〔韓國林學會誌 No. 43, p 14~19 (1979)(論文)〕

論　文

^{13}C-NMR에 依한 알카리 모델리그닌의 着色構造에 關한 硏究*1

尹　炳　虎*2

Identification of the chromophoric structure in alkali model lignin by ^{13}C-NMR*1

Byung Ho Yoon*2

펄프化中의 리그닌의 着色構造를 究明하기 爲하여 바니릴알콜을 1規定濃度의 苛性소다로 90~180分間 窒素下에 處理한 後 空氣中에 8時間 酸化하여 크로마토그라피로 着色物質을 分離했다.

이 物質은 ^{13}C-NMR스펙트로로는 着色構造에 基因하는 케미칼쉬플트는 보이지 않았다. 한편, 炭素를 ^{13}C으로 라벨한 바니릴알콜 —〔카비놀 —^{13}C〕을 上記와 같은 處理로 生成된 着色物質은 퀴논메티드로 歸屬시킬 수 있는 101.7과 104.6 ppm에 케미칼쉬플카 나타나므로 α炭素에關與하는 着色構造임을 確認했다.

On treatment with sodium hydroxide at 165° for 1.5~3hr followed by air oxidation. vanillyl alcohol afforded colored materials.

^{13}C-NMR spectrum of the material did not show any absorptions assigned to the carbons of chromophoric structures, but gave the valuable information on the chemical structures of the condensation products. On the other hand, the colored material specifically labelled by ^{13}C at the benzylic position was prepared by alkali treatment of vanillyl alcohol-〔carbinol-^{13}C〕 followed by air oxidation, and ^{13}C-NMR spectra of the material exhibited absorptions at 101.7 and 104.6 ppm due to the carbons of a quinonemethide structure, indicating that the quinomethide unit would be one of the important types of chromophores in which benzylic carbon of vanillyl alcohol was included.

INTRODUCTION

As reported in Part I of this series[3], treatment of vanillyl alcohol, which serves as a model for uncondensed units in lignin, in alkaline media afforded diguaiacylmethane and various condensation products with C_5-C_α linkage. This findings lead us to assume that these products are the possible source of the chromophoric structure of model alkali lignin[1,2]. The present work deals with experiments illustrating the chromophoric structures of the colored materials from vanillyl alcohol mainly by ^{13}C-NMR spectroscopy.

EPERIMENTAL

1. Alkaline cooking of vanillyl alcohol.

A solution of 0.504g of vanillyl alcohol in 10ml of IN. sodium hydroxide solution was heated in a sealed glass tube for 1.5 hour under nitrogen. After cooling, half volume of the reaction solution was acidified with 4N. hydrochloric acid under nitrogen and extracted with ethyl acetate. The organic phase was

*1. Received for Publication July 20, 1979.

*2. 江原大學校 農科大學, College of Agriculture, Kang Woen University.

dried over sodiumsulfate and evaporated to give noncolored material. Another half volume of the reaction mixture was then oxidized in air with vigorous stirring for 8 hour at room temperature. The dark solution was acidified with 4N. hydrochloric acid and extracted with ethyl acetate. After evaporation of the ethyl acetate solution dried over sodium sulfate, colored material (0.224g) was obtained. At the next step of experiment the colored material was chromatographed on slight ly deactivated silica gel. Elution with chloroform, ethyl acetate and methanol gave three fractions (0.133g, 0.059g and 0.008g, respectively).

2. Treatment of creosol with formaldehyde in alkaline solution.

To a soution of 1.0g of creosol in 10ml of 1N sodium hydroxide was added 0.6g of formalin (HCHO, 37%). The mixture was heated at 160° for 3 hour and then cooled. After acidification to pH 2, the aqueous solution was extracted with ethyl acetate and the organic phase was dried over sodium sulfate. The solution was concentrated to give an oil, which was purified by chromatography on silica gel using *n*-hexane and acetone (5 : 1; v/v) as eluent to afford 42mg of 2,2′-dihydroxy-3,3′-dimethoxy-5,5′-dimethyl-diphenylmethane VI [13),14)]; m.p. 132-133°.

