Light-induced yellowing of selectively ^{13}C-enriched dehydrogenation polymers (DHPs). Part 1. Side-chain ^{13}C-enriched DHP (α, β, and γ-^{13}C)

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SUMMARY: Light-induced yellowing has been studied using side-chain (α, β, and γ) ^{13}C-enriched DHP (dehydrogenation polymer) and quantitative solution state ^{13}C NMR spectroscopy. The DHP was formed from ^{13}C-enriched coniferin using an enzymatic system consisting of β-glucosidase, glucose oxidase, and peroxidase in a pH 6 buffer solution. The DHP was applied to filter paper, irradiated with UV/VIS light and the photodegraded DHP was extracted and analyzed. The NMR study revealed a drastic decrease in the amount of coniferyl alcohol end groups with the formation of end groups of the vanillin type and small amounts of end groups of the vanillic acid type. The results indicated a moderate formation of α-carbonyls, however, no significant decrease in the amount of β-O-4 structures in the extractable part of the irradiated DHP could be established, based on difference spectra corresponding to the ^{13}C-enriched DHP.

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Aging in the form of light-induced yellowing is the main reason why mechanical pulps have limited use in high-quality paper grades. The lignin in mechanical pulps undergoes photooxidation when subjected to ultraviolet and visible light causing a brightness loss and a yellow tone in the paper, leading to unacceptable aging performance of the product. There are both economic and environmental advantages to be gained if photoyellowing can be inhibited or if the yellowing rate can be slowed down so that mechanical pulp can be used to a larger extent in high-quality paper products. The treatment to retard or slow down photoyellowing must preferably be cheap and safe in order to be industrially applicable. Although recent research has given valuable insight into the mechanism of light-induced yellowing (Gratzl 1985; Heitner 1993; Leary 1994; Davidson 1996; Forsskåhl 2000) there is still some controversy, for instance, regarding the nature of the initially formed colored structures (*ortho*- and/or *para*-quinones) and the contribution of β-aryl ether cleavage to color forming reactions.

The study of lignin reactions during technically important processes is obstructed by the fact that there are no applicable methods for quantitative isolation of lignin from pulp or paper material in a chemically unchanged form. The use of lignin model compounds representative of single substructures in native lignin has been widely employed for the study of, for example, photoyellowing reactions. The advantage of this method is that the identification of the product mixture is relatively straight-forward compared to when many different types of inter-unit linkages are present, such as in milled wood lignin, MWL. One disadvantage could be that simple lignin models might not react in the same way as when integrated in a lignocellulosic material. Furthermore, many investigations have been conducted with lignin models in solution and with high-energy UV-lamps as the irradiation source which may influence the outcome of the experiment and make it difficult to relate the results to an actual case with carbohydrates present and with lower-energy UV/VIS light as the irradiation source (sunlight and indoor lighting). A thorough review of the photochemistry of lignin model compounds has recently been published (Lanzalunga, Bietti 2000).

Dehydrogenation polymers (DHPs) can be seen as high-molecular weight lignin model compounds. It is generally accepted that the structure of DHP differs from that of milled wood lignin, which is considered to be the lignin preparation that best represents native lignin. The amount of end groups (especially of the coniferyl alcohol type) and the amount of structures of the β-β and β-5 type is higher and the amount of β-O-4 structures is lower in DHP than in milled wood lignin (Nimz, Lüdemann 1976; Brunow, Lundquist 1980; Terashima et al. 1995, 1996a). However, the major types of structural units in lignin are present also in DHP, although not necessarily in the same proportions. Dehydrogenation polymers are produced by enzymatic dehydrogenation of for example coniferyl alcohol, usually by hydrogen peroxide/peroxidase or oxygen/laccase (Freudenberg, Richtzenhain 1943; Freudenberg 1968). Another possibility is to start with coniferin (6-glucoside of coniferyl alcohol) and an enzymatic system consisting of β-glucosidase, glucose oxidase, and peroxidase, in which case no external hydrogen peroxide is needed since it is formed during the oxidation of liberated glucose (Terashima et al. 1995, 1996a). The preparation of selectively ^{13}C-enriched DHP by using ^{13}C-enriched precursors renders the possibility to produce difference spectra (^{13}C-enriched DHP - unenriched DHP) and ideally it is possible to track the chemical changes of the single ^{13}C-enriched carbon by

^{13}C NMR spectroscopy, in solution or solid state. Studies of selectively ^{13}C-enriched DHP (enriched at the side-chain, position 4 in the aromatic ring, and at the methoxyl carbon) have previously been published (Gagnaire, Robert 1977; Ellwart et al. 1981; Lewis et al. 1987; Botto 1988) and as an example, ^{13}C-enriched DHP has been used to investigate microbiological degradation of lignin (Haider et al. 1985).

In this study, which is a continuation of previous studies of light-induced yellowing using specifically α-, β-, and γ-^{13}C-enriched cell wall dehydrogenation polymers (CW-DHPs) and solid state ^{13}C NMR spectroscopy (Parkås et al. 2001, 2002), specifically α-, β-, and γ-^{13}C-enriched ,dehydrogenation polymers (DHPs) of the traditional kind (without cell walls) have been prepared and ,applied on filter paper. The DHP-impregnated sheets were then subjected to accelerated light-induced yellowing and the structure of the extractable photo-degraded DHP was subsequently determined by quantitative ^{13}C NMR spectroscopy in solution state.

