Studies on the Cooking Mechanism of Wood. XIV.[1] *On the UV-Absorption Spectrum, Phenolic Hydroxyl Group and Carboxyl Group of Thiolignin*

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UV-Absorption Spectrum

UV-absorption spectrum of lignosulphonic acid is not so different from that of the lignin in wood[2], the minimum at about 260 mμ being rather deep and the maximum at 280 mμ comparatively sharp. This fact suggests that the structure of the building unit of lignosulphonic acid is at least optically almost uniform. UV-absorption spectrum of thiolignin has been investigated by Enkvist in order to estimate the amount of the phenolic hydroxyl group[3]. The spectra were very different from that of lignosulphonic acid and were very diffuse.

Curve (I) of Fig. 1 is the absorption spectrum of thiolignin, prepared by an experimental cook under the condition similar to that of the industrial cooking. The spectrum is very diffuse, indicating that the structure of the building units is not very uniform. The absorption around 280 mμ, calculated on methoxyl basis, is high in comparison with that of lignin or lignosulphonic acid. As the methoxyl content of thiolignin is in general not so low, except those prepared under very drastic condition or the fraction suffered an intense disintegration, the comparatively high absorbancy at this wave-length region must not be due to demethoxylation.

As will be reported later[4], benzyl alcoholic group, i. e. X group and Z group of lignin, must be exposed to high temperature alkali during the kraft cooking. Model experiments done by Leopold[5] in connection with nitrobenzene oxidation of lignin show that models with benzyl alcoholic groups (I) and (II) were split by hot alkali, resulting in a formation of carboxyl group and double bond conjugated to benzene nucleus.

Merewether recognized the existence of some amount of carbonyl group in Eucalyptus regnans thiolignin[6]. As will be reported

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[1] Part XIII, This Bulletin, 29, 245 (1956).

[2] P. Lange, *Svensk Papperstidn.*, **47**, 262 (1944); **48**, 241 (1945); **50**, No. 11 B, 130 (1947); G. Aulin-Erdtman, *Tappi.*, **32**, 160 (1949).

[3] T. Enkvist and B. Alfredsson, *Svensk Papperstidn.*, **54**, 185 (1951).

[4] Part XVI, to be published in this Bulletin.

[5] B. Leopold, *Acta Chem. Scand.*, **4**, 1523 (1950).

[6] J. Merewether, *Aust. J. Sci. Res., Ser.* A, **1**, 241 (1948).

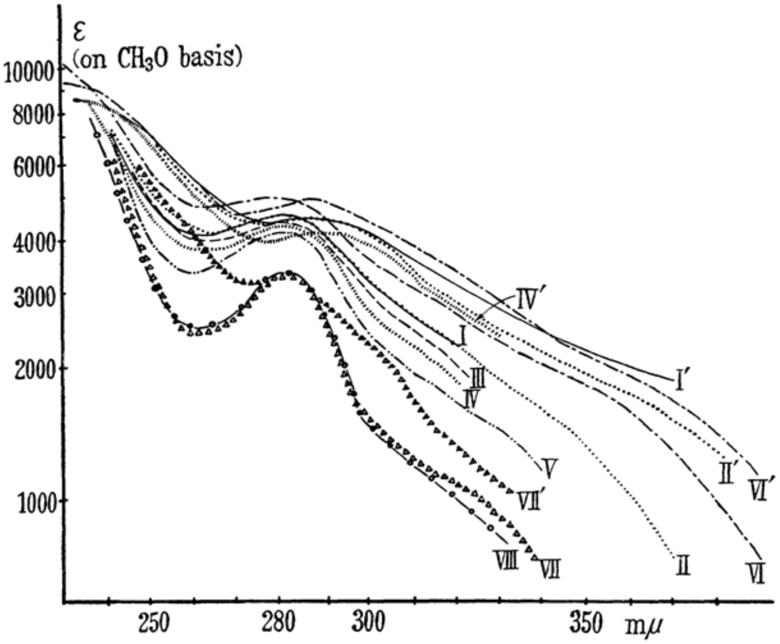
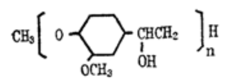


Fig. 1. UV-absorption spectrum of thiolignin, thiolignin treated in various ways, native lignin and dioxane lignin in neutral medium and in 1 N KOH.

