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Estimation of Free Phenolic Groups in Lignin

by

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Estimation of Free Phenolic Groups in Lignin*

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A method has been developed for the determination of free phenolic groups in softwood lignin. It is based on the colour reaction occurring between 1–nitroso 2–naphthol and eugenol.

The colour reaction of 1–nitroso–2–naphthol with a variety of substances containing one or two guaiacyl nuclei (representing probable structural principales of lignin) has been investigated.

The number of free phenolic hydroxyls per methoxyl group in milled soft wood lignin was determined. Satisfactory agreement was obtained with results reported in the earlier literature.

INTRODUCTION

Since knowledge of the number of free phenolic groups in lignin is essential for a further elucidation of its structure, various methods have been used for this determination. Although some of the earlier results are conflicting [1,2,3], some investigators obtained similar results, using different methods [4,5,6].

In the present communication, a rapid and simple method for the determination of free phenolic hydroxyls in lignin is developed.

The method is based upon the colour reaction between 1–nitroso–2–naphthol and eugenol, which is used in the spectrophotometric determination of eugenol [7].

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First it was necessary to study the influence upon the colour reaction of various structural features which have been proposed for lignin. Thus, the colour reaction was investigated between 1–nitroso–2–naphthol and various appropriate lignin model compounds containing hydroxyl groups, carbonyl groups, and ethylenic double bonds on the guaiacyl propane side chains. The number of free phenolic hydroxyls per methoxyl group in milled soft wood lignin was then determined.

RESULTS AND DISCUSSION

In a previous report from this laboratory [7], it was suggested that the colour reaction of eugenol with 1–nitroso–2–naphthol in the presence of hydrochloric and nitric acids was caused by the presence of a free phenolic hydroxyl group.

In the present work, experiments were carried out with substances containing substituted hydroxyl groups in a 4–substituted guaiacyl nucleus, i.e., 4–methyl-veratrol, and 4–methyl-cresol; thus, it was clearly shown that this colour reaction must be due to the presence of free phenolic hydroxyl groups.

As expected from information in the literature [8], no colour reaction occurred with 4,6–dimethyl-guaiacol.

Under the conditions employed, the colour reaction does not take place unless the phenolic hydroxyl group, and the ortho- and meta- positions of the p-substituted guaiacyl nucleus are free.

In order to estimate the free phenolic hydroxyl groups in softwood lignin using this method, it was necessary that the colour should be formed solely from the p-substituted guaiacyl nuclei while no other structural elements on the side chain should have any influence.

To examine the possible effects on the colour reaction, of substituents in the position para to the phenolic hydroxyl group of the guaiacyl nucleus, experiments were performed with a variety of lignin model compounds containing one or two guaiacyl nuclei.

The colour reactions were carried out according to the procedure described earlier for the estimation of eugenol [7]. The beha-

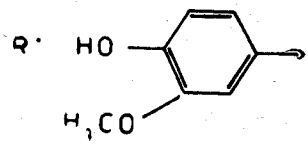
viour of all substances examined was very similar to that of eugenol.

The absorption curves obtained for the colours resulting from the reactions of various lignin model compounds containing one or two guaiacyl nuclei show a maximum at 505 mμ, with very similar shapes.

The absorption values obtained showed that the colour formed during the reaction with 1–nitroso–2–naphthol followed Beer's law throughout the entire range of concentrations of model compounds used.

Table: I

Molar Absorptivities of Model Compounds Containing One Guaiacyl Nucleus.



