

## Estimation of Phenolic Hydroxyl Groups in Lignin

### I. Periodate Oxidation of Guaiacol Compounds

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A method for the estimation of free phenolic groups in softwood lignin has been developed. The method is based on the finding that the action of sodium periodate upon guaiacol results in rapid oxidative demethylation, methanol being liberated in a yield of about 0.9 mole per mole of guaiacol. A variety of substances containing one or two guaiacyl nuclei and representing the probable structural principles of lignin have been investigated with regard to their behaviour towards periodate. Substituents in the positions *para* and *ortho* to the phenolic hydroxyl group of guaiacol in general were found to be without influence upon the methanol formation. A few exceptions, which, however, are of minor importance to the lignin problem, are pointed out.

As a result of these model experiments, it is concluded that any amount of methanol (moles of methanol per methoxyl group) obtained on periodate oxidation of softwood lignin preparations, when multiplied by 1.1, will give the number of phenolic hydroxyl groups per methoxyl in the lignin preparation examined.

One of the main problems of lignin chemistry is to elucidate the way in which the guaiacyl propane monomers (in softwood lignin) or the guaiacyl and syringyl propane monomers (in hardwood lignin) are linked to each other to form the lignin molecules. Previous work (*cf.*<sup>1</sup>) suggests that different types of linkages are involved, *viz.*,

a) alkyl aryl ether linkages connecting the propane side-chain of one monomer with the aromatic nucleus of the following one. The alkyl carbon atom can be either the  $\alpha$ -carbon (type  $a_1$ ) or possibly the  $\beta$ -carbon (type  $a_2$ ) of the side-chain;

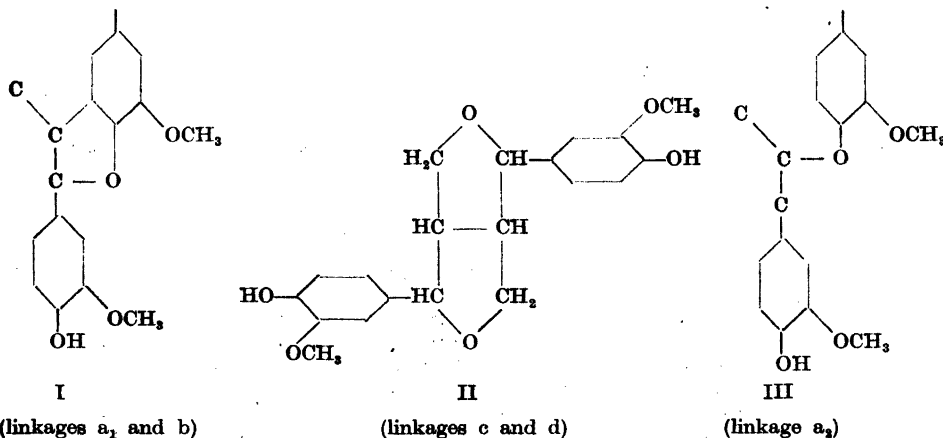
b) carbon-carbon linkages between the side-chain of one monomer and the aromatic nucleus of the following one;

c) alkyl ether linkages between two side-chains;

d) carbon-carbon linkages between two side-chains.

A combination of types  $a_1$  and b is present in the coumaran ring structure (I), whereas a combination of types c and d is to be found in pinoresinol (II). Both structures have been shown to be formed as intermediates in the bio-

synthesis of lignin from coniferyl alcohol, and the coumaran structure at least is assumed to constitute an essential building element in the final lignin molecule<sup>1</sup> (*cf.*, however,<sup>2</sup>). It has been proposed that alkyl ether linkages (c) of the benzyl alkyl ether type are involved in sulphonation, alcoholysis, and other typical reactions of lignin<sup>3,4</sup>. Finally, the occurrence in lignin of alkyl aryl ether linkages of type  $a_2$  (*iso*-propyl aryl ether linkage, III) has been discussed on the basis of model experiments<sup>5,6</sup>.\*



As yet, conclusive information regarding the relative amounts of the various kinds of linkages in lignin is missing. Obviously, for a further elucidation of this problem, a knowledge of the number of free phenolic groups in lignin must be of fundamental interest. If this number were known ( $n$  phenolic groups per phenylpropane monomer), the number of the alkyl aryl ether bonds ( $a_1$  plus  $a_2$ ) would also be known ( $1-n$ ).

Moreover, a determination of any change in the number of phenolic groups taking place if lignin is subjected to reactions such as sulphonation and alcoholysis, would be of great value for the understanding of the mechanism of these reactions and the structure of the reacting groups.

The need of correct data concerning the phenol content of lignin was early realized, and a variety of methods for its estimation have been used in the past; they yielded, however, widely differing figures (*cf.* the summaries on lignin<sup>7,8</sup>). Conflicting values have also been reported very recently. Whereas Freudenberg<sup>9,1</sup>, using the reaction of phenolic hydroxyl groups with 2,4-dinitrofluorobenzene and other methods, found 0.5–0.6 phenolic hydroxyls per methoxyl group (in lignosulphonic acid), Aulin-Erdtman<sup>10,11</sup>, as a result of her extensive spectrophotometric investigations, concludes that lignosulphonic acid contains 0.17–0.25 phenolic groups per methoxyl. Values of 0.23–0.24, likewise obtained spectrographically, have been reported by Goldschmid<sup>12</sup>.

\* Note added in proof: The formation of linkages of type  $a_2$  (III) on enzymatic dehydrogenation of coniferyl alcohol was recently demonstrated by K. Freudenberg, H. Schlüter and W. Eisenhut *Naturwiss.* 41 (1954) 576.

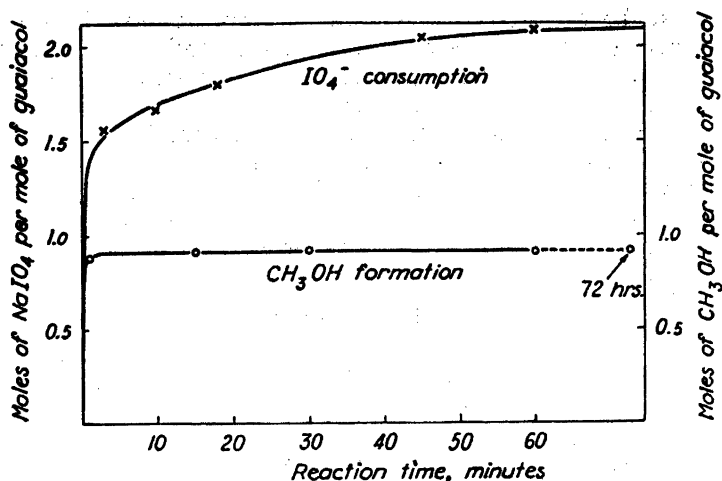


Fig. 1. Consumption of periodate and formation of methanol on periodate oxidation of guaiacol. (0.5 millimole of guaiacol and 3.5 millimoles of sodium metaperiodate in 50 ml of water, at 4°.)

