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The Presence of Cinnamyl Alcohol Groups in Lignin

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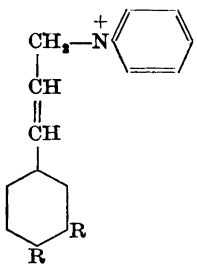
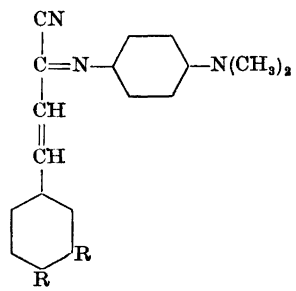
A colour reaction has been worked out for cinnamyl alcohol groups. It involves a transformation of these groups into *p*-dimethylamino-anilides of styryl-glyoxylonitriles (II). By this method the presence of a small amount of cinnamyl alcohol groups in spruce lignin has been indicated.

In 1896 Klason [1] found that coniferin is easily sulphonated when heated with an acid sulphite solution. This reaction was attributed to the presence of the cinnamyl alcohol group in coniferin. Partly because of this finding he suggested that the sulphonatable structures in lignin consist of cinnamyl alcohol groups. Later investigations [2] showed, however, that the main parts of these structures most probably are phenyl-carbinols and their ethers. On the other hand, the possibility of the presence of a small amount of cinnamyl alcohol groups in lignin has not been excluded.

This possibility may also be compatible with the modern view that gymnosperm lignin is formed by oxidative polymerization of coniferyl alcohol [3]. It may be possible that during such a process some of the coniferyl alcohol molecules are built into the lignin molecules without a change of their allyl alcohol side chains, thus forming cinnamyl alcohol groups in lignin.



Ia, R = H
b, R = OCH_3



IIa, R = H
b, R = OCH_3

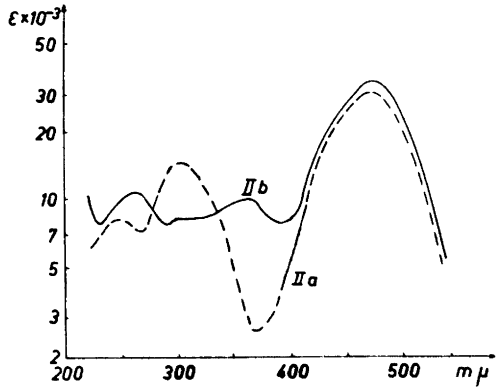


Fig. 1. The extinction curves for the *p*-dimethylamino anilides of styryl glyoxylonitrile (IIa) and for its 3,4-dimethoxy derivative (IIb). Alcoholic solution.

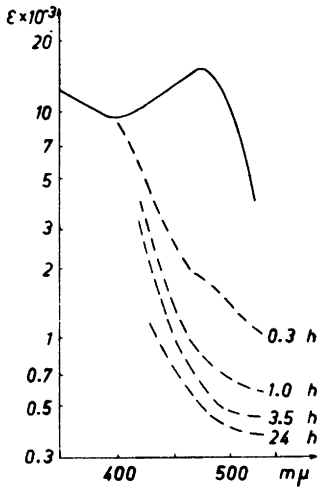


Fig. 2. Extinction curves. Alcoholic solution. The ε-values were calculated on the basis of the amount of (Ib) used.

Continuous line. The unfractionated reaction products from the reaction of (Ib) with *p*-nitrosodimethyl-aniline and potassium cyanide.

Dotted lines. The unfractionated products after being kept in 1/15 N alcoholic hydrochloric acid for varying times at room temperature.

We have therefore tried to find a colour reaction for these groups in lignin. It was found that cinnamyl alcohol and its 3,4-dimethoxy derivative could be transformed into the strongly red coloured *p*-dimethylamino-anilides of styryl-glyoxylonitriles (IIa and b, respectively) in a two-step process *via* the N-cinnamyl-pyridinium salts (Ia and b, respectively).