Experimental

Coniferin synthesis

Coniferin, α-, β-, and γ-^{13}C-enriched as well as unenriched, was prepared according to a previously published method by Terashima and coworkers (Terashima et al. 1996b). The ^{13}C enrichment degrees at the labeled sites of the coniferins were approximately 99%, 88%, and 86% for α-, β-, and γ–enriched, respectively as determined from solution state ^{1}H NMR.

DHP preparation

Dehydrogenation polymers, ^{13}C-enriched as well as unenriched, were prepared from coniferin mainly according to Method A described by Terashima and coworkers (1995). Coniferin (300 mg unenriched or 150 mg ^{13}C-enriched + 150 mg unenriched) was dissolved in 30 ml phosphate buffer of pH 6 (0.1 M) in an Erlenmeyer flask. β-Glucosidase (60 u, almonds, Sigma-Aldrich, Sweden), glucose oxidase (70.5 u, *Aspergillus niger*, Sigma-Aldnch, Sweden), and peroxidase (69 u, horse-radish, Sigma-Aldrich, Sweden) was added and the temperature was kept at 31-32°C during the entire DHP-formation period of 77 hours. After 24 hours, the same amount of the three enzymes was added again and the pH was adjusted to 6 every 24 hours with dilute NaOH. After completed DHP formation, the suspensions were centrifuged and the solid residues were freeze-dried. Purification of the DHP was performed by dissolution of the material soluble in 1,2-dichloroethane (DCE)/ethanol (EtOH, 99.5%) (2:1, 2.25 ml) and precipitation in dry ether (34 ml). The DHP was collected by centrifugation, a small amount of petroleum ether was added and after re-centrifugation, the DHP was dried. The yield of purified DHP averaged 75.9 mg (corresponding to about 48% of the coniferyl alcohol moiety in the added coniferin). The structure of the formed DHP was subsequently investigated by solution state ^{13}C NMR spectroscopy.

Application of DHP on filter paper

Air-dried, extracted (1,2-dichloroethane/ethanol; 2:1) filter papers (Analytical grade 00A, diameter 5.5 cm, Munktell Filter AB Sweden) were impregnated with the DHPs dissolved in a small amount of DCE/EtOH (2:1) until the desired weight increase was reached. In the case of the ^{13}C-enriched samples, equal amounts of ^{13}C-enriched DHP and unenriched DHP were mixed and applied. The amount of DHP on the filter papers was determined by weighing the sheets in air-dry state. The amount of applied DHP to each filter paper was approximately 47 mg and one filter paper for each labeled position was used. The sheets were always kept in the dark when not handling them.

Accelerated light-induced yellowing and determination of optical properties

Accelerated light-induced aging was performed in a SUNTEST CPS (Hereus HANAU) equipped with a xenon lamp and filters (ultraviolet and window glass) excluding light with a wavelength shorter than 310 nm. The spectral characteristics of the light source have been described elsewhere (Paulsson, Ragauskas 1998). The sheets were irradiated for equal times on both sides and the optical properties were measured after fixed times. ISO-brightness and color changes according to the CIE-LAB color scale (L*-, a*-, and b*-values) were measured after 0, 1, 4, and 21 hours of irradiation on each side of the sheet with an Elrepho 2000 spectrophotometer. For measuring of R_∞, an opaque stack of unextracted filter papers was used as the background for all samples. Optical properties were measured on both sides of the sheets after each irradiation period.

Extraction of the photo-aged DHP

The sheets were cut into smaller pieces and extracted with DCE/EtOH (2:1, 5 ml), for 2x24 h + 1x48 h with new extraction solvent after each period (extractions were performed under dark conditions). The combined extracts were evaporated to dryness on a rotary evaporator and the weight of the extracted DHP was determined. On average, 42 mg of photooxidized DHP was isolated. This constitutes approximately 89% of the applied weight of the DHP (the molecular weight could, however, differ from the original).

Table 1. Optical properties of unirradiated and irradiated DHP-impregnated filter papers. UE = unenriched, α, β, γ = ^{13}C-enriched in positions α, β, and γ, respectively.

Irradiation time on each side (h)	Brightness (%ISO)				b*			
	UE	α	β	γ	UE	α	β	γ
0	55.6	52.6	54.3	53.3	21.5	23.2	22.1	22.4
1	54.8	53.1	53.4	54.6	20.1	20.8	20.5	20.0
4	52.6	50.9	50.6	52.6	20.7	21.7	21.7	20.8
21	38.4	35.5	36.6	36.3	28.2	29.7	29.1	29.3

Solution state ^{13}C NMR spectroscopy

The ^{13}C NMR data for DHPs were obtained with a Bruker DPX-250 spectrometer (62.9 MHz carbon) fitted with a 5 mm quadranuclear probe. The sample concentration was 40 mg DHP in 400 µl of DMSO-d_6. Quantitative ^{13}C relaxation delay was 15 seconds and sample temperature was 300 K. 3800 transients (16K data points) were