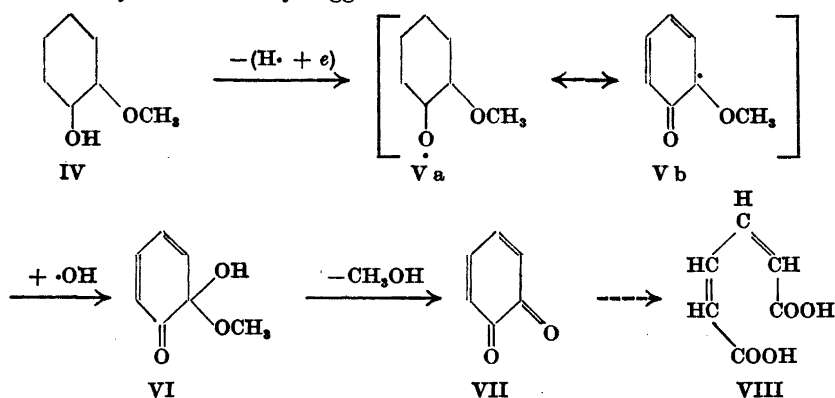
In this laboratory, a new method for the estimation of free phenolic groups in lignin has been developed. The present communication deals with an examination of the method using lignin model substances, and in following papers some results obtained with various lignin preparations will be reported. Preliminarily it may be stated that, with comparable lignin preparations (lignosulphonic acids), our method yielded values similar to those given by Aulin-Erdtman<sup>11</sup> and Goldschmid<sup>12</sup>.

The new method is based upon the behaviour of phenols, especially guaiacol and substituted guaiacols, towards periodic acid. Whereas this reagent has been extensively used for many years in the cleavage of C—C linkages in  $\alpha$ -glycols,  $\alpha$ -ketols and related structures, very little attention has been paid to its action upon phenols. The only work covering the latter problem to be found in the literature is contained in two short communications by Pennington and Ritter<sup>13,14</sup>. These authors found that carbohydrate-free preparations of lignosulphonic acids consumed periodic acid, the products recovered after the oxidation containing less methoxyl than the original material. Methanol was detected in the reaction mixture. It was also shown that various phenols, including guaiacol and some substituted guaiacols such as vanillyl alcohol, vanillic acid, and ferulic acid, were attacked by periodic acid. In the two last-mentioned cases amorphous oxidation products were formed, the methoxyl contents of which were considerably lower than those of the original acids.

In connection with the synthesis of guaiacylglycerol, Adler and Yllner<sup>15</sup> investigated the periodate breakdown of the glycerol side-chain of this substance. As could be expected from the results of Pennington and Ritter, a simultaneous attack upon the guaiacyl nucleus occurred. A comparison with the behaviour of guaiacol revealed that in both cases a very rapid initial consumption of periodic acid, followed by a slower further oxidation, took place.

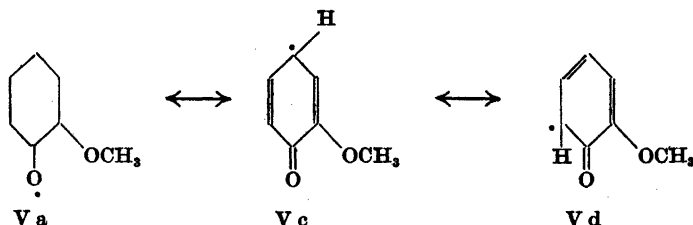
It seemed to be of interest to examine whether the periodate oxidation of these substances involved any formation of methanol, similar to that observed by Pennington and Ritter in the case of lignosulphonic acid. Surprisingly enough, it was found that no less than 90—92 % of the amount of methoxyl groups present were rapidly liberated as methanol, when guaiacol in aqueous solution was treated at 4° C with an excess of sodium periodate. The total amount of methanol was formed during the initial, rapid phase of periodate consumption (Fig. 1). Under the conditions used (4°, dark room), the methanol formed was stable against the excess periodate present during a period of several days (*cf.*<sup>16</sup>).

Regarding the mechanism of the oxidative methanol formation, the following sequence of reactions may be tentatively suggested:



The primary attack of periodate upon guaiacol will result in the formation of the mesomeric radical V. This view regarding the initial step in the oxidation of phenols by various oxidants has been put forward by Pummerer<sup>17</sup> and has been generally accepted (*cf.*<sup>18</sup>). Recently, it has received further experimental support by the isolation of the comparatively stable 2,4,6-tri-*tert.*-butylphenoxy radical<sup>19</sup> from the dehydrogenation of the corresponding phenol with lead dioxide and similar oxidants. In a second oxidation step, the radical V, in its mesomeric form V b, may react with an hydroxyl radical, yielding the hemiacetal VI. This will spontaneously lose one mol. of methanol, thus being converted into o-quinone (VII), which finally undergoes periodate oxidation to *cis-cis*-muconic acid (VIII). The last-mentioned product has been isolated from the reaction mixture.

Since the yield of methanol is 90—92 %, the reaction sequence proposed above appears to be the main pathway of the oxidation. Only 8—10 % of the guaiacol used is oxidized in a different way, obviously not including the hypothetical intermediate VI, which is supposed to be the immediate source of the methanol. These side-reactions might be explained by the assumption that the further oxidation of the aroxyl radical, to an extent of 8—10 %, arises from the mesomeric forms c and d:



As has been shown recently by Wessely<sup>20</sup>, the action of lead tetraacetate upon guaiacol results in the formation of methoxy-*p*-quinone in a yield of 57 %. This oxidant thus seems to attack mainly the mesomeric form V c.

The scope and the mechanism of the oxidative cleavage of *o*- (and *p*-) hydroxyaryl ethers as well as the reaction of other types of phenols with periodic acid are being further studied in this laboratory.