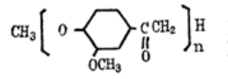
neutral	1 N KOH	curves	lignin
I alcohol	I′ 80% alcohol	————————	thiolignin
II water	II′ water	· · · · · ·	after sulfonation
III alcohol		— — — — —	after reduction with $NaBH_4$
IV 〃	IV′ 80% alcohol	················	after reduction with Raney alloy
V 〃		—··—··—··	IV after reduction with Pd-H_2
VI 〃	VI′ 〃	—·—·—·—·	thiolignin prepd. from dioxane lignin
VII 〃	VII′ 〃	△△△△△△	dioxane lignin
VIII 〃		—○—○—○—	native lignin

later[4], the carbonyl group contained in thiolignin is expected, at least partly, to be conjugated with benzene nucleus, being formed at the same position, where the sulphur atom is situated, after it was split off by alkali. It is reported also that acetovanillone with such a carbonyl group is obtained from the kraft waste liquor but not from that of the soda cooking[7].

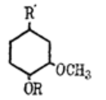
Experiments done by Leopold[5] include also reactions between high temperature alkali and model compounds with such conjugated carbonyl as those of group (III) and (IV). Formation of the carboxyl group directly connected with of benzene nucleus was observed.



I n=2
II n=3



III n=2
IV n=3



	R	R′
V	CH_3	$-COOH$
VI	H	$-CH=CH_2$
VII	OH	$-COOH$
VIII	OH	$-COCH_3$

$$\text{I or II} \xrightarrow[180^{\circ}\text{C}]{2\,\text{N NaOH}} \text{V}+\text{VI}$$

$$\text{III or IV} \xrightarrow{\prime\prime} \text{V}+\text{VII}+\text{VIII}$$

Not much attention has been paid to the existence of carboxyl group in thiolignin. Merewether showed, however, the existence of carboxyl groups in a fraction containing no sulphur, separated from Eucalyptus regnans thiolignin by solvent fractionation[8].

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[7] T. Enkvist, M. Moilanen and B. Alfredsson, *Svensk Papperstidn.*, **52**, 53 (1949); D. Brink, R. Hossfeld and W. Standstrom, *J. Am. Chem. Soc.*, **71**, 2275 (1949).

[8] J. Merewether, *Aust. J. Sci. Res., Ser.* A, **2**. 117 (1949).

Based on the model experiments discussed above and on the mechanism of the kraft cooking which will be reported later[4], the existence of the carbonyl group and the double bond conjugated with benzene nucleus and the carboxyl group directly connected to the nucleus seems to be quite probable, and it is conceived that such structures in the side chain influence the absorption spectrum of thiolignin, making it diffuse and the absorption deep. It appears, therefore, to be very interesting to investigate the influence of such treatment on the absorption spectrum of thiolignin, that the carbonyl group or double bond is expected to be eliminated.

Curve (II) is the absorption of thiolignin prepared by sulphonating (I) according to the Enkvist's method[9]. No appreciable change of the absorption was observed. (III) is the absorption of (I), measured after the reduction with sodium boronhydride, a reduction which is reported to be sufficient to reduce the carbonyl group to the hydroxyl group. The change of the absorption was not very appreciable. (IV) is the absorption of the lignin prepared from (I) by reducing it with Raney alloy and alkali. This reduction is reported to be sufficient to reduce the carbonyl group conjugated with benzene nucleus to methylene[10].

As seen in the figure, this reduction lowers the absorption at about 300 mμ very much, probably because of the complete elimination of the carbonyl group. As this lignin is expected to be completely desulphurized, one can reduce this lignin with palladium black, a catalyst which is very sensitive to sulphur. (V) represents the spectrum of thiolignin (I) reduced with Raney alloy and palladium successively. Here, the spectrum is very similar to that of native or dioxane (curves (VII) and (VIII)) lignin, all of the sulphur atom, carbonyl group and double bond in the thiolignin being eliminated by these treatments. Native lignin was isolated according to Brauns[11] and dioxane lignin according to Freudenberg[12].