Results

Optical properties of DHP-impregnatedfilter paper before and after irradiation

The optical properties of the DHP-impregnated sheets before and after irradiation are shown in *Table 1*. The optical properties are given as average values for both sides of the sheets. The brightness decreased, on average, from 54% before irradiation to 37% after irradiation for 21 hours on each side corresponding to an average PC (Post Color)-number due to irradiation of approximately 34. The variation in initial brightness may be due to some difficulties in applying the DHPs evenly to the filter papers. During the same period of time (2x2 1 hours), the b* -value increased on average from 22 to 29, i.e. the sheets indeed yellowed upon irradiation. Furthermore, during irradiation, the a* -values increased somewhat and the L* -values decreased to some extent (not included in *Table 1*). The changes in optical properties observed during irradiation are what could be expected from previous studies of light-induced yellowing of high-yield pulps (see, e.g. Paulsson et al. 1996). The optical properties (ISO-brightness and b* -values) of extracted filter paper without DHP applied and aged for the same period of time, did not change significantly during the irradiation period of 2x21 hours. Since the sheets were cut into smaller pieces, the optical properties after irradiation and extraction were not determined. A later complementary study has been performed to determine the extent of color remaining in the sheets after irradiation and extraction (without cutting them) and to try to conclude whether or not the amount of material remaining in the sheets after extraction had to do with the irradiation. About 55 mg of unenriched DHP was added to each of two filter papers (brightness 91.0%, b*-value 2.4). The resulting brightness was, in average, 52.7% and the b*-value was 25. When extracted without prior irradiation, the brightness rose to 89.5% and the b*-value decreased to 3.2. Furthermore, the whole weight of the applied DHP was recovered in this case. After irradiation (2x21 h), the brighness decreased to 35.0 and the b*-value increased to 30, but this time, the extraction did not result in restored optical properties (resulted in a brightness of 54.0% and a b*-value of 20). The amount of extracted DHP, in this case, consituted 93% of the applied weight. This clearly shows that the failure to recover the whole quantity of the DHP is due to the irradiation, since the DHP can be extracted quantitatively from the sheets prior to irradiation, and that colored materials, not soluble in DCE/EtOH, are formed during photoyellowing.

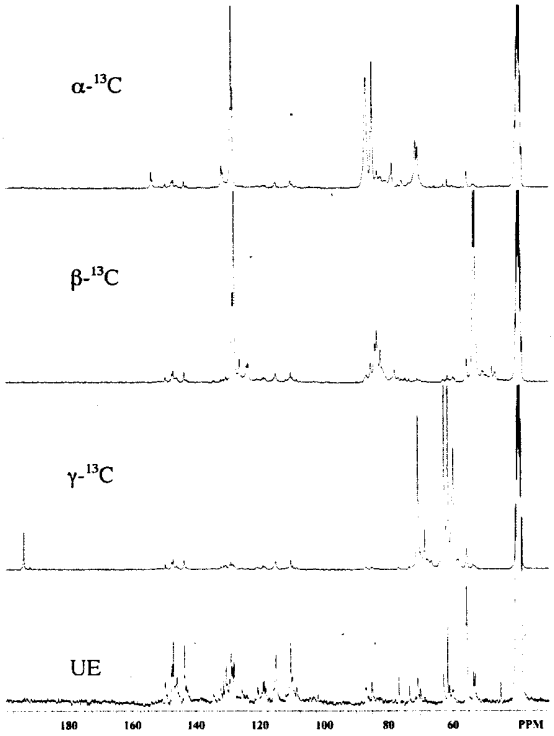

Fig 1. Quantitative ^{13}C NMR spectra of unirradiated α-, β-, γ-^{13}C DHP and unenriched (UE) DHP.

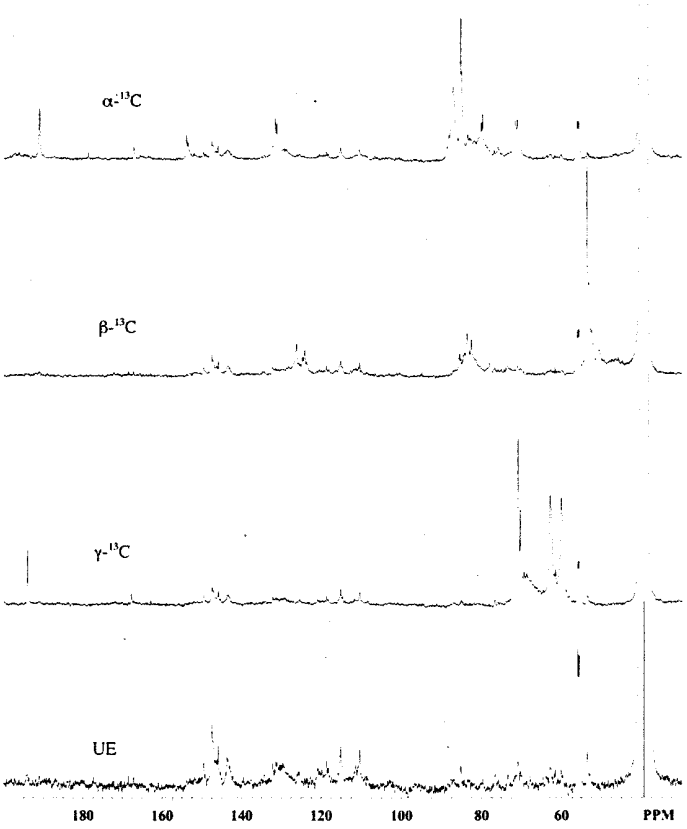

Fig 2. Quantitative ^{13}C NMR spectra of irradiated (2x21 h) α-, β-, γ-^{13}C DHP and unenriched (UE) DHP.

