-7 and C-8, and the alkyl chain of the pyran ring attached to C-4a). γ-Tocopheryl-methyl ether (**4**, Figure 1) can be regarded as a model trap in which only the electronic contributions are active because the methyl substituent is sterically innocent, exerting no influence on the neighboring non-substituted aromatic position. Methyl ether **4** reacts with hydroxyl radicals about one order of magnitude faster than with hydroperoxyl, alkylperoxyl, and alkoxyl radicals, taking the yields of the respective trapping products as a rough indicator of the trapping rates.

In the case of bulkier alkoxy substituents, the electronic effects act in conjunction with the steric factors. The high selectivity of **2** is thus also caused by the steric effect of the *iso*-propoxy substituent as compared to the methoxy group. On the other hand, the *iso*-propyl group is still small enough to allow hydroxyl radicals to approach the neighboring aromatic position. A *tert*-butyl structure as in γ-tocopheryl-*tert*-butyl ether (**5**, Figure 1), carrying only one CH_3 group more than **2**, has apparently lost this property, as hydroxyl radicals attack this molecule almost randomly, with only a slight preference for the 5 position remaining.

The trapping reagent offers two additional advantages. First, it possesses only one aromatic position free for hydroxylation, which limits the number of hydroxylation products and facilitates analysis. Second, the spin trap and its reaction products are strongly lipophilic molecules due to the isoprenoid C_{16} side chain of tocopherols, and are thus readily extractable by apolar solvents, such as petroleum ether or *n*-hexane, even from very complex systems. They can thus be retrieved from complex matrices also in the presence of other aromatic compounds, which, because of their higher hydrophilicity, are not extracted into the apolar solvent. For analysis, the hydroxylation product is separated by adsorption onto basic alumina and subsequently oxidized in a quantitative reaction to α-tocored (**6**), a deeply red-colored *ortho*-quinone, which is then determined spectrophotometrically.

Besides the trapping reaction, the spin trap undergoes some side reactions, mainly loss of the 2-propyl moiety followed by deoxygenative coupling of the liberated phenol, but also conversion to undefined products. The ratio between trapping reagent and by-products ranges between 4 : 1 and 3 : 1, independent of the degree of conversion. Although the by-products are extracted as well because they possess the lipophilic side chain, they are readily separated from the hydroxylated product as they lack the free phenolic hydroxyl group and thus elute much faster from the alumina column.

Hydroxyl Radical Trapping in Alkali Cellulose during Aging

Aging of the alkali cellulose was performed at two temperatures, 35°C and 40°C, over a time of 19 h. The spin trap was added during shredding of the

alkali cellulose. After 10 min, a sample was taken as a blank, further sampling was done after 3, 5, and 19 h. The samples were extracted with petroleum ether to remove all tocopherol products. The trapping product was separated, oxidized, and analyzed by UV spectroscopy as described in the experimental section. This way, the presence of hydroxyl radicals during ripening of alkali cellulose was proven for the first time. The α-tocored (**6**) content corresponds to the amount of hydroxyl radicals trapped at the respective time (see Figure 2). It should be noted that this number is an integral value, reflecting the amount of hydroxyl radicals trapped from the beginning of the aging process to a specific time, and is not a "snapshot" of the radical concentration at the respective point of time.

A quantification of the total amount of generated hydroxyl radicals cannot be performed (as it cannot with any other trapping method) because hydroxyl radicals are an overly aggressive chemical species, reacting with any structure in their immediate environment. However, the aging reactions in the absence and presence of the radical trap can be compared to yield relative values. Possible differences in the aging kinetics are thus attributable to the trapped hydroxyl radicals, with the "missing amount" of OH radicals being equal to the amount of hydroxylation product isolated.

Analysis of the trapping product showed that OH radical production was quite intense in the initial phase of aging. In the later stages, radical production gradually slowed down.[25] About 15% of the reagent was consumed within the first 3 h, nearly 30% at the end of the aging procedure, that is after 19 h. Thus, about one half of the trapped OH radicals was generated in the first sixth of the overall aging time. Figure 2

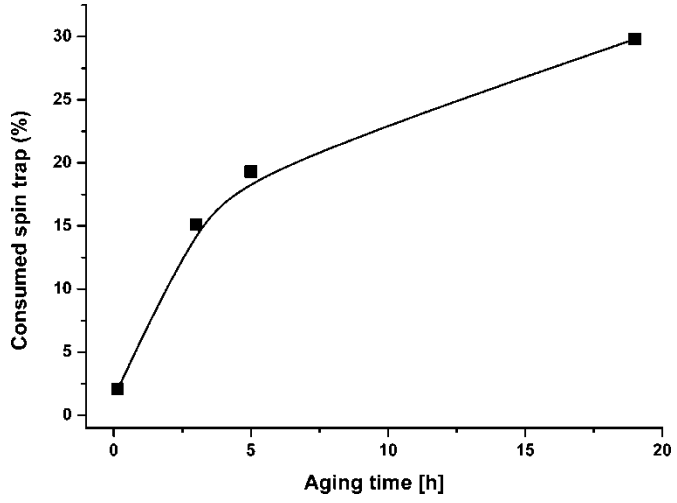

Figure 2. Hydroxyl radical trapping in aging alkali cellulose. The amount of trapping product as a percentage of consumed spin trap *versus* reaction time.