The first step was accomplished at room temperature by treating the alcohols with tosyl chloride dissolved in pyridine. The pyridinium salts (Ia and b) were obtained in good yields. The second step was carried out according to the method used by Kröhnke [4] to transform N-phenacyl-pyridinium bromide into the *p*-dimethylamino-anilide of benzoyl-glyoxylonitrile. The pyridinium salts (Ia and b) were treated at room temperature with a water-alcohol solution of *p*-nitrosodimethyl-aniline and potassium cyanide. The nitriles (IIa and b) were obtained, after chromatographic purification, in yields of 46 and 26 %, respectively.

On the other hand, when coniferyl alcohol was treated with tosyl chloride and pyridine no pyridinium salt was obtained. It is hardly probable, however, that lignin contains such reactive structures as *p*-hydroxy-cinnamyl alcohol groups because they would have been easily oxidized during the lignin biosynthesis. This failure is therefore of no great importance in the present case.

The extinction curves for the two nitriles (IIa and b) had strong maxima at 475 mμ, ε being 29 800 for (IIa) and 32 700 for (IIb) (Fig. 1).

The light absorption of the unfractionated reaction products obtained from the pyridinium salt (Ib) had $\varepsilon_{max} = 15\,500$ at 475 mμ (Fig. 2), calculated on the basis of the amount of pyridinium salt used. This absorption was considerably higher than that which corresponded to the yield of the nitrile (IIb). Therefore some coloured compounds other than this nitrile were formed by the Kröhnke synthesis. This inference must be considered if the colour reaction is used for the photometric estimation of the amount of cinnamyl alcohol groups.

The glyoxylonitrile derivatives are easily destroyed by acid [4] with the disappearance of the extinction maximum at 475 mμ. Fig. 2 shows that after treatment of the unfractionated mixture with 1/15 N hydrochloric acid at room temperature for 1 h the absorption at 475 mμ had decreased to 5 % of its original value.

The great reactivity of cinnamyl alcohol structures makes their presence in isolated lignin preparations unlikely even if the original lignin contained such structures. The colour reaction was therefore performed on extracted wood slices, the thickness of which was about 20 μ. If not otherwise stated, they were freshly prepared from a newly cut spruce tree. The slices were first treated with a solution of tosyl chloride in pyridine, and then, after careful washing, with one of *p*-nitrosodimethyl-aniline and potassium cyanide in water-alcohol. After another very careful washing, the light absorption of the slices was determined with a Beckman spectrophotometer. The light scattering was minimized by wetting the slices with benzene.

The suitability of this method was tested on slices containing an approximately known amount of cinnamyl alcohol groups derived from the cinnamyl *aldehyde* groups of lignin. The procedure was as follows.

First, the slices were heated with a neutral sulphite solution for 20 h at 135°C. In this way the cinnamyl alcohol groups which might have been present in lignin were destroyed by transforming them into alkali stable sulphonic acid structures.

The conditions for the sulphite treatment were chosen from a study of 3,4-dimethoxy-cinnamyl alcohol. Fig. 3 shows that this compound was almost completely sulphonated when heated at 135°C for about 20 h with a sulphite solution of pH 6.

During the sulphonation of the alcohol, its UV absorption curve changed considerably (Fig. 4). The 265 mμ maximum was shifted to 280 mμ and its ε value decreased. The absorption in the vicinity of 300 mμ disappeared. These changes indicated that the aliphatic double bond became saturated. Accordingly, the sulphonation probably involved an addition of sulphite to the aliphatic double bond of the alcohol.