Substance		ε_{max}
Eugenol	R—CH_2—CH=CH_2	1672
Isoeugenol	R—CH=CH—CH_3	1680
β-Hydroxydihydroeugenol	R—CH_2—CHOH—CH_3	1668
Guaiacyl-acetone	R—CH_2—CO—CH_3	1670
Ferulic acid	R—CH=CH—COOH	1676
4–Methyl-guaiacol	R—CH_3	1670
Vanillyl alcohol	R—CH_2—OH	1688
Vanillin	R—COH	—
Acetovanillone	R—CO—CH_3	

As seen from Table I, the molar absorptivity values of the model compounds containing one guaiacyl nucleus fall within the narrow range of 1668 to 1688. Substituents in the position para to the phenolic hydroxyl group were generally found to be without influence on the colour reaction. However, compounds containing a carbonyl group in the α-position of the side chain, i.e., vanillin

and acetovanillone, do not react with 1–nitroso–2–naphthol. This exception however is of minor importance to the lignin problem, since it is well established that carbonyl groups in lignin are generally not in the α-position of the side chains [9,10]. Thus, it appears reasonable to conclude that the molar absorptivity of compounds containing one free phenolic hydroxyl group on a guaiacyl nucleus will not differ appreciably from 1670.

The results obtained with a number of lignin model compounds containing two guaiacyl nuclei are presented in Table II.

Table: II

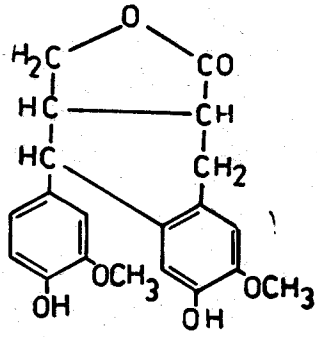
Molar Absorptivities of Model Compounds Containing Two Guaiacyl Nuclei

Substance	ε_{max}
Conidendrin (I)	1680
Iso-lariciresinol (II)	1695
Dehydrodiconiferyl alcohol (III)	1665
Dehydrodiisoeugenol (IV)	1680
Pinoresinol (V)	3326
Lariciresinol (VI)	3360
Seco-iso-lariciresinol (VII)	3345

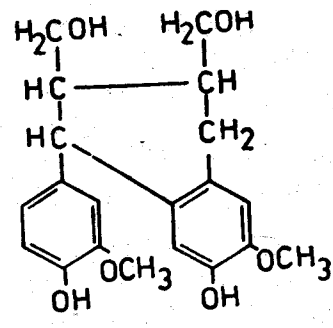
Although neither conidendrin nor iso-lariciresinol are appropriate models for lignin, their behaviour may be of some interest, since it may elucidate certain structural influences on the colour reaction.

The molar absorptivity values of the colours formed during the reaction of 1–nitroso–2–naphthol with conidendrin (I) and isolariciresinol (II), which contain two guaiacyl nuclei and two free phenolic hydroxyl groups, are 1680 and 1695 respectively. But the experimental results would indicate the presence of just one free phenolic hydroxyl group in each compound.

However the behaviour of these substances is to be expected, since in one of the guaicyl nuclei, the position meta to the phenolic hydroxyl group is not free.

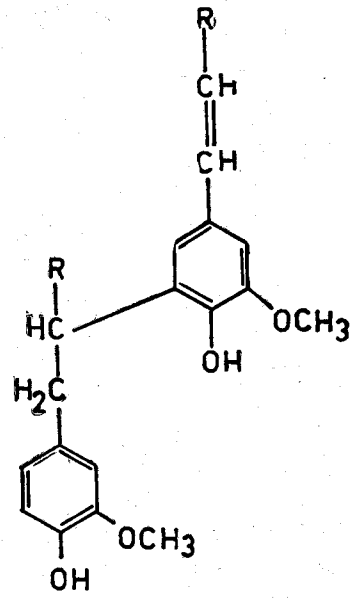


I



II

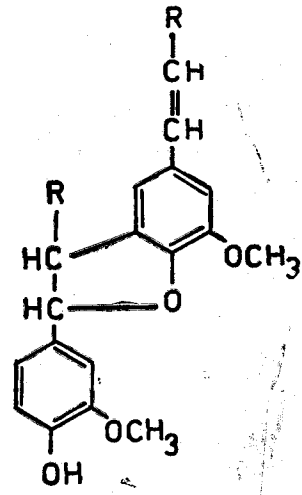
Evidence was obtained which supports the presence of phenylcoumaran structures(III-IV) in milled-wood lignin [11]. The "closed" phenylcoumaran structure contains but one free phenolic hydroxyl group. However, under the experimental conditions used for the colour reaction, the closed system could be transformed into the open form (VIII). In addition to the 4–propyl-guaiacyl nucleus, the open form also contains 4,6 di-substituted guaiacyl elements, and two free phenolic hydroxyl groups.