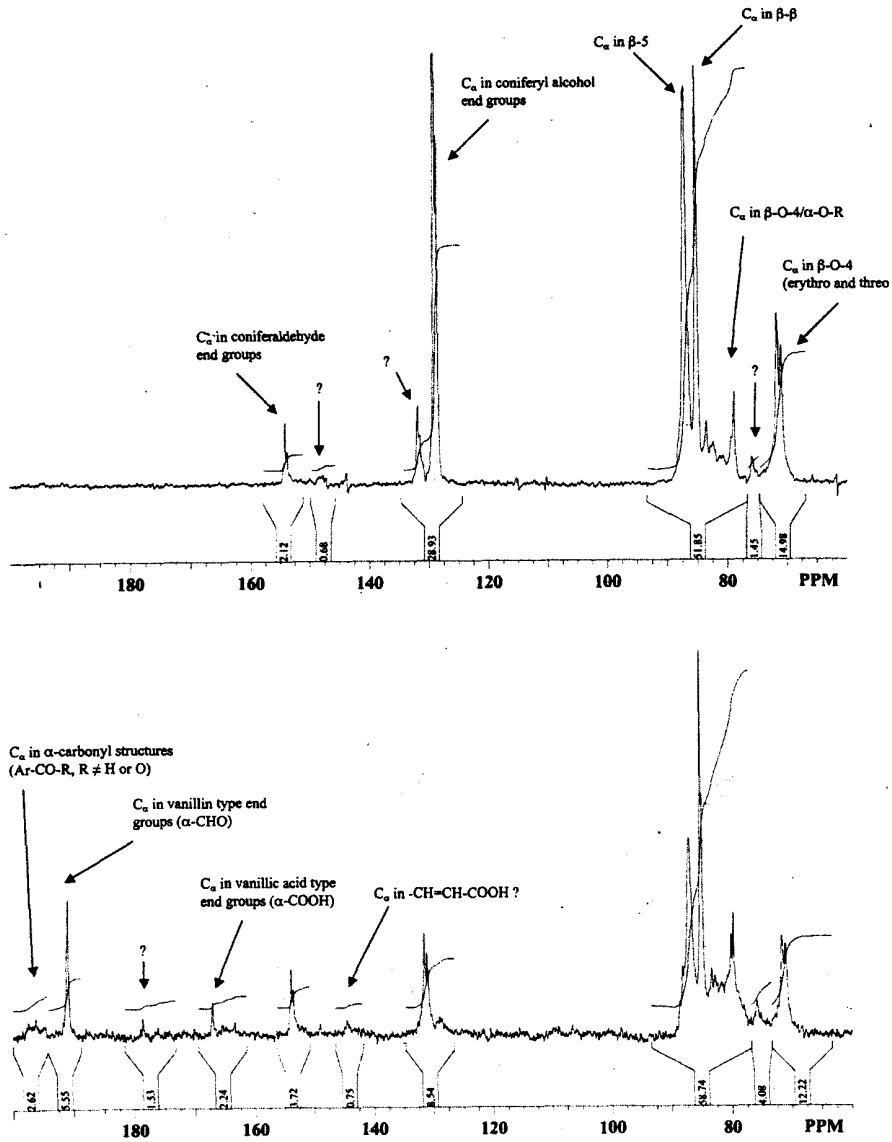

Fig 3. Difference spectra (^{13}C-ennched - unenriched) of unirradiated (top) and irradiated (2x21 h, bottom) α-^{13}C-DHP

^{13}C NMR analysis

Fig 1 shows the ^{13}C NMR spectra of α-, β-, and γ-^{13}C-enriched as well as unenriched DHP before irradiation and Fig 2 shows the corresponding spectra of the extractable part of the DHP after irradiation for 21 hours on each side of the filter paper. The difference spectra presented in the following figures are produced by subtracting the spectra of unenriched DHP from the spectra of ^{13}C-enriched DHP (^{13}C-enriched - unenriched). The relative signal areas in the difference spectrum correspond to the fractions of ^{13}C-labeled carbons in different substructures. It should be noted that since not all of the DHP applied on the filter papers was extractable after irradiation. the NMR-analysis is of the soluble part only (approximately 89% of the applied weight, cf. Experimental section). Studies aimed at analyzing the non-extractable part of the DHP by solid state ^{13}C NMR spectroscopy are underway. It has to be acknowledged that during photodegradation some loss of label might occur, which could influence the interpretation of the results. We have, however, no evidence that such loss does occur. The assignment of the signals is mainly based on the database published by Ralph and co-workers (1998).