The sulphite treatment of the slices would also transform the cinnamyl aldehyde structures of lignin more or less completely into the hydrosulphonic acid derivative (III) and its sulphite addition product (IV) [5]. The carbonyl group in structure (III) causes the sulphonic acid groups of (III) and (IV) to be split off easily by alkali with reforming of the double bond. To regenerate

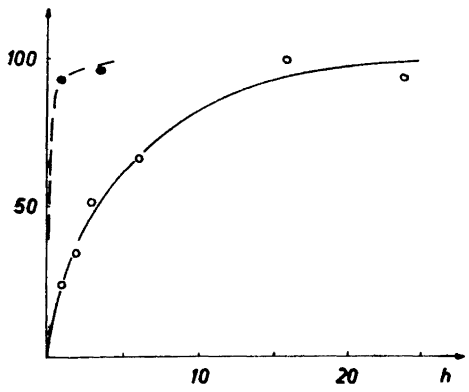


Fig. 3. The rate of sulphonation of 3,4-dimethoxy-cinnamyl alcohol (yield of sulphonic acid versus heating time). Temperature of the reaction 135°C. Total concentration of SO_2, 5 %.
— — — Sulphite solution at pH 3
———— Sulphite solution at pH 6
The yield was determined as described for similar experiments with vanillyl alcohol [6].

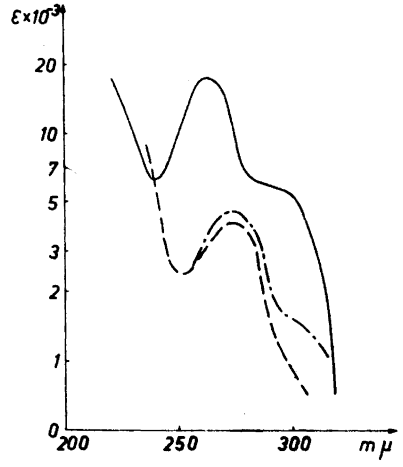
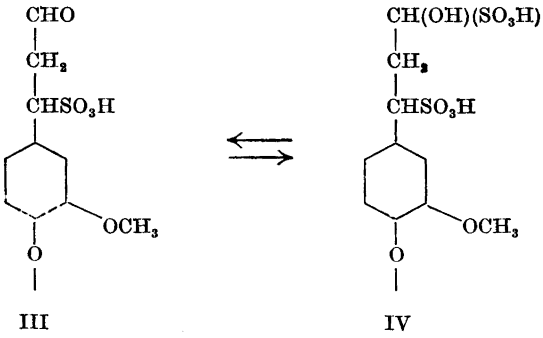


Fig. 4. The extinction curves for 3,4-dimethoxy-cinnamyl alcohol and its sulphonated products.
———— 3,4-Dimethoxy-cinnamyl alcohol
— — — Sulphonated at pH 6
— — — — Sulphonated at pH 1.5

the cinnamyl aldehyde structures the slices were therefore immersed in a sodium hydroxide solution.



Finally, the slices were treated with an aqueous sodium borohydride solution, to reduce the cinnamyl aldehyde groups of lignin to cinnamyl alcohol groups. As the yield of such a reduction is high, the amount of cinnamyl alcohol groups formed should be about the same as the amount of cinnamyl aldehyde structures originally present in lignin. Adler and Ellmer [5] have

estimated that spruce lignin contains one such aldehyde group per 40—60 phenylpropane monomers.

The presence of the cinnamyl alcohol groups in the treated slices was clearly demonstrated since the slices turned red when subjected to the colour reaction, and the absorption curve for the coloured slices had a distinct maximum at 475 mμ (Fig. 5). If the coloured slices were treated with acid the colour and the maximum disappeared (Fig. 5) in agreement with the acid labile nature of the glyoxylonitrile structures. (The wavelength of the maximum obtained on different slices varied between 475 ± 5 mμ. This uncertainty depended probably on the superposition of the absorption due to the aromatic structures of lignin, which caused the extinction to increase sharply at lower wavelengths as is shown in Fig. 5.)

As described above, slices which have been treated only with sulphite or with sulphite and alkali should contain no cinnamyl alcohol groups. In accordance with that no maximum was obtained when these slices were subjected to the colour reaction (Fig. 5).