shows the amount of hydroxylated product isolated, that is, of hydroxyl radicals trapped, as percentage of the starting material introduced. This allowed the interesting conclusion that hydroxyl radicals were mainly active in the initial phases of aging, but less involved in later stages. This appears reasonable as alkalization of cellulose under oxygen is known to produce a variety of low-molecular weight (aromatic) compounds[26] and semi-stable radicals, all of which act as sacrificial substrates trapping hydroxyl radicals. These compounds either originate from cellooligosaccharides and xylooligosaccharides already contained in the starting pulp, or from low-molecular weight compounds generated from cellulose or hemicelluloses degradation (β-alkoxy elimination, etc.). The concentration of these species will be low in early aging phases, so that hydroxyl radicals predominantly react with the trap. In later stages, reaction with the degradation products would become increasingly competitive with the trapping process, so that less hydroxylated spin trap is formed.

This observation agreed very well with the observed cellulose chain degradation (see Figure 3). The DP loss was pronounced in the early alkalization stage, and became slower with longer reaction times, parallel to the activity of hydroxyl radicals as detected through the trapping approach. The radicals present (this applies to other radicals as well) appeared to attack the alkali cellulose mainly in the early phases of the aging procedure. In later phases, reactions of the radicals with low-molecular weight products became more and more dominant, so that in the later stages of aging, cellulose degradation could be assumed to be caused more and more by ionic rather than radical processes. Determination of the ratio between overall homolytic and overall heterolytic reactions—as performed recently for the cellulose/*N*-methylmorpholine-*N*-oxide/water system[27]—would be able to provide a more comprehensive picture of the ionic *versus* radical processes responsible for cellulose degradation in aging alkali cellulose, including their dependence on aging time.

Figure 3 shows a clear difference between the cellulose degradation in the absence of any additive and in the presence of the radical trap. The presence of γ-tocopheryl isopropyl ether (**2**) in aging alkali cellulose slowed down chain cleavage and thus impeded the rate of the DP loss.[28] This is indirect evidence of hydroxyl radicals being present during ripening of alkali cellulose because trap **2** scavenges only OH radicals. Further, the amount of trapped OH radicals can be correlated to the lack of chain degradation. It should be noted that this does not mean that the OH radicals, which hydroxylated the trap, directly attack and cleave the cellulose chain in the absence of the trap. Instead, they initiate a complex set of reactions, which as a whole result in the chain degradation observed in the absence of the trap. These processes, for instance, might comprise generation of secondary radicals that attack the cellulose, or introduction of oxidized functionalities into the cellulose chain followed by base-induced cleavage (β-elimination).

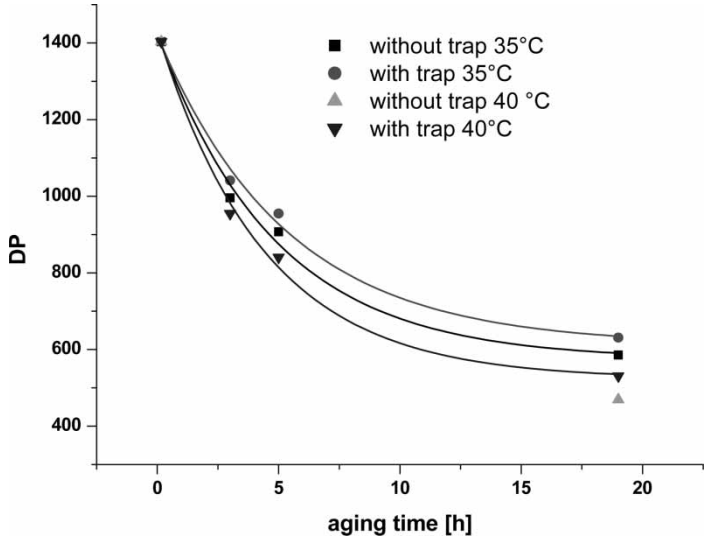

Figure 3. DP loss in the absence and presence of the OH-selective spin trap γ-tocopheryl-*iso*-propyl ether (**2**) in the aging of alkali cellulose. The data points are the average of three independent experiments with double runs of the DP measurements, the errors being below 4.5%.

The contribution of OH radicals to the overall cellulose degradation is comparatively small; by far the larger part must be attributed to the action of different radicals, reactive oxygen species, and heterolytic processes. The aging temperature had the expected strong effect; the cellulose degradation proceeded faster with increasing temperature (Figure 3). The effect of the hydroxyl trap was in principle the same at both temperatures investigated,[29] 35°C and 40°C: the DP loss was slowed down.