In contrast with guaiacol, veratrol was found to be completely stable in the presence of sodium periodate under the experimental conditions used. It is interesting to note that, in this respect, periodic acid differs from other specific oxidants, like percarboxylic acids, which have been shown to oxidize not only pyrocatechol<sup>21</sup> — with the formation of muconic acid — but also veratrol<sup>22</sup>, with the formation of dimethyl muconate.

These findings seemed to indicate that the determination of methanol formed on periodate oxidation of lignin might offer a possibility of estimating the amount of phenolic guaiacyl residues present in softwood lignin. It was obvious, however, that correct values would not be obtained unless the following conditions were fulfilled:

- 1) All types of phenolic guaiacyl groups in lignin, irrespective of their way of substitution, yield similar amounts of methanol;
- 2) Methanol is formed solely from the phenolic nuclei, and not from other structural elements in lignin.

The following experiments constitute an examination of point 1, *i.e.*, a study of the methanol formation from various guaiacol derivatives related to possible structural elements in lignin. The validity of point 2 will be discussed in a subsequent paper.

Table 1 gives the yields of methanol obtained from a series of guaiacol derivatives. The oxidation was carried out with an excess of sodium periodate either in aqueous solution or in 80 % acetic acid. After precipitation of iodate and unconsumed periodate with a lead nitrate solution, methanol was distilled off and determined by oxidation with permanganate and colorimetric measurement of the formaldehyde obtained (*cf.* exptl. part). Several (generally 3 to 6) samples of each substance were oxidized for various periods of time as shown for guaiacol in Fig. 1. The rate of methanol formation was slightly lower in 80 % acetic acid than in water. In the case of guaiacol, for instance, the maximum value was reached after a reaction time of 1—2 minutes in aqueous solution (*cf.* Fig. 1) and after 5—6 minutes in the acetic acid. The final methanol values, however, were practically equal in both types of experiments (*cf.* substances IV, XI, and XXIX, Table 1). The methanol figures given in Table 1 represent the average of the values obtained after various times of reaction, excluding values relating to the rapid initial phase (*cf.* Fig. 1). In some cases the periodate consumption was followed and found to proceed in a generally similar way in both solvents, the total periodate consumption in the slower phase, however, being somewhat greater in acetic acid than in water.

The substances in Table 1 have been divided into four groups, A—D.

*Group A* comprises substances in which the guaiacol nucleus is substituted in the 4-position, with no double bond conjugated with the phenolic ring present. In addition to some guaiacyl carbinols (IX—XI, XVI), a guaiacyl

Table 1. Formation of methanol on periodate oxidation of guaiacol derivatives.

Substance	Method of preparation	Moles of CH <sub>3</sub> OH per mole of substance	
		in water	in 80 % acetic acid
<i>Group A:</i>			
Guaiacol (IV)	—	0.91	0.92
Vanillyl alcohol (IX)	23	0.88	—
$\alpha$ -(4-Hydroxy-3-methoxyphenyl)-ethanol (X)	cf. exptl. part	0.88	—
$\alpha$ -(4-Hydroxy-3-methoxyphenyl)- <i>n</i> -propanol (XI)	24	0.94	0.91
Vanillyl ethyl ether (XII)	cf. exptl. part	0.89	—
Barium 1-(4-hydroxy-3-methoxyphenyl)- <i>n</i> -propane-1-sulphonate (XIII)	»	0.92	—
Eugenol (XIV)	—	—	0.89
1-(4-Hydroxy-3-methoxyphenyl)-2-propanone (XV)	25	—	0.88
Isoeugenolglykol $\beta$ -guaiacyl ether (XVI)	26	—	0.90
Dehydro-diisoeugenol (XVII)	27	—	0.92
Dihydro-dehydro-diisoeugenol (XVIII)	28	—	0.93
Dehydro-diconiferyl alcohol (XIX)	29	—	0.93
<i>d</i> -Pinoresinol (II)	30	—	0.93 *
<i>Group B:</i>			
6- <i>n</i> -Propylguaiacol (XX)	31	—	0.93
Barium 3-methoxy-4-hydroxy-5- <i>n</i> -propylbenzyl sulphonate (XXI)	cf. exptl. part	0.88	—
<i>o</i> -Eugenol (XXII)	32	—	0.87
<i>o</i> -Vanillyl alcohol (XXIII)	cf. exptl. part	0.87 **	—
<i>o</i> -Homovanillyl alcohol (XXIV)	»	—	0.88
Barium dehydro-divanillyl di-sulphonate (XXV)	»	0.80 *	—
1-(4-Hydroxy-3-methoxyphenyl)-2-(6-hydroxy-5-methoxy-3-propylphenyl)propane (XXVI) ***	33	—	0.89 *
<i>Group C:</i>			
<i>trans</i> -Isoeugenol (XXVII)	34	—	0.69
Coniferyl alcohol (XXVIII)	29	—	0.72
Coniferyl aldehyde (XXIX)	cf. exptl. part	0.85	0.88
Ferulic acid (XXX)	—	—	0.92
<i>Group D:</i>			
Vanillin (XXXI)	—	—	0.76 ****
4-Acetoguaiacone (XXXII)	cf. exptl. part	—	0.77
4-Propioguaiacone (XXXIII)	»	—	0.74
Vanillic acid (XXXIV)	35	—	0.76

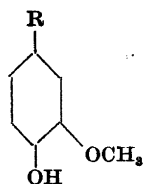
\* Based on one phenolic equivalent of the substance.

\*\* After a reaction time of 1 minute (cf. p. 327).

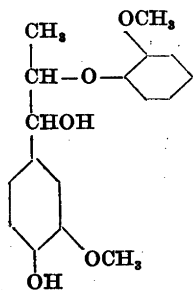
\*\*\* Kindly submitted by Dr. Gunhild Aulin-Erdtman.

\*\*\*\* Not constant after 8 days (cf. Fig. 2).

