Abstract

Lignin was extracted from wood by means of anhydrous acidic ethanol, and spectral changes suggested a mechanism involving both alkylation of lignin and formation of unconjugated keto groups in it. By measuring alkoxy content, alkylation was found to have taken place mainly at benzyl alcohol and benzyl ether groupings, in agreement with the assumption that cleavage of benzyl ether bonds had occurred during ethanolytic extraction.

Rates of reaction of compounds of the phenylpropan-1,3-diol type with 2,2-dialkoxypropanes in acidic dioxane were faster than with ethanol under the same conditions. Isolation of 5-phenoxyl-4-phenyl-2,2-dimethyl-1,3-dioxane from reaction of 1-phenyl-2-phenoxypropan-1,3-diol with 2,2-dimethoxypropane indicated that such 1,3-dioxanes were also formed with other model compounds and with lignin. These results and consideration of mechanisms appeared to favour the hypothesis that dissolution of lignin from wood required cleavage of benzyl ether bonds, some of which may bind lignin to carbohydrate.
SOLVOLYTIC EXTRACTION OF LIGNIN FROM WOOD

by

CHWEE-HAR CHEW, B.Sc. (Nanyang U. Singapore)

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Department of Chemistry
McGill University
Montreal, Canada

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CHAPTER I

A REVIEW OF THE STRUCTURAL FEATURES OF LIGNIN
INTRODUCTION

Lignin is a polymeric component of wood which, like cellulose, occurs in the cellular structure of all higher plants. It was first discovered by Payen (1) in 1838, who treated wood with nitric acid and strong alkali, and designated those cell wall components which were removed as "matières ligneuse" or "matières incrustantes". The term lignin was first applied to Payen's "incrusting material" by Schulze (2) in 1851, and has been retained to the present time.

Since natural lignin is apparently highly cross-linked, and at best only slightly soluble in most common solvents, dissolving it usually requires chemical treatments. In fact, harshly degradative treatments form the basis of commercial sulphite and kraft pulping, and they produce waste liquors which contain altered and degraded lignins, as well as a variety of compounds of low molecular weight. Since these by-product materials are potentially available in vast quantities, attempts to utilize them have been the subject of much research, of which only a small proportion can be termed successful. Perhaps the main impediment to the utilization of lignin is the highly variable character of the material and its complex chemical structure. Therefore, study of the structure of lignin is important not only because of its chemical interest, but also because it may provide knowledge of mechanisms of pulping processes, and thus lead to improved bleaching and pulping methods, as well as to the commercial utilization of by-products from lignin.

It is still impossible to draw a precise organic-chemical
structure of the lignin macromolecule. But in recent years, new instruments and techniques have facilitated intensive studies which have greatly increased our knowledge of detailed structural features. A review of the many publications related to this problem which have appeared (3-8) in recent years indicates that in the field of wood chemistry, many problems have been solved: for instance, the organic-chemical structure of cellulose, and of many hemicelluloses, are well established. Moreover, we now also have a picture of lignin, which, even without the fine details, permits us to understand many of its reactions, and even its biosynthesis. Yet, one problem has defied over a hundred years of effort: the nature of the forces which bind lignin and carbohydrate together.

The purpose of the work described in this thesis is to seek evidence concerning any chemical bond or bonds that may exist between lignin and carbohydrate. Almost any valid additions to knowledge that this research might provide would have both theoretical and practical consequences of considerable importance.

Before discussing the present work, it may be helpful to outline some of the relevant details of the basic structure of lignin as now understood. Therefore, a brief review on this subject is introduced in the following paragraphs to lay the groundwork for the subsequent discussion.

QUANTITATIVE ESTIMATION OF FUNCTIONAL GROUPS IN LIGNIN MOLECULE

Recent work on the quantitative determination of composition has
been largely based on the study of Björkman lignin isolated from finely divided wood (9, 10), since its structural features are generally considered to be very close to those of the lignin which occurs in plant tissue (designated as protolignin or native lignin). Adler (7) has derived the following average formula for a phenylpropane or C₉ unit from Björkman lignin: $C_{9}H_{8.85}O_{2.37}(OCH_{3})_{0.96}$.

The oxygen represents a variety of functions, such as bonded and free phenolic hydroxyl groups, aliphatic hydroxyl, ether and carbonyl groups. Acetylation, spectroscopic measurements (11, 12), and oximation experiments (7) indicated that the oxygen atoms are distributed as shown in Table I-1. From these analyses, the empirical formula of Björkman spruce lignin can be represented in more detail as follows:

$C_{9.76}(\text{phenolic OH})_{0.29}(\text{aliphatic OH})_{0.86}(\text{carbonyl O})_{0.18}(\text{alkylaryl-ether O})_{0.71}(\text{dialkyl ether O})_{0.33}(OCH_{3})_{0.96}$

The Carbonyl Groups in Lignin

The occurrence of carbonyl functions in lignin was first detected by Klason (13) in 1922, but detailed studies of the quantity and nature of such carbonyl groups have not been attempted until recently. Oximation of Björkman lignin with hydroxylamine hydrochloride at room temperature, and titration of the hydrochloric acid liberated, indicated the presence of 0.20 CO/OCH₃ (14). The same value was obtained from the increase of hydroxyl groups found when the lignin was reduced with sodium borohydride (15). Examination of the spectral changes after reduction of lignin with
TABLE I-1

Distribution of Non-Methoxyl Oxygen Atoms
in Björkman Spruce Lignin
(per C₉ unit)

<table>
<thead>
<tr>
<th></th>
<th>Total O</th>
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<tr>
<td>Phenolic OH</td>
<td>1.00</td>
<td>0.29</td>
</tr>
<tr>
<td>free</td>
<td></td>
<td>0.71</td>
</tr>
<tr>
<td>bonded (as ether)</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Aliphatic OH</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Carbonyl O</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Dialkyl ether O</td>
<td>0.33</td>
<td>2.37</td>
</tr>
<tr>
<td>(by difference)</td>
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</table>
sodium borohydride (16) suggested that the total number of $0.20 \text{ CO/OCH}_3$ is distributed as follows:

\[
\begin{array}{cccc}
\text{HCO} & \text{HCO} & \text{C} & \text{C} \\
\text{CH} & \text{CH} & \text{C} & \text{C} \\
\text{CH} & \text{CH} & \text{O} & \text{O} \\
\text{OCH}_3 & \text{OCH}_3 & \text{OCH}_3 & \text{OCH}_3 \\
<0.01 & 0.03 & <0.01 & 0.06 & 0.10
\end{array}
\]
(by difference)

Similar results regarding the number of carbonyl-containing chromophores were obtained by spectrochemical investigation of Björkman lignin hydrogenated over a palladium-barium sulphate catalyst (17).

In contrast to these results, Gierer and Söderberg (18) had earlier obtained a value of $0.41-0.44 \text{ CO/OCH}_3$ from the volumetric borohydride method. Reinvestigation of this method by Marton et al. (15) yielded a value of $0.20-0.24 \text{ CO/OCH}_3$ once air had been excluded from the reaction apparatus. This is in reasonable agreement with the results obtained from the hydroxylamine method. However, the formula of Björkman spruce lignin, mentioned in the previous section contains only $0.18 \text{ CO}$ per $0.96 \text{ OCH}_3$ (i.e. $0.188 \text{ CO/OCH}_3$); the lower figure may due to incomplete oxidation of the total carbonyl groups, since it is known that $\beta$- and $\gamma$-carbonyls are oxidized much faster than $\alpha$-carbonyls (18).
Although a vast amount of research has been conducted on various aspects of the chemistry of lignin since its discovery, its detailed structure, as mentioned earlier, is yet incomplete. However, the studies of degradation products isolated from various chemical reactions, performed either on whole wood or on isolated lignin, provide information with respect to simple units which are thought to be basic building stones.

The long-suspected presence of phenylpropane units in lignin was first actually demonstrated by Phillips (19) in 1932, who isolated a phenylpropane derivative, dihydroeugenol (VII) from alkali lignin from corn cobs which had been subjected to destructive distillation under reduced pressure in an atmosphere of carbon dioxide. High pressure catalytic hydrogenation of aspen methanol lignin (20), extractive-free spruce and maple woods (21, 22) gave a good number of phenylpropane (IV-VI) and cyclo-hexyl (I-III) derivatives, as shown in Figure I-1. From lignins which had been treated with sodium in liquid ammonia at low temperature, Shorygina (23) was able to isolate dihydroeugenol (VII) and 1-guaiacyl-propanol-2 (VIII) in good yields from the reaction products. Hibbert and co-workers (24, 25, 26) isolated guaiacyl derivatives (IX-XII) from the ethanolysis products of spruce lignin. From maple lignin, three corresponding syringyl derivatives (XIII-XV) were isolated in addition to the guaiacyl derivatives mentioned.

As to how these phenylpropane units are linked to each other
FIGURE 1-1 Phenylpropane Products from the Degradation of Lignin (27)
in the lignin macromolecules, the evidence indicates two main kinds of
linkages between them: ether bonds and carbon-to-carbon bonds. The former
are either acid- or alkali-labile, and can be cleaved when treated with
different chemical reagents; the latter linkages are highly resistant
towards chemical degradation, and may probably be the main factor pre­
venting the conversion of lignin to monomeric units during the reactions
of ethanolysis, hydrogenation, etc.

Figure 1-2 summarizes some current views on the nature of
intermonomeric linkages in lignin (27, 28). It is necessary to point out
that not all of these linkages have been experimentally proven to exist:
some of them have been proposed to account for the chemical behaviour
of lignin in such reactions as ethanolysis, sulphonation, hydrogenolysis,
alkaline nitrobenzene oxidation, permanganate oxidation, etc.

Finally, the study of the biogenesis of lignin has made an
invaluable contribution towards the understanding of the initial steps
of this process as well as of the structural aspects of lignin. As early
as the eighteenth century, Klasson (29), and Tiemann and Haarmann (30)
expressed their opinion that sprucewood lignin may be formed in nature
from coniferin (XXVIII), a glucosidic compound present in the cambial
sap. Erdtman (31), in 1933, suggested that the biogenesis of lignin may
be very similar to the dehydrogenative dimerization of isoeugenol (XXIX),
from which dehydrodiisoeugenol (XXX) was formed by the action of mushroom
enzymes (32, 33, 34).

In recent years, these early views have been developed by
FIGURE I-2 Postulated Intermonomeric Linkages in Lignin (27, 28)
biochemical studies, especially by Freudenberg. He was the first to demonstrate that radioactive D-coniferin was a biological precursor of lignin (35, 36). Kratzl (37) also isolated radioactive lignin from spruce to which radioactively-labelled D-coniferin had been administered. Moreover, the monomers obtained from the ethanolysis products of such lignin were also found to be strongly radioactive (37, 38). These findings indicated that the coniferyl alcohol liberated from the hydrolysis of D-coniferin by β-glucosidase in cambium cells was polymerizable to a lignin-like material (39). In an attempt to obtain this material, Freudenberg has done a series of in vitro experiments involving treatment of "primary lignin building stones" with air in the presence of a mushroom oxidase (40). Of the compounds tested, the dehydrogenation product of coniferyl alcohol (so-called DHP) showed extensive similarities to spruce protolignin and Brauns' native lignin (41).

The interpretation of the mechanism of the polymerization is
that coniferyl alcohol is first dehydrogenated by the enzymes, and forms
a highly reactive quinone methide radical, which is a resonance hybrid of
various canonical forms (XXXI a to d). Combination of these radicals in
different ways will produce a variety of carbon-carbon and carbon-oxygen
bonds, such as are thought to constitute the secondary building stones
of lignin. Indeed, Freudenberg did obtain several dimeric compounds
(as shown in Figure I-3) from an intermediate stage of the dehydrogenative
reaction. Furthermore, some of these dimers (XXXII-XXXIV) have also been
found in spruce cambium sap and isolated from spruce lignin either directly
in crystalline form (42) or as crystalline derivatives (43). Nevertheless,
others of the dimers have not actually been isolated, but were proposed to
account for the isolation of degradation products from lignin; for in-
stance, 3,4,5',6'-tetramethoxybiphenyl-6,3'-dicarboxylic acid is believed
to arise from structures such as dehydrobisconiferyl alcohol (XXXV), and
4,3'-dicarboxy-2,5',6'-trimethoxydiphenyl ether from the diconiferyl ether
type. The presence of 1,2-diguaiacylpropane-1,3-diol (XLII) among the
intermediates of artificial lignin has so far been indicated only by
Dehydrodiconiferyl alcohol/aldehyde

Dehydrobisconiferyl alcohol

Lignenolide

DL-Pinoresinol (and epimer)

Hypothetical diconiferyl ether

Pinoresinolide

Hypothetical cyclolignane

α-Conidendrin

FIGURE I-3 Dilignols
(cont'd. Figure I-3)

XLI
Hydroxymatairesinol

XLII
1,2-Diguaiacylpropane-1,3-diol

XLIII
Hypothetical coniferyl alcohol guaiacyl ether
chromatography (44, 45). However, it also appeared among the products of mild hydrolysis of spruce lignin, and was isolated in crystalline form (42, 46). Coniferyl alcohol guaiacyl ether (XLIII) has not yet been isolated from the intermediate stage, but it accounts for the isolation of 6,3',4'-trimethoxy-4-carboxydiphenyl ether from the alkaline treatment of lignin, followed by methylation and oxidation (47). The lignenolide (XXXVIII) may undergo a condensation between its double bond and position 2- or 6- of the nucleus remote from the double bond to form a cyclolignan (XXXIX), which, on degradation, can give rise to benzene-pentacarboxylic and tricarballylic acids. The former acid was obtained by Reed and Purves (47) in a yield of 0.2% of the lignin. These two acids can also originate from the acidolysis of α-conidendrin (XL), formed from the acidolysis of hydroxymatairesinol (XLI) (48). These alternative interpretations need further investigation.

Upon treating the above mentioned dimeric compounds with dehydrogenating enzymes, "dehydrogenation polymers" (DHP) were obtained, and some of these dimeric compounds were also reported to be present in cambial sap of wood (38). From these facts, one can say that the formation of lignin is a result of oxidation and condensation of coniferyl alcohol by certain plant enzymes. Since the dimeric intermediates are also phenols, it is possible for them to undergo further enzymatic dehydrogenation to produce other radicals, and these might then combine together, or with the monomeric radicals. Therefore, trimeric, tetrameric, or polymeric intermediates may be formed. Freudenberg has claimed to have identified six such trimers (49, 50), two tetramers (49, 51), one penta- and one hexa-lignol (50), shown in Figure I-4.
It seems reasonable to believe that the formation of lignin \textit{in vivo} and \textit{in vitro} are very similar, since many of the products formed in the intermediate stages of the dehydrogenation process have also been found among the degradation products from lignin subjected to mild hydrolysis.

In order to construct a constitutional scheme for the lignin molecule, it is necessary to understand not only the nature of the bonds which link each monomeric unit, but also the frequency with which each type of monomeric unit occurs in the natural lignin. Therefore, much effort has been devoted to the study of model compounds which represent groups thought to be present in lignin. By performing some specific reactions on a given model compound, one hopes to discover experimental analogies for the presence or absence of that particular structural element, and information about its frequency in the natural lignin. By this principle, the frequency of different structural elements, for example, guaiacylglycerol-\(\beta\)-arylether (5, 52, 53, 54), pinoresinol (47, 55), phenylcoumaran (56), diphenyl structures (57, 58), etc. were estimated as outlined in Table I-2. Based on these analytical data, together with the biogenesis studies, several structural formulae for lignin have been drawn and modified from time to time. One of the latest versions is shown in Figure I-5 (59). However, it should be remembered that this scheme is neither a true structure nor the structural formula in the proper sense; it is only a sort of "averaged-out" picture of the groupings to be found in a typical portion of a macromolecule of lignin, and accounts for the various reactions of lignin.
A TRILIGNOLS

Guaiacylglycerol-β-dehydrodiconiferyl ether

Bisguaiacylglycerol-β-coniferyl ether

Guaiacylglycerol pinoresinol ether (epimer)

Guaiacylglycerol-diguaiacyl propanediol (two isomers)

FIGURE 1-4 Oligolignols (49, 50, 51)
**A TRILIGNOLS**

- Dehydrotriconiferyl alcohol

- Guaiacylglycerol-\(\beta\)-coniferyl-\(\alpha\) dehydrodiconiferyl ether

**B TETRA-LIGNOLS**

- Guaiacylglycerol-\(\beta\)-coniferyl-\(\alpha\) dehydrodiconiferyl ether

- Bisdehydropinoresinol

(cont'd. Figure 1-4)
C PENTA- AND HEXA-LIGNOLS

Guaiacylglycerol-β-ether of L

Hexalignol

(cont'd. Figure I-4)
<table>
<thead>
<tr>
<th>Basic Units in Lignin</th>
<th>Found</th>
<th>Calcd from Formula (Fig. I-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Arylglycerol-β-aryl ether</td>
<td>0.25</td>
<td>0.17-0.23</td>
</tr>
<tr>
<td>2 Phenylcoumaran</td>
<td>0.16-0.18</td>
<td>0.12</td>
</tr>
<tr>
<td>3 Pinoresinol</td>
<td>0.10-0.20</td>
<td>0.12</td>
</tr>
<tr>
<td>4 Diphenyl</td>
<td>0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>5 Condensations at C₅</td>
<td>0.45</td>
<td>0.43</td>
</tr>
<tr>
<td>6 Condensations at C₆</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>7 Condensations at C₂</td>
<td>0.04</td>
<td>----</td>
</tr>
<tr>
<td>8 Benzyl alcohol</td>
<td>0.20</td>
<td>0.22</td>
</tr>
<tr>
<td>9 Benzyl ether</td>
<td>0.23</td>
<td>0.28</td>
</tr>
<tr>
<td>10 α-Carbonyl</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>11 β-Carbonyl</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td>12 Coniferaldehyde</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>13 Coniferyl alcohol</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>
FIGURE I-5 Constitutional Model of Spruce Lignin (59)
THE LIGNIN-CARBOHYDRATE LINKAGES

Origin of the Problem

With the background knowledge of some of the basic structural features of lignin, we can now turn to the great controversial problem of the lignin-carbohydrate bonds.