EXPERIMENTAL

General

Chemicals were purchased from commercial suppliers and used without further purification. All solvents used were HPLC grade. Thin layer chromatography (TLC) was performed on silica gel 60 plates (5 × 10 cm, 0.25 mm) with fluorescence detection using UV light at 254 nm. Column chromatography was performed on silica gel G60 (40–63 μm). ^{1}H NMR spectra were recorded at 300.13 MHz, ^{13}C NMR spectra at 75.47 MHz with $CDCl_3$ as the solvent and TMS as the internal standard. Resonances are given in ppm. ^{13}C peaks were assigned by means of APT, HMQC, and HMBC

spectra. The resonances of the isoprenoid side chain of tocopherol[30] are not listed, as they are not affected by modifications of the chroman ring.

Synthesis of γ-Tocopheryl-*iso*-propyl Ether (2)

Under an argon atmosphere, γ-tocopherol (6.25 g, 15 mmol) dissolved in toluene (50 mL) was added to a suspension of NaH (0.25 g, 10.5 mmol) in toluene (20 mL). The mixture was stirred for 1 h at room temperature and 2-bromopropane (1.00 g, 8.15 mmol) in 10 mL of toluene was added dropwise with stirring. The yellow, cloudy mixture was heated at 50°C for 1 h with stirring, cooled to room temperature, and filtered. The residue was suspended in EtOH to destroy excess NaH, and was discarded. The filtrate was washed with water, dried over $MgSO_4$, and the solvent was evaporated *in vacuo*. The residue was dissolved in *n*-hexane (5 mL) and chromatographed on silica gel (*n*-hexane/toluene; 9 : 1, vol) to yield **2** (6.04 g, 13.1 mmol, 87.7%) as a colorless oil. 6-Isopropoxy-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-chroman, ^{1}H NMR (r.t.): δ 6.34 (s, 1H, ^{Ar}H), 4.85 (sept, 1H, Me_2HC-O), 2.65 (t, 2H, H-4), 2.12 (s, 3H, H-8b), 2.09 (s, 3H, H-7a), 1.64 (m, 2H, H-3), 1.28 (d, 6H, Me_2HC-O). ^{13}C NMR: δ 147.6 (C-8a), 145.9 (C-6), 126.1 (C-8), 122.5 (C-7), 117.8 (C-4a), 113.6 (C-5), 75.5 (C-2), 69.5 (Me_2HC-O), 31.0 (C-3), 24.1 (C-2a), 22.8 (C-4), 21.4 (Me_2HC-O), 12.3 (C-8b), 11.9 (C-7a). Anal. calcd. for $C_{31}H_{54}O_2$: C 81.16, H 11.86; found: C 81.11, H 12.03.

Aging of Alkali Cellulose

Bleached beech sulfite pulp (33 g, o.d.) was suspended in 18.9% NaOH at a NaOH-to-cellulose ratio of 18 : 1. After pressing off the alkaline solution, the press cake was immediately shredded after addition of 0.5 g of hydroxyl trap dissolved in 10 mL of diethyl ether to provide an even distribution of the trap within the alkali cellulose. The large surface area after shredding guarantees a uniform reaction with atmospheric oxygen. The alkali cellulose was agitated in a tumbler at 35°C. Cellulose samples were taken 10 min after adding the trap, and again after 3, 5, and 19 h of aging and extracted with petroleum ether. After washing out the alkali, the viscosity of the regenerated cellulose sample was analyzed.[31] For comparison a blank run employing a sample without the spin trap under otherwise identical conditions was performed.

Analysis and Quantification of Trapping Products

The petroleum ether extracts obtained during the aging experiments were washed with 1M HNO_3, dried over $MgSO_4$, and the solvent was removed

in vacuo. The residue was adsorbed on basic alumina, and the by-products were eluted with petroleum ether. Elution with toluene/MeOH (20:1, vol) provided the hydroxylated product 5-hydroxy-6-*iso*-propoxy-γ-tocopherol (**3**). The solution was evaporated to dryness and dissolved in 5 mL of ethanol. $AgNO_3$ solution (5 M, in 2 M HNO_3) was added to a total volume of 10 mL. The mixture was kept 20 min at 50°C, filtered, and analyzed by UV spectrometry (420 nm). Oxidation to α-tocored (**6**) proceeded quantitatively without side reactions. Calibration was performed with an authentic sample of **6**, giving a linear calibration plot in the range between 0.1 mM to 1 M.

CONCLUSIONS

A spin trap selective for hydroxyl radicals that can operate under strongly alkaline conditions (concentrated NaOH) was synthesized. Employing this trap, the presence of OH radicals during aging of alkali cellulose—as performed in the "pre-ripening" step of viscose production—was demonstrated for the first time. Hydroxyl radicals contribute only to a minor degree to the overall cellulose degradation. The action of OH radicals is especially pronounced in the earlier phase of the aging reaction, in later stages the effect of OH radicals is increasingly attenuated by competitive reactions with (aromatic) carbohydrate degradation products. Addition of hydroxyl-selective trapping agents might prove to be a way to lower the contribution of the extremely reactive and thus non-selective OH radicals to the overall cellulose chain degradation, favoring more selective radical or ionic degradation processes.

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