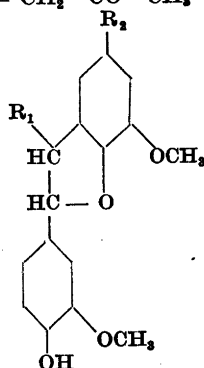
Group A:



- IX: R = CH<sub>2</sub>OH
- X: R = CHOH-CH<sub>3</sub>
- XI: R = CHOH-CH<sub>2</sub>-CH<sub>3</sub>
- XII: R = CH<sub>2</sub>OC<sub>2</sub>H<sub>5</sub>
- XIII: R = CH(SO<sub>2</sub>Ba<sub>0.5</sub>)-CH<sub>2</sub>-CH<sub>3</sub>
- XIV: R = CH<sub>2</sub>-CH=CH<sub>2</sub>
- XV: R = CH<sub>2</sub>-CO-CH<sub>3</sub>

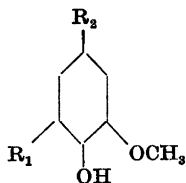


XVI

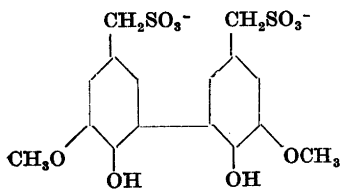


- XVII: R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub> = CH=CH-CH<sub>3</sub>
- XVIII: R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub> = CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>
- XIX: R<sub>1</sub> = CH<sub>2</sub>OH, R<sub>2</sub> = CH=CH-CH<sub>2</sub>OH

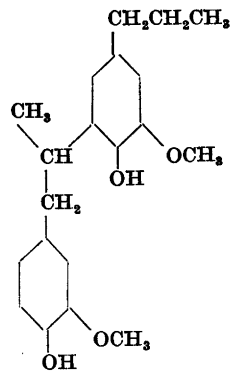
Group B:



- XX: R<sub>1</sub> = CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>, R<sub>2</sub> = H
- XXI: R<sub>1</sub> = CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>, R<sub>2</sub> = CH<sub>2</sub>(SO<sub>2</sub>Ba<sub>0.5</sub>)
- XXII: R<sub>1</sub> = CH<sub>2</sub>-CH=CH<sub>2</sub>, R<sub>2</sub> = H
- XXIII: R<sub>1</sub> = CH<sub>2</sub>OH, R<sub>2</sub> = H
- XXIV: R<sub>1</sub> = CH<sub>2</sub>-CH<sub>2</sub>OH, R<sub>2</sub> = H

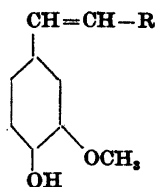


XXV



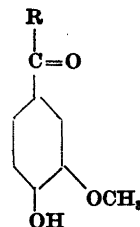
XXVI

## Group C:



- XXVII: R = CH<sub>3</sub>  
 XXVIII: R = CH<sub>2</sub>OH  
 XXIX: R = CHO  
 XXX: R = COOH

## Group D:



- XXXI: R = H  
 XXXII: R = CH<sub>3</sub>  
 XXXIII: R = CH<sub>2</sub>-CH<sub>3</sub>  
 XXXIV: R = OH

alkyl ether (XII), and a sulphonic acid (XIII), it includes the "dimeric" lignin models XVI (a guaiacyl-propane- $\beta$ -aryl ether), XVII—XIX (coumarans), and *d*-pinoresinol (II).

The behaviour of all these substances was very similar to that of guaiacol itself, the rates of methanol formation being practically the same as for guaiacol. The final amounts of methanol ranged from 0.88 to 0.94 moles per guaiacyl nucleus, with a mean value for all substances of 0.91.

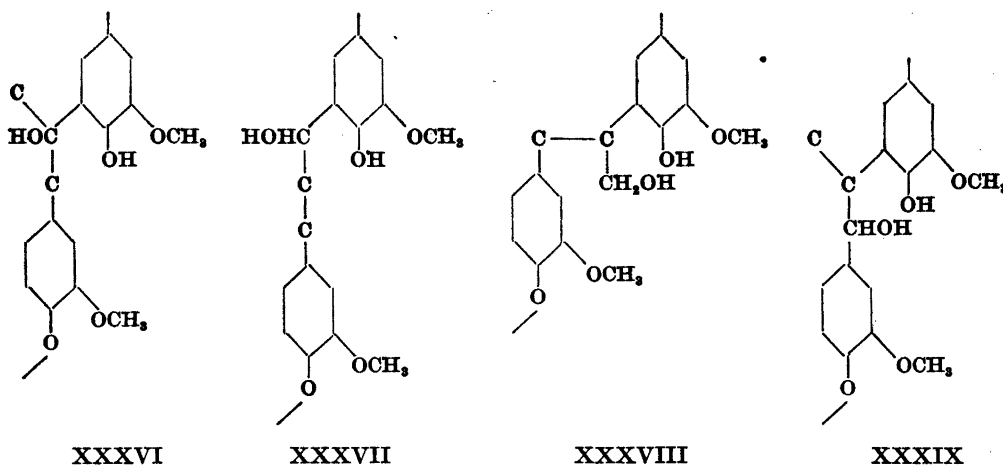
It may be noted here that in the periodate oxidation of the carbinols IX, X, and XI the side-chains are partially split off yielding the corresponding aliphatic aldehydes in yields of 50–60 % of the theoretically possible amounts<sup>36</sup>.

*Group B.* In addition to (phenolic or etherified) 4-propylguaiacol residues lignin contains 4,6-substituted guaiacol elements. Predominantly, the phenolic hydroxyl group and the 6-substituent will be joined by ring-closure, as in dehydro-diconiferyl alcohol (XIX), but there are also indications of the occurrence, in lignin, of minor amounts of phenolic structures like those represented by the upper ring of formula XXVI (*cf.* Richtzenhain<sup>37</sup>, Aulin-Erdtman<sup>11</sup>). Furthermore, the presence of 6,6'-diguaiacyl structures, represented here by model substance XXV, has been discussed, although recent spectrophotometric evidence indicates<sup>11</sup> that their amount (in liginosulphonic acids) must be very low. Aulin-Erdtman<sup>33</sup> has shown that the two neighbouring phenolic groups in such substances have highly different ionization constants, and it could be supposed that this might influence their reactivity towards periodic acid.

Similarly, it appeared possible that the dehydrogenation of the phenolic hydroxyl group in *o*-vanillyl alcohol (XXIII) might be influenced by intramolecular hydrogen bonding. In lignin, the presence of *o*-guaiacyl carbinol groupings is, however, highly improbable. Tertiary carbinols of type XXXVI seem not to be present in lignin in appreciable amounts<sup>1</sup>. And also, the occurrence of carbinols of type XXXVII, represented by *o*-vanillyl alcohol (XXIII), would be hardly conceivable on the basis of the present views regarding the biosynthesis of lignin.