These experimental results suggest to us that the absorption spectrum of thiolignin is diffuse and the absorption is higher in thiolignin than that of lignin in situ due to the presence of the carbonyl group and the double bond conjugated with benzene nucleus in thiolignin. Absorbancy shown by curve (V) around 280 mμ region is as yet higher than that of native lignin, probably because carboxyl group exists in lignin (V) as yet. As shown in Fig. 2, vanillic acid has very large absorption around 260–290 mμ region.

As stated previously, thiolignin (I) was prepared from wood powder and the thiolignin investigated by Enkvist was also prepared from wood. Absorption spectrum of thiolignin prepared from lignin without the existence of carbohydrate is not reported as yet. It is known that especially hemicellulose suffers a severe degradation during the alkaline cooking of wood. As such compounds, Sohn isolated reductic acid (V)[13] and Enkvist identified methylcyclopentenolone VI[14].

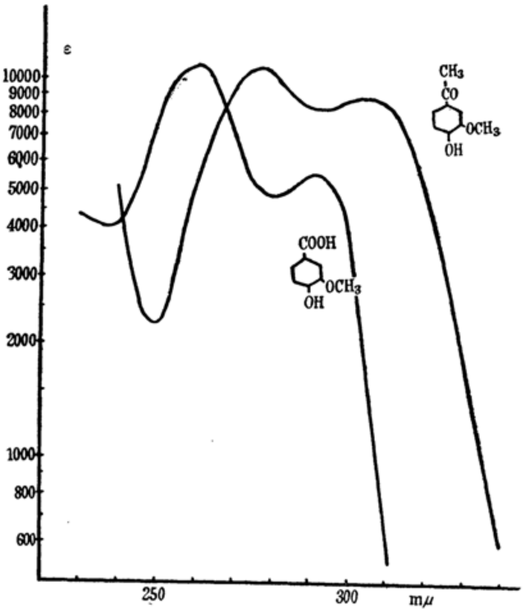

Fig. 2. UV-Absorption spectrum of vanillic acid and acetovanillone (in alcohol).

Migita and Kanda report that the color of kraft pulp is due not only to lignin but also to substances originating from hemicellulose[15]. Richtzehain assumed the condensation of lignin with some substances originating from carbohydrate during the kraft cooking in order to explain his results, that thiolignin gives a comparatively high yield of isohemipinic acid, when it is methylated with dimethyl sulphate and oxidized with permanganate[16]. It will, therefore, be con-

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[9] T. Enkvist, B. Alfredsson and E. Hägglund, *Svensk Papperstidn.*, **55**, 588 (1952).

[10] D. Papa, E. Schwenk and B. Whitman, *J. Org. Chem.*, **7**, 587 (1942).

[11] F. Brauns, *J. Am. Chem. Soc.*, **61**, 2120 (1939).

[12] W. Stumpf and K. Freudenberg, *Angew. Chem.*, **62**, 537 (1951).

[13] A. Sohn, *Das Papier*, **4**, 379 (1950).

[14] T. Enkvist, B. Alfredsson, M. Merikallio, P. Paakbonen and O. Jarvela, *Acta Chem. Scand.*, **8**, 51 (1954).

[15] N. Migita, T. Kanda, S. Mochizuki and H. Nishimura, *Japanese Tappi.*, **8**, 277 (1954).

[16] H. Richtzenhain, *Svensk Papperstidn.*, **53**, 644 (1950).

ceivable that the absorption spectrum of thiolignin differs from that of the lignin in situ, because of the condensation of the substances originating from wood carbohydrate with lignin. Thiolignin was prepared, therefore, from Freudenberg's dioxane lignin under the ordinary kraft cooking condition and its spectrum was measured. The spectrum (VI) represents, however, a typical thiolignin spectrum, thus indicating that the influence of wood carbohydrate on thiolignin spectrum may not be very large and the spectrum of thiolignin must be explained by the structure of the thiolignin itself alone.