VIII

It has been demonstrated that 4,6–di-substituted guaiacol structures do not react with 1–nitroso–2–naphthol to give a colour reaction. However, in phenylcoumaran systems, whether open or closed, there is only one phenolic hydroxyl group available for the colour reaction. This is obviously of no importance in the application of the method to lignin.

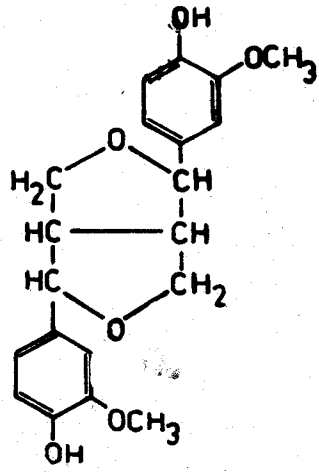
As can be seen from Table II, the results obtained with dehydrodiconiferyl alcohol (III) and dehydrodiisoeugenol (IV) also indicate the presence of only one free phenolic hydroxyl group.



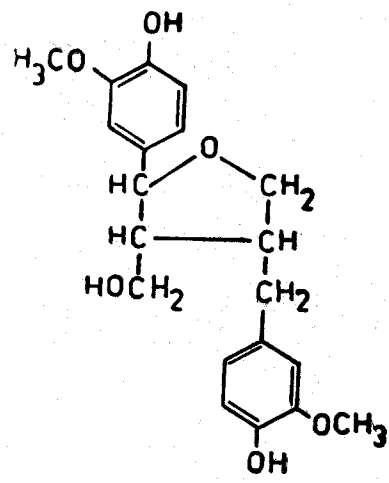
III R = CH_2OH

IV R = CH_3

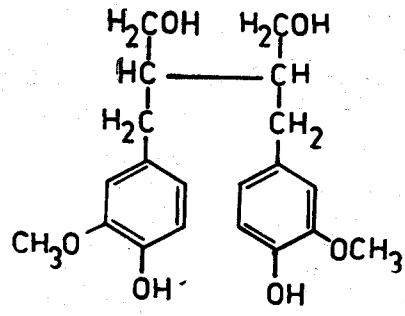
The colours formed with the compounds containing two guaiacyl nuclei and two free phenolic hydroxyl groups, i.e, pinoresinol (V), lariciresinol (VI) and seco-iso-lariciresinol (VII), showed molar absorptivities of 3340, which is twice as high as the values obtained for the molar absorptivities of the model compounds containing but one free phenolic hydroxyl group.



V

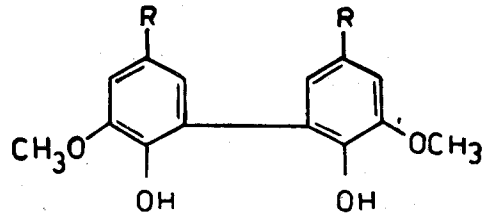


VI



VII

Compounds containing the 6,6′–diguaiacyl structure (IX) give no colour reaction, since the ortho-positions of the guaiacyl nuclei are not free. This is of minor importance in the lignin problem however, because the amount of such structures in lignin is very low [12].



IX

In view of the similarity of the wavelengths of maximum absorption and of the ε_{max} values of the various structures shown in Tables I and II, substituents in the position para to the phenolic hydroxyl group of guaiacol were found to be without effect on the colour reaction. Thus, it would appear reasonable to assume that the molar absorptivity of the free phenolic hydroxyls present in softwood lignin will not differ appreciably from those of the model compounds.

The colour reaction of spruce milled-wood lignin with 1–nitroso–2–naphthol was carried out under conditions similar to those used for the model compounds. The absorption spectrum of the colour formed exhibited a maximum at 505 mμ.