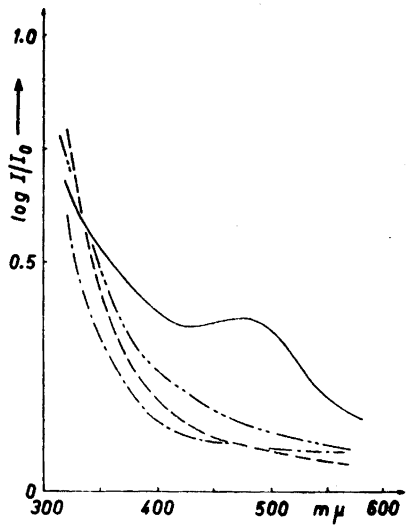


Fig. 5. Absorption curves on spruce wood slices first pretreated and then subjected to the colour reaction. Weight of the slices, 1.6 mg per cm². For the treatment conditions, see Table 1.
– – – – Pretreated with sulphite.
— — — — Pretreated with sulphite and sodium hydroxide.
——— Pretreated with sulphite, sodium hydroxide and sodium borohydride.
— – — – The last slice after treatment with hydrochloric acid.

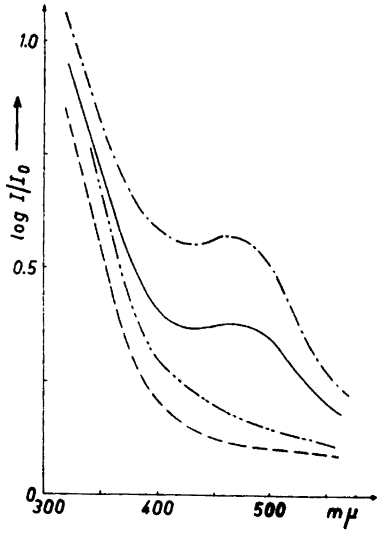


Fig. 6. Absorption curves on spruce wood slices subjected to the colour reaction. Weight of the slices, 1.6 mg per cm².
——— No pretreatment
— — — — The same slice after treatment with hydrochloric acid
— — — — Pretreated with sodium borohydride
— – — – The last slice after treatment with hydrochloric acid

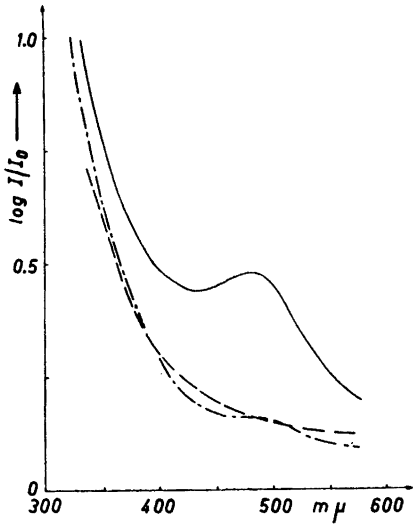


Fig. 7. Absorption curves on spruce wood slices stored for 6 months and then subjected to the colour reaction. Weight of the slices, 2.0 mg per cm².
- – – – – No pretreatment
——— Pretreated with sodium borohydride
— — — The last slice after treatment with hydrochloric acid

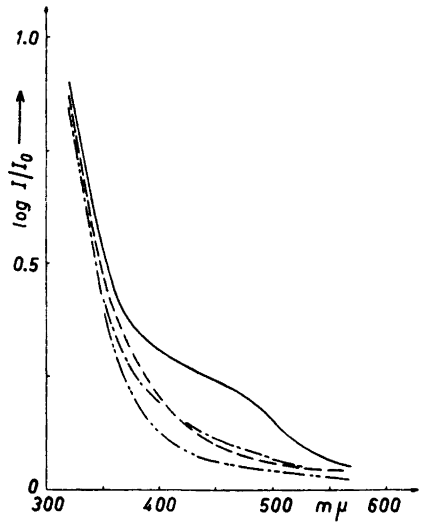


Fig. 8. Absorption curves for Brauns lignin subjected to the colour reaction. Concentration: 10 mg lignin in 50 ml of dioxan.
– – – – No pretreatment
– – – – The same material after treatment with hydrochloric acid
——— Pretreated with sodium borohydride
— — — The last material after treatment with hydrochloric acid

Thus, this experiment showed that even a small amount of cinnamyl alcohol groups in lignin can be demonstrated by this method.