Summarizing the results, it was shown that the UV-absorption spectrum of thiolignin must be explained by assuming the existence of the carbonyl group, the double bond conjugated with the nucleus and the carboxyl group attached directly to the nucleus in thiolignin.

Estimation of the Amount of the Phenolic Hydroxyl Group and the Carboxyl Group of Thiolignin

Enkvist measured the amount of the phenolic hydroxyl group of thiolignin spectroscopically[17]. His method was, however, the old method of Aulin-Erdtman, which is not sensitive enough even when applied to simple model compounds[18].

Absorption spectra of thiolignin (I), shown in Fig. 1, which were taken in neutral and alkaline media, indicate that this lignin contains only 0.22–0.18 phenolic hydroxyl group per methoxyl group when calculated by the Aulin-Erdtman's new method[19].

The content of the phenolic hydroxyl group of thiolignin has been estimated by several different methods, and the values obtained are much greater. Enkvist titrated with sodium ethylate in ethylenediamine solution or with hydrochloric acid after addition of barium chloride to the sodium hydroxide solution of the lignin[20]. According to these methods, equivalent weight was about 200–300. Methylation with diazomethane is also used. Values measured by this method, hitherto reported, are shown in the table. The amount 0.55–0.9 per methoxyl group, being about the same as those obtained by titration methods.

As stated previously, the UV-absorption spectrum of thiolignin is rather complex,

	MeO %	After CH_2N_2 methylation MeO %	After CH_2N_2 methylation followed by hydrolysis MeO %	COOH+OH* per MeO	OH* per MeO	OH* per MeO spectroscopically measured	Literature
Alkali lignin A (spruce)	14.9	22.2		0.55			21)
Alkali lignin B (spruce)	14.0	24.8		0.87			21)
Thiolignin (spruce)	14.6	24.8		0.79			22)
Thiolignin (spruce)	12.9	23.1		0.89			3)
Alkali lignin (oat straw)	14.8 14.7	23.5 22.4					23)
Thiolignin (Eucalyptus)	21.1	27.1					24)
Native lignin (spruce)	14.8	21.4		0.49		0.4 −0.32	25)
Dixane lignin (Picea jezoensis)	15.56	17.98	17.23	0.16	0.12	0.2 −0.16	
Thiolignin I (Picea jezoensis)	15.31	23.3	20.81	0.58	0.40	0.225 −0.18	
Thiolignin prepd. from dioxane lignin	14.55						

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17) T. Enkvist, B. Alfredsson, *Svensk Papperstidn.*, **54**, 185 (1951).

18) G. Aulin-Erdtman, *Tappi.*, 32, 160 (1949).

19) G. Aulin-Erdtman, *Svensk Papperstidn.*, **55**, 745 (1952).

20) T. Enkvist, *Svensk Papperstidn.*, **51**, 225 (1948).

21) H. Marshall, F. Brauns and H. Hibbert, *Can. J. Res.*, **13B**, 103 (1935).

22) C. Ahlm, *Paper Trade J.*, **113**, No. 13, 115 (1941).

23) M. Phillips and M. Goss. *J. Biol. Chem.*, **114**, 557 (1936).

24) F. Lahey and J. Merewether, *Austr. J. Sci. Res.*, **A 1**, 112 (1948).

25) F. Brauns, *J. Am. Chem. Soc.*, **61**, 2120 (1939).

and the building unit seems to be not very uniform. It is, therefore, as yet open to question, whether it is justifiable or not to estimate spectroscopically the amount of the phenolic hydroxyl group of such ununiform substance as thiolignin. Diazomethylation method seems to be much more reliable in such cases.