The phenolic hydroxyl content of this lignin sample was calculated by using the absorptivity value of the sample (Amax) and the average ε_{max} value for the model compounds (1670), as follows:

Moles of phenolic hydroxyl per 1000 ml solution containing 100 mg spruce milled-wood lignin (61.94 % C,5.9 % H, 14.79 % OCH_3) = Amax (0,204)x1000 /1670=0.122 m-mole OH /100 mg lignin. On the other hand, the sample of lignin contained 14.79 % or 14.79 /31=0.47 m-mole OCH_3 per 100 mg of lignin. Consequently the lignin sample contained 0.122 /0.47 = 0.255 mole of phenolic hydroxyl per OCH_3 group.

CONCLUSIONS

The colour reaction does not take place only with compounds containing an α-carbonyl group on the side chain of the guaiacyl

nucleus. But, this is of practically no importance in the application of the method to lignin, since α-carbonyl groups do not seem to be present in lignin in appreciable amounts. However in the following paper, results obtained after the reduction of the α-carbonyl groups present in lignin with lithium borohydride will be reported.

The entire spectrophotometric determination of free phenolic hydroxyl groups, including the construction of the complete absorption curve, can be performed in about one hour. Routine determinations involving only the measurement of the peak of the curve can be performed in a matter of minutes.

This rapid and accurate determination of the free phenolic hydroxyl group content could be extremely helpful in a study of lignin degradation reactions.

EXPERIMENTAL

Reagents:

A solution of 1–nitroso–2–naphthol (recrystallised from ethyl alcohol) in 95 % ethyl alcohol at a concentration of 0.1 % was used.

Nitric acid solution was prepared by diluting chemically pure nitric acid (sp.gr. 1.42) with distilled water to 2.5 N.

Concentrated hydrochloric acid (sp.gr. 1.19) was used.

Model compounds were purified by recrystallization or distillation twice in vacuo under a nitrogen atmosphere, and their purity was checked by paper chromatography. For the colour reaction, 96 % ethyl alcohol solutions at concentrations ranging from 45–50 μg/ml were prepared.

The milled-wood lignin was obtained from blue spruce wood as described in the literature [12].

The procedure for the colour reaction is as follows:

The colour reaction was carried out according to the procedure used earlier for the determination of eugenol [7]. In one of two Pyrex test tubes, was placed a 5 ml sample of a solution containing a model compound, and in the other, 5 ml ethyl alcohol. To each tube, 1 ml of 1–nitroso–2–naphthol solution, 0.25 ml

of nitric acid solution and 1.5 ml of hydrochloric acid were added. The contents of the tubes are well mixed by shaking, and then heated in a water bath maintained at 85 ∓ 0.5°C. Subsequent to the beginning of the development of the orange-red colour, the tubes were kept in the bath for an additional 30 seconds, after which they are kept at room temperature for 30 seconds, and then cooled in a ice-water bath.

Spectra were measured with a Beckmann quartz Model DU spectrophotometer, using 1 cm-quartz cells, compared with a blank treated in an identical manner.

Acknowledgment

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ÖZET

Konifer ağaçların odunlarından hazırlanan ligninde serbest fenolik hidroksil gruplarının çabuk tayini için bir metod geliştirilmiştir. Metot, 1–nitroso–2–naftol ile eugenol arasındaki reaksiyona dayanmaktadır.

Bir veya iki guayasil grubu ihtiva eden model bileşiklerin 1–nitroso–2–naftol ile verdiği renk reaksiyonu incelenmiş ve renk reaksiyonu üzerine guayasil halkasında, fenol grubuna nazaran para yerinde bulunan sübstitüentlerin tesiri olmadığı gösterilmiştir. Ayrıca molar absorptivitenin yalnız serbest fenol grubunun sayısına bağlı olduğu tesbit edildiğinden, metot, ligninde serbest fenolik hidroksil grubunun tayininde kullanılabilmiş ve literatürde verilen değerlere yakın neticeler elde edilmiştir.

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