3. Synthesis of vanillyl alcohol-α-^{13}C (vanillyl alcohol-[carbinol-^{13}C]) from acetic acid-1-^{13}C.

Vanillin-α-^{13}C (vanillin-[carbinol-^{13}C]) was synthesized from gvaiacol and acetic acid-1-^{13}C (^{13}C content 90%) by the same method described for preparation of vanillin-α-^{14}C. 16) The ^{13}C content of it was estimated to be about 88% from the IR sbsorptivity of ^{13}C=O at 1650cm^{-1} and ^{12}C=O at 1680 cm^{-1}, and the Mass spectrum. Reduction of 108mg of vanillin-α-^{13}C in 28ml of methanol with 85mg of sodium borohydride for 1 hour at room temperature afforded 93mg (85.8%) of vanillyl alcohol-α-^{13}C.

4. Alkaline cooking of vanillyl alcohol-α-^{13}C.

Vanillyl alcohol-α-^{13}C was treated with alkali in the same manner described above except heating for longer time (3 hour), followed by air oxidation to give the ^{13}C labelled colored material.

5. Sodium borohydride reduction of the ^{13}C labelled colored material.

A solution of 16mg of the ^{13}C labelled colored material in 4ml of methanol was reduced with 8mg of sodium borohydride at room temperature for 2 day. After destruction of excess sodium borohydride with acetic acid, the solution was diluted with water and extracted with ethyl acetate. The extract, after being dried over sodium sulfate, was concentrated *in vacuo* to give 13mg of reduction products.

6. Distribution of molecular weight of noncolored and colored materials.

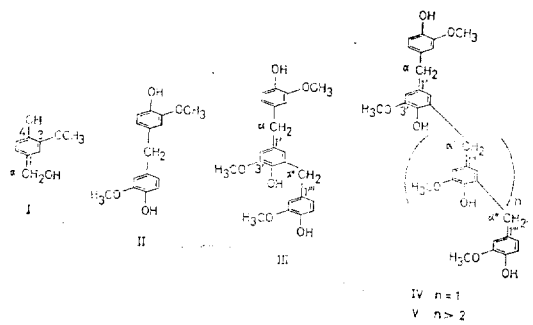
Gel-filtration was carried out in the same way as described in the previous paper[6)].

7. Measurement of UV and ^{13}C-NMR spectra.

UV spectrum was measured in solvents of dioxane and 1N sodium hydroxide using a Shimadzu MPS-50 spectrophotometer. ^{13}C NMR spectrum was recorded on a Jeol JNM-FX 100 pulse Fouriertransform instrument (25.05 MHz). CD_3OD was employed as solvent with TMS as the internal reference.

RESULTS and DISCUSSION

The ^{13}C NMR chemical shifts in CD_3OD for condensation products[3)], which were obtained by treatment of vanillyl alcohol with alkali. are shown in Table 1. By comparison of the chemical shifts of I and II with that of III and IV, it is clear that the chemical shifts of the aromatic carbon atoms depend on the substiuents in the aromatic rings and therefore the



( )

Table 1. ^{13}C NMR spectra of noncolored (top) and colored (middle) materials, and methanol eluted fraction (bottom) of colored material.

Compound	Car. 1,‴	1′,1″	2,2‴	2′,2″	3,3‴	3′,3″	4,4‴	4′,4″	5,5‴	5′,5″	6,6‴	6′,6″	OCH^3	Cal. α	α′,α″
Ⅰ	133.7		111.7		148.5		146.5		115.6		120.7		56.0	65.1	
Ⅱ	134.4		113.1		148.5		145.3		115.8		122.0		56.1	41.8	
Ⅲ	134.0 134.3	133.4	113.1 113.3	110.5	148.2	148.3	145.1 145.2	143.0	115.6	128.9	122.0 122.1	123.5	56.1 56.2	41.8	35.7
Ⅳ	133.9 134.3	133.1 133.3	112.9 113.1	110.3 110.5	148.0 148.1 148.2		144.8 145.0	142.6	115.4 115.5	128.3 128.8	121.8 121.9	123.4 123.5	56.0 56.1	41.7	35.6
noncolored material	133.2 ≀ 134.7		113.3	110.7	148.3		145.3	142.8	128.0 ≀ 128.3 115.8	128.9 ≀ 129.1	122.2	123.7	56.2	41.9	35.9 30.2

Car.=aromatic carbon, Cal.=aliphatic carbon.