This question arose from the very year that Payen (1) published his view of wood as a physical mixture, with the cellulose mechanically embedded in the "incrusting material". Almost simultaneously, Schleider (60) published his experimental results that cellulose in plants did not give the customary blue colour reaction with sulphuric acid and iodine, such as given by isolated cellulose, and concluded that the reason for this lack of reaction must be that cellulose in plant tissue is somehow chemically combined. Erdmann (61), in 1866, furthered this view by postulating that lignin is chemically bound to carbohydrate, forming "glycolignose". These early views stand in sharp contrast to each other, and both have had numerous adherents throughout the history of lignin chemistry.

Nowadays, we understand the controversy and confusion of the problem mainly caused by many ill-founded statements in the literature published during the past hundred years since the discovery of lignin. Many of the early wood chemists were anxious to earn the credit for providing evidence and a solution. Oscillation of individual opinion between either view during the careers of many prominent wood chemists
was not uncommon, while other chemists, due to the common lack of knowledge of the three major components of wood, often perpetrated ideas which were totally misleading, and created further complications. Furthermore, misquotation of original sources, and lack of specification of experimental technique employed by the numerous workers have added even more confusion.

Exhaustive surveys of the literature related to this problem have appeared in many reviews (3, 62, 63, 64), but, unfortunately, they have not always been critical. Although a comprehensive review of pertinent literature on the lignin-carbohydrate linkages will not be repeated here, some details of previous researches relevant to the present work will be briefly sketched.

The Morphological Structure of the Cell Wall

It is now well-known that three main classes of polymers --- cellulose, hemicelluloses and lignin --- constitute the plant cell walls. It is also known that most of the lignin is located in the middle lamella, i.e., the intercellular region where there is no cellulose. Ritter (65) studied the cytological distribution of lignin in red alder and western white pine by the standard Klason analytical method, and obtained the results shown in Table I-3. The low methoxyl contents indicated that the preparations were not pure, but it seemed clear that the lignins in the cell wall and in the middle lamella differed from each other, since the methoxyl contents of the lignins were quite different, though they had
TABLE I-3

Distribution of Lignin Between Cell Wall and Middle Lamella, by Ritter (65)

<table>
<thead>
<tr>
<th>Species</th>
<th>Red Alder</th>
<th></th>
<th>Western White Pine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell wall</td>
<td>Middle lamella</td>
<td>Total</td>
</tr>
<tr>
<td>Lignin (% of O.D. wood)</td>
<td>7.3</td>
<td>19.5</td>
<td>26.8</td>
</tr>
<tr>
<td>Lignin (% of total lignin)</td>
<td>27.3</td>
<td>72.7</td>
<td>----</td>
</tr>
<tr>
<td>Methoxyl (%)</td>
<td>4.8</td>
<td>13.6</td>
<td>----</td>
</tr>
</tbody>
</table>
been isolated under the same conditions.

By ultraviolet microscopy, Lange (66) found that, in spruce, 70\% of the lignin occurs in the middle lamella and is continuously distributed in the fibre walls with very rapidly diminishing concentration from the middle lamella to the lumen but never reaching zero. However, Procter, Yean and Goring (67) recently have found that the concentration of lignin across the secondary wall is constant, in contrast to Lange's finding. Nevertheless, in considering the lignin in the middle lamella, clearly, only hemicellulose, and not cellulose, can possibly be associated with it. This may also be true in the secondary wall, although there is a large amount of cellulose present. If, however, there are chemically bonded lignin and carbohydrate compounds in the middle lamella and the secondary wall, they do not necessarily have the same compositions.

Evidence for the Association of Lignin with Hemicelluloses

After studying the chlorination of poplar wood under anhydrous conditions, Traynard and co-workers (68) reported that three fractions of chlorolignins had been obtained by successive extractions with (i) cold ethanol, (ii) hot ethanol, and (iii) cold alkali. Part of the first and third fractions were acetylated and then purified by chromatographic adsorption on a column of alumina. All of these five fractions were then hydrolyzed by N hydrochloric acid. Parallel paper chromatograms showed that in each case, free sugars were absent from the unhydrolyzed sample and were present after hydrolysis. The sugars found comprised
mainly xylose, with smaller amounts of arabinose and galactose. Traynard concluded that lignin must be tightly bound to xylans. Similar results were obtained by Merewether (69) in studies on the ethanolysis of *Eucalyptus regnans*, and by Kawamura and Higuchi (70) who performed the acidolysis of beech wood with 1% sulphuric acid and dioxane. One point deserving comment in these experiments is the uncertainty of the assumption of complete physical separation of carbohydrate and the isolated lignins in their unhydrolyzed samples. Details of experimental procedures, of solvent system used to separate the partially acetylated lignin and polyxylose (70), were not clearly demonstrated in the original publication. Although, from these results one cannot say precisely that lignin is chemically bound to hemicelluloses, they clearly showed that these polymers are closely associated with each other.

Perhaps the most obvious evidence of the closer intimacy of the association of lignin with hemicelluloses, rather than with cellulose, comes from analyses of the so-called lignin-carbohydrate complexes that have been described in several papers (71, 72, 73, 74, 75, 76). In all cases, the analyses of these complexes showed that the carbohydrates were hemicelluloses, mainly xylan in birch, mannan in spruce and pine (Table 1-4).

**The Lignin-Carbohydrate Complexes**

The study of isolated lignin-carbohydrate complexes, may, in the end, provide the best proof of the existence of lignin-carbohydrate
TABLE I-4

Average Carbohydrate Composition of the Lignin-Carbohydrate Complex Obtained by Björkman (73)

<table>
<thead>
<tr>
<th>Relative Amount, %</th>
<th>Spruce</th>
<th>Pine</th>
<th>Birch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactan</td>
<td>10</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Glucan</td>
<td>17</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Mannan</td>
<td>50</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>Araban</td>
<td>4</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Xylan</td>
<td>19</td>
<td>15</td>
<td>86</td>
</tr>
</tbody>
</table>
bonds, provided it can be shown that whatever bonds are formed are not artifacts.

Reviewing the literature reveals the common fact that lignin-carbohydrate complexes can be extracted from wood only after it has been finely subdivided in a ball mill, except in the work of Hayashi and Tashi (77) who isolated a complex from wheat straw which had been treated with 50% acetic acid for five days, and then extracted with dioxane. Grohn (78), in 1951, reported that solubility of a standard wood flour (95% passing an 0.1 mm sieve) in methanol was increased from 0.6-0.9% (corresponding to 2-3% of the lignin) to 3.1-4.5% (corresponding to 9-11% of the lignin) after grinding in a falling-ball mill for 60 hours. His results thus suggested that separation of lignin could be facilitated by simple mechanical action, and that breakage of chemical bonds was probably unnecessary. Yet, in 1952, Brauns and Seiler (79) reported that neither 95% ethanol nor dioxane would extract lignin from wood beaten to colloidal dimensions. They had extracted native lignin (in the usual small yield) from wood meal, then had beaten the residual meal to such an extent that part of it went into colloidal suspension. The wood was regenerated as a fine powder which had the same chemical composition as the original. The particle size of the powdered wood was so small that it dissolved completely in cuprammonium solution. This result is in conflict with that of Grohn.

Nevertheless, Björkman (9, 73, 74, 80) later demonstrated that prolonged grinding of wood meals first in a Lampén mill (48 hours), and
then in a vibrational ball mill (48 hours) in the presence of toluene, resulted in the dissolution in moist dioxane of 50% of the lignin. This extracted lignin is the so-called milled-wood lignin (MWL) or Björkman lignin. Subsequent extraction of the wood meal with dimethylformamide or dimethyl sulfoxide, after dioxane, yielded additional material which Björkman called "lignin-carbohydrate complexes" (LCC). In the interpretation of the linkage of lignin and carbohydrate in these complexes, it was considered significant that additional milling had the effect of causing further degradation, so that moist dioxane could extract a lignin fraction with a substantially reduced carbohydrate content. Lindgren (81) has studied Björkman's "lignin-carbohydrate complexes" by electrophoresis. He separated the complexes into two fractions: a slow-moving fraction was entirely carbohydrate, and another fraction contained both lignin and carbohydrate. This latter fraction, in turn, moved more slowly than pure lignin. These results suggested a chemical linkage between lignin and hemicellulose. Wang (75) has repeated Lindgren's work both with the Björkman type of complexes (74) and with a complex (LXC) obtained by extracting milled wood with hot water. He obtained results similar to those of Lindgren, and further pointed out that the complexes were not hydrolyzed by alkali since the electrophoresis was conducted in an alkaline medium. If, however, the complexes were first subjected to mild acid hydrolysis, complete separation of lignin and carbohydrates occurred as demonstrated on the electrophotogram.

Nevertheless, it is recognized (82) that under milling conditions covalent bonds break and reform, with free radicals as intermediates.
Phenolic compounds, such as lignin react readily with free radicals and cations, therefore, further evidence is needed to show that the chemical bonds between the lignin and carbohydrate complexes are not actually artifacts.

Grohn and Schierbaum (83) investigated the milling of wood and found that about $34\%$ of milled spruce or poplar wood became soluble in cold water. The residual wood, after extraction, was easily susceptible to the action of a cellulytic enzyme and, subsequently, an additional portion of the enzyme-degraded wood became soluble in cold water. A total of $54\%$ of the wood was rendered soluble by the combined action of milling and enzymatic hydrolysis.

Pew and Weyna (71, 72) also found that undamaged spruce wood was almost completely resistant to the action of cellulytic enzymes, and was still quite resistant after the wood had been ground to pass an 80-mesh screen. However, with wood subjected to vibratory milling, a grinding period of only ten minutes resulted in the removal of two-thirds of the carbohydrate in two consecutive enzymatic digestions. When the wood was ground for eight hours, the enzyme-induced solubility of carbohydrate increased to $96\%$. Although the effect of grinding was obviously profound, hydrolysis with hydrochloric acid did not enhance the susceptibility of wood to the enzyme. On the other hand, treatment with swelling agents, such as sodium hydroxide, pyridine, butylamine and ammonia, had the effect of increasing susceptibility, so that $80\%$ of carbohydrate could be removed by digestion. Furthermore, if the wood was first ground for ten minutes
and then treated with swelling agents before digestion, 95% of the carbohydrate was removed. The residue was nearly pure lignin, and was insoluble in organic solvents or even in alkali. Here we see that although this lignin was nearly free of carbohydrate, and well ground, it remained insoluble. Pew thus concluded that "spruce lignin as it exists in wood, regardless of its association with other wood components, is a highly insoluble substance and is not likely to dissolve in any solvent with which it does not react chemically". If this statement is true, then current methods available for the isolation of lignin, no matter how mild the conditions, must involve some reaction with lignin. Of course, the reaction is not necessarily such as to break a lignin-carbohydrate bond --- it may involve degradation within the lignin molecule. Indeed, Pew (72) believes his experiments indicated that lignin is only physically associated with the carbohydrate in a form such as that of a "snakecage resin" (84).

From these results, it is clear that lignin is closely associated with hemicellulose, and highly insoluble. Grinding of wood has a pronounced effect on the separation of lignin from wood, but the mechanism of the extraction after grinding is by no means clearly understood. One would expect, of course, that during milling, oxidation might occur, and lignin might suffer depolymerization, together with some chemical changes. The extraction with water or moist dioxane after grinding may, in the end, be very similar to an acidolytic extraction of lignin from wood, since the grinding of wood may generate some organic acids, which would catalyse the liberation of lignin and, in the Björkman procedure, the milled-wood
is extracted with dioxane-water for a very long time. It is well-known that heating wood with water alone will liberate formic and acetic acids (85, 86), so that the process is a mild acid hydrolysis rather than a simple extraction of water-solubles. Various workers have reported that heating wood with water results in an increase in the alcohol-solubility of the wood (87, 88, 89). Moreover, there is some evidence to indicate that the lignin in solution after mild pre-hydrolysis is primarily in the form of a water-soluble complex which consists of lignin and carbohydrate (87, 90, 91). Traynard (87) has studied further the nature of this water-soluble complex by heating poplar wood with water at 140° for eight hours. The hydrolysate was found to contain carbohydrate, methanol, furfural, formic and acetic acids, together with a complex. On addition of acetone to the hydrolysate, carbohydrates were precipitated and separated, the solution which contained the complex (9.7% OCH₃, 50.5% C) yielded lignin and reducing sugars on hydrolysis.

Since complexes of lignin and carbohydrates appear to have been isolated under widely different conditions, many lignin chemists generally accept that complexes must exist in the wood. The nature of their linkages has been the subject of much study and has produced several hypotheses. Some authors have expressed the belief that the linkage was of the ester type (61, 92, 93, 94, 95, 96, 97, 98, 99, 100), some have thought it took the form of an acetal link (13, 101, 102, 103, 104), some suggested that it might be glycosidic (70, 77, 105, 106, 107, 108, 109), and others have proposed a bond of the benzyl ether type (4, 53, 54, 110, 111, 112, 113).
The Ester Linkage

As early as 1867, Erdmann (61) published his experimental results on the extraction of lignin from wood by cooking in alkali, and offered the explanation that in the natural wood substance, carboxyl groups of the lignin had been chemically combined with carbohydrate in ester linkages. In fact, the lignin that Erdmann obtained was an alkali lignin, and the carboxyl groups had been formed by the oxidative cleavage of the phenylpropane side chains of lignin. Thus, because of insufficient knowledge of lignin at that time, Erdmann misinterpreted his results. Nevertheless, he originated the idea which laid the groundwork for subsequent researches along this line, although more recent work has considered that any ester bond between lignin and carbohydrate might involve carboxyl groups from the latter, rather than the former. For example, in 1947-8, Sarkar and co-workers (96, 97) conducted experiments on jute, and found that treatment with 0.25% sodium hydroxide at room temperature practically doubled the acid number of the jute, as measured by the silver-ion exchange method. They concluded that the additional free acid might have resulted from the hydrolysis of ester linkages between lignin and polyuronides. Very similar results were obtained by Bhattacharjee and Callow (98) who found that treating de-ashed jute fibre with 0.25% sodium hydroxide resulted in an increase in carboxyl content from 11.4 to 22.5 milliequivalent per 100 grams of jute, and they, too, explained this increase as due to the hydrolysis of an uronic ester. Sarkar (97), working again with jute, Tachi and Yamamori (114) working with beech and elm, obtained further results that the carboxyl content was higher in
holocellulose than in the original jute or wood, and once more concluded that in plant tissue the carboxyl groups were esterified with lignin.

In 1950, Foster, Schwerin and Cohen (99) found that uronic acid could be liberated much faster from holocellulose than from wood. On treating *Eucalyptus regnans* wood with N sulphuric acid at 91–92°, they observed that 1.45% of uronic acid anhydride was hydrolyzed in three hours, whereas treating holocellulose under the same conditions, resulted in the hydrolysis of 2.33% of uronic acid anhydride in one hour. From these results, they suggested that in wood, the uronic acid could be bound to either lignin or carbohydrate or both by an ester link.

Later, Harris (100) found that after treatment with cold 17.5% sodium hydroxide, 70% of the lignin of maple and aspen, and 24% of the lignin of spruce became extractable with methanol. He considered this fact to be evidence of an ester link between lignin and carbohydrate; however, the solubilization of lignin may also be due to degradation of lignin itself.

In 1954, Stewart and McPherson (115) pretreated holocellulose with 5% aqueous sodium hydroxide at room temperature for four days, then cooked it with methanol at 150°. The extract, after removal of Klasson and acid-soluble lignins, was fractionated by paper chromatography. Xylose, galactose, a trace of mannose, 4-O-methyl-D-glucuronic acid and "glycose uronides" were found, but similar treatment of wood did not yield the "glycose uronides". They concluded that lignin polyuronides existed in wood. Unfortunately, the composition and structure of the "glycose
uronides" was not rigorously established.

The conclusion of the existence of ester bond between lignin and uronic acid was revised by Wang (75) recently, who made an extensive study of the ammonolyses of ester links in wood and found, that in birch, about 70% of the glucuronic acid units in its xylan fraction were esterified in their native state. However, periodic acid oxidation indicated that the group to which the uronic acids were esterified was not lignin, but xylose, either on the same chain as the uronic acid or on the adjacent ones.

At about the same time, Brownell (76) isolated a lignin-carbohydrate complex by subjecting wood to ball-milling under dry conditions, then partitioning the ball-milled wood in a liquid-liquid system. He found that the complexes were stable for a long time in aqueous sodium thiocyanate at room temperature, but decomposed to lignin and carbohydrate when heated at 100 ° for 15 minutes in 1% aqueous sodium hydroxide. From the stability of these complexes, he judged that the linkage was of an ester type. However, an attempted ammonolysis of this "ester bond" failed to liberate lignin.

The Glycosidic Linkage

A review of the literature has revealed that most of the linkages which have been thought to exist between lignin and carbohydrate were acid-labile. One example is the glycosidic bond, a bond which is more readily hydrolyzed in acid than in alkali. Hibbert and his co-workers (105)
investigated this possibility as early as 1940, by studying models representing the glycosides known to be widely present in plants. They studied the rates of hydrolysis in acid and alkali, respectively, of a series of one aliphatic and several phenolic glycosides (Figure I-6) under a variety of conditions. They concluded that the phenyl glycoside linkage is a plausible type for the lignin-carbohydrate complex in wood.