The reaction was next performed on slices which had not been pretreated in any way but by extraction. A maximum was again obtained at about 475 mμ (Fig. 6). It disappeared when the coloured slices were treated with acid (Fig. 6). Accordingly, the presence of cinnamyl alcohol groups in lignin was indicated.

Slices which have been exposed to air during six months gave no maximum (Fig. 7). As cinnamyl alcohols are easily oxidized this could be expected. Stored Brauns lignin also gave no maximum (Fig. 8). After reduction with sodium borohydride, however, the presence of the cinnamyl alcohol groups formed was demonstrated in both materials (Figs. 7 and 8), even if the maximum obtained was not so distinct in the case of Brauns lignin.

The question now arises as to whether the 475 mμ maximum on coloured wood slices could be due to a type of group other than cinnamyl alcohol. Conceivable types might be phenyl and benzoyl-carbinols as the corresponding pyridinium salts (N-benzyl and N-phenacyl-pyridinium salts, respectively) are also transformed into anilides of glyoxylonitriles (phenyl and benzoyl-

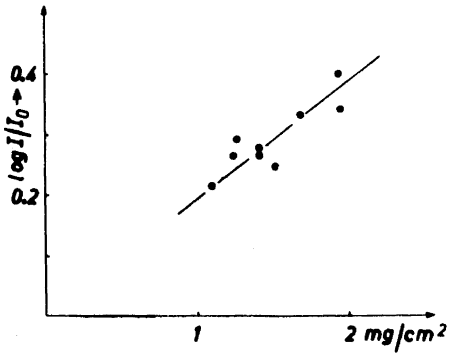


Fig. 9. The absorption at 475 mμ of coloured slices versus their weight per cm².

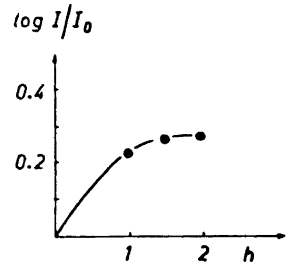


Fig. 10. The absorption at 475 mμ of coloured slices versus the time of treatment with the solution of *p*-nitrosodimethylaniline and potassium cyanide. The weight of the slices, 1.1 mg per cm².

glyoxylonitriles) by nitroso-dimethylaniline and potassium cyanide [4]. Furthermore, it was found that the anilide of benzoyl-glyoxylonitrile had a maximum in its extinction curve at 478 mμ (ε, 32 700) which was close to that of the nitriles obtained from cinnamyl alcohols. The maximum of the anilide of phenyl-glyoxylonitrile was determined to be at 458 mμ (ε, 21 200).

Only primary carbinols can be transformed into this type of nitrile as the central carbon atom in them is linked both with a double bond to a nitrogen atom and with a single bond to a nitrile group. If the 475 mμ maximum is caused by the presence in lignin of phenyl or benzoyl-carbinol structures lignin should then contain phenylmethane or phenylethane monomers, respectively. This does not seem probable according to the modern view of the lignin structure.

Another possibility is, however, not excluded. The maximum may be due to some type of structure which during the performance of the colour reaction is transformed into chromophoric groups structurally completely different from the anilides of glyoxylonitriles. However, the agreement between the maximum wavelength of the coloured slices and that of the model compounds, the destruction of the chromophoric groups by acid, and the inhibition of the colour reaction by pretreatment with sulphite and by air make it probable that the maximum is due to the presence of cinnamyl alcohol structures in lignin.