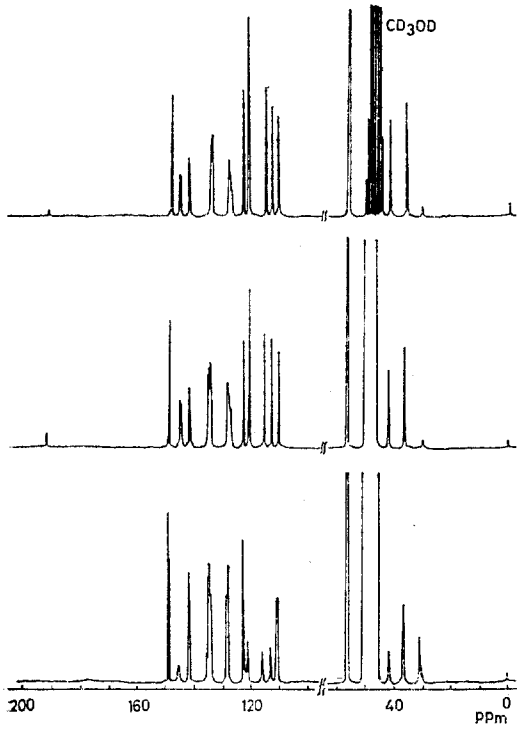
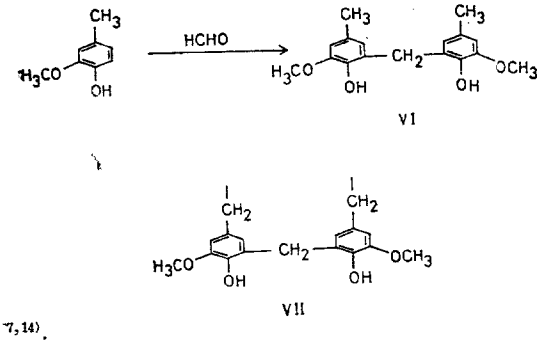


Fig. 1; ^{13}C NMR spectra of noncolored (top) and colored (middle) materials, and methanol eluted fraction (bottom) of coored material.

aromatic carbon atoms at both ends are easily distinguished from others. The methoxyl carbons possess almost constant chemical shift at 56.1 ppm without influence on substituents. On the other hand, the aliphatic carbons at α-position are affected by the hydroxyl groups at the ortho position and showed an upfield shift of about 5 ppm.

The ^{13}C NMR spectrum of the noncolored material, which was prepared from vanillyl alcohol by treatm ent with sodium hydroxide at 165° for 1.5 hours, is shown in Fig. 1. By comparison with models we assigned the signals (Table 1) at 192.7 (CHO), 148.3 (C-3, -3′, -3″ and -3‴), 145.3 (C-4 and -4‴), 142.8 (C-4′ and -4″), 134.7-133.2 (C-1, -1′, -1″ and -1‴), 129.1-128.9 (C-5′ and -5″), 123.7 (C-6′ and -6″), 122.2 (C-6 and -6‴), 115.8 (C-5 and -5‴), 113.3 (C-2 and -2‴), 110.7 ppm (C-2′ and -2″). In high field region the signals at 56.2, 41.9 and 35.9 ppm correspond to methoxyl carbons, C-α, and C-α′ and -α″, respectively. The signal (30.2 ppm) at higher field of the remaining two carbon atoms appears upfielb some 5 ppm relative to corresponding C-α′ and C-α″, suggesting that two hydroxyl groups situate at ortho position of the methylenic carbon. Another peak (128.3-128.0 ppm) lowed the assignment of the aromatic carbon attached to the newly formed aliphatic carbon. The formation of the aliphatic carbon is suggested to proceed via the addition of formaldehyde, which was liberated in the formation of diguaiacylmethane units, to the aromatic carbon at C-5 position, followed by dehydration condensation with other aromatic nucleus as illustrated by the well-known phenol-formaldehyde type condensation[10]. Accordingly this behaviour of the eliminated carbon is analogous to that of the γ-carbons of lignin side chain in alkaline pulping processes



7,14).