The phenyl glycosidic linkage was studied extensively by Kawamura and Higuchi (70, 107, 116, 117, 118, 119) on beech wood, and by Hayashi and Tachi (77, 108, 109) on wheat straw. On treating beech dioxane lignin with "Taka Diastase" --- an enzyme which acts specifically on glycosidic bonds --- Kawamura and Higuchi (107) found that xylose was liberated, and suggested that lignin and xylan were glycosidically linked. Also, the authors isolated an "acetyl xylo-lignin" from the chloroform extract of beech wood pretreated with a mixture of acetic acid - acetic anhydride (2:1) containing a trace of sulphuric acid. The "acetyl xylo-lignin", containing 27.2% Klason lignin and 15.8% pentosan, was then extracted with chloroform and acetone. The "acetyl xylo-lignin" was saponified with 0.1N sodium hydroxide and resolved by ascending paper chromatography with a mixture of n-butanol-acetic acid-water (4:1:1). The lignin spots (R_f=1.0) were cut and eluted with acetone. Hydrolysis of the lignin with acid followed by paper chromatography indicated the presence of xylose. Complete methylation, followed by hydrolysis with 3% hydrochloric acid, and paper chromatography yielded 2,3-di-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-xylose. Kawamura and Higuchi (118) considered that the presence of trimethyl sugars in the hydrolyzate
and the corresponding \( \beta \)-d-glucoside, and acetovanillone-\( \beta \)-cellubioside.

FIGURE I-6 Phenylglycoside Models Studied by Hibbert (105)
provided convincing evidence for the existence of a phenolic glycosidic linkage in wood. However, the trimethyl xylose could well be derived from entrained xylobiose. A similar result was obtained by Hayashi and Tachi (108) on wheat straw, but they could not completely de-esterify the "acetyl xylo-lignin" with 0.1 N sodium hydroxide as Kawamura and Higuchi had done. Furthermore, Hayashi and Tachi (108) found evidence for what they considered to be a transglycosidation reaction whereby xylan was transferred from a lignin-xylan complex to cellulose under alkaline conditions. The simultaneous increase of phenolic content of the lignin was taken as evidence for the hydrolysis of phenyl glycosidic bonds.

The Acetal Linkage

A type of acid-labile linkage that has received considerable attention is the acetal bond. Without experimental support Klason (101) suggested in 1920 that the acrolein end-group of lignin might form an acetal bond with hemicellulose. In 1925, Holmberg and Runius (102) found that new ethoxyl groups were introduced into lignin isolated by ethanolysis of spruce wood. To account for this newly introduced ethoxyl group, they suggested that lignin might be combined with carbohydrate through an acetal link, with the lignin as the carbonyl containing component. Berlin (104) arrived at a similar conclusion from the results of oxidations of wood with hydrogen peroxide.

Early in 1942, Maeda and Kobayshi (103), from a study of the
sulphite cooking process of larch wood, suggested that protolignin does not contain free carbonyl or acidic hydroxyl groups, but that such groups were produced by heating lignin with dilute alkali and acid. To check this, Nokihara and Tanaka (120), in 1952, subjected wood, holocellulose, and viscose pulp to ethanolysis in the presence of hydroxylamine. To explain the three different reaction rates that were found, it was postulated that in wood, the formation of the lignin oxime was preceded by the hydrolysis of some other compounds. As indicated earlier (p. 5), Maeda and Kobayashi's view can only be partly true, for, in fact, protolignin contains α-carbonyl groups, as revealed by spectroscopic studies. On the other hand, the β-carbonyl group is formed only on hydrolytic isolation of lignin. Bolker (121, 122) has found by differential infrared spectroscopy that the lignin in wood and pulp contained no non-conjugated carbonyl. Only spectra of isolated lignins showed evidence of the presence of such a group. These observations led Bolker to suggest that the unconjugated carbonyl group arose only because of the process of isolation, and, therefore, in wood or in pulp it might be masked by combination with other groups. The simplest explanation was that masking occurred through the combination of the carbonyl group with carbohydrate in an acetal linkage (Figure I-7), as earlier proposed by Holmberg. These observations, if not the conclusions, have been confirmed by Michell, Watson and Higgins (123).

The Benzyl Ether Linkage

Of course, the production of the carbonyl group in isolated
FIGURE I-7 Plausible Acetal Linkages Between Lignin and Carbohydrates
lignin can alternatively be explained by the hydrolysis of a benzyl ether linkage between lignin and carbohydrate (Figure 1-8), as proposed by Freudenberg on the basis of his biosynthetic experiments (112). The possible existence of such a bond had been foreshadowed by the work of Berg and Holmberg (113) in 1935 on the alkylation of lignin with ethanol in the presence of a trace of mineral acids. They suggested that the dissolution of lignin in ethanolic hydrogen chloride involved transetherification of a benzyl ether group, either between lignin and carbohydrate or within lignin itself (LXII). This suggestion has been supported by several experimental studies on model compounds (4, 53, 54, 110, 111).

The work of Freudenberg (112) on the enzymatic dehydrogenation of coniferyl alcohol in aqueous methanol solution also gave evidence suggesting the existence of such a bond. Freudenberg has demonstrated that the same biosynthetic reaction which produces benzyl ether bonds in lignin is capable of binding carbohydrates to the lignin. It is known that a quinonemethide derived from coniferyl alcohol combines readily with cane sugar to form a benzyl ether compound (LXII), and it is distinctly possible that such a reaction can occur \textit{in vivo}.

\begin{center}
\includegraphics[width=0.5\textwidth]{LXII.png}
\end{center}
FIGURE 1-8 Plausible Benzyl Ether Linkage Between Lignin and Carbohydrates, and Its Hydrolysis
CONCLUSIONS

On summarizing the above discussion, it seems reasonable to accept that in plant tissue lignin is more closely associated with hemicelluloses rather than with cellulose. However, whether the association is chemical or physical has never been settled. But if the bond is of chemical type, then it seems that it must be acid-labile and not alkali-labile judging from most of the experimental evidence.

In order to solve this problem, considerably more work must be done. One approach is to seek direct evidence of a chemical link. Another approach is to study the mechanisms of different methods of separating lignin from carbohydrate. The former approach has led us to perform alcoholysis studies, as described in the following chapter. The latter approach was made recently by Bolker and Terashima (124) in their study of the isolation of lignin with 2,2-dimethoxypropane in acidic dioxane, which, in turn, suggested the other part of the present research, as described in chapter III.
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CHAPTER II

STUDIES ON THE ETHANOLYSIS OF LIGNINS AND WOOD
INTRODUCTION

Since, in the field of wood chemistry, the nature of bonds between lignin and other wood components has been a matter of debate for many decades, and since new evidence as to the possible nature of the bond had recently been found, we undertook to apply alcoholysis reactions to isolated lignins and to wood. It seemed likely that the application of new physical methods of analysis might yield new and valuable answers.

The older literature leaves little doubt that several reactions take place in the alcoholysis of lignin. Part of the lignin condenses with alcohol to give a soluble alcohol lignin; a small part of the lignin undergoes alcoholysis with simultaneous degradation or depolymerization to monomolecular lignin building units, followed by condensation of some of them with the alcohol; yet another part polymerizes with itself, and gives rise to a less reactive lignin which also condenses with alcohol and remains insoluble in the solvent. The present study deals only with the major product, the soluble alcohol lignin.

The Origin of the Alcoholysis Problem

In 1925, Holmberg and Runius (1) treated spruce wood with ethanolic hydrogen chloride (1 N) for five hours at reflux temperature and obtained an ethanol lignin with 64.6% carbon, 6.3% hydrogen, 13.1% methoxyl and 6.2% ethoxyl, corresponding to the formula $C_{36}H_{30}O_8(OCH_3)_4(OCH_2CH_3)_2$ or, $C_9H_{7.5}O_2(OCH_3)(OC_2H_5)_{0.5}$ as the average ethanol lignin.
building unit. Holmberg interpreted the result as indicating that a transacetalation reaction had occurred between alcohol and the carbonyl groups of lignin. Later, he suggested an alternative explanation (2, 3): etherification and transetherification of benzyl alcohol and benzyl ether groups. Each of these views found its supporters and formed the basis of a controversy. Brauns (4) supported the carbonyl theory and assumed that the carbonyl groups of lignin reacted to form acetal (XVII) or enol ether (XVIII) groups. Adler and Gierer (5) rejected the carbonyl theory since they found "native lignin" could be methylated even after its carbonyl groups had been removed by reduction with sodium borohydride, and they supported the second explanation proposed by Holmberg as illustrated by the transformation of XIX to XX.
Early Work on the Ethanolysis of Lignin

In 1929, Campbell (6) studied in detail the action of ethanol-hydrochloric acid on Sitka spruce under various conditions, and found that two main reactions took place: hydrolysis of carbohydrates, and reaction of alcohol with lignin. According to Campbell, the absence of water from the system favoured depletion of the carbohydrates, the maximum effect on the lignin in wood being secured by the use of an oven-dry woodmeal with a concentrated solution of hydrogen chloride in absolute ethanol.

The ethanolysis reaction was extended and revised by Hibbert and his colleagues (7, 8, 9), who performed a series of experiments on spruce and maple woods. They isolated a variety of monomolecular lignin building units, known as "Hibbert's ketones" (7, 8). In an attempt to determine the mechanism of ethanolysis, Hewson, McCarthy and Hibbert (9) modified the method by using stepwise removal and replacement of the ethanol-hydrogen chloride medium as extractant. They used finely divided wood in order to minimize physical factors such as penetration, etc. The technique was successful in removing nearly all the lignin from the wood, and consideration of the yields of ethanol-soluble lignin, ethanol-insoluble lignin, and "distillable oils" (i.e., the low-molecular weight monomers obtained from the ether-soluble fraction), led to the conclusion that delignification had occurred by hydrolysis of the lignin polymer or of a lignin-carbohydrate complex. Although the use of finely powdered wood was considered as one factor facilitating the solubilization of wood
components, another factor was the increased solubility of the etherified lignin products. Polymerization and condensation reactions accompanying ethanolysis gave rise to insoluble forms of lignin which exerted an important role in its removability. Thus the authors considered that the basis of the ethanolysis mechanism was the net effect of simultaneous progressive depolymerizations and polymerizations.

Merewether (10) has studied the lignin of *Eucalyptus regnans* obtained by ethanolysis. He boiled the pre-extracted woodmeal with absolute ethanol containing 2% hydrogen chloride for 48 hours. After several precipitations from dioxane-ether, a dioxane-ether insoluble ethanol lignin-A was isolated, which had an equivalent weight in the order of magnitude of 1400, as determined by potentiometric titration, corresponding to the molecular formula, C_{59}H_{38}O_{10}(OCH_{3})_9(OC_2H_5)_2(OH)_7, in which two carbonyl groups were present, as shown by the formation of a bisphenyl-hydrazone and a bis-p-nitrophenylhydrazone. Of the seven hydroxyls present, two were sufficiently acidic to react with diazomethane. Furthermore, of the two acidic hydroxyls, at least one was enolic, as revealed by a positive spot test for enols. On refluxing the ethanol lignin-A with 12% hydrochloric acid, only one ethoxy group was split off, but with 20% hydrochloric acid, complete de-ethylation took place (11). It was further found that de-ethylated ethanol lignin-A contained only one carbonyl group and nine hydroxy groups, i.e., one carbonyl group less and two hydroxy groups more than in the original ethanol lignin-A. According to Merewether (11) these facts favoured the theory that the ethoxy introduced by ethanolysis was ether rather than acetal in nature. Since one would expect an acetal to be readily hydrolyzed by 12% hydrochloric acid, and since the carbonyl
content did not increase on hydrolysis, and instead two new hydroxyl groups were formed, the evidence did not favour the acetal theory. These findings were further supported by Merewether's later research on the ethanolysis of an isolated alkali lignin of *Eucalyptus regnans* (12).

In order to further elucidate the reaction mechanism, Sarkanen, Arlt and Schuerch (13) investigated the ethanol lignin isolated from Norway spruce by the use of 0.2 N hydrogen chloride in a 4:1 mixture of chloroform-ethanol at 60°. They found that this ether-insoluble ethanol lignin had a methoxyl to carbon ratio close to theoretical for a propylguaiacyl polymer and their average empirical formula was \( C_{9.8}H_{0.02.6}(OCH_3)^{0.95}(OC_2H_5)^{0.41} \). Furthermore, the average ethoxyl to phenolic hydroxyl ratio in this ethanol lignin was 1.16, near to the theoretical for simple alcoholysis of alkyl aryl ethers. By subjecting this ether-insoluble ethanol lignin to a series of re-ethanolyses in a chloroform-ethanol mixture for various period of time at 65°, Sarkanen and Schuerch (14) found that the ethoxyl content of the product reached a maximum at about 0.55 per lignin building unit. They also found that with the introduction of ethoxyl groups, new phenolic groups were formed which reached a constant value of 0.55 to 0.65 per monomer. Since each of the propylguaiacyl monomers contains one phenolic hydroxyl, either free or as a phenolic ether group, their ethanol lignin still contained 0.35-0.45 of phenolic ether groups per monomer unit which could not be cleaved by the ethanolysis reaction. A study of the rate of re-ethanolysis of these phenolic ether groups in the ethanol lignin suggested that ethanol lignin, and perhaps spruce protolignin contained at least three different
phenolic ether linkages between the monomeric units. Of these, one was resistant to ethanolysis, and the others were cleaved at different rates. However, details as to the nature of these phenolic ether linkages were not worked out, except that ultraviolet spectra of the lignins indicated that unconjugated phenolic groups and phenol ethers with α-carbonyl groups were formed during the ethanolysis reaction.

Most of the work on ethanolysis discussed so far was done chemically by obtaining quantitative analytical results on isolated lignins and their derivatives. Recently, infrared spectroscopy has been used extensively by many chemists as a physical means to approach the problem of the structure of lignin. Bolker (15, 16) has reported, from spectroscopic evidence, that in wood and pulps, the bond between lignin and carbohydrate was one that generated a non-conjugated β-keto group in the lignin on isolation by hydrolysis. However, scantiness of basic knowledge of the infrared spectra of lignins causes some difficulties in precise interpretation. Therefore, it seemed worthwhile to attempt to combine the two available methods, and to examine the changes in spectra of lignins and in the quantitative analytical results brought about by various chemical treatments.

Aim of Present Study

Considered as lignin-carbohydrate bonds, both the hypothetical acetal and benzyl ether bonds (Figure II-1) can account for the formation of unconjugated β-keto groups in lignin isolated by hydrolysis. In order
to differentiate between these two, if either exists, as the potentially reactive group in the hydrolysis reaction, experiments as illustrated in Figure II-2 were done. It was expected that alcoholysis would introduce two moles of alkoxy groups for every mole of acetal groups, and one mole of alkoxy for every mole of benzyl ether groups (Figure II-1). Thus by careful determination and comparison of the alkoxy groups introduced in each lignin preparation, and by spectroscopic investigation, a significant answer might be obtained.
A. Acidolysis

\[
\begin{align*}
&\text{Carbohydrate} + H_3O^+ \\
&\xrightarrow{H_2O} \text{Carbohydrate} + &
\end{align*}
\]

B. Alcoholysis

\[
\begin{align*}
&XXI \xrightarrow{ROH/H^+} \text{XVII} \\
&XXII \xrightarrow{ROH/H^+} \text{XX}
\end{align*}
\]

FIGURE II-1 Hydrolytic Reactions of Lignin-Carbohydrate Bonds
FIGURE II-2 Flow Sheet of Reactions

Alcohol-Benzene Pre-extracted Black Spruce Woodmeal

1. EtOH/HCl
2. Dioxane/H$_2$O/HCl
3. NaBH$_4$
4. EtOH/HCl
5. KOH
6. Dioxane/H$_2$O/HCl
7. NaBH$_4$
8. EtOH/HCl
9. NaBH$_4$
10. NaBH$_4$
11. NaBH$_4$
12. Dioxane/H$_2$O/HCl
13. NaBH$_4$
14. NaBH$_4$
15. NaBH$_4$

I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
RESULTS AND DISCUSSION

Infrared Spectra of Lignins

Figure II-3 shows the infrared spectra of black spruce. The upper curve (a) is the spectrum of wholewood after alkaline treatment, which removed the acetyl groups of the uronides, the second curve (b) is the same wood after having been washed with dilute hydrochloric acid, the third curve (c) is Tappi holocellulose (17) from the same wood, the fourth curve is the difference spectrum (b and c), and represents mainly lignin. The bottom curve is the spectrum of wholewood reduced with sodium borohydride. In the differential spectrum of spruce lignin, the intense band at 1680 cm\(^{-1}\) is the same as that noted by Bolker and Somerville (16) and by Kolboe and Ellefsen (19). This absorption band can be attributed to carbonyl functions, conjugated with a benzene ring. For a coniferous lignin such as that of spruce, it would indicate either a coniferyl aldehyde structure or guaiacylpropane with a keto group alpha to the benzene ring. The band can also be due to an aromatic carboxylic group, or a conjugated carboxyl group in the side chain of a benzene ring such as in cinnamic acid, \(\beta\)-methoxycinnamic acid or ferulic acid (18).

Since sodium-borohydride-reduced-wood does not show this absorption, it has been assigned as the conjugated carbonyl function (16, 18, 19, 20, 21, 22). This is in agreement with the quantitative analyses of milled-wood lignin which contains 0.03 coniferaldehyde units per methoxyl group, and about 0.06 carbonyl groups alpha to the benzene ring (23).
FIGURE II-3 Infrared Spectra of Preparations of Black Spruce Wood
Since the alkali-treated-wood did not absorb at 1730 cm$^{-1}$, but, after the wood was washed with dilute hydrochloric acid, a band at 1730 cm$^{-1}$ appeared in the spectrum, and was also observed in the differential spectrum, it indicated the presence of carboxyl groups in lignin.