The main part of the aliphatic hydroxyl groups in lignin are probably primary carbinol groups<sup>1</sup>. Therefore, the presence of phenolic structures





like XXXVIII appears possible, although, as mentioned above, their frequency in lignin seems to be low<sup>11</sup>. Furthermore, structures like XXXIX cannot be excluded. In order to examine whether *ortho* side-chains with aliphatic hydroxyl groups in the  $\beta$ -position, such as in XXXVIII and XXXIX, may have any effect on the oxidation of the guaiacyl grouping, homo-*o*-vanillyl alcohol (XXIV) was synthesized as a model substance.

The periodate oxidation of the majority of the substances of group B exhibited no peculiarities. 6-*n*-Propylguaiacol (XX) yielded methanol at the same rate and in the same amounts as did guaiacol, and, similarly, the values obtained with substances XXI, XXII, XXIV, and XXVI were on the same level as those presented in group A. The oxidation of the diphenyl derivative XXV was slightly retarded, the final methanol value being reached shortly after a reaction time of five minutes. The extent of side-reactions was somewhat increased which is indicated by the comparatively low yield of methanol, *viz.*, 0.80 mole per guaiacyl nucleus. Possibly, the increased electron density at the *ortho*-linkage between the two phenolic nuclei favours attack of hydroxyl radicals upon the linked ring carbon atoms.

The behaviour of *o*-vanillyl alcohol (XXIII) was unlike that of all the other substances examined. The methanol value given for XXIII in Table I refers to a reaction time of only one minute. After a few further minutes of reaction a colourless crystalline solid began to deposit, the amount of which increased during the following hours. As the precipitate appeared, methanol determinations yielded values which were considerably lower (down to 0.4 moles of CH<sub>3</sub>OH per mole of XXIII) than the initial value recorded in Table I. It then was found that an aqueous suspension of the isolated precipitate, when boiled at pH 4.5, yielded a distillate which contained methanol.

The elementary composition of the crystalline solid, m. p. 171–172°, was C<sub>8</sub>H<sub>8</sub>O<sub>3</sub>, which agrees to vanillyl alcohol minus two atoms of hydrogen. It contained 20.36% OCH<sub>3</sub> (calc. OCH<sub>3</sub> 20.40). Probably the substance is a dimerization product, C<sub>16</sub>H<sub>16</sub>O<sub>6</sub>. Its structure as well as that of similar products obtained from other *o*-hydroxybenzyl alcohols is being further investigated.

The properties of the solid oxidation product of XXIII explain the observation mentioned above that the methanol values became lower when the amount of deposited

product increased. The deposited product containing the "loosely bound" methoxyl group was removed in the precipitation with lead nitrate, and, therefore, methanol was lost.

The behaviour of XXIII suggested the possibility that, in the periodate treatment, comparatively stable intermediates containing methoxyl groups in some "loosely bound" form might be similarly formed from other guaiacol compounds, although only in the case of XXIII was the intermediate sparingly water-soluble and therefore separated from the reaction mixture. If this were true, serious difficulties might arise in the application of the periodate method to the estimation of phenolic groups in lignin, especially in lignosulphonic acids. Oxidized material, carrying "loosely bound" methoxyl groups, might be totally or partially removed in the lead nitrate precipitation step, and methanol would thus be lost.

This possibility prompted experiments with the aim of examining whether or not the methanol which is obtained, when the filtrate from the lead precipitate is distilled, is generally present in a free state after completed periodate oxidation. Three representatives of group A were investigated in this respect. It was found that, after oxidation of guaiacol and carbinol XI in aqueous solution as well as of XVII in 80% acetic acid the total methanol content could be distilled off from the reaction mixtures at 3 mm Hg (boiling temperature about 0°). This result, together with the fact that crystals of *cis-cis*-muconic acid deposit from an aqueous solution containing guaiacol and sodium periodate at 4°, constitute sufficient evidence in favour of the view that, generally, methanol is liberated from the guaiacyl nuclei in the oxidation step, and that the different behaviour of *o*-vanillyl alcohol is unusual. This question will be further discussed in a forthcoming publication.

*Group C.* It is well established that side-chains with a double-bond in conjugation with a phenolic nucleus are not present to any appreciable extent in lignin. This is obvious especially from spectrophotometric data<sup>11</sup>, which indicate that mildly prepared lignin products contain less than 0.03—0.04 groups of this type per methoxyl group. On the basis of a specific colour reaction, Lindgren and Mikawa<sup>2</sup> state that small amounts of coniferyl alcohol groupings may be present in native lignin. As yet, however, there is no definite information as to whether such groups may be phenolic or etherified. The coniferyl aldehyde groups demonstrated in lignin<sup>38,39</sup> in an amount of one such group per 40—60 methoxyl groups, have been shown to occur predominantly in the etherified state<sup>11</sup>. Since lignin is devoid of carboxylic groups, the behaviour of the unsaturated substances XXVII—XXIX is of minor importance in the lignin problem.

*Isoeugenol* and coniferyl alcohol (XXVIII) reacted rapidly with periodate. Methanol formation, however, did not reach the normal level, the maximum values being about 0.7 mole of methanol per mole. This is obviously due to oxidative dimerization to dehydro-*diisoeugenol* (XVII) and dehydro-*diconiferyl alcohol* (XIX), respectively, taking place in competition with the oxidative demethylation and resulting in the blocking of phenolic groups. (*Cf.* the formation of XVII<sup>30</sup> and XIX<sup>29</sup> by ferric chloride oxidation of XXVII and XXVIII, respectively.)

Coniferyl aldehyde (XXIX), in which the C=C bond is stabilized by further conjugation with a carbonyl group, probably will not undergo oxidative dimerization and yielded a normal methanol value. The same was true for ferulic acid (XXX).