α-^{13}C-enriched DHP

Fig 3 presents the difference spectra corresponding to unirradiated (top) and irradiated (21 hours on each side of the filter paper, bottom) α-^{13}C-enriched DHP. The integrals are included in the figure for easier comparison, the total area under the individual integrals was set to 100. The peaks that can be discerned in the unirradiated a-^{13}C-enriched DHP are the following (cf. *Fig 3* and *Table* 2): C_α in arylglycerol-β-aryl ether structures (*erythro* and *threo*) and, if present, β-1 structures, C_α in β-O-4/α-O-R structures, C_α in β–β structures, C_α in β-5 structures, C_α in coniferyl alcohol end groups, and C_α in coniferaldehyde end groups. The peak at 83.5 is assigned to C_α in dibenzodioxocin structures and is consistent with NMR data of model compounds. 2-D C-H correlation spectra run on the acetylated, unirradiated DHP also indicated the presence of structures of the dibenzodioxocin type. The signal at 130-132.5 ppm (the α-carbon of an unsaturated side-chain) is, at present, not conclusively assigned. This type of unassigned signal was also reported by Gagnaire and Robert (1977) in their study of α-^{13}C-enriched DHP (a broad signal centered at 132 ppm). It can be seen that the DHP has the structural features previously reported, i.e. with more end groups of the coniferyl alcohol type and with more β-β and β-5 and fewer β-O-4 structures than milled wood lignin. After irradiation (*cf. Fig 3 bottom spectrum*) it can be seen that the most drastic change is an almost complete removal of coniferyl alcohol end groups, while the amount of end groups of the coniferaldehyde type has increased slightly. A slight decrease in the area corresponding to C_α in arylglycerol-β-aryl ether structures (70-72.5 ppm) can also be noted. The decrease in this region can be due to other chemical changes in the vicinity of the a-carbons than β-ether cleavage. New peaks that appear are assigned to C_α in end groups of the vanillin (191 ppm, α-CHO) and vanillic acid (minor peak at 167 ppm, α-COOH) type. Furthermore, formation of a-carbonyls (Ar-CO-R, R ≠ H or O) upon irradiation is indicated by the presence of a broad resonance centered at about 196 ppm in the difference spectrum of irradiated α-^{13}C-enriched DHP.

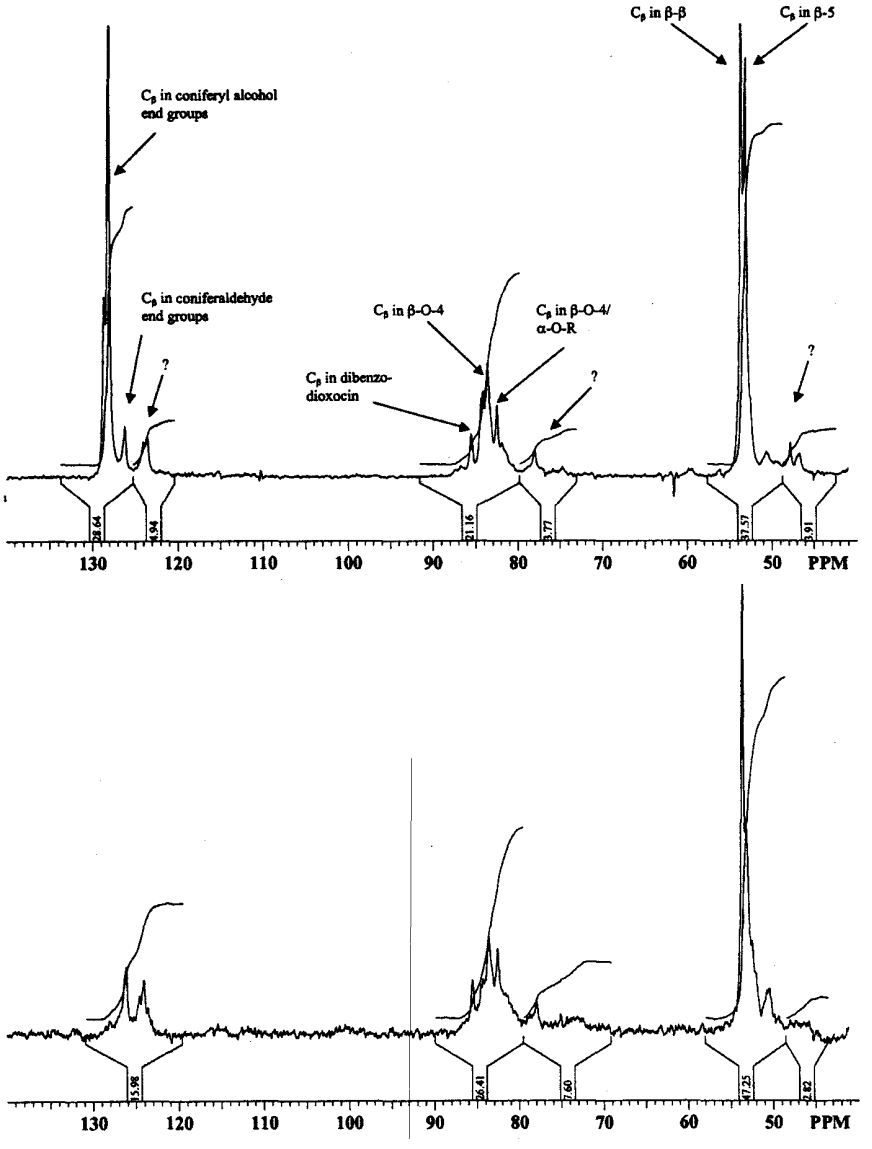

Fig 4. Difference spectra (^{13}C-enriched-unenriched) of unirradiated (top) and irradiated (2x21 h, bottom) β-^{13}C DHP.