**Isolated Lignin from Unreduced Spruce Wood**

In the spectrum of spruce dioxane lignin (Figure II-4) an absorption band at 1720 cm$^{-1}$ was observed in addition to the band at 1680 cm$^{-1}$. The band at higher frequency could be due to carbonyl or carboxyl groups bonded to aliphatic or arylaliphatic chains without conjugation to a benzene ring such as in XXIV-XXVII. On treatment of spruce dioxane lignin with sodium borohydride (Figure II-5), the band at 1680 cm$^{-1}$ disappeared and the one at 1720 cm$^{-1}$ was greatly depressed. Only a shoulder remained and it was not removed, even after repeated reduction with the borohydride. Upon alkaline treatment of the dioxane lignin followed by reduction with sodium borohydride, the 1720 cm$^{-1}$ absorption became un reducible, in agreement with Hergert (24). Thus it was attributed mainly to a readily enolizable aryl β-keto group.
FIGURE II-4  Infrared Spectra of Black Spruce Ethanolysis Lignins
FIGURE II-5 Infrared Spectra of Reduced Black Spruce Lignins
assignment supported by ultraviolet spectra and the chemical evidence of Adler (25) --- and to a lesser extent to unconjugated carboxyl groups (26, 27). Sarkanen, Chang and Allan (28) have investigated a series of milled-wood lignins from coniferous species and found that most of them seemed to contain esters of conjugated aromatic acids as demonstrated by infrared absorption at 1715 cm\(^{-1}\) such as in vanillate and ferulate. Pine lignin appeared to contain both acetyl groups and other ester groups as indicated by the absorptions at 1735 and 1715 cm\(^{-1}\) in its infrared spectrum. They suggested that in lignin, structures of the types XXVIII and XXIX might be present and pointed out that such structures were compatible with the current view of lignin biogenesis as outlined by Kratul and Okabe (29). However, more experiments are needed to clarify this point.

\[
\begin{align*}
\text{XXVIII} & \quad \text{(Lignin)} \\
\text{XXIX} & \quad \text{(Lignin or H)}
\end{align*}
\]

On refluxing spruce dioxane lignin with anhydrous ethanol-hydrogen chloride (3%) for a reaction time of two hours, ethoxylation occurred which gave rise to 0.47 ethoxy1 per methoxyl group. The infrared spectrum of this product (Figure II-4) indicates that the absorption at 1680 cm\(^{-1}\) was less intense than, and the one at 1720 cm\(^{-1}\) was about the same as, in dioxane lignin. Moreover, the spectrum was the same as that of spruce ethanol lignin (V) which contained 0.53 ethoxy1 per methoxyl group. Treating the ethanol lignin with aqueous alkali did
not change the spectrum (VI), but hydrolysis with acid gave intensified bands at 1680 and 1720 cm$^{-1}$ (VII), identical to those of spruce dioxane lignin, and the responses of these two absorption bands after treatment with sodium borohydride were similar to those of dioxane lignin.

Based on the present spectroscopic evidence, it seemed acceptable to a first approximation that ethanolysis of lignin was the result of transacetalation wherein the lignin acted as a carbonyl compound and formed lignin acetals or enol ethers, as proposed early by Holmberg (1) and Brauns (4). In support of this idea, Brauns (32) cited the infrared study of Jones (33), who had noted that the absorption band in the spectrum of native lignin at 1663 cm$^{-1}$ was absent from the spectrum of native lignin methylated with methanol-hydrochloric acid.

Adler and Gierer (5), in contrast to Jones' work, found that the carbonyl band in the infrared spectrum of native lignin did not disappear upon methylation with methanol-hydrochloric acid. Hergert (24) studied the spectra of native lignin treated with ethanol-hydrochloric acid and of ethanol-hydrochloric acid lignin and found absorption bands at about 1650 cm$^{-1}$ and 1710-1715 cm$^{-1}$. The 1710 cm$^{-1}$ absorption was appreciably stronger than in the original native lignin, while the 1650 cm$^{-1}$ band was slightly less intense. The controversy between these investigators may be due to the different conditions which they used in the alcoholysis experiments. The experimental results seem to be time-dependent. Pólín et al. (18) have observed that with a reaction time of thirty minutes, Norway spruce lignin after reaction with methanol-
hydrochloric acid (2%, 65°), showed both absorption bands at 1680 and 1720 cm\(^{-1}\) in its infrared spectrum; as the reaction time increased, the absorption at 1680 cm\(^{-1}\) became less intense; for a reaction time of 16 hours, it disappeared completely, while the band at 1720 cm\(^{-1}\) became stronger. The present investigation agreed with these latter results.

The non-alkylation of carbonyl groups in lignin has been further indicated by studies of model compounds. Adler and Marton (34) found that guaiacyl acetone (XXX), propioguaiacone (XXXI) and the corresponding veratryl derivatives were not methylated by methanol-hydrogen chloride, but the related ketols (XXXII, XXXIII) took up new methoxyl and formed ketol ethers. The rates of reaction were different: XXXII reacted rapidly at room temperature, while under the same conditions, XXXIII was only partially converted to ketol ether after several days of treatment, and XXXIV was completely non-reactive. Only ketol XXXV, \(\omega\)-hydroxyguaiacyl...
acetone, reacted with loss of carbonyl function and took up more than one new methoxyl group. On the other hand, Adler and Delin (35) have found that veratrylglycerol-β-guaiacyl ether (XXXVI) was completely methylated after a reaction time of two to three days at room temperature, or two to three hours at reflux temperature. Coniferyl alcohol took up 0.7 new methoxyl groups by similar treatment. These results suggest that reaction of lignin with alcohol-hydrogen chloride mainly involves alkylation of α-carbinol group of the propane side chain, while the carbonyl group is non-reactive.

However, if ethoxylation does not take place on the carbonyl function, then another interpretation must be advanced to explain the changes in the absorption bands at 1680 and 1720 cm\(^{-1}\) in the infrared spectra of ethoxylated dioxane lignin (II) and ethanol lignin (V) (Figure II-4). One of the possible interpretations is a shift of the absorption band at 1680 cm\(^{-1}\) to 1720 cm\(^{-1}\) on ethanolysis as illustrated in Figure II-6.

Cinnamic aldehyde (XXXVII), on methylation with a mixture of methanol-hydrochloric acid (2%, 65\(^°\), 6 hours), took up 5.4% of methoxyl and 0.3% of bound chlorine (18); these changes corresponded to 30% reaction of the double bond. The absorption band at 1680 cm\(^{-1}\) was indeed much weaker, and a new band at 1720 cm\(^{-1}\) was formed simultaneously. However, as found by Adler (25, 30), spruce native lignin contains, at most, 0.03 coniferyl aldehyde units, which therefore can contribute only a small fraction to the absorption band at 1680 cm\(^{-1}\); the largest part must be due to carbonyl groups alpha to the benzene ring. On ethanolysis, they would shift from the alpha (XLI) to the beta position (XLIII) and do so rather easily,
FIGURE II-6  Plausible Reaction Mode of Ethanolysis of Lignin (18, 31)
especially when there is a hydroxyl or ether group adjacent to the carbonyl; the mechanism of this shift has been outlined by Hibbert (31) in his studies on ethanolysis (Figure II-6).

Assignments of bands other than in the carbonyl-carboxyl region have mostly been established (16, 19, 20, 21, 22, 36, 37, 38). As shown in Figures II-4 and 5, bands at 3430-3440 cm\(^{-1}\) are due to hydroxyl groups, both phenolic and alcoholic, and their frequency indicates that they are hydrogen-bonded. The absorption bands at 3100-2800 cm\(^{-1}\) represent various types of C-H bonds. In dioxane lignin a shoulder at 2970 cm\(^{-1}\) is due to CH\(_2\) antisymmetric stretching vibration as well as to OCH\(_3\), which also give rise to the band at 2835 cm\(^{-1}\). Ethanol lignin has a more intense band at 2970 cm\(^{-1}\), a frequency characteristic of OC\(_2\)H\(_5\) groups (39). On acid hydrolysis of ethanol lignin (VII) the band at 2970 cm\(^{-1}\) decreases in intensity. The sharp band at 2940 cm\(^{-1}\) in lignin spectra is caused by CH\(_3\) and CH\(_2\) vibrations.

The two bands at 1515 and 1603 cm\(^{-1}\) are characteristic of the C=C stretching vibrations of benzene rings. Absorptions at 1466 and 1456 cm\(^{-1}\) are related to C-H deformation vibrations in CH\(_2\) and CH\(_3\) groups. The doublet configuration is characteristic of softwood lignins (16). The band at 1425 cm\(^{-1}\) has been assigned to C-H bending vibrations in methoxyl groups, an assignment supported by the observation that the band is stronger in hardwoods than in softwoods (16, 40).

Based on deuteration of Braun's native lignin, Kolboe and Ellefsen (19) have found that the band at 1370 cm\(^{-1}\) was shifted to 980 cm\(^{-1}\) in the
deuterated lignin, thus it was assigned as an O-H deformation vibration.

The band at 1275 cm\(^{-1}\) has been assigned to the C-O-C asymmetric stretching vibration of aryl ether linkages and the one at 1226 cm\(^{-1}\) to aryl-alkyl ether linkages (40).

The assignment of bands at 1000-1200 cm\(^{-1}\) is less certain. However, they are likely due to vibrations involving oxygen, since both paraffinic and aromatic structures yield only weak absorptions here.

The bands at 1146 and 1037 cm\(^{-1}\) have been assigned to dialkyl ether linkages (16). The weak absorption band at 1090 cm\(^{-1}\) in dioxane lignin has been attributed to aryl-alkyl ether linkages; it becomes quite intense in ethanol lignin.

The two bands at 860 and 820 cm\(^{-1}\) represent the 1,2,4- substitution pattern of a benzene ring, and are typical of softwood lignin. The weak shoulder at 940 cm\(^{-1}\) has not been assigned; it is possibly due to OH out-of-plane deformation vibration (24). In ethanol lignin, a shoulder at 880 cm\(^{-1}\) appears, possibly due to C-H out-of-plane bending vibration (41).

Bolker and Marraccini (42) have reported that the infrared spectrum of acid-hydrolyzed ethanol lignin showed sharp bands at 1125, 892 and 873 cm\(^{-1}\). A similar result was obtained by Bolker and Terashima (43) on isolation of lignin from spruce by 2,2-dimethoxypropane-HCl-dioxane and they concluded that it might represent an ether or acetal bond. However, in the present investigation, the spectrum of acid-
hydrolyzed ethanol lignin was identical with the spectrum of dioxane lignin (Figure II-4). To solve this discrepancy, we have examined the spectra of 2,2-dimethoxypropane lignins from spruce and birch, and found that after further purification of the isolated lignins, the intensities at these frequencies decreased. Moreover, the spectrum of pure liquid dioxane showed intense absorptions at 1122, 1083, 887 and 873 cm\(^{-1}\). Consequently, it was reasonable to believe that the three unexpected absorption bands in lignin were due to residual dioxane in the lignin sample --- an idea raised by Michell (44). A similar phenomenon has also been observed by Reznikov, Pilipchuk and Solov'ev (36).

**Isolated Lignin from Reduced Spruce Wood**

If the conclusion suggested by infrared spectra is true, that in whole-wood \(\beta\)-carbonyl groups are protected and \(\alpha\)-carbonyls are free, then on treating whole-wood with sodium borohydride, only the \(\alpha\)-carbonyl groups should be reducible. We, therefore, reduced spruce wood with sodium borohydride and isolated both dioxane (X) and ethanol (XII) lignins from it. Figure II-7 shows that the lignins had no absorption at 1680 cm\(^{-1}\) at all, but the band at 1720 cm\(^{-1}\) was found in both lignins and its intensity decreased upon treatment with borohydride (XIII). Acid hydrolysis resulted in an increased intensity of the 1720 cm\(^{-1}\) absorption, and the intense band could be diminished by reduction with sodium borohydride. These results strengthened the conclusions obtained from unreduced wood that the band at 1680 cm\(^{-1}\) was exclusively due to the \(\alpha\)-carbonyl group, and the band at 1720 cm\(^{-1}\) was largely due to \(\beta\)-carbonyl groups formed on
FIGURE II-7 Infrared Spectra of Lignins from Reduced Black Spruce Wood
isolation, and to a lesser extent, to carboxyl groups. Ethanolysis involves mainly ethoxylation of α-carbinol groups, the carbonyl function being non-reactive towards the reagent. This conclusion was further supported by quantitative studies of the ethoxyl group introduced in each lignin preparation.

Quantitative Studies

Table II-1 shows the quantitative data for each lignin preparation, the left hand set of columns represents lignins obtained as illustrated in Figure II-2; the middle set of columns indicates the chemical compositions; the right hand set of columns gives the empirical formula of lignin calculated per C₉ unit, which shows that the hydrogen contents of the lignins obtained from reduced wood (X-XV) are higher than those obtained from unreduced wood (I-VIII). It was not possible to explain this observation.

In order to interpret the analytical results, theoretical values for ethoxyl have been calculated. These calculations were based on the currently accepted distribution of functional groups in lignin (see Chapter I), and on two assumptions:

(i) that, as Adler and Marton (34) found for model compounds, the carbonyl groups of lignin do not form ketals with ethanol-hydrogen chloride under the conditions of ethanolysis, and

(ii) that, lignin and carbohydrate are linked through benzyl ether groups.

Based on these assumptions, Table II-2 shows the quantities of functional
<table>
<thead>
<tr>
<th>Lignin Prepn.</th>
<th>%C</th>
<th>%H</th>
<th>(%\text{OCH}_3)</th>
<th>(%\text{OC}_2\text{H}_5)</th>
<th>(\frac{\text{OC}_2\text{H}_5}{\text{OCH}_3})</th>
<th>(\text{C})</th>
<th>(\text{H})</th>
<th>(\text{O})</th>
<th>(\text{OCH}_3)</th>
<th>(\text{OC}_2\text{H}_5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>62.6</td>
<td>5.60</td>
<td>14.7</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>7.92</td>
<td>2.88</td>
<td>0.90</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>66.6</td>
<td>6.60</td>
<td>14.1</td>
<td>9.41</td>
<td>0.47</td>
<td>9</td>
<td>9.06</td>
<td>2.12</td>
<td>0.88</td>
<td>0.40</td>
</tr>
<tr>
<td>III</td>
<td>61.3</td>
<td>5.65</td>
<td>14.8</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>8.19</td>
<td>3.08</td>
<td>0.93</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>64.9</td>
<td>6.22</td>
<td>14.3</td>
<td>7.68</td>
<td>0.37</td>
<td>9</td>
<td>7.79</td>
<td>2.29</td>
<td>0.90</td>
<td>0.33</td>
</tr>
<tr>
<td>V</td>
<td>64.8</td>
<td>6.35</td>
<td>14.6</td>
<td>11.2</td>
<td>0.53</td>
<td>9</td>
<td>7.53</td>
<td>2.20</td>
<td>0.96</td>
<td>0.51</td>
</tr>
<tr>
<td>VI</td>
<td>63.4</td>
<td>6.09</td>
<td>14.5</td>
<td>10.3</td>
<td>0.49</td>
<td>9</td>
<td>7.41</td>
<td>2.48</td>
<td>0.97</td>
<td>0.48</td>
</tr>
<tr>
<td>VII</td>
<td>63.3</td>
<td>6.01</td>
<td>13.8</td>
<td>2.78</td>
<td>0.14</td>
<td>9</td>
<td>8.33</td>
<td>2.70</td>
<td>0.85</td>
<td>0.12</td>
</tr>
<tr>
<td>VIII</td>
<td>63.1</td>
<td>6.14</td>
<td>13.1</td>
<td>3.21</td>
<td>0.17</td>
<td>9</td>
<td>8.64</td>
<td>2.73</td>
<td>0.81</td>
<td>0.14</td>
</tr>
<tr>
<td>IX</td>
<td>66.1</td>
<td>7.03</td>
<td>14.0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>10.1</td>
<td>2.18</td>
<td>0.80</td>
<td>0</td>
</tr>
<tr>
<td>XI</td>
<td>63.5</td>
<td>6.88</td>
<td>13.6</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>10.2</td>
<td>2.62</td>
<td>0.82</td>
<td>0</td>
</tr>
<tr>
<td>XII</td>
<td>65.7</td>
<td>6.80</td>
<td>14.9</td>
<td>12.8</td>
<td>0.59</td>
<td>9</td>
<td>9.01</td>
<td>1.93</td>
<td>0.98</td>
<td>0.57</td>
</tr>
<tr>
<td>XIII</td>
<td>65.4</td>
<td>6.98</td>
<td>12.4</td>
<td>8.83</td>
<td>0.49</td>
<td>9</td>
<td>9.28</td>
<td>2.19</td>
<td>0.77</td>
<td>0.38</td>
</tr>
<tr>
<td>XIV</td>
<td>64.8</td>
<td>6.33</td>
<td>13.4</td>
<td>3.52</td>
<td>0.19</td>
<td>9</td>
<td>8.68</td>
<td>2.42</td>
<td>0.81</td>
<td>0.15</td>
</tr>
<tr>
<td>XV</td>
<td>64.3</td>
<td>6.46</td>
<td>13.8</td>
<td>2.64</td>
<td>0.13</td>
<td>9</td>
<td>9.07</td>
<td>2.49</td>
<td>0.84</td>
<td>0.11</td>
</tr>
</tbody>
</table>

**TABLE II-I** Chemical Composition of Lignins
groups (A to G) in lignin which should be potentially reactive in the ethanolation reaction. Units A, D, E, F and G should be etherified, and C should be esterified on ethanolation. On reduction with sodium borohydride, the coniferyl aldehyde unit (B) in lignin should be reduced to A, and should react with ethanol-hydrogen chloride. The carbonyl group of unit D, on ethanolation, should convert to a carbinol group and should etherify at this position rather than at the γ-hydroxyl group. There should be no change in the ethoxyl content before and after reduction, with respect to unit D. According to this interpretation, as Table II-2 shows, despite some exceptions which will be discussed below, the agreement of the theoretical and calculated values was remarkably good.