Since thiolignin is expected to contain some amount of carboxyl group as mentioned before, one can not expect, to obtain exact values simply from the increase of the methoxyl group by diazometylation. The content of the phenolic hydroxyl group must be calculated from the difference between the methoxyl group content of the thiolignin, methylated with diazomethane followed by alkaline hydrolysis, and that of the original untreated thiolignin, the difference of the content of the methoxyl group before and after the hydrolysis being expected to show the amount of the carboxyl group in the thiolignin. The content of the phenolic hydroxyl group of thiolignin (I) estimated according to this method is 0.4 per methoxyl group, and the amount of the carboxyl group is 0.18 per methoxyl group.

As reported previously, lignin in wood contains only about 0.14 phenolic hydroxyl group per methoxyl group; the phenolic hydroxyl group increases, therefore, very much during the kraft cooking. Because of the presence of this phenolic hydroxyl group and the carboxyl group, thiolignin is very easily soluble in alkali.

Added in proof. According to the recent investigation by J. Lindberg and T. Enkvist (*Suomen Kemistilehti*, **B 28**, 23 (1955)) the spectroscopic method of the detection of the phenolic groups due to G. Aulin-Erdtman could not be used in the case of orthovanillic acid, salicylic acid, 5-carboxyvanillin and 5-carboxyvanillic acid. As it might not be improbable that thiolignin contains some amount of pherolic hydroxyl groups with a carboxyl group in its ortho position, their findings might provide us one of the reasons why herspectroscopic method of the estimation of phenolic hydroxyl groups in lignin was not suitable in the case of thiolignin preparations.

Experimental

Preparation of Thiolignin I.—Extractives free Picea jezoensis wood powder (550 g.) was cooked with 3.5 1. of white liquor of 20% sulphidity with 4% active alkali. Temperature was raised to 165° within two hours and kept at this temperature for one hour. The black liquor was acidified, filtered and the crude thiolignin obtained was dissolved in a mixture of acetone (200 ml.) and alcohol (40 ml.) and precipitated with ether. After repeated reprecipitation, the yield was 38 g.

Anal. C, 61.33; H, 5.64; S, 2.42; CH_3O, 15.31%.

Sulphonation of Thiolignin I at Neutral pH.—To 3 g. of thiolignin I was added sulphite cooking acid of pH 8 (80 ml.) and the whole was heated at 105° for two hours in a quickly rotating autoklave. Thiolignin went into solution almost completely. Sulphonic acid was separated as Ba salt as usual and analyzed. CH_3O, 14.05; SO_3H/CH_3O, 0.119.

Reduction of Thiolignin I with Sodium Borohydride.—Five grams of thiolignin was dissolved in 100 ml. of phosphate buffer, which can dissolve lignin but has as low pH as possible. 1.5 g. of $NaBH_4$ was added and kept at room temperature over-night. CH_3O, 15.08%.

Reduction of Thiolignin with Raney Alloy and Alkali)—Three grams of thiolignin was dissolved in 20 ml. of 10% alkali and was reduced with 12 g. of fine Raney alloy powder at 80–90°. CH_3O, 15.10%.

Reduction with Palladium and Hydrogen.—0.1 g. of palladium chloride was dissolved in 20 ml. of water slightly acidified with hydrochloric acid, 500 mg. of thiolignin reduced with Raney nickel added, and the lignin was dispersed by agitation. Palladium was reduced with hydrogen and precipitated on lignin, which was separated and washed by centrifuge. The precipitate thus obtained was hydrogenated in 100 ml. of alcohol for two hours at room temperature.

Hydrolysis of the Thiolignin methylated with Diazomethane.—The lignin wasl gently refluxed with 1 N alcoholic KOH for two hours.

Kraft Cooking of Dioxane Lignin.—Three hundred milligrams of dioxane lignin was dissolved in white liquor of 20% sulphidity with 4% active alkali (5 ml.) and was heated as in the case of wood powder. CH_3O, 14.55%.

Summary

It was shown that the UV-absorption spectrum of thiolignin must be explained by assuming the existence of the carbonyl group, the double bond conjugated with the nucleus and the carboxyl group attached directly to the nucleus. The amount of the phenolic hydroxyl group and the carboxyl group of thiolignin were estimated by methylating with diazomethane and subsequently hydrolyzing the methylated lignin with alkali.

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