As might be expected, on treatment with formaldehyde in alkaline media, creosol afforded the compound VI and, in fact, the ^{13}C NMR chemical shift of the methylenic carbon of VI was in good agreement with that (30.2 ppm) of the carbon described above. Therefore, the condensation products from vanillyl alcohol with alkali would have the partial structure VII in addition to the diguaiacylmethane unit and the elemental structure with C_5-Cα linkage. After alkaline cooking followed by air oxidation in alkaline media, vanillyl alcohol afforded the colored material. The ^{13}C NMR spectrum (Fig. 1) of the material did not show any specific absorption which was concerned with a chromophoric structure, indicating that the proportion of the chromophoric structure are very low on the whole. To concentrate the structural units, the colored material was subjected to column chromatography on slightly deactivated silica gel. Elution with chloroform, ethyl acetate and methanol gave three fractions. In these fractions ethyl acetate eluted part of the colored material was dark brown and methanol eluted fraction almost dark. Unfortunately no specific absorption of the chromophoric structure was detectable even in the ^{13}C NMR spectrum (Fig. 1) of the methanol eluted material. However, the intensity ratio of peaks at 113.3, 115.8, 122.2 and 145.3 ppm for C-2 and -2‴, C-5 and -5‴, C-6 and -6‴, and C-4 and -4‴, respectively, in the aromatic ring situated at both ends diminished to a great extent, suggesting that the material would contain more high-molecular-weight molecules.

Gel-filtration on sephadex G-15, as shown in Fig. 2, exhited that the air oxidation of the alkaline cooking products from vanillyl alcohol in alkaline solution

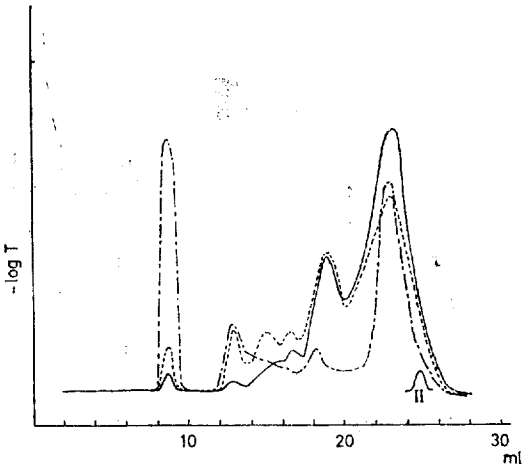


Fig. 2; Gel-filtration curves of noncolored and (——) colored (·····) materials, and methanol eluted fraction (— · — ·) of colored material.

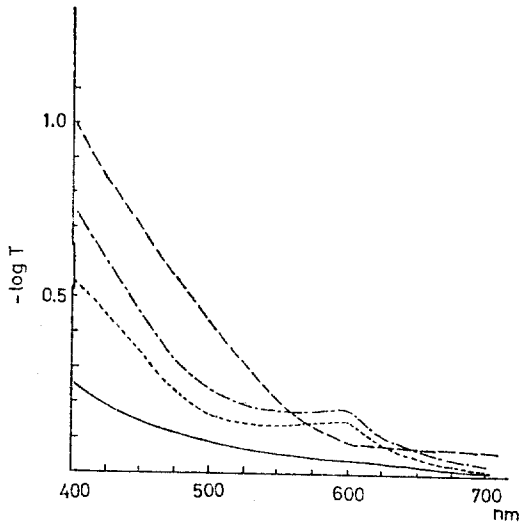


Fig. 3; Visible region absorption spectra (0.074% concentration) of colored material at pH 7 (——) and 12(— · — ·), $\Delta\varepsilon_i$ curve(·····), and methanol eluted fraction (-----) of colored material.

brought about polymerization to a small txtent and the methanol eluted fraction contained more polymers in agreement with the results of the ^{13}C NMR spectrum.