The small peak at rouund 144 ppm may originate from the Ca in ferulic acid type end groups (Ar-CH=CH-COOH), while the small peak at 178 ppm is unassigned at present. A build-up os intensity can be noted in the region of 78-90 ppm as a whole and an increase in the relative signal area of the unassigned peak at 130-132.5 ppm.

β-^{13}C-enriched DHP

Fig 4 shows the difference spectra corresponding to unirradiated (top) and irradiated (21 hours on each side of the filter paper, bottom) β-^{13}C-enriched DHP. Assigned peaks are (*see also Table 2*): C_β in β-5 structures, C_β in β-β structures, C_β in β-O-4/α-O-R structures, C_β in β-O-4 structures (arylglycerol-β-aryl ether structures), C_β in coniferaldehyde end groups, and C_β in coniferyl alcohol end groups. The peak at 85-86 ppm is assigned to the β-carbon of dibenzodioxocin structures (cf. the section on α-^{13}C DHP). Not conclusively assigned peaks can be seen at 46-51 ppm, ~78 ppm, and 123-125 ppm. No resonance that can be clearly attributed to C_β in β-1 structures can be discerned in the spectrum, if present the amount is at a sub-detectable level.

As in the case of α-enriched DHP, it is evident that the amount of coniferyl alcohol end groups decreases to almost zero upon irradiation (*cf: bottom spectrum in Fig 4*) and that the amount of coniferaldehyde end groups seems to increase somewhat. There is a build-up of intensity in the broad area containing β-O-4 structures (80-87 ppm) together with an increased broadening of the region, and it is hard to conclude how the arylglycerol β-aryl ethers have been affected due to the overlapping signals. However, it seems that no significant degradation of β-ethers have occurred. The signal area in the region of 49-58 ppm has increased, and this is mainly due to the growth of the area of the unassigned signal at 50-52 ppm. Furthermore, the relative area of the unassigned peak at 123-125 ppm has increased during irradiation.

γ-^{13}C-enriched DHP

Fig 5 presents the difference spectra corresponding to unirradiated (top) and irradiated (21 hours on each side of the filter paper, bottom) γ-^{13}C-enriched DHP. Identifiable signals

Table 2. Assignments and peak positions of the major resonances in the difference spectra corresponding to α-, β-, and γ-^{13}C-DHP

	Chemical shift (ppm)		
Type of structure	C_α	C_β	C_γ
Arylglycerol-β-aryl ethers (*egthro* and *threo*)	70-72.5	83-85	59-61
β-O-4/ α-O-R	78-80	82-83	59-61
6-5	86-88	~53	62-63
β-β	84-86	~53.8	70.5-71.5
Coniferaldehyde end groups	153-155	125.5-127	~194
Coniferyl alcohol end groups	128-130	127-129.5	61-62
Dibenzodioxocin[a]	~83.5	85-86	59-61
Vanillin type end groups	~191		
Vanillic acid type end groups	~167		
α-Carbonylic structures[b]	~196		