When the second assumption was replaced by an alternative theory — that lignin and carbohydrate are joined by acetal bonds — the theoretical value of ethoxyl content in ethanol lignin, (V) and (XII) should have been 0.66 and 0.69 per methoxyl group, both higher than the previously calculated values, 0.53 and 0.59, respectively, and than any values actually found.

On the other hand, if assumption (ii) is replaced by the hypothesis that there is no covalent bond between lignin and carbohydrate, (i.e., unit G in Table II-2 is not present, and the formation of unconjugated carbonyl groups in lignin during isolation may originate from the hydrolysis of α-ether bonds within lignin itself (Table II-2, unit F)), then two discrepancies will arise:

(i) the ratio of \( \text{OC}_2\text{H}_5/\text{OCH}_3 \) in ethoxylated dioxane lignin (II) should be less than 0.46 —— predicted by the benzyl ether hypothesis ——
(1) Lignin in Spruce Wood

\[
\begin{align*}
A & : \text{H}_2\text{COH} & & \text{CHO} & & \text{COOH} & & \text{H}_2\text{CO}- \text{C} & & \text{COH} \\
B & : \text{CHO} & & \text{CHO} & & \text{COOH} & & \text{H}_2\text{CO}- \text{C} & & \text{COH} \\
C & : \text{COOH} & & \text{CH} & & \text{CH} & & \text{CO} & & \text{OCH}_3 \\
D & : \text{H}_2\text{CO}- \text{C} & & \text{C} & & \text{CO} & & \text{OCH}_3 & & \text{OCH}_3 \\
E & : \text{H}_2\text{CO}- \text{C} & & \text{C} & & \text{CO} & & \text{OCH}_3 & & \text{OCH}_3 \\
F & : \text{H}_2\text{CO}- \text{C} & & \text{C} & & \text{CO} & & \text{OCH}_3 & & \text{OCH}_3 \\
G & : \text{C} & & \text{CO} & & \text{OCH}_3 & & \text{OCH}_3 & & \text{OCH}_3 \\
\end{align*}
\]

\[
\begin{align*}
0.03 & & 0.03 & & \leq 0.05 & & \leq 0.06 & & 0.20 & & \text{per OCH}_3 \\
\end{align*}
\]

(2) Spruce Dioxane Lignin (I)

\[
\begin{align*}
A & : \text{H}_2\text{COH} & & \text{CHO} & & \text{COOH} & & \text{H}_2\text{CO}- \text{C} & & \text{COH} \\
B & : \text{CHO} & & \text{CHO} & & \text{COOH} & & \text{H}_2\text{CO}- \text{C} & & \text{COH} \\
C & : \text{COOH} & & \text{CH} & & \text{CH} & & \text{CO} & & \text{OCH}_3 \\
D & : \text{H}_2\text{CO}- \text{C} & & \text{C} & & \text{CO} & & \text{OCH}_3 & & \text{OCH}_3 \\
E & : \text{H}_2\text{CO}- \text{C} & & \text{C} & & \text{CO} & & \text{OCH}_3 & & \text{OCH}_3 \\
F & : \text{H}_2\text{CO}- \text{C} & & \text{C} & & \text{CO} & & \text{OCH}_3 & & \text{OCH}_3 \\
G & : \text{C} & & \text{CO} & & \text{OCH}_3 & & \text{OCH}_3 & & \text{OCH}_3 \\
\end{align*}
\]

\[
\begin{align*}
0.03 & & 0.03 & & \leq 0.05 & & \leq 0.06 & & 0.20 & & 0.12 & & \text{per OCH}_3 \\
\end{align*}
\]

\[
\begin{align*}
\text{Lignin} & : \text{H}_2\text{CO} & & \text{C} & & \text{CO} & & \text{OCH}_3 \\
\text{Carbohydrate} & : \text{C} & & \text{CO} & & \text{OCH}_3 & & \text{OCH}_3 \\
\end{align*}
\]

\[
\begin{align*}
\frac{\text{OC}_2\text{H}_5}{\text{OCH}_3} & \text{ Theor.} & \text{ Found} \\
0.00 & 0.00 \\
\end{align*}
\]

---

TABLE II-2 Acceptors for \text{OC}_2\text{H}_5 \text{ Groups in } \text{C}_2\text{H}_5\text{OH}/\text{HCl Ethylation}
(3) Ethoxylated Spruce Dioxane Lignin (II)

<table>
<thead>
<tr>
<th></th>
<th>A'</th>
<th>B'</th>
<th>C'</th>
<th>D'</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>0</td>
<td>≤ 0.05</td>
<td>≤ 0.06</td>
<td>OC$_2$H$_5$/OCH$_3$</td>
</tr>
</tbody>
</table>

(4) Reduced Spruce Dioxane Lignin (III)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>C</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.32</td>
<td>0</td>
<td>0</td>
<td>OC$_2$H$_5$/OCH$_3$</td>
<td></td>
</tr>
</tbody>
</table>

Theor. 0.46  Found 0.47

Theor. 0.0  Found 0.0

TABLE II-2 (cont'd.)
(5) Ethoxylated-Reduced Spruce Dioxane Lignin (IV)

<table>
<thead>
<tr>
<th>A'</th>
<th>C'</th>
<th>E'</th>
<th>( \text{OC}_2\text{H}_5/\text{OCH}_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>( \leq 0.05 )</td>
<td>0.38</td>
<td>0.49/0.37</td>
</tr>
</tbody>
</table>

(6) Spruce Ethanol Lignin

<table>
<thead>
<tr>
<th>A'</th>
<th>B</th>
<th>C'</th>
<th>D'</th>
<th>E'</th>
<th>( \text{OC}_2\text{H}_5/\text{OCH}_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>( \leq 0.05 )</td>
<td>0.06</td>
<td>0.42</td>
<td>0.56/0.53</td>
<td></td>
</tr>
</tbody>
</table>

(7) Alkali-Hydrolyzed Spruce Ethanol Lignin (VI)

<table>
<thead>
<tr>
<th>A'</th>
<th>B</th>
<th>C</th>
<th>D'</th>
<th>E'</th>
<th>( \text{OC}_2\text{H}_5/\text{OCH}_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>0</td>
<td>0</td>
<td>0.06</td>
<td>0.42</td>
<td>0.51/0.49</td>
</tr>
</tbody>
</table>

(8) Acid-Hydrolyzed Spruce Ethanol Lignin (VII)

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>( \text{OC}_2\text{H}_5/\text{OCH}_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.06</td>
<td>0.26</td>
<td>0.12</td>
<td>0.10 per OCH₃</td>
<td>0.0/0.0</td>
</tr>
</tbody>
</table>

(9) Lignin in Reduced Spruce Wood (IX)

<table>
<thead>
<tr>
<th>A</th>
<th>C</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>( \text{OC}_2\text{H}_5/\text{OCH}_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>0.05</td>
<td>0.26</td>
<td>( &gt; 0.12 )</td>
<td>0.10 per OCH₃</td>
<td>0.0/0.0</td>
</tr>
</tbody>
</table>

(10) Spruce Ethanol Lignin from Reduced Wood (XII)

<table>
<thead>
<tr>
<th>A'</th>
<th>C'</th>
<th>E'</th>
<th>( \text{OC}_2\text{H}_5/\text{OCH}_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>( \leq 0.05 )</td>
<td>0.48</td>
<td>0.59/0.59</td>
</tr>
</tbody>
</table>

(11) Acid-Hydrolyzed Spruce Ethanol Lignin from Reduced Wood (XIV)

<table>
<thead>
<tr>
<th>A</th>
<th>C</th>
<th>E</th>
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</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.06</td>
<td>0.19</td>
<td>0.59/0.59</td>
</tr>
</tbody>
</table>

**TABLE II-2 (cont'd.)**
because the amount of unit F in dioxane lignin (I) must be less than 0.12 due to conversion into unit G'; but the experimental value of the \( \text{OC}_2\text{H}_5/\text{OCH}_3 \) was 0.47.

(ii) the theoretical values of \( \text{OC}_2\text{H}_5/\text{OCH}_3 \) in ethanol lignin, (V) and (XII) should be 0.46 and 0.49 (unit G is not present), but both were lower than the experimental values, 0.53 and 0.59, respectively.

Based on this analysis the validity of the hypothesis of non-existence of covalent bonds between lignin and carbohydrate seemed improbable.

The experimental value for ethoxylated reduced dioxane lignin (IV) was somewhat lower than its theoretical value, probably because of incomplete ethoxylation. The acid-hydrolyzed ethanol lignin, (VII) and (XIV) should theoretically contain no ethoxyl groups, but exhibited traces on analysis. Similar results have also been reported by Bland et al. (45) and Bolker and Terashima (43). Regarding the inconsistency between the theoretical and found ethoxyl contents of acid-hydrolyzed ethanol lignins, we first thought it might be an artifact of the analysis, and that lignins which have undergone extraction and hydrolysis might contain side-chain groupings capable of undergoing retroaldol reactions in the presence of hydriodic acid to release acetaldehyde which might then become reduced and converted to ethyl iodide. Some authors have reported that 1,2-diethylene glycol (46), glycols, polyhydric alcohols and hydroxy-alkyl compounds (47, 48, 49) and sugars (50), all containing no alkoxyl groups, yielded, upon boiling with hydriodic acid, a small quantity of
alkyl iodide, and gave rise to false alkoxyl values. This apparent
alkoxy content might result from ethylene iodide or ethyl iodide
according to equations 1-4,

\[ \text{ROCH}_2\text{CH}_2\text{OH} + 3\text{HI} \rightarrow \text{RI} + \text{ICH}_2\text{CH}_2\text{I} + 3\text{H}_2\text{O} \quad (1) \]
\[ \text{ICH}_2\text{CH}_2\text{I} \rightarrow \text{CH}_2=\text{CH}_2 + \text{I}_2 \quad (2) \]
\[ \text{CH}_2=\text{CH}_2 + \text{HI} \rightarrow \frac{1}{2}\text{CH}_2\text{CH}_2\text{I} \quad (3) \]
\[ \text{Sample} + \text{HI} \rightarrow \text{HCHO} \xrightarrow{\text{HI}} \text{CH}_2=\text{CHI} + \text{X} \quad (4) \]

We therefore checked several compounds such as cinnamaldehyde, conifer-
aldehyde, ferulic acid, phenyl propanol, glycerol and 1,2-propanediol,
but none of them gave apparent ethyl iodide as analyzed by the gas
cromatographic method.

Another possibility to explain the residual ethoxyl content in
the acid-hydrolyzed ethanol lignins was that it might have arisen from
incomplete hydrolysis in 3% hydrochloric acid. Merewether (11) has
found that on refluxing ethanol lignin from Eucalyptus regnans with 12%
hydrochloric acid, one ethoxyl group was split off, but on hydrolysis
with 20% hydrochloric acid, complete de-ethylation took place. We have
changed the acid concentration from 3% to 12% and to 20%, keeping the
reaction time of one hour. Analyzing the resulting hydrolyzed lignins
showed that the ethoxyl to methoxy ratio decreased from 0.16 to 0.14
and to 0.10. This result indicated that the introduced ethoxyl groups
in lignin might differ in their reactivity towards acid. This difference
in reactivity might arise from the nature of substituents at the \( \beta \)-carbon
of the propane side chain. It was not possible to confirm this point
experimentally.
CONCLUSIONS

With respect to the long-debated question of the lignin-carbohydrate bond, the first question that must be answered is whether it exists at all. If present knowledge of the distribution of functional groups in lignin is correct, then the quantitative results described in this chapter rule out the possibility of non-existence of such a bond.

They also rule out the possibility that the bond is an acetal group formed between the hydroxyl groups of carbohydrates and a keto group of lignin. Of the possibilities considered, the only one that fits the experimental results is the benzyl ether bond. Most of the ethoxyl groups introduced into lignin on isolation by ethanolysis, or by treatment of isolated lignin with ethanol, can be accounted for in terms of ethoxylation of free benzyl alcohol and benzyl ether groups within lignin; the rest in terms of ethoxylation of terminal carboxyl groups and primary hydroxyls.

The infrared spectra of ethoxylated lignins and their derivatives can be interpreted in accordance with this view.
EXPERIMENTAL

Preparation of Extractive-Free Woodmeal

Black spruce woodmeal of approximately 40 mesh size with unknown history and background was first extracted with a 1:2 azeotropic solution of ethanol and benzene in a Soxhlet apparatus for 24 hours according to the Tappi method (51) for removal of extraneous materials. The greater part of the benzene left in the meal was then removed by soaking the bag containing the meal in ethanol for one hour, then squeezing out the ethanol with the entrained benzene. This treatment was followed by extraction with hot ethanol for 24 hours. The bag of meal was then removed from the extractor and washed with hot running tap water for 48 hours. It was next soaked in distilled water for four hours and the meal was first air-dried, then dried in a vacuum oven over P₂O₅ for 48 hours at 50°. It was stored in screw-capped bottles.

Anal. Klason lignin, 28.4%; moisture, 2.01%.

Treatment of Woodmeals with Sodium Hydroxide

Sixty grams of woodmeal was mixed with a solution of sodium hydroxide (500 ml. containing 1.2 g. NaOH). The mixture was heated at 65 ± 5° with continuous stirring for 14 hours. It was then filtered, and washed thoroughly with dilute hydrochloric acid and then with water until free of acid. The meal was air-dried, then dried in a vacuum oven over P₂O₅.
Anal. Klason lignin, 28.0%; ash, 1.1%; Tappi holocellulose, 72.9%; acetyl, 0%.

Analytical Procedures

The following analyses were carried out according to the procedure described in "Testing Methods of the Technical Association of the Pulp and Paper Industry, New York (1961)"

Klason lignin content   T13m-54
Moisture content       T210m-58
Ash content            T15m-58
Holocellulose          T9m-58

Elemental analyses were done by Beller Mikroanalytisches Laboratorium of Göttingen, West Germany and Schwarzkopf Microanalytical Laboratory of Woodside, N.Y.

Alkoxyls Determination

Methoxyl and ethoxyl were determined by a slight modification of the method developed by Cobler, Samsel and Beaver (52).

A precisely weighed quantity (20-30 mg.) of lignin was suspended in a solution of phenol (2 g., A.R. grade) and HI (6 ml., 57%, Fisher Scientific Co.) and the mixture was heated to 150° for one hour in an atmosphere of nitrogen. The liberated alkyl iodides were distilled through a washing device containing water to a trap containing 2,2,5-trimethylhexane (1 ml.) cooled by a dry-ice-acetone mixture (Figure II-8).
Fifty microliters of this solution was used for analysis on the gas chromatograph (Perkin-Elmer Vapor Fractometer, model 154) on a column of diisodecylphthalate (Perkin-Elmer Column-A) at 75°. Curves for the quantitative measurement of ethyl and methyl iodides had previously been prepared from the following as standards: methyl iodide, ethyl iodide, \textit{p}-methoxyphenol, \textit{p}-ethoxyphenol, vanillin, 4-hydroxy-3-ethoxybenzaldehyde.

The conditions used for gas chromatographic analyses of samples were as follows:

- Column temperature: 75°
- Power: 50 watts
- Column pressure: 20 PSIG
- Gas flow: 120 cc/min. He
- Recorder range: 8
- Sampling: 50 \( \mu \)l.
- Scanning speed: 120 in./hr.
- Retention time:
  - \( \approx \) 2 min. (MeI)
  - \( \approx \) 4 min. (EtI)

\textbf{Lignin Preparation}

(i) \textit{Dioxane Lignin}

Dioxane lignin was prepared from black spruce wood-meal (40 mesh) which had been freed of soluble materials. The procedure used for isolation was based on the method of Pepper et al. \cite{53, 54} with some
FIGURE II-8

Apparatus for the Determination of Alkoxy Groups
Two hundred grams of extractive-free, vacuum-dried wood was placed in a three-necked flask and extracted with four liters of 0.2 N hydrogen chloride in dioxane-water (9:1 v/v) under an atmosphere of nitrogen at 87 ± 2° in an oil bath. At the end of one hour, the reaction mixture was allowed to cool to 35-40°. It was filtered and the wood residue was washed with neutral dioxane-water (9:1 v/v) until the washings were colourless. The combined filtrates and washings were reduced to a volume of about 200 ml. under reduced pressure.

The concentrate was then pipetted slowly into 16 liters of rapidly stirred water, and the lignin precipitate was separated from the solution by centrifugation. It was washed with distilled water several times until the washings were free of acid, and the product was then freeze-dried. Yield 9.6 grams.

Anal. Klason lignin, 89.6% (moisture free); C, 62.6; H, 5.60; OCH$_3$, 14.7.

(ii) Ethanol Lignin

Ethanol lignin was isolated by the method of Hibbert (7). Thirty grams of extractive-free, dry spruce woodmeal and 600 ml. anhydrous ethanol containing 3% hydrogen chloride were placed in a three-necked flask. The contents were refluxed (~78°) for two hours under an atmosphere of nitrogen.
The mixture was cooled, and filtered, and the residual woodmeal was washed with hot anhydrous ethanol (100 ml.). The combined ethanol liquors were concentrated to a volume of about 50 ml. under reduced pressure at 50°, and the concentrate was then pipetted into vigorously stirred distilled water (3 liters). A light tan precipitate was obtained which was separated by centrifugation and was washed with water until the pH of the washings was 6. The product was freeze-dried. Yield, 2.7 grams.

Anal. C, 64.8; H, 6.35; OCH₃, 14.6; OC₂H₅, 11.2.

Reduced Spruce Woodmeal

One hundred grams of spruce woodmeal (60 mesh) was suspended in 1 l. of 0.1 N aqueous sodium hydroxide solution. To this mixture, 151.3 grams of sodium borohydride was added, and the mixture was set aside for 72 hours at room temperature with occasional stirring. The reduced woodmeal was filtered by suction and was washed with water until the washings were free of alkali. After preliminary drying in air, the residual wood was dried in a vacuum oven over P₂O₅ at 50°. The woodmeal was almost white in colour.