As illustrated in Fig. 3, the UV spectra measured in dioxane (0.074% concentration) would suggest the presence of quinonemethide structure[8] in the colored material.

It was difficult to obtain the informations on the

chromophoric structures of the colored material by a simple method of concentration of chromophoric structural units followed by ^{13}C NMR measurement. In consideration of the estimation[7,8,11,17] that quinon emethide stuctures have held a very important position on the chromophoric structures of alkali lighin, vanillyl alcohol specifically labelled with ^{13}C at benzylic position was used as a experimental material.

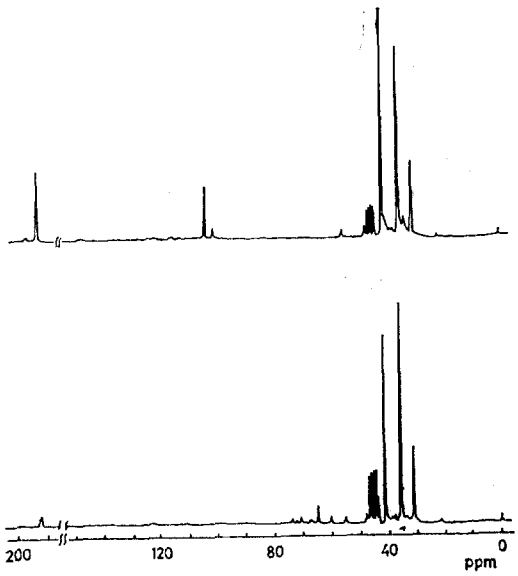


Fig. 4; ^{13}C NMR spectra of ^{13}C labelled (top) and its $NaBH_4$reduced (bottom) materials.

On treatment with alkali for somewhat longer time (3 hour) followed by air oxidation in the similar way as that of nonlabelled vanillyl alcohol, vanillyl alcohol-α-^{13}C gave the colored material. In the ^{13}C NMR spectrum, as shown in Fig. 4, of the material, we assigned the three big peaks at 41.8, 35.9 and 30.1 ppm to C-α, C-α' and the methylenic carbon of VII, respectively. In low field region, the chemical shifts of carbonyl carbons of aldehyde and keto, such as a benzophenone, groups appear at 193.1~192.8 and 196.2 ppmm, respectively. In aromatic and olefinic field regions two peaks at 101.7 and 104.6 ppm, both of which were not detected in the ^{13}C NMR spectra of the non-labelled colored materials, were observed and would be assigned to quinone-methide structures in terms of the chemical shifts. On the bases of the chemical structures of the cond-

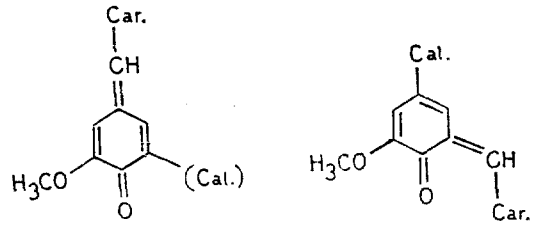


Fig. 5; Te estimated chromphoric structures of coloredhmaterial. Car.=aromatic carbon, Cal. =aliphati carbon.

ensation products[3] obtained by treatment of vanillyl alcohol with alkali, the structural units, as shown in Fig. 5, could be estimated in interpreting the observed absorptions of the chromophoric carbon. As expected, in the ^{13}C NMR spectrum (Fig. 4) of the reduction products of the ^{13}C labelled colored material with sodium borohydride[9] in methanol solution, these two peaks disappeared, although some of the carbonyl carbon remained without reduction, and shifted to higher ficld. Therefore, these results will indicate the presence of the quinonemethide structures, as shown in Fig. 5, possible chromophoric units formed by alkaline cooking of vanllyl alcohol followed by air oxidation.