*Group D.* As for the substances of group C, the carbonyl compounds collected in group D are of secondary importance as lignin models. It is true that lignin contains a certain number of carbonyl groups. In addition to the

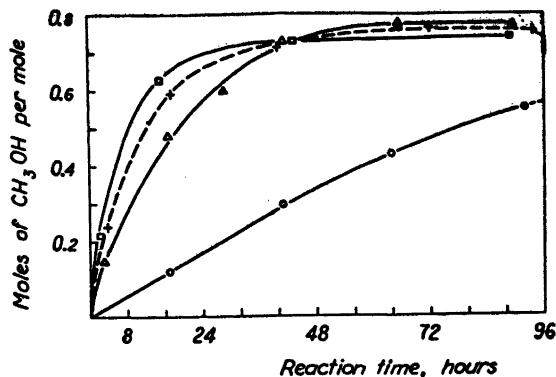


Fig. 2. Formation of methanol on periodate oxidation of ○ ——— ○ vanillin, △ ——— △ 4-acetoguaiacone, □ ——— □ 4-propioquaiacone, × ——— × vanillic acid. (0.5 millimole of substance and 3.5 millimoles of sodium metaperiodate in 50 ml of acetic acid-water (4 : 1), at 4°.)

coniferyl aldehyde end groups (0.02—0.025 per  $\text{OCH}_3$ <sup>38</sup>), about 0.1 CO per  $\text{OCH}_3$  has been shown to be present in Brauns' "native lignin"<sup>40</sup> and in ligno-sulphonic acid<sup>41</sup>, both from spruce. These carbonyl groups, however, are mainly not in the  $\alpha$ -position of the side-chains<sup>42,40</sup>. Consequently, neither vanillin nor the ketones XXXII and XXXIII are appropriate models for the majority of the carbonyl-containing phenolic elements in lignin. Their behaviour may be, however, of some interest, as it may illustrate the structural influences upon the course of the periodate oxidation of guaiacols.

The carbonyl compounds examined are oxidized at a much slower rate than the substances of groups A—C (Fig. 2). In the oxidation of vanillin, methanol was still being formed after one week's reaction, and in the case of the ketones XXXII and XXXIII the methanol values became constant only after about 48 hours. Vanillic acid (XXXIV) behaved in a similar way. It appears likely that this decreased activity towards periodate is due to the +I and +E effects exerted by the substituents and resulting in a diminished electron density at the phenolic hydroxyl group.

The final methanol values obtained in the case of the ketones and vanillic acid are definitely lower than those from the rapidly oxidized substances (except *isoeugenol* and *coniferyl alcohol*, see above). Since neither  $\alpha$ -carbonyl groups nor carboxylic groups seem to be present in lignin in appreciable amounts, this fact is of practically no importance for the application of the method to lignin. In this connection, it may also be pointed out that a carbonyl group in the  $\beta$ -position of the side-chain, as in *guaiacylacetone* (XV), does not decrease the yield of methanol.

#### CONCLUSIONS

As an average for the yield of methanol from the various model substances in group A a value of 0.91 moles of methanol per mole is obtained. On the basis of this result and of the preceding discussion regarding the behaviour of

the other model substances, it appears justified to adopt this value as the yield of methanol per phenolic guaiacyl nucleus which can be expected on periodate oxidation of softwood lignin preparations. Consequently, any methanol value obtained ( $\text{CH}_3\text{OH}/\text{OCH}_3$ ), when multiplied by 1.1, will give the number of phenolic hydroxyl groups per methoxyl in the lignin preparation examined.

In addition to the guaiacyl elements, softwood lignin contains very small amounts of syringyl and *p*-hydroxyphenyl elements<sup>43</sup>. Preliminary experiments have shown that pyrogallol-1,3-dimethylether is partially demethylated by periodate, the yield of methanol being slightly higher than that obtained from guaiacol. Phenolic elements of the *p*-hydroxyphenyl type, which may be present in lignin, are of course not determinable by the periodate demethylation method. The amount of such methoxyl-free elements, however, seems not to exceed 4 % of the total amount of phenyl propane units, and if it is assumed that one fourth of the *p*-hydroxyphenyl elements is phenolic, any figure obtained with the periodate method will be too low by an amount of, at most, 0.01 phenolic groups per methoxyl. In other words, the traces of syringyl and *p*-hydroxyphenyl groups present in softwood lignin will not have any appreciable influence on the estimation of phenolic groups.

## EXPERIMENTAL

*Periodate oxidation. 1. In aqueous solution.* A solution of 0.5 millimole of the substance in 25 ml of water was kept for about 10 minutes at 4° in a dark-room. (Of substances containing two phenolic nuclei, 0.25 millimole was used.) A solution of 3.5 millimoles of sodium metaperiodate,  $\text{NaIO}_4$ , in 25 ml of water, cooled to 4°, was added. The reaction was stopped by the addition of 25 ml of 20 % lead nitrate solution. The precipitate (lead iodate and periodate) was filtered off and washed thoroughly with water.

From the filtrate (about 200 ml) about 100 ml were distilled off in a slow stream of nitrogen and the distillate was collected in an ice-cooled receiver containing about 50 ml of water. In order to remove traces of iodine, the distillate was passed through a column (13 × 200 mm) of an anion exchange resin (Dowex 2), which had been saturated with bisulphite ions. The column was washed with water, and the filtrate made up to a final volume of 300 ml.

Formaldehyde which is formed in the oxidation of vanillyl alcohol (IX) and which would interfere with the subsequent methanol determination, is completely retained by the bisulphite-saturated resin<sup>44</sup> in the same operation.

*2. In 80 % acetic acid.* Substances which were sparingly soluble in water, were oxidized in 80 % acetic acid. Glacial acetic acid *pro analysi*, 99–100 % (E. Merck, Darmstadt), which was shown to be free of methanol, was used.

The solution of the substance (0.5 millimole) in 25 ml of glacial acetic acid was mixed with 25 ml of a solution of sodium metaperiodate, prepared by dissolving 0.14 mole of periodate in 400 ml of water and adding acetic acid to a volume of 1 litre. The oxidation was carried out at 4° in a dark-room. At the end of the reaction time 25 ml of a 20 % aqueous lead nitrate solution was added, the mixture filtered and the precipitate washed with about 50 ml of acetic acid and subsequently with about 75 ml of water.

The combined filtrates were distilled as described above. As it was found that acetic acid, when oxidized with permanganate under the conditions used in the following oxidation step, gave rise to formaldehyde, acetic acid contained in the distillate had to be removed. For this purpose the distillate was made alkaline by the addition of an excess of 50 % sodium hydroxide solution, and redistilled. The final distillate was made up to a volume of 300 ml.

If formaldehyde was formed during the periodate oxidation, the final distillate was passed through a bisulphite-saturated anion exchange resin (see above), and the filtrate made up to a volume of 300 ml.