Dioxane Lignin from Reduced Spruce Woodmeal

The procedure for preparing dioxane lignin from reduced woodmeal was the same as for the unreduced woodmeal. The yield of lignin was 1.1 grams from 50 grams of woodmeal.

Anal. C, 66.1; H, 7.03; OCH₃, 14.0.
Ethanol Lignin from Reduced Spruce Woodmeal

The procedure for preparing ethanol lignin from reduced woodmeal was the same as for the unreduced woodmeal. The yield of lignin was 2.5 grams from 50 grams of woodmeal.

Anal. C, 65.7; H, 6.80; OCH₃, 14.9; OCH₂, 12.8.

Chemical Changes of the Lignin Samples

(1) Reaction of Lignin with Ethanol-Hydrogen Chloride

Lignin (300 mg.) was dissolved in purified dry dioxane (5 ml.). Anhydrous ethanol containing 3% hydrogen chloride (20 ml.) was added and the solution was boiled under reflux (78°) for two hours under an atmosphere of nitrogen. After the reaction mixture was cooled, it was filtered by suction, and the flask and residue were washed with ethanol (5 ml.). The filtrate and washings were combined and concentrated under reduced pressure to a volume of about 10 ml. It was then pipetted into distilled water (100 ml.) with vigorous stirring. The precipitated lignin was centrifuged and washed with water several times until the pH of the washings was 5-6. Then the lignin was freeze-dried. The analytical data are given in Table II-1.

(2) Reduction of Lignin with Sodium Borohydride

Lignin (500 mg.) was dissolved in purified dioxane-water (9:1, v/v, 20 ml.). To this solution, 0.1 N sodium hydroxide solution (7 ml.)
was first added, and then sodium borohydride (200 mg.). The mixture was
shaken for ten minutes, and it became a clear solution. Water (20 ml.)
was added to the solution and it was allowed to stand at room temperature
for ten days. At the conclusion of the reduction, the pH of the solution
was adjusted to 4 by means of dilute hydrochloric acid, which precipitated
the lignin. It was centrifuged and washed until acid-free, then dried
at 40° over P₂O₅ in a vacuum oven.

(3) Reaction of Lignin with Acid

Lignin (300 mg.) was dissolved in purified dioxane (15 ml.) and
aqueous HCl (3% by weight, 15 ml.) was introduced. This milky mixture
was refluxed for one hour whereupon it became a clear solution. After
cooling, the solution was filtered and concentrated under reduced
pressure until it became cloudy. The concentrate was poured into 150
ml. of vigorously stirred water. The precipitated lignin was centri-
fuged and was washed with water until free of acid, and finally dried
over P₂O₅ in a vacuum oven.

(4) Reaction of Lignin with Sodium Hydroxide

Lignin (300 mg.) was dissolved in 0.1 N sodium hydroxide
solution (90 ml.), and was allowed to set at room temperature for 24
hours. The mixture was acidified to pH 4 with dilute hydrochloric acid.
The precipitated lignin was centrifuged and was washed with water and
finally was dried over P₂O₅ in a vacuum oven.
Isolated lignin samples were examined in clear, pressed disks (16 mm. diameter) containing 1.6 mg. of lignin in 400 mg. of KBr.

Differential spectra were determined on wood which had first been analyzed for their Klason lignin and Tappi holocellulose contents. The wood samples were ground through the 40-mesh screen of a Wiley mill, and then were ball-milled for 5 minutes in a stainless steel Wig-L-Bug. Five milligrams of wood was incorporated into 400 mg. KBr pellets. A corresponding amount of holocellulose from the same wood, as determined by the previous analysis, was made up into a 400 mg. KBr pellet for use in the reference beam.

Spectra were determined on a Unicam SP-100 prism-grating spectrophotometer.
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CHAPTER III

REACTIONS OF LIGNIN MODEL COMPOUNDS WITH
ETHANOL AND WITH 2,2-DIMETHOXYPROPANE
IN DIOXANE-HYDROGEN CHLORIDE
INTRODUCTION

As part of a study aimed at determining the nature of any covalent bonds that might link lignin and hemicelluloses, Bolker and Terashima (1) recently took a new approach to this subject. They first assumed that the bond between lignin and carbohydrate might take the form of an acetal, as proposed by Bolker's early spectroscopic studies (see Chapter I), and reasoned that if acetal bonds could undergo transacetalation with alcohols, they should also be able to do so with ketones; i.e., it should be possible to extract wood with an anhydrous mixture of dioxane, ketone or aldehyde, and hydrogen chloride, and obtain a reasonable yield of lignin. In their preliminary experiment, they chose benzaldehyde as the reactant, but the yield of lignin was exceedingly low. Later, they found that by using a mixture of anhydrous acidic dioxane and 2,2-dialkoxypropane, it was possible to extract lignin from wood (spruce, birch and Japanese red pine) at a higher rate and in better yield than corresponding acidic mixtures of dioxane and water, methanol, ethanol or acetone. In addition, the infrared spectra of the isolated lignins suggested the presence of acetal (or ketal) groups; new alkoxyl groups were also introduced. From these results, they proposed that the extraction of lignin required the breaking of covalent bonds between lignin and carbohydrate, which might be in the acetal form as in formula I.

\[
\begin{align*}
\text{CARBOHYDRATE} & \xrightarrow{R_2C(OR)\_2} \text{CARBOHYDRATE} \\
\text{I} & \xrightarrow{H^+} \text{II} + \text{CARBOHYDRATE}
\end{align*}
\]
In order to investigate the mechanism of the dissolution of lignin from wood, Bolker and Terashima studied reactions of model compounds. In an unpublished preliminary experiment, they found that 

$$1-(4\text{-benzyloxy}-3\text{-methoxyphenyl})-2-(2\text{-methoxyphenoxy})\text{-propan-1,3-diol (III)}$$

reacted with 2,2-dimethoxypropane-dioxane-HCl when refluxed for 2.5 hours in an atmosphere of nitrogen; whereas the diacetate (IV) remained intact. On the other hand, if 2,2-dimethoxypropane was replaced by methanol or ethanol in the reaction mixture, both model compounds underwent reaction, giving rise to alpha and beta keto reaction products as revealed by infrared spectra. This information led to the present investigation of the relative rates of reactions of compounds similar to III towards alcohol and 2,2-dimethoxypropane, respectively.

In the present study, the model compounds, 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-ethanol-1 (V) and its ethyl (VI) and methyl (VII) ethers, 1-phenyl-2-phenoxypropan-1,3-diol (VIII), 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-propan-1,3-diol (IX), 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-propan-1,3-diol (X), 1-ethoxy-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-propanol-3 (XI) and 1-ethoxy-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-propanol-3 (XII) were synthesized, and their reactions towards 2,2-dimethoxypropane and alcohol were investigated.
V $R = H$
VI $R = \text{C}_2\text{H}_5$
VII $R = \text{CH}_3$

VIII $R = R_1 = H, R_2 = \text{OH}$
IX $R = R_1 = \text{OCH}_3, R_2 = \text{OH}$
X $R_1 = R_2 = \text{OH}, R = \text{OCH}_3$
XI $R = R_1 = \text{OCH}_3, R_2 = \text{OC}_2\text{H}_5$
XII $R = \text{OCH}_3, R_1 = \text{OH}, R_2 = \text{OC}_2\text{H}_5$
RESULTS AND DISCUSSION

Syntheses of Model Compounds

1-(3,4-Dimethoxyphenyl)-2-(2-Methoxyphenox)Ethan-1 (V) and the Ethyl (VI) and Methyl (VII) Ethers

The model compounds V-VII were synthesized by the method of Gierer and Norein (2). Compound V was obtained in a yield of 83.5%, and had a melting point of 129.5-130.3° (lit. m.p. 133-4° (2)). The methyl ether (VII) was obtained in a yield of 74% (m.p. 86-7°, identical to the reported melting point (2)). The n.m.r. spectrum (CCl₄) of this product had three singlets at 6 3.21, 3.69, and 3.71 with a ratio of 1:2:1, corresponding to four methoxyls; the first signal originated from the side-chain methoxyl, and the other three from the three methoxyls attached to the phenyl rings. In addition, there was a doublet at 6 3.93, corresponding to two protons at the terminal carbon atom, and a triplet at 6 4.32, which originated from the proton at the carbon atom alpha to the phenyl ring. The seven phenyl protons appear at 6 6.70.

The ethyl ether (VI) was obtained by treating V with a solution of ethanol-hydrochloric acid (0.5%) at reflux temperature. The yield was 67%, the melting point 57.5-58.5°, has not previously been reported in the literature. The n.m.r. (CCl₄) spectrum was similar to that of the methyl ether (VII), except that the five protons of the ethoxy group appeared at 6 1.08 (triplet, CH₃) and 3.34 (quartet, OCH₂), displacing the signal at 6 3.21 in the spectrum of methyl ether (VII).
Arylglycerol-8-Aroxy1 Ether Series (VIII-XII)

The model compounds VIII-XII were synthesized by appropriate modifications of methods already used by Adler et al. (3) and Kratzl et al. (4). Figure III-1 shows an outline of the sequence of reactions employed. Compound VIII has been synthesized by Freudenberg and Müller (5) by applying the Claisen reaction to benzaldehyde and phenoxyacetic ester as starting materials, and reducing the product. The present synthetic method gave a better yield.

The intermediate (XVI, $R=R_1=H$) has been prepared earlier by Kroehnks and Ahrenhols (6) and Temnikova and Dneprovskii (7) either by treating a solution of bromoacetophenone and potassium iodide in ether with a mixture of phenol, sodium and potassium carbonate, or nucleophilic displacement of dibenzoylbromomethane by phenoxide ion. The yield was 76%, at most. In the present method, a mixture of 3-bromoacetophenone, phenol and potassium carbonate in a mole ratio of 1:2:2 in dry acetone was refluxed for one hour. The product was isolated in a yield of about 94% and had a melting point of 72-72.5° (lit. m.p. 73° (7)).

Successful preparation of compound XVII ($R=R_1=H$) depends on the mole ratio of the two reagents --- formalin and 3-phenoxyacetophenone --- used in the reaction. It was found in a preliminary experiment that a large excess of formalin in the reaction mixture (mole ratio 2:1) led to the introduction of two hydroxymethylene groups at the carbon atom adjacent to the carbonyl group. This new compound, m.p. 90-90.5° was identified as 2-phenoxy-2,2-dihydroxymethyl-1-phenyl-ethanone-1 (XXIII,
FIGURE III-1 Synthetic Scheme for Model Compound VIII-XII
Figure III-2) according to the following observations:

(a) The elemental analyses gave a molecular formula of $C_{16}H_{16}O_4$, in agreement with the proposed structure.

(b) The infrared spectrum (KBr) had absorption bands at 3320 cm$^{-1}$ (broad, OH) and 1680 cm$^{-1}$ (conjugated C=O).

(c) The n.m.r. spectrum ($CDCl_3$) exhibited a singlet at $\delta$ 3.12 (area 2) which was exchangeable with $D_2O$ and corresponded to two hydroxyls. Another singlet at $\delta$ 4.31 (area 4) corresponded to the two terminal CH$_2$ groups, and multiplets at $\delta$ 7.14 (area 5), 7.66 (area 3) and 8.46 (area 2) corresponded to ten phenyl ring protons.

(d) The peak at the highest m/e value in the mass spectrum (Figure III-3) lay at 224, and the next at 212. Both peaks existed even in the low voltage scans. Isotopic analyses performed on these peaks gave reasonable values fitting the formulae $C_{15}H_{12}O_2$ and $C_{14}H_{12}O_2$, respectively. The fragmentation pattern (Figure III-3) indicated that these compounds had structures XXIV (Figure III-2) and XVI (R=R'=H, figure III-1), respectively. Thus the compound (XXIII) was probably decomposed in the inlet of the spectrometer into its component parts. In the present case, the expected compound (XXIII) decomposed to give fragment XXIV with elimination of a mole of water and a mole of formaldehyde. This is supported by the presence of intense peaks at m/e 30 and 18 in the spectrum. This phenomenon is very common particularly for diols and triols (8). In this light, the mass spectrometric results support the proposed structure (XXIII).
FIGURE III-2 Decomposition of XXIII
During Mass Spectrometric Analysis
FIGURE III-3 Mass Spectrum of 2-Phenoxy-2,2-dihydroxymethyl-1-Phenyl-Ethanone-1
To return to the problem of preparing the desired compound (XVII, R=R′=H), the proportion of formalin was reduced to one-half of that in the preliminary experiment, and the product isolated, m.p. 76-7°, gave an empirical formula of C_{15}H_{14}O_{3}, in agreement with that of the desired compound. The n.m.r. spectrum (CDCl₃) of this new compound had a singlet at δ 2.83 (area 1), exchangeable with D₂O, and corresponding to a hydroxyl proton, a doublet at δ 4.10 (area 2) corresponding to the two protons at the terminal carbon atom, a triplet at δ 5.50 (area 1) corresponding to the proton at C₂, and multiplets centered at δ 7.10 (area 5), 7.45 (area 3) and 8.10 (area 2) corresponding to the phenyl ring protons. In addition, absorption bands at 3480 and 1680 cm⁻¹ in the infrared spectrum, indicated the presence of hydroxyl and carbonyl groups. After the compound was reduced with sodium borohydride, the carbonyl absorption band disappeared and the intensity of hydroxyl group absorption at 3480 cm⁻¹ increased; two hydroxyl protons appeared at δ 2.78 in the n.m.r. spectrum (CDCl₃). This reduced compound (VIII) melted at 74-5°, identical to the reported melting point (5).

Compound (IX) was prepared according to the method of Adler (3). In the transformation of XVI to XVII (R=R′=OCH₃, Figure III-1), a white crystalline material, m.p. 67.5-69° was obtained. However the reported melting point of this product (XVII, R=R′=OCH₃) was 114-5° (3). The elemental analyses gave a formula of C_{18}H_{22}O₇ (expected C_{18}H_{20}O₆) which contains a mole of water in excess of the formula of the desired product, and suggests the incorporation of water of crystallization. This suggestion is supported by the presence of absorption bands at 3420 and 3240 cm⁻¹.
FIGURE III-4

Infrared Spectrum of 1-Phenyl-2-Phenoxypropan-1,3-Diol
in the infrared spectrum (Figure III-5), characteristic of the absorptions of water of crystallization (9). The hydrate had a n.m.r. spectrum identical to that recorded as No. 675 for the anhydrous compound in the Varian catalogue of n.m.r. spectra. After reduction, the compound gave the desired 1,3-diol (IX). Etherification of IX in anhydrous ethanol containing 0.5% hydrogen chloride gave the ethyl ether (XI).

Compound (X) was prepared according to the method of Terashima (10) --- a modification of the method of Kratzl et al. (4). Again, a hydrate of XVII (R=OCH₃, R¹=OCH₂Ph, Figure III-1) was formed instead of the anhydrous product as indicated by the absorption bands of water of crystallization (9) at 3502, 3320 and 1638 cm⁻¹ (Figure III-6). The compound was reduced with sodium borohydride in dioxane, acetylated, de-benzylated, and acetylated again to give the tri-acetate (XXII), m.p. 101-103.5° (reported m.p. 106-8° (3)). De-acetylation of XXII gave a syrup of X, which was then converted into the ethyl ether (XII) (see Experimental).

Isolation and Identification of Reaction Products from the Reactions of 2,2-Dimethoxypropane and Model Compounds

According to Bachman (11), Delephine (12), McElvain and Curry (13), reactions of alcohols and acetals or ketals result in an interchange of alkoxy groups. Recently, Jansson (14) has found that transacetalation occurred when 1,1-dimethoxyethane was treated with dialkyl formals in the presence of catalytic amounts of acid. In the present study, we obtained a compound (XXV) in a yield of 83% from the reaction of 1-phenyl-2-
FIGURE III-5

Infrared Spectrum of 3-Hydroxy-2-(2-Methoxyphenoxy)-Propioveratrone
FIGURE III-6
Infrared Spectrum of 3-Hydroxy-2-(2-Methoxyphenoxy)-Propiovanillone Benzyl Ether
phenoxypropan-1,3-diol (VIII) and 2,2-dimethoxypropane-dioxane-HCl (0.2N) after two hours at 90° in an atmosphere of nitrogen. The compound (XXV) was easily hydrolyzed to the original compound (VIII) in a weakly acidic aqueous medium. The infrared spectrum (Figure III-7) suggested that the compound was 5-phenoxy-4-phenyl-2,2-dimethyl-1,3-dioxane (XXV). There is a group of absorptions at 1168 (doublet), 1130, 1090-1080 (doublet), 1055 and 1028 cm⁻¹ which are characteristic of acetals and ketals and are due to the C-O-C-O-C group (15, 16, 17). In addition, there are at least two distinct absorptions, one at 1375-1380 cm⁻¹ (doublet of nearly equal intensities) and the other at 842 cm⁻¹ arising from the C-H deformation and skeletal vibrations of the gem-dimethyl group, (CH₃)₂C (18, 19). The molecular weight of this compound (XXV) was found to be 284 by mass spectroscopic analysis, in agreement with elemental analyses which gave an empirical formula of C₁₈H₂₀O₃. The fragmentation pattern (Figure III-8) seems consistent with the structure proposed (XXV).