It has been known[2,4,5,11] the presence of ortho-quinone structure as a chromophoric structure of

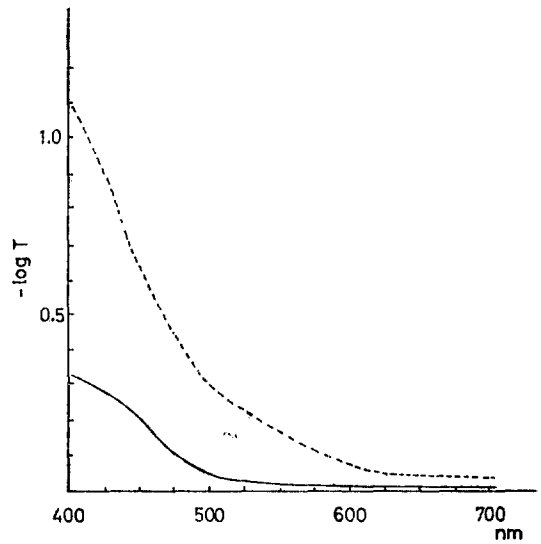


Fig. 6; Visible region absorption spectra of colored materials in 50ml of lN NaOH soln. from vanillyl (50mg) and *p*-hydroxy benzyl (50mg) alcohols.

alkali lignin and, in particular, vanillyl alcohol was easily demethlated[1] by alkaline cooking, suggesting that the ortho quione unit would play an important role of the chromophoric structure derived from vanilly alcohol. In fact, comparison between the UV spectra, as shown in Fig. 6, of the colored materials from vanillyl alcohol and p-hydroxy benzyl alcohol indicated that the catechol unit was responsible for 60-70% of the chromophoric structure from vanillyl alcohol. In these alkaline solutions free radicals,[15] in addition to anions of condensation products would give rise to chromophoric systems to a great extent.

Acknowledgement. I am grateful to Prof. N. Terashima and S. Yasuda of Nagoya Univ. for providing this work and for valuable discussions.

REFERENCES

1) A. Hayashi, K. Kinoshita and A. Taniguchi, Mokuzai Gakkaishi, 21, 480 (1975)
2) A. Hayashi, K. Knoshita, A. Taniguchi, and T. Nakamura, ibid., 21, 485 (1975)
3) B.H Yoon, M. Okada, S. Yasuda and N. Terashima, Mokuzai Gakkaishi, in press (1979)
4) C. Steelink, T. Reid and G. Tollin, J. Am. Chem. Soc., 85, 4048 (1963)
5) G. Chirkin and D. Tishchenko, C. A., 61, 9668 (1964)
6) H. Araki and N. Terashima, Mokuzai Gakkaishi, 23, 370 (1977)
7) J. Gierer, Svensk Papperstidn., 70, 571 (1970)
8) J.M. Harkin, Advan. Chem. ser., 59, 65 (1966)
9) J. Polcin and W.H. Rapson, Pulp paper Mag. Can., 72, T103 (1971)
10) K.H. Ekman, Tappi, 48, 396 (1965)
11) K. Iiyama and J. Nakano, Japan Tappi, 27, 530 (1973)
12) K. Iiyama, Doctor thesis, The Univ, of Tokyo (1975)
13) K. Kratzl, I. Wagner and O. Ettingshausen, Holzforsch. Holzverwert., 24, 32 (1972)
14) M. Tamao and N. Terashima, Mokuzai Gakkaishi, 16, 284 (1970)
15) K.V. Sarkanen and C.H. Ludwig, "Lignins", Wiley-Interscience, New York, p327 (1971)
16) N. Terashima, N. Suganuma, H. Araki and T. Kanda, Mokuzai Gakkaishi, 22, 450 (1976)
17) S. Rothenberg and P. Luner, Advan. Chem. Ser., 59, 90 (1966)