[a]The α,β-etherified side-chain carbons in the dibenzodioxocin structures

[b]Apart from vanillin and vanillic acid type end groups

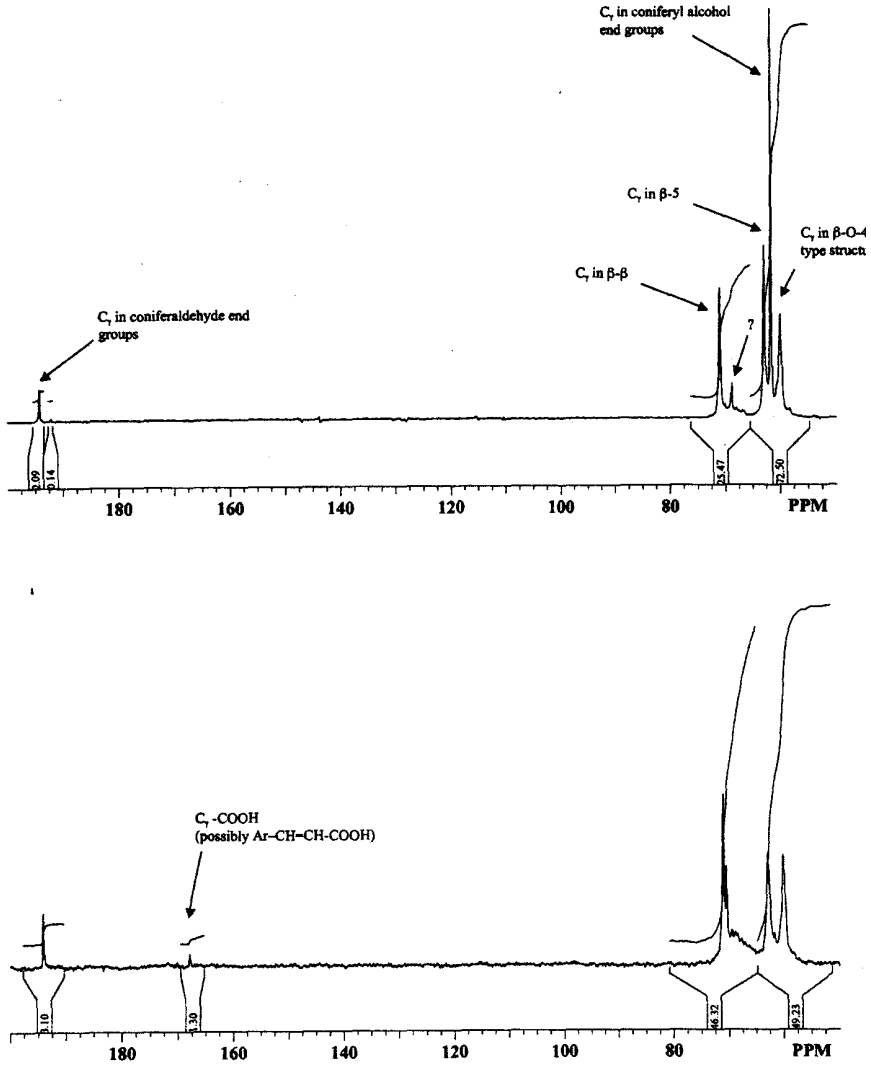

Fig 5. Difference spectra (^{13}C-enriched - unenriched) of unirradiated (top) and irradiated (2x21 h, bottom) γ-^{13}C DHP.

are: C_γ in arylglycerol-β-aryl ether structures (+α-etherified β-ethers including dibenzodioxocin structures), C_γ in coniferyl alcohol end groups, C_γ in β-5 structures, C_γ in β-β structures, and C_γ in coniferaldehyde end groups. Based on this spectrum, we cannot be sure whether or not β-1 structures are present at a detectable level, since the C_γ in these structures would appear at more or less the same shift as the C_γ in coniferyl alcohol and β-5 structures (around 62.2 ppm). An unidentified peak occurs centered at 68.8 ppm. After irradiation (*cf. Figure 5 bottom spectrum*), the peak corresponding to coniferyl alcohol end groups almost completely disappears and the peak corresponding to coniferaldehyde end groups increases slightly, in accordance with the results obtained with α- and β-^{13}C-enriched DHP. The relative peak area in the region of arylglycerol-β-aryl ether structures, i.e. 59-61 ppm, is essentially unchanged during irradiation. New peaks occur at 168 ppm (possibly γ-COOH) and at 70.4 ppm (unidentified at present).

Discussion

The behavior of end groups of the coniferyl alcohol and coniferaldehyde type during photoyellowing has been described previously, and it has been shown that both types are reactive during photoyellowing (Gellerstedt, Pettersson 1975; Pan et al. 1992; Jaeger et al. 1993; Wang et al. 1995; Agarwal, McSweeny 1997). The suggested photoproducts include structures, such as vanillin and vanillic acid end groups. Vanillin and vanillic acid have, furthermore, been identified in the acetone extracts of irradiated sheets made from spruce groundwood pulp (Holmbom et al. 1992). The results from the present investigation corroborate that these types of photoproducts are formed, since signals corresponding to vanillin type end groups, as well as vanillic acid type end groups could be found after irradiation (cf. paragraph regarding α-^{13}C-enriched DHP above). It seems as if the amount of vanillic acid end groups formed is only minor when compared to the amount of vanillin end groups formed. Results obtained from photoaging experiments on ^{13}C-enriched Cell Wall-DHP (formed on differentating xylem from spruce) indicated that both the amount of coniferaldehyde and the amount of coniferyl alcohol end groups decreased upon irradiation, as judged by solid state ^{13}C NMR spectroscopy (Parkås et al. 2002). In the work by Pan and coworkers (1992), the amount of coniferaldehyde end groups increased slightly during photoyellowing, as judged by thioacidolysis of photoaged, bleached TMP samples. To what extent end-group degradation contributes to color formation during photoyellowing is not clear, but Castellan and coworkers (1991) showed that color was formed during the irradiation of paper sheets impregnated with coniferyl alcohol or the corresponding non-phenolic model (3,4-dimethoxycinnamyl alcohol). One explanation for the increased amount of coniferaldehyde end groups might be the photooxidation of coniferyl alcohol end groups, however, Jaeger and coworkers (1993) did not detect coniferaldehyde after irradiation of lignin models of the coniferyl alcohol type.

It was shown early on that 2-aryloxy-1-aryl-1-propanone structures (α-carbonylic β-ethers) are sensitive to light and that they may form colored photoproducts when the C_β-O bond is cleaved and phenoxy radicals are formed (Gierer, Lin 1972; Castellan et al. 1988, 1989; see also Lanzalunga, Bietti 2000). However, the amount of these structures is quite low in native lignin as compared to the arylglycerol-β-aryl ether structures (i.e., β-O-4

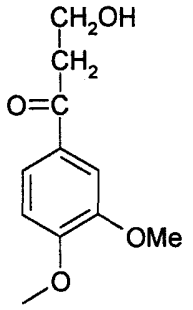

Fig 6. α-Carbonylic photoproduct suggested to be formed via cleavage of a-carbonylic β-ethers (see for example Fukagawa, Ishizu 1991) or via the "ketyl radical pathway" (Schmidt, Heitner 1993).