Attempts to isolate reaction products from other four models (IX-XII) in 2,2-dimethoxypropane reactions were not made because of the limited quantities available. However, judging from the similarity of the infrared spectra of the crude products from the reactions of 2,2-dimethoxypropane and the other four models (IX-XII) to that of 1-phenyl-2-phenoxypropan-1,3-diol (VIII), it is a reasonable conclusion that all of the five model compounds underwent similar reactions with 2,2-dimethoxypropane, i.e., the introduction of the isopropylidene group into the diol structure.
FIGURE III-7

Infrared Spectrum of 5-Phenoxy-4-Phenyl-
2,2-Dimethyl-1,3-Dioxane
FIGURE III-8  Mass Spectrum of 5-Phenoxy-4-
Phenyl-2,2-Dimethyl-1,3-dioxane

[Mass Spectrum Graph]

Relative Abundance, per cent

m/e
Isolation and Identification of Reaction Products from Reactions of Ethanol and Model Compounds

In a preliminary experiment, we have performed alcoholysis experiments on 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-ethanol-1 (V) and have obtained 1-ethoxy-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-ethane (VI) in a yield of 67.2% after purification. This compound underwent trans-etherification with methanol-hydrochloric acid under the same conditions (0.5% HCl, reflux temperature, 2.5 hours, N₂) to give the corresponding methyl ether (VII); both methyl (VII) and ethyl (VI) ethers were readily hydrolyzed to the free hydroxyl compound (V) in acidic aqueous dioxane. Under the same conditions of alcoholysis, 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-propan-1,3-diol (IX) gave 1-ethoxy-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-propanol-3 (XI) in a yield of 71%. Similarly, 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-propan-1,3-diol (X) gave the corresponding ethyl ether (XII) with ethanol at room temperature in a yield of 72%. These results indicate that alcoholysis involved the introduction of an alkoxy group at the carbon atom alpha to the benzene ring.

As was mentioned in Chapter II, ethanolysis experiments on spruce and maple woods were early done by Hibbert and co-workers (20, 21). By the use of absolute ethanol containing 3% hydrochloric acid as solvolytic reagent, after refluxing spruce woodmeal for 40 hours, they obtained several guaiacylpropane derivatives (so-called Hibbert's ketones, see Chapter I, p. 7) from the solution which remained after the lignin had been precipitated by adding water. Since then, extensive studies
have been conducted on related monomers and dimers of lignin model compounds, such as veratryl alcohol and diveratryl ether (22), veratryl-glycerol (23) and its β-guaiacyl ether (3), dehydrodiconiferyl alcohol (24), 3-hydroxy-1-(3,4-dimethoxyphenyl)-2-propanone (25) and 1-acetoxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone (26). It was found that behaviour of these model compounds towards acidic ethanol was very similar to that of lignin itself. Lindgren (22) obtained ethyl veratryl ether from the reactions of veratryl alcohol, diveratryl ether, respectively, with acidic ethanol. Adler (27) studied the reactions of guaiacyl veratryl ether, ethyl veratryl ether and veratryl alcohol with methanol-hydrogen chloride, and found that in all cases, methyl veratryl ether was formed. All of these results clearly revealed that the principal reaction in alcoholysis is alkoxylation at the benzyl-carbon atom.

Having identified the final reaction products of the two reactions --- alcoholysis and reaction with 2,2-dimethoxypropane ---- we then proceeded to study the extents of reaction at selected intervals of time by measuring, in the ethanolysis reaction, the amount of ethoxyl introduced into or cleaved from the compound, and in the acetal-exchange reaction, the amount of C-methyl groups introduced.

Comparison of Rates of Reaction of Model Compounds

Figures III-9, 10, 11 illustrate reaction rates of model compounds with 2,2-dimethoxypropane and ethanol, respectively. The percentage of completion of reaction was calculated by assuming that
FIGURE III-9 Rates of Reaction of Model Compounds with Anhydrous 2,2-Dimethoxypropane/Dioxane/HCl (0.2N), 90°, N₂
Figure III-10 Rates of Reaction of Model Compounds with Anhydrous EtOH/Dioxane/HCl (0.2N), 90°, N₂
FIGURE III-11 Rates of Reaction of Model Compounds with Alcohols and with 2,2-Dimethoxypropane
the final product is only the isopropylidene derivative (e.g. XXV) of the corresponding models in the ketalysis reaction; and the ethyl or methyl ether derivative in the alcoholysis reaction. Possible side reactions were assumed to be negligible. Of course, some side reactions occur, especially in the ethanolysis process, where the ethyl or methyl ether first formed tends to undergo cleavage and rearrangement to give Hibbert's ketones at reflux temperature. However, at the early stage of reaction, say within one hour, the assumption seems to hold.

As shown in Figure III-11, the reaction rates of model compounds (VIII-XII) with 2,2-dimethoxypropane were faster than with ethanol under the same conditions in all cases. The difference was great in the case of 1-phenyl-2-phenoxypropan-1,3-diol (VIII) but decreased when the aromatic ring carried substituents.

In ethanolysis experiments (Figure III-10), 1-phenyl-2-phenoxypropan-1,3-diol was the least active species in the series studied, and it reacted only to the extent of about 30% in a reaction period of 8 hours. However, the rate was higher when the para position of the benzene ring was occupied by a substituent, such as an alkoxy or hydroxyl group, and about 87% of the starting material was consumed for the model 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propan-1,3-diol (X). In addition, free benzyl alcohols (IX, X) reacted somewhat faster than the corresponding benzyl ethers (XI, XII). On the other hand, the effect of substituents on the rate was not very pronounced in the ketalysis reaction, as shown in Figure III-9.
It is significant to note that the reactivity of these models towards ethanol-hydrochloric acid parallels the sulfonation process. Erdtman (28), Lindgren (29) and Mikawa (30, 31) have studied the reactivity of sulfonatable groups in lignin and have classified them as follows:

1. Phenolic benzyl alcohol groups or their corresponding ethers (designated as X groups), sulfonated rapidly in almost neutral solutions (pH 5-6).
2. Guaiacyl carbinols etherified at the phenolic hydroxyl groups (designated as Z groups), sulfonated slowly in neutral solution but rapidly in acidic sulfite solutions.
3. Guaiacyl carbinols etherified at both the phenolic and benzylic hydroxyl groups (designated as B groups), sulfonated only in acidic medium.

Based on the rate studies of the present models in the ethanol-hydrochloric acid and sulfonation reactions, a reaction mechanism as illustrated in Figure III-12 can be proposed to explain the results obtained. The first step is an acid-catalyzed protonation of the benzylic hydroxyl group to form its conjugated acid. Elimination of water or alcohol gives rise to the benzylic carbonium ion, then attack of the reagent leads to the final product. In the overall reaction, formation of the benzylic carbonium ion is a slow process, i.e., the rate determining step, thus the difference in rate of reactions among the five models (VIII-XII) arises from the differences in stabilities of carbonium ions derived from the corresponding parent compounds, the predicted order of stabilities being XXVIII-a ≫ XXVIII-b ≫ XXVIII-c.
FIGURE III-12

Proposed Mechanism of Ethanolysis Reaction
Therefore the rates of ethanolation of X and XII are faster than those of IX and XI respectively, which are in turn faster than that of VIII. This seems contrary to what would be predicted on the basis of the positive inductive effect of an R-group at the phenolic oxygen atom, for it would stabilize carbonium ion XXVIII-b rather than XXVIII-a. The effect of R-group may apply in an inert solvent or gas phase reaction, but in aqueous or other polar solutions, hydrogen bonding between solvent molecules and reactants occurs, and probably overshadows the inductive effect of the R-group. This assumption is supported by Hammett's sigma values—the substituent constant, a measure of the electron-donating or electron-withdrawing power of the substituent. For para-phenolic hydroxyl sigma is -0.357 and is much greater than for the corresponding phenolic ethers OCH$_3$, OC$_2$H$_5$, and OC$_6$H$_5$. Their sigma values are -0.268, -0.250, and -0.028, respectively (32, 33). In the present experiments, the solvent, dioxane, may interact with models X and XII in a way illustrated by XXX, wherein the hydrogen atom of the phenolic group is pulled by

\[
\begin{align*}
\left(\begin{array}{c}
\text{C}^+ \\
\text{OH}
\end{array}\right) & > \left(\begin{array}{c}
\text{C}^+ \\
\text{OR}
\end{array}\right) > \left(\begin{array}{c}
\text{C}^+ \\
\text{O}
\end{array}\right) \\
\text{XXVIII-a} & \quad \text{XXVIII-b} & \quad \text{XXVIII-c}
\end{align*}
\]

Stability decreases
the oxygen atom in the dioxane molecule. The phenolic oxygen atom becomes partially electron-rich, and thus favourable towards better resonance. No such effect occurs in phenolic ethers. On the other hand, the difference in rate between benzylic hydroxyl (IX, X) and benzylic ether (XI, XII) can readily be explained partly by the steric effect of R-groups and partly by the fact that the ease of elimination of HOH is greater than that of ROH.

From this discussion, one can probably generalize most of the solvolytic reactions of lignin in a common scheme as outlined in Figure III-13. Formation of benzylic carbonium ions is the key step in these solvolytic reactions.

In contrast, judging from the similarity of the reaction rates of 2,2-dimethoxypropane-hydrochloric acid with the five models studied in the present work (Figure III-9), the mechanism of this latter reaction must be different from ethanolysis, or, at least, formation of the benzylic carbonium ion is not the rate-determining step in the overall reaction. An alternative route can be proposed to explain the results.

It is well-known that acetals or ketals are easily hydrolyzed by dilute acids and the reaction mechanism has been worked out (34-39). Formation of the carbonium ion (XXXIII) is a slow step, and the ion itself is stabilized by the resonance effect of the OR'-group (XXXIII-a ↔ XXXIII-b). This carbonium ion (XXXIII) is probably the active species in the ketalysis reaction under discussion, where, presumably, it attacks
Common Scheme of the Solvolytic Reactions of Lignin

**FIGURE III-13**

- Acidolysis
- Alcoholysis
- Sulfonation
- Thioglycolic acid
- Nitrosation
the hydroxyl groups in the propyl side chain (Figure III-14). This suggested reaction pathway for transketalsation is supported by some earlier evidence in the literature (14, 40, 41, 42, 43, 44, 45, 46). Piantadosi et al. (45) have prepared cyclic glycerol acetals by trans-acetalation of glycerol with acetals. They have isolated an intermediate at a stage when one-half of the theoretical quantity of alcohol was evolved. This intermediate was a mixed ethyl-glycerol acetal when glycerol and diethyl acetal were used as starting materials. Thus Piantadosi et al. concluded that the trans-acetalation reaction progressed via a mixed acetal stage (XXXVIII).

More recently, Hampton (47) has suggested that the diol or triol and the carbonyl compound were the primary reactants, with the acetal acting as a dehydrating agent as indicated in equations (1) and
Proposed Mechanism of the Trans-ketalation Reaction
(2). If this is the case, reaction to the right in equation (1) should be favoured until no diol is left, since the water produced in the equilibrium is subsequently consumed in the hydrolysis reaction. However, in actual practice, complete consumption of the diol is never found. Furthermore, as shown in still more recent work (46), addition of methanol to the reaction mixture decreases the yield of cyclic acetal.

\[
\begin{align*}
\text{H}_2\text{COH} + \text{H}_2\text{COH} &\to \text{H}_2\text{COH} + \text{H}_2\text{COH} + \text{ROH} \\
\text{R}_1\text{C}(\text{OR})_2 + \text{H}_2\text{COH} &\to \text{H}_2\text{COH} + \text{H}_2\text{COH} + \text{ROH}
\end{align*}
\]

XXXVIII

\[
\begin{align*}
\text{H}_2\text{C} &\to \text{H}_2\text{CO} \\
\text{H}_2\text{C} &\to \text{H}_2\text{COH}
\end{align*}
\]

XXXIX

(1)
This is difficult to explain by Hampton's view, but is readily accounted for by the transacetalation mechanism.

From the discussion, we see that two competitive reactions exist in the ketalysis process; one is the formation of the benzylic carbonium ion (XXVIII), and the other is the formation of the tertiary carbonium ion (XXXIII). Bearing in mind that the reaction rates of 2,2-dimethoxypropane with the present models are faster than with ethanol, and that no great differences in rate were found among these five models in the ketalysis reaction, it is reasonable to say that formation of XXXIII is faster than formation of XXVIII, probably because of steric factors. The species XXXIII thus controls the rate of reaction. On the other hand, in the ethanolysis reaction, formation of the ethyl carbonium ion is less favoured because of the lack of resonance stabilization, and, therefore, formation of the benzylic carbonium ion controls the rate of reaction. Consequently, the nature of substituents on the benzene ring greatly affects the rate of reaction.

Correlation of Model Compound Studies with Lignin

The problem of the mechanism of dissolution of lignin from wood may possibly be explained on the basis of the present model compound studies. Of the many hypotheses (see Chapter I), the following three seem to be most probable:

(1) breakage of acetal bonds between lignin and carbohydrates (Figure III-15),
(ii) breakage of benzyl ether bonds between lignin and carbohydrates (Figure III-16), and
(iii) no bond need be broken between lignin and carbohydrates, but lignin itself is degraded by side reactions.

In consideration of the first possibility, transketalation occurs between the acetal bond of lignin-carbohydrate and 2,2-dimethoxypropane or ethanol as shown in Figure III-15, and new methoxyl or ethoxyl groups are introduced into the lignin molecule. Although, the simple model, 2-methyl-2-benzyl-1,3-dioxolane studied by Bolker and Terashima (48) did react faster with 2,2-dimethoxypropane than with ethanol, similar to the behaviour of lignin itself, however, as pointed out by the authors, these rates were still relatively low (extent of reaction: 27% in ketalysis, 3% in methanolysis in a reaction period of 8 hours). Furthermore, similar models with substituents on the benzene ring, which are better models to represent lignin, have not yet been studied. Their rates of reaction may be quite different, and therefore it is impossible to draw a conclusion from this single reaction. Moreover, if this type of reaction (Figure III-15) is involved, one would expect to isolate lignin with a high methoxyl or ethoxyl content and carbohydrate with an isopropylidene residue. However, as shown in Table III-1, spruce 2,2-dimethoxypropane lignin contains 14.4% of methoxyl groups, whereas spruce dioxane lignin contains 14.7% and spruce ethanol lignin, 14.6%. These values agree with each other within reasonable limits. Therefore, this mode of reaction seems not to occur, but, reaction of the 1,3-diol groups is far more probable as noted by the new experiments with model
FIGURE III-15

Reactions of Hypothetical Acetal Bond Between Lignin and Carbohydrate with 2,2-Dimethoxypropane and with Ethanol
### Table III-1

Analysis of Lignins

<table>
<thead>
<tr>
<th>Lignin Preparation</th>
<th>% C</th>
<th>% H</th>
<th>% OCH$_3$</th>
<th>% OC$_2$H$_5$</th>
<th>% C-CH$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spruce Dioxane lignin</td>
<td>63.8</td>
<td>5.96</td>
<td>14.7</td>
<td>0</td>
<td>1.46</td>
</tr>
<tr>
<td>*Spruce 2,2-dimethoxypropane lignin</td>
<td>62.2</td>
<td>6.29</td>
<td>14.4</td>
<td>trace</td>
<td>4.76</td>
</tr>
<tr>
<td>*Spruce 2,2-diethoxypropane lignin</td>
<td>61.1</td>
<td>6.47</td>
<td>12.6</td>
<td>6.38</td>
<td>6.09</td>
</tr>
<tr>
<td>Spruce ethanol lignin</td>
<td>64.8</td>
<td>6.35</td>
<td>14.6</td>
<td>11.2</td>
<td>3.76</td>
</tr>
</tbody>
</table>

* from ref. (1)
If reaction of 1,3-diol groups (VIII, IX, X) and the corresponding alkoxy-hydroxyl groups (XI, XII) is the sole, or principal reaction in the ketalysis process, and induces greater dissolution of lignin from wood than the ethanolysis process, then the possibilities are either that cleavage of benzyl ether bonds (XLIV, Figure III-16) occurs, or that no bond is cleaved between lignin and carbohydrates. As illustrated in Figure III-16, the α-carbon of the phenylpropane side chain may be linked to carbohydrate in the form of a benzyl ether (XLIV) and may be broken by solvolytic reagents, forming the isopropylidene derivatives of lignin (XLV) in the ketalysis reaction, or forming the ethyl benzyl ether (XLVI) in the ethanolysis process, in agreement with the present model compounds studied. This possibility is also supported by the evidence that lignins isolated by 2,2-dimethoxypropane and 2,2-diethoxypropane contain 4.76% and 6.09% of C-methyl groups, values which are higher than that of ethanol or dioxane lignin, whose C-methyl contents are 1.46% and 3.76%, respectively. It is clear that the C-methyl groups in ethanol lignin arise from the "Hibbert's ketones" formed in the ethanolysis reaction.

Of course, the latter argument also allows the possibility that the dialkoxypropanes extract lignin from wood without breaking any lignin-carbohydrate bonds at all, but merely by solubilizing the lignin by masking the hydroxyl groups on the phenylpropane side chains. As in the model compounds studied, isopropylidene groups are introduced into lignin by ketalysis. They block the hydroxyl groups in the lignin
Reactions of Benzyl Ether Bond with 2,2-Dimethoxypropane and with Ethanol
molecule and reduce the possibility of other side reactions such as recombination or repolymerization with carbohydrate or with lignin itself. Thus the liberation of lignin from wood is favoured. However, this explanation itself is not adequate to account for the great difference in solubility of lignin in ketalysis and other solvolysis processes. Furthermore, as noted by Terashima and Bolker (1), extraction of periodate lignin with 2,2-dimethoxypropane gave lignin only in small yield (11%), while extraction of woodmeal by the same method gave lignin in high yield, this observation is difficult to explain by the "no-bond" hypothesis. From the present investigation, we cannot exclude the latter hypothesis completely, but it seems less probable than the hypothesis of breakage of a benzyl ether bond between lignin and carbohydrates.

However, if the primary mechanism for the isolation of lignin by means of dialkoxypropane involves the introduction of an isopropylidene group and the blocking of the hydroxyl groups of the side chain, then the ethoxyl groups found in the spruce 2,2-diethoxypropane lignin (Table III-1) can only be explained by the assumption that they originated from a secondary reaction in which the diethoxypropane partly hydrolyzed in the course of reaction to produce ethanol, which, in turn, participated in an ethanolysis reaction.