**Determination of methanol.** The procedure of Ahlén and Samuelson<sup>44</sup> was followed with slight modifications. To a mixture of 25 ml of the distillate, obtained according to procedure 1 or 2 above, and 5 ml of an aqueous solution of phosphoric acid (165 ml  $H_3PO_4$ ,  $d = 1.75$ , per litre), 5 ml of potassium permanganate (40 g  $KMnO_4$  per litre) were added at 25°. After exactly 10 minutes, 10 ml of a mixture of oxalic and sulphuric acid (40 g  $H_2C_2O_4$ ,  $2H_2O$  and 40 ml of conc.  $H_2SO_4$  per litre) were added. The solution became colourless after about 10 minutes. The carbon dioxide evolved was removed by shaking, and 5 ml of the solution were mixed with 5 ml of a freshly prepared 2 % aqueous solution of chromotropic acid and 25 ml of conc. sulphuric acid. This mixture was heated in a loosely stoppered Erlenmeyer flask for 45 minutes in a boiling water bath. The extinction of the solution was measured at  $570 m\mu$  against a blank solution prepared in a similar way from 25 ml of distilled water.

A standard curve was obtained with aqueous solutions of methanol of known concentration, which were treated as described above. At least up to a concentration of 30 mg of methanol per 300 ml the amount of formaldehyde formed is a linear function of the methanol content.

The accuracy of the method may be illustrated by the following example (periodate oxidation of guaiacol, cf. Fig. 1).

Time of periodate oxidation, hrs	1/60	1/4	1/2	1	6	14	38	72
Moles of $CH_3OH$ per mole of guaiacol	0.89	0.92	0.92	0.91	0.92	0.93	0.92	0.91

**cis-cis-Muconic acid from guaiacol.** From the red-brown aqueous solution obtained when guaiacol was oxidized with periodate under the conditions given above, a carboxylic acid could be isolated by extraction with ethyl acetate. The crystalline product, after recrystallization from water, had a wide melting point range and probably consisted of a mixture of the geometric isomers of muconic acid.

If the concentration of guaiacol was increased to 0.8 millimole per 25 ml of water and the amount of periodate kept unchanged, a small amount of nearly colourless prismatic needles deposited from the reaction mixture after some hours at 4°. The crystals were collected and washed with little water; m. p. 187°, no depression with *cis-cis*-muconic acid, m. p. 187–188°, prepared from phenol or pyrocatechol and peracetic acid<sup>21</sup>.

***a*-(4-Hydroxy-3-methoxyphenyl)-ethanol (= Apocynol) (X).** Sodium borohydride (0.45 g) was added to a solution of 4-acetoguaiacone (4 g) and sodium hydroxide (1.2 g) in water (60 ml). After three days at 60° a sample of the mixture no longer gave any precipitate with 2,4-dinitrophenylhydrazine. The solution was neutralized at room temperature with carbon dioxide, and extracted with ether. Evaporation of the dried ether solution yielded 3.7 g of a crystalline product, which was recrystallized by dissolving in ethylacetate (without heating!) and precipitation with hexane. M. p. 101–102° C (101° according to Finnemore<sup>45</sup>).

**Vanillyl ethyl ether (XII).** A 4 % solution of dry hydrogen chloride in abs. ethanol (521 g) was added dropwise during 3 hours to a stirred, ice-cooled solution of vanillyl alcohol (30 g) in abs. ethanol (500 g). After 16 hours at room temperature, somewhat less than the equivalent amount of sodium hydroxide (15 g NaOH in 50 ml  $H_2O$ ) was added and the solution finally neutralized with a saturated sodium bicarbonate solution. Ethanol was distilled off and the residue extracted with chloroform. The chloroform solution was dried over  $Na_2SO_4$ , and the solvent removed by distillation. The residual oil was distilled, b. p. 124–125° at 4 mm;  $n_D^{20} = 1.5297$ . Yield 85 %. (Found: C 65.6; H 7.59; alkoxy calc. as  $OCH_3$  33.9. Calc. for  $C_{10}H_{14}O_3$ : C 65.9; H 7.74; alkoxy calc. as  $OCH_3$  34.1.)

**Barium 1-(4-hydroxy-3-methoxyphenyl)-*n*-propane-1-sulphonate (XIII)\*.** Guaiacyl ethyl carbinol (XI) (1 g) was sulphonated with an aqueous solution (50 ml) containing 14.3 g NaOH and 50 g  $SO_2$  per litre. The mixture (pH 1.4) was heated to 135° during 3 hours and kept at this temperature for a further 3 hours. Excess of  $SO_2$  was removed *in vacuo*, the solution neutralized with barium carbonate and filtered. The filtrate was

\* This preparation was carried out by Mr. E. Eriksoo.

passed through a cation exchange resin and sulphur dioxide removed *in vacuo*. The solution was reneutralized with calcium carbonate and the precipitate ( $\text{CaSO}_4$ ) filtered off. Residual sulphite was precipitated by the addition of saturated calcium hydroxide solution to pH 8–9. The filtered solution was concentrated *in vacuo* in a carbon dioxide atmosphere to about 40 ml, the concentrate filtered and again passed through a cation exchange resin. Finally, the solution was neutralized with barium carbonate, filtered and concentrated *in vacuo* to a small volume. The barium salt (XIII) deposited and was recrystallized by dissolution in isopropanol-water (85 : 15) and addition of ethyl ether. Colourless needles. (Found:  $\text{OCH}_3$ , 9.55; S 10.47; Ba 21.4. Calc. for  $\text{C}_{10}\text{H}_{11}\text{O}_5\text{SBA}_{0.5}$ :  $\text{OCH}_3$ , 9.85; S 10.18; Ba 21.8.)

*5-n-Propylvanillyl alcohol*. 5-Allylvanillin<sup>22</sup> (5 g), dissolved in 96 % ethanol (25 ml) was shaken with platinum oxide catalyst (0.05 g) in a hydrogen atmosphere at a pressure of 4 kg/cm<sup>2</sup>. One mole of hydrogen per mole of the substance was rapidly consumed. Since 2,4-dinitrophenylhydrazine produced a precipitate with a sample of the solution, only the allyl group had been hydrogenated. A further mole of hydrogen was consumed after the addition of 0.5 ml 1 M  $\text{FeCl}_3$ , and the dinitrophenylhydrazine test became negative. The catalyst was filtered off and the solvent removed *in vacuo*. The residual oil was dissolved in ether, the solution dried over  $\text{Na}_2\text{SO}_4$  and the solvent evaporated. The product crystallized from benzene-hexane in needles, m. p. 45–47°. Yield 4.5 g (88 %). (Found:  $\text{OCH}_3$ , 15.97; Calc. for  $\text{C}_{11}\text{H}_{16}\text{O}_3$ :  $\text{OCH}_3$ , 15.81.)