The data accounted for in Figs. 5—7 could be used to estimate approximately the concentration in lignin of the cinnamyl alcohol groups. In Table 1 the optical density at 475 mμ of the coloured slices (column 2) and of the same slices after decolourization with acid (column 3) have been put together. (The data in Figs. 5 and 6 have been determined on slices which had a weight of 1.6 mg per cm². On the other hand, the slices used for Fig. 7 had a weight of 2.0 mg. By using the fact that the absorption was roughly proportional to the weight per cm² of the slices (Fig. 9) the latter data were recalculated to correspond to a weight of 1.6 mg per cm².)

The optical density due to the glyoxylonitrile structures has been taken as the difference (Column 4) between the values in Columns 2 and 3 (Δ density). The table shows that the Δ density for the cinnamyl alcohol groups originally present in lignin was about 0.25. About the same value was obtained for the cinnamyl alcohol groups formed by reduction of the aldehyde structures with sodium borohydride. Consequently, the amount of cinnamyl alcohol structures in lignin is about the same as that of the cinnamyl aldehyde groups, *e. g.* one group per about 50 phenylpropane monomers.

If the slices were assumed to be optically homogeneous materials an approximate value of the concentration could be calculated in a simple way by using Beer's law. In this way it was estimated that lignin contained one cinnamyl alcohol group per 180 phenylpropane monomers. (The ε-value was assumed to be the same as that of the unfractionated reaction products from (Ib), which was $\varepsilon = 15\,500$ for $\lambda_{max} = 475$ mμ. The absorption due to the glyoxylonitrile structures was taken as the main value of Table 1, log $I_0/I = 0.22$.)

The difference between these two values may be caused by an incomplete reaction of the cinnamyl alcohol groups because of the failure of the reagents to penetrate the wood slices completely. As this obstacle ought to affect to a lesser degree the value obtained by a comparison with the cinnamyl aldehyde concentration, the latter value is assumed to be the more correct one.

Table 1. The optical density at 475 mμ of coloured and of acid decolourized slices.

Pretreated with [a]	Opt. density [b] for		Δdens. [d]	Structure [e]
	colour sl.	decol. sl. [c]		
no reagent	0.38	0.12	0.26	alc.
$NaBH_4$	0.57	0.17	0.40	alc. + ald.
sulphite	0.10	—	—	—
sulphite, alkali	0.10	—	—	—
sulphite, alkali and $NaBH_4$	0.38	0.15	0.23	ald.
air	0.13	—	—	—
air, $NaBH_4$	0.38	0.15	0.23	ald.

a) The conditions were:
$NaBH_4$; the slices were immersed in an aqueous sodium borohydride solution for 4 h.
Sulphite; the slices were heated with a sulphite solution (pH 6.1; total SO_2, 5 %) for 19 h at 135°C.
Alkali; the slices were immersed in N sodium hydroxide solution for 0.5 h.
Air; the slices were exposed for air during 6 months.
b) Determined as log I_0/I corrected to a slice weight of 1.6 mg per cm^2.
c) The coloured slices were immersed in N hydrochloric acid for about 15 h.
d) The difference of the values in columns 2 and 3.
e) The group in lignin which have been transformed into the glyoxylonitrile structure. Cinnamyl alcohol = alc; cinnamyl aldehyde = ald.

EXPERIMENTAL

The reaction of the model alcohols with tosyl chloride and pyridine

3,4-Dimethoxycinnamyl alcohol (2 g) and tosyl chloride (2 g) were dissolved in pyridine (3 ml). The solution was kept at room temperature for 2 days. Sodium hydroxide solution (2 N, 5 ml) was then added and the solution was concentrated *in vacuo* to a small volume. By repeated water additions and concentrations the solution was practically freed from pyridine. Dimethoxycinnamyl-pyridinium picrate was precipitated from the solution by addition of picric acid dissolved in alcohol. The product was recrystallized from aqueous alcohol. Obtained 2.9 g (yield 72 %), m. p. 139—141°. (Found: OCH_3 13.4. Calc. for $C_{22}H_{20}O_9N_4$: OCH_3 12.8.) The picrate was transformed into the perchlorate by using an anion exchanger. M. p. 106—107°. (Found: OCH_3 17.7; N 3.8. Calc. for $C_{16}H_{15}O_6NCl$: OCH_3 17.5; N 3.9.)