structures with α-OH) and there are several theories on how these latter structures might react during light-induced yellowing. The cleavage of arylglycerol β-aryl ether bonds has been suggested to contribute to the color formed during light-induced yellowing (Schmidt, Heitner 1993). It was estimated that up to 70% of the color formed during photoyellowing could be derived from these structures. The reaction pathway is suggested to start with the abstraction of the α-hydrogen at the benzylic position by a lignin-based radical, followed by the homolytic cleavage of the C_b-O bond. This reaction would lead to a phenoxy radical and an enol. The formed enol could subsequently form aromatic ketones (α-carbonyls) via tautomerization. Others have also reported β-O-4 ether cleavage during light-induced yellowing both by model compound studies in solution and solid state and by investigations on mechanical pulp lignin (Pan et al. 1992; Argyropoulos, Sun 1996, see also Lanzalunga, Bietti 2000). It seems that lignin model compounds representative of α-OH β-O-4 structures are relatively stable during irradiation, except for when photosensitizers like a-carbonyl compounds or peroxides are present (Lanzalunga, Bietti 2000).

We observed a slightly decreased relative signal area of C_α in arylglycerol-β-aryl ethers, not necessarily due to cleavage of the β-ether bonds. Furthermore, we could observe the formation of α-carbonyls in the case of α-^{13}C DHP The formed α-carbonyls might originate from oxidation of benzylic groups by photoexcited carbonyls (Scaiano 1973; Schmidt et al. 1990; Francis et al. 1991). Direct transformation from benzylic alcohol groups to carbonyl groups under the influence of light has, however, been shown to be slow (Balsells, Frasca 1982; Castellan et al. 1988; Omori et al. 1991). The signal at around 196 ppm could also correspond to the α-carbonylic product resulting from the cleavage of β-ethers either after oxidation to an a-carbonylic β-ether, as described above, or according to the "ketyl radical pathway". The structure of the suggested product can be seen in *Fig 6*. The β-carbon and the γ-carbon in the photoproduct (*Fig 6*) in DMSO-d_6 would appear at around 40.9 and 57.1 ppm, respectively, according to NMR data of the model compound 3-hydroxy-1-(3,4-dimethoxyphenyl)-1-propanone. We find no conclusive peaks corresponding to this structure in the difference spectra of irradiated β- and γ-^{13}C-enriched DHP (*see Figs 4* and *5*, note that the signal of the β-carbon is very close to DMSO which makes it difficult to draw conclusions on small peaks). However, there are two small -CH_2- peaks at 41.0 and 41.4, respectively, in the DEPT-spectrum of irradiated β-^{13}C-DHP (unacetylated in DMSO), not present in the unirradiated DHP, as well as two small peaks at 57.1 and 57.2 in the corresponding spectrum of γ-^{13}C-DHP. This indicates that at least a small amount of this type of ketone is formed and the presence of two peaks is probably due to different substitution patterns in the aromatic rings. The α-carbonyl carbon in this structure would appear at 197.4 ppm, which matches with our α-carbonyl peak in the difference spectrum of irradiated α-^{13}C-DHP. In other words, we cannot be certain about the nature of all of the formed α–carbonyls, at present. A 2-D NMR study on the acetylated photodegraded ^{13}C-enriched DHPs might be helpful in this respect.

Our results suggest that no extensive cleavage of β-ethers occurred as a result of irradiation for 2x21 hours, an irradiation time that has to be considered extensive. The preponderance of color is formed rather quickly (about 33% of the color is formed within the first 4 hours of a 20-hour irradiation with the accelerated equipment used in the present study) when a paper sample based on mechanical pulp is subjected to UV/VIS light (cf. Paulsson et al. 1995). This indicates that the contribution of β-ether cleavage to the formed color is small, at least in this particular case with DHP-impregnated filter paper. However, it has to be understood that not all of the DHP was extractable after irradiation and that the precursors to these non-extractable photoproducts might, in part, be β-ethers. As previously mentioned, investigations of the non-extractable part of the DHPs with solid state ^{13}C NMR spectroscopy are underway and although the quantity of photo-aged DHP left on the filter papers is small, some information regarding the structure may be obtained.

We have not been able to satisfactorily relate the color formed during the irradiation of side-chain ^{13}C-enriched DHPs to formed chromophores. For this purpose, ^{13}C-enrichment of the carbons in the aromatic ring of the DHP may prove to be useful. Results from similar experiments using ring-1, ring-3, ring-4, and ring-5-^{13}C-enriched DHPs will be presented in the next part of this series.

Conclusions

The brightness of filter papers with applied DHP decreased on average from 54 to 37%, and the b*-value increased during the irradiation period of 2x21 hours (21 hours on each side of the filter paper), which is in agreement with the general behavior of mechanical pulps subjected to UV/VIS light. About 89% of the applied weight of DHP could be extracted after the irradiation. The coniferyl alcohol end groups were almost completely removed upon irradiation, while the amount of coniferaldehyde end groups appeared to increase somewhat. Among the identified photoproducts are end groups of the vanillin and vanillic acid types and small amounts of an end-group, possibly of the ferulic acid type. The results indicated a slight formation of a-carbonyls, but no significant decrease in the relative amount of β-ether structures in the extractable part of the irradiated DHP

could be established, as judged from difference spectra corresponding to the ^{13}C-enriched DHP. The exact nature of the α-carbonyls formed was not determined.

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