The free phenol alcohol was unstable at room temperature; it turned slightly yellow and became liquid after a few days. After a few weeks the product had crystallized again. Recrystallization from ethanol-water yielded the diphenylmethane described below.

*Bis-(4-hydroxy-3-methoxy-5-n-propylphenyl)-methane*. Attempts to prepare 5-n-propylvanillyl alcohol from 6-n-propylguaiaicol (XX) by the Lederer-Manasse reaction yielded the corresponding diphenylmethane. A mixture of 6-n-propylguaiaicol (5 g), sodium hydroxide (6 g  $\text{NaOH}$  in 30 ml  $\text{H}_2\text{O}$ ) and formalin (8 ml) was kept at 40–45° for one week. Neutralization with carbon dioxide yielded a white precipitate, which after washing with petroleum ether was recrystallized from ethanol-water. Needles, m.p. 124–125°; yield 1.4 g. (Found: C 73.3; H 8.26;  $\text{OCH}_3$ , 18.22. Calc. for  $\text{C}_{21}\text{H}_{26}\text{O}_4$ : C 73.2; H 8.19;  $\text{OCH}_3$ , 18.02.)

*Barium 3-methoxy-4-hydroxy-5-n-propylbenzyl sulphonate (XXI)*. Freshly prepared 5-n-propylvanillyl alcohol was heated at 135° for three hours with an aqueous solution of sodium sulphite and sodium bisulphite of pH 6.6 and a total  $\text{SO}_3$  content of 5 %. The sulphonic acid formed was isolated as described above for XIII. Needles from isopropanol-water (85 : 15)-ethyl ether. (Found:  $\text{OCH}_3$ , 9.42; Ba 20.8. Calc. for  $\text{C}_{11}\text{H}_{15}\text{O}_5\text{SBA}_{0.5}$ :  $\text{OCH}_3$ , 9.46; Ba 20.9.)

*o-Vanillyl alcohol (XXIII)*. *o*-Vanillin was reduced catalytically as described by Carothers and Adams<sup>23</sup> for other hydroxybenzaldehydes. The oily product crystallized in needles from benzene-hexane. Yield 91 %, m. p. 60–61° (60–62° according to Eliel<sup>46</sup>).

*o-Homovanillyl alcohol (XXIV)*. *o*-Eugenol (5.0 g) was treated in ethyl acetate solution with a stream of oxygen containing 0.8–1.0 % of ozone, as described by Hahn and Schales<sup>47</sup> for the ozonisation of safrol. Hydrogenation with Pd- $\text{CaCO}_3$  catalyst and bisulphite extraction yielded 4.0 g of a crystalline bisulphite addition product. This was dissolved in water and the solution extracted with ether to remove impurities. Sodium hydroxide (4 g) and sodium borohydride (1 g) were added to the aqueous phase. The mixture was kept over night at 50°, neutralized with carbon dioxide and extracted with ethyl ether. The ether solution was dried over  $\text{Na}_2\text{SO}_4$  and the ether evaporated, yielding a colourless, viscous oil. Yield: 1.6 g. (Found:  $\text{OCH}_3$ , 18.37; Calc. for  $\text{C}_9\text{H}_{13}\text{O}_3$ :  $\text{OCH}_3$ , 18.45.)

*Dehydrodi-vanillyl alcohol*. Dehydrodi-vanillin (8.2 g), dissolved in sodium hydroxide (6.5 g  $\text{NaOH}$  in 60 ml  $\text{H}_2\text{O}$ ) was reduced with sodium borohydride (1.0 g) at room temperature. After 16 hours, long needles of the sodium phenolate had deposited. These were collected, dissolved in water (200 ml), and the free phenol was precipitated with acetic acid. Recrystallization from ethyl acetate yielded 6.42 g (76 %) of a crude product, which was purified by recrystallization from acetone-ethanol (1:1). Prisms, m. p. 187–190° (rapid heating). (Found: C 62.7; H 6.11;  $\text{OCH}_3$ , 20.6. Calc. for  $\text{C}_{14}\text{H}_{18}\text{O}_6$ : C 62.7; H 5.93;  $\text{OCH}_3$ , 20.3.)

*Barium dehydrodi-vanillyl disulphonate (XXV)*. The sulphonation of dehydrodi-vanillyl alcohol was carried out as described for the preparation of the sulphonate XIII.

The barium salt deposited from the final aqueous concentrate and was completely precipitated by the addition of ethanol and ether. Reprecipitated from aqueous *isopropanol*-ethyl ether. (Found: OCH<sub>3</sub>, 10.74; Ba 23.8. Calc. for C<sub>14</sub>H<sub>16</sub>O<sub>10</sub>S<sub>2</sub>Ba : OCH<sub>3</sub>, 10.89; Ba 24.1.)

*Coniferyl aldehyde* (XXXIX) was prepared according to Pauly and Feuerstein<sup>49</sup> with a few modifications\*. The condensation of methoxymethyl vanillin with acetaldehyde was carried out in a borate buffer solution at pH 11.5. Coniferyl aldehyde was obtained from its methoxymethyl ether by heating the latter in a solution of 0.1 % conc. H<sub>2</sub>SO<sub>4</sub> in 50 % acetic acid for 1 hour at 80° in a nitrogen atmosphere.

*4-Acetoguaiacone* (XXXII)\*\*. The substance was prepared from guaiacol, acetic acid, and boron fluoride, following the procedure used by Kästner<sup>48</sup> for the synthesis of *p*-hydroxyacetophenone. Its purification was carried out according to Reichstein<sup>60</sup>. Yield 50 %.

*4-Propioguaiacone* (XXXIII). From guaiacol, propionic acid, and boron fluoride. Yield 60 %, m. p. 58° (cf. Kratzl<sup>61</sup>).

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\*\* Substances XXXII and XXXIII were prepared by Mr. S. Delin.

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