Cinnamyl alcohol (1.4 g) and tosyl chloride (1.9 g) were dissolved in pyridine (2 ml). The solution was kept at room temperature for 5 h. Perchloric acid solution (N, 20 ml) was added by which the cinnamyl-pyridinium perchlorate precipitated. It was collected and purified by precipitating from its methanol solution by ether addition. M. p. 70.5—71.5°, undepressed by authentic sample. Yield 60 %.

The synthesis of the *p*-dimethylamino-anilide of styryl-glyoxylonitrile (IIa) and its 3,4-dimethoxy derivative (IIb)

The pyridinium bromide (Ia or b) (1 g), *p*-nitrosodimethylaniline (0.6 g), and potassium cyanide (0.4 g) were dissolved in a mixture of water (2.5 ml) and alcohol (8 ml). The solution turned red. After being kept at room temperature for 1 h the solution was added to water (5 ml). The mixture obtained was extracted with benzene. The extract was passed through a column of alumina, and the column developed with benzene. The anilide was isolated from the coloured zone which moved most rapidly. It was recrystallized from alcohol.

The dimethylamino-anilide of styryl-glyoxylonitrile was obtained from N-cinnamyl-pyridinium bromide in a yield of 46 %. M. p. 116—117°. (Found: C 78.7; H 6.2; N 15.0. Calc. for $C_{18}H_{17}N_3$: C 78.5; H 6.2; N 15.3.)

The dimethylamino-anilide of 3,4-dimethoxy-styryl-glyoxylonitrile was obtained from N-(3,4-dimethoxycinnamyl)-pyridinium bromide in a yield of 26 %. M. p. 158—159°. (Found: C 71.7; H 6.4; N 12.5; OCH_3 18.9. Calc. for $C_{20}H_{21}O_3N_3$: C 71.7; H 6.3; N 12.5; OCH_3 18.5.)

The colour reaction performed on lignin samples

Wood slices. Radial slices about 20 μ thick of freshly cut spruce wood were extracted with hot water and benzene-alcohol (1:1). Some of the slices were then pretreated in accordance with the conditions given in Table 1. The slices were immersed in a solution of tosyl chloride (2 g) in pyridine (20 ml) for 24 h at room temperature. To facilitate penetration, the reaction mixture was placed in a desiccator which was repeatedly evacuated. After washing successively with benzene, alcohol, dilute hydrochloric acid and flowing water, the slices were immersed for 1.5 h (*cf.* Fig. 9) in a solution of *p*-nitrosodi methyl-aniline (1.1 g) and potassium cyanide (0.9 g) in aqueous alcohol (20 ml, 85 % v/v). The penetration was aided as before. The wood slices were then again carefully washed with alcohol and benzene. While wet with benzene they were pressed between two quartz plates, and their optical density was determined with a Beckman spectrophotometer.

Brauns lignin. Brauns lignin (10 mg) was dissolved in a solution of tosyl chloride (35 mg) in pyridine (300 mg), and left for 2 days at room temperature. Water was added and the precipitated lignin was washed well with water. The tosylated lignin was then

dissolved in dioxan-alcohol (0.5 ml, 1:1 v/v). To this solution *p*-nitrosodimethyl-aniline (65 mg) and potassium cyanide (52 mg) dissolved in water-dioxan (1.5 ml, 4:1 v/v) were added. The solution was kept at room temperature for 1 h. Water was added and the lignin suspension obtained was exhaustively extracted with benzene. The lignin was removed from the suspension by passage through a layer of fine sand. The sand was washed well with water, and then the lignin was extracted from it with dioxan. The optical density of the dioxan solution was determined with a Beckman spectrophotometer.

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