So far, we have only investigated model compounds with free hydroxyls at position 1 and 3 of the phenylpropane side chain (VIII, IX, X) and their benzyl ethers (XI, XII). If the simple alkyl group in XI and XII were to be replaced by a carbohydrate residue, the same reaction
might occur, but the rate of reaction might be very different. This point required further investigation but was beyond the scope of the present work.

In considering the lignin molecule itself, many factors must be taken into account, and allowance should be made for the fact that model experiments suggest only approximations. Nevertheless, the present work provides a close examination of the problem of the linkage of lignin to carbohydrate, and the results obtained appear to favour the possibility of a benzyl ether bond between them. However, the possibility cannot be completely excluded that the two polymers constitute an intimate physical mixture, and there are no chemical bonds of any kind between them.
Melting points were determined on a calibrated Fisher-Johns apparatus, unless otherwise specified.

Elemental and C-methyl analyses were done by Beller Mikroanalytisches Laboratorium of Göttingen, West Germany, and Schwazkopf Mikroanalytical Laboratory of Woodside, New York. Ethoxyl and methoxyl contents were determined by a gas chromatographic method as described in Chapter II.

Infrared spectra were recorded on an Unicam Spectrometer model SP-200G. N.m.r. spectra were recorded on a Varian A-60 Spectrometer, with tetramethylsilane as an internal standard. Ultraviolet spectra were recorded on a Beckman model DU Spectrophotometer. Mass spectra were determined by the Morgan-Schaffer Corp., Montreal, on a Hitachi Perkin-Elmer RMU 60 Mass Spectrometer operating at 70 ev.

**Syntheses of Model Compounds**

1) Synthesis of 1-Phenyl-2-Phenoxypropan-1,3-Diol (VIII)

(A) ω-Phenoxyacetophenone (XVI, R=R₁=H)

To a solution of ω-bromoacetophenone (19.6 g.; 0.1 moles) in dry acetone (150 ml.), phenol (18.8 g.; 0.2 moles) and anhydrous potassium carbonate (27.6 g.; 0.2 moles) were added. The mixture was refluxed for one hour with vigorous stirring, then cooled, diluted with distilled water
(400 ml), and extracted with chloroform. The chloroform extract was washed with aqueous sodium hydroxide (2 N) to remove excess phenol, then with water, and was dried over anhydrous sodium sulphate. After removal of the chloroform, the residue was a yellowish oil which solidified on standing. It was recrystallized twice from methanol; white needles (19.9 g., 93.8%), m.p. 72-72.5° (lit. m.p. 73° (5)).

Anal. Calcd. for C\textsubscript{14}H\textsubscript{12}O\textsubscript{2} (M.W., 212): C, 79.2; H, 5.45; O, 14.8; found: C, 79.2; H, 5.40; O, 15.1. N.m.r. signals (CDCl\textsubscript{3}) at δ, 5.12 (CH\textsubscript{2}), 7.20 and 7.90 (aromatic 10 H).

(B) α-Phenoxo-β-Hydroxypropiophenone (XVII, R=R\textsubscript{1}=H)

Formalin (7.5 ml. of 40%; 0.09 moles) and anhydrous potassium carbonate (1.0 g.) were added to a suspension of α-phenoxoacetophenone (16 g.; 0.075 moles) in ethanol (40 ml). The mixture was heated at 35° with efficient stirring for 0.5 hours, then diluted with water and extracted with chloroform. The chloroform extract was dried and distilled off, and a pale yellowish oil remained. It was precipitated from ether-petroleum ether (b.p. 30-60°) several times, finally yielding a white crystalline product (6 g., 33%), m.p. 76-77°.

Anal. Calcd. for C\textsubscript{15}H\textsubscript{14}O\textsubscript{3} (M.W., 242): C, 74.4; H, 5.82; O, 19.8; found: C, 74.2; H, 5.95; O, 20.2. N.m.r. signals (CDCl\textsubscript{3}) at δ, 2.83, disappeared after D\textsubscript{2}O exchange (OH), 4.10 (doublet, CH\textsubscript{2}), 5.50 (triplet, CH), 7.10 (phenyl, 5H), 7.45 and 8.10 (phenyl, 3:2). Infrared spectrum showed absorptions at 3480 cm\textsuperscript{-1} (broad, OH), 3050, 2930, 2870 cm\textsuperscript{-1} (CH), 1680 cm\textsuperscript{-1} (C=O), 1600 and 1500 cm\textsuperscript{-1} (phenyl C=C). This com-
pound has not been reported in the literature.

(C) 1-Phenyl-2-Phenoxypropan-1,3-Diol (VIII)

Five grams of α-phenoxy-β-hydroxypropiophenone (0.02 moles) was dissolved in ethanol (25 ml), and sodium borohydride (0.4 g., 0.01 moles) was added slowly with stirring. Stirring was continued at room temperature for five hours, and the clear solution was then poured into water (150 ml) and extracted with chloroform. After drying with anhydrous sodium sulphate, the chloroform was removed under vacuum, and a colourless oil (5 g.) remained. It was dissolved in ether and then was precipitated by adding petroleum ether (b.p. 30-60°). A second precipitation gave white needles, m.p. 74-5° (lit. m.p. 74-5° (5)).

Anal. Calcd. for C15H16O3 (M.W., 244): C, 73.7; H, 6.60; O, 19.6; found: C, 73.6; H, 6.64; O, 19.8. N.m.r. signals (CDCl3) at 6, 2.78 (2OH), 3.70 (quartet, CH2), 4.44 (multiplet, CH), 5.04 (multiplet, CH) and 6.95-7.45 (phenyl 10H). Infrared spectrum is shown in Figure III-4.

(D) Preparation of 2-Phenoxy-2,2-dihydroxymethyl-1-Phenyl-Ethanone-1 (XXIII)

Early attempts to prepare α-phenoxy-β-hydroxypropiophenone (XVII, R=R'1=H) failed when the conditions described in the following paragraph were used. A by-product was obtained instead of the desired product.

Formalin (10 ml of 40%, 0.12 moles) and anhydrous potassium
carbonate (0.8 g.) were added to a suspension of \( \omega \)-phenoxyacetophenone (10 g.; 0.04 moles) in ethanol (30 ml). The mixture was heated at 30° with stirring for one hour, then diluted with water (20 ml), neutralized with dilute hydrochloric acid (1:3) and extracted with chloroform. The extract was dried and the chloroform was distilled off. A pale yellowish oil remained. It was dissolved in boiling petrolum ether (b.p. 60-110°) and, on being allowed to cool with efficient stirring, yielded a white crystalline product. Yield, 6.5 g., m.p. 90-3°.

Anal. Calcd. for \( C_{16}H_{16}O_4 \) (M.W., 272): C, 70.6; H, 5.91; O, 23.5; found: C, 70.5; H, 5.74; O, 23.5. The infrared spectrum showed absorption bands for both hydroxyl and carbonyl at 3320 and 1680 cm\(^{-1}\), respectively. N.m.r. signals (CDCl\(_3\)) were found at \( \delta \) 3.12 (singlet, disappeared after D\(_2\)O exchange, OH), 4.31 (singlet, CH\(_2\)), 7.14, 7.66 and 8.46 (multiplet, phenyl H) in the ratio of 2:4:5:3:2. Mass spectrometric analysis indicated a parent peak at m/e 224.

2) Synthesis of 1-(3,4-Dimethoxyphenyl)-2-(2-Methoxyphenoxy)-Propan-1,3-Diol (IX)

The method used for this synthesis was that of Adler (3).

(A) Acetoveratrine (49) (XIV, \( R=\text{R}_1=\text{OCH}_3 \))

This compound was prepared from acetovanillone and dimethyl sulphate. Yield, 96%, m.p. 48-9° (m.p. of pure product 52-3° (50)). It was used in the next step without further purification.
(B) 2-Bromoacetoveratrone (XV, $R=R_1'=OCH_3$)

The yield was 73.5% of theoretical, m.p. 81-2° (lit.m.p. 80-1° (49)).

(C) $\omega$-(2-Methoxyphenoxy)-Acetoveratrone (XVI, $R=R_1'=OCH_3$)

The yield was 71% of theoretical, and the product consisted of white shiny crystals, m.p. 91.5-2.5° (lit.m.p. 90-2° (3)).

(D) 3-Hydroxy-2-(2-Methoxyphenoxy)-Propioveratrone (XVII, $R=R_1'=OCH_3$)

The yield was 50% of theoretical; white crystals, m.p. 67.5-9° (lit.m.p. 114-5° (3)).

Anal. Calcd. for $C_{18}H_{22}O_7$ (M.W., 350): C, 61.7; H, 6.29; O, 32.0; found: C, 60.9; 61.2; H, 6.32, 6.05; 0, 32.0, 32.5. The empirical formula $C_{18}H_{22}O_7$ contains a mole of water in excess of the expected formula, $C_{18}H_{20}O_6$ (M.W., 332), which suggests that water of crystallization might exist in the product, and is confirmed by the infrared absorption bands at 3420 and 3240 cm$^{-1}$ (9) (Figure III-5).

This result explains the low melting point observed. N.m.r. signals (CDCl$_3$) at δ, 3.46 (OH), 3.83 (OCH$_3$), 3.93 (OCH$_3$), 3.92 (OCH$_3$), 4.10 (doublet, CH$_2$), 5.41 (triplet, CH), 6.89, 7.66 and 7.84 (phenyl, 7H). The spectrum is identical with No. 675 shown in the Varian catalogue of n.m.r. spectra. UV absorption at $\lambda_{max}$ 280 (ε=10,960), 310 (ε=8017).
In a modification of Adler's procedure (3), the reaction was done in dioxane instead of in ethanol. The substitution was made because in preliminary experiments partial ethoxylation occurred when ethanol was the solvent. In the modified preparation the yield was 92.7%. The carbonyl absorption at 1690 cm⁻¹ in the infrared spectrum of the starting material disappeared completely and the hydroxyl absorption at 3470 cm⁻¹ was more intense in the product.

Anal. Calcd. for C₁₈H₂₂O₆ (M.W., 334): C, 64.7; H, 6.63; O, 28.7; found: C, 65.1; H, 6.64; O, 28.5. The infrared spectrum is shown in Figure III-17. N.m.r. signals (CDCl₃) at δ, 3.47 (multiplet CH₂&OH), 3.88 (3 OCH₃), 4.10 (CH), 4.95 (CH), 6.95 (phenyl 7H). UV absorption at λ_max 280 (ε =7,709). The identity of this compound was further confirmed by mass spectrometry (Figure III-18).

3) Preparation of 1-Ethoxy-1-(3,4-Dimethoxyphenyl)-2-(2-Methoxyphenoxy)-3-Propanol (XI)

A solution of compound IX (500 mg.) in dry ethanol containing 0.5% hydrogen chloride was boiled for 2.5 hours under an atmosphere of nitrogen. Then the mixture was neutralized with solid sodium bicarbonate, diluted with water and extracted with chloroform. The chloroform extract was dried with anhydrous sodium sulphate. Evaporation of the chloroform left a pale yellowish oil. It was purified by dissolving it in benzene and filtering through an alumina column. Evaporation of the benzene gave
FIGURE III-17
Infrared Spectrum of 1-(3,4-Dimethoxyphenyl)-2-(2-Methoxyphenoxy)-Propan-1,3-Diol
FIGURE III-18 Mass Spectrum of 1-(3,4-Dimethoxyphenyl)-2-(2-Methoxyphenoxo)-Propan-1,3-Diol

\[
\begin{align*}
\text{H}_2\text{COH} & \quad \text{H}_2\text{COH} \\
\text{HCO} & \quad \text{OCH}_3 \\
\text{HO} & \quad \text{OCH}_3 \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\text{IX} & \\
\end{align*}
\]
a colourless oil. Yield, 385 mg. (71% of theoretical).

Anal. Calcd. for C₂₀H₂₆O₆ (M.W., 362): C, 66.3; H, 7.18;
OCH₃, 25.7; OC₂H₅, 12.4; found: C, 66.8; H, 7.21; OCH₃, 25.3; OC₂H₅, 12.8.

N.m.r. signals (CDCl₃) at δ, 1.19 (triplet, CH₃), 3.45 (quartet, OCH₂),
3.10 (OH), 3.83 (CH, overlap with OCH₂), 3.90 (3 OCH₃), 4.17 (multiplet,
CH₂), 4.58 (doublet, CH), 6.97 (multiplet, phenyl 7H).

The structure was further confirmed by mass spectrometric analysis.

4) Synthesis of 1-(3,4-Dimethoxyphenyl)-2-(2-Methoxyphenoxy)-Ethanol-1 (V)

Sodium borohydride (2.5 g.; 0.066 moles) was added to a suspension
of 1-(2-methoxyphenoxy)-acetoveratrone (XVI, R=H, R₁=OCH₃, 10 g.; 0.033
moles) in ethanol (70 ml). The mixture was shaken at room temperature,
and the ketone dissolved. After 24 hours, there was a white precipitate
in the flask, and it was filtered, washed with water (150 ml), and dried.
Its m.p. was 126-8°. The filtrate was extracted with chloroform twice
(30 ml each), and the combined chloroform extracts were dried over
anhydrous sodium sulphate. On evaporation of the chloroform, a pale
yellow solid was obtained. Both solid products were combined and re-
crystallized together from hot acetone. Yield, 8.35 g. (83.5% of theoretical),
m.p. 129.5-130.3° (lit.m.p. 133-4° (2)). The infrared spectrum
of the product indicated disappearance of the carbonyl absorption at
1680 cm⁻¹ with appearance of an hydroxyl absorption at 3520 cm⁻¹.

The ethyl ether (VI) of compound V was obtained by refluxing it
(6 g.) with 0.5% of ethanolic hydrochloric acid (70 ml) for 2.5 hours
in an atmosphere of nitrogen. After cooling the solution and evaporating it to dryness, a white oily substance remained in the flask. It was purified by fractional precipitation from warm n-hexane, and white crystals were obtained. Yield, 4.4 g. (67%), m.p. 57.5-8.5°. N.m.r. signals (CCl₄) at 6, 1.08 (triplet, CH₃), 3.34 (quartet, CH₂), 3.63, 3.68 (two singlets, OCH₃), 3.85 (multiplet, CH₂), 4.40 (triplet, CH), 6.69 (phenyl, 8H). The infrared spectrum indicated the disappearance of the hydroxyl absorption at 3520 cm⁻¹.

**Trans-etherification of l-Ethoxy-l-(3,4-Dimethoxyphenyl)-2-(2-Methoxyphenoxy)-Ethane with Methanolic Hydrogen Chloride**

A solution of 250 mg. of l-ethoxy-l-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-ethane (VI) in anhydrous methanol containing 0.5% hydrogen chloride (6 ml) was refluxed under an atmosphere of nitrogen for 2.5 hours. The mixture was allowed to cool and was evaporated to dryness. A white precipitate (VII) remained and was recrystallized from n-hexane. Yield, 185 mg. (74%), m.p. 86-7° (lit.m.p. 86-7°(2)). N.m.r. signals (CCl₄) at 6, 3.21 (singlet, OCH₃), 3.69 (singlet, 2 OCH₃), 3.71 (singlet, OCH₃), 3.93 (doublet, CH₂), 4.32 (triplet, CH), 6.70 (singlet, phenyl 7H).

**Acid Hydrolysis of l-Ethoxy-l-(3,4-Dimethoxyphenyl)-2-(2-Methoxyphenoxy)-Ethane (VI)**

The ether (VI, 100 mg.) was dissolved in dioxane (5 ml), and aqueous hydrochloric acid (3%, 5 ml) was added to the solution which was
then boiled (reflux) for one hour. On evaporation of the solvent to
dryness, a pale yellowish residue was obtained. It was recrystallized
from acetone, and gave white crystals. Yield, 50 mg. (57%), m.p. 132.5-3°,
whose infrared spectrum was identical to that of V.

5) Synthesis of 1-(4-Hydroxy-3-Methoxyphenyl)-2-(2-Methoxyphenoxy)-
Propan-1,3-Diol (X)

The method of Kratzl et al. (4), modified by Terashima (10) was
used for the synthesis.

(A) Acetovanillone Benzyl Ether (51) (XIV, R=OCH₃, R¹=OCH₂C₆H₅)

Acetovanillone (100 g.; 0.6 moles) and redistilled benzyl
chloride (150 ml; 1.3 moles) were dissolved in ethanol (1.5 l), and
sodium hydroxide (37 g.; 0.92 moles) was then added. The mixture was
boiled (reflux) for 1.5 hours, then poured into water (2 l), and the
yellow oil which separated was extracted with ether several times until the
ether extract became colourless. The combined ether extracts were washed
with water twice, dried with anhydrous sodium sulphate, distilled under
reduced pressure, and a pale yellow solid was obtained. It was recrystal-
lized from hot petrolun ether (b.p. 30-60°), yielding white needle-like
crystals. Yield, 124.7 g. (81%), m.p. 86-6.5° (lit.m.p. 86-6.5° (51)).

(B) ω-Bromoacetovanillone Benzyl Ether (XV, R=OCH₃, R¹=OCH₂C₆H₅)

A solution of bromine (62.5 g.; 0.39 moles) in chloroform
(500 ml) was added dropwise with vigorous stirring to a solution of acetovanillone benzyl ether (100 g.; 0.39 moles) in chloroform (1 l) at room temperature. Air was introduced to expel the hydrogen bromide evolved during the course of reaction. The reaction mixture was washed immediately with aqueous sodium bicarbonate and then with water until the aqueous layer was colourless. The chloroform solution was dried with anhydrous sodium sulphate and evaporated under reduced pressure, and the residual orange coloured oil solidified on standing. It was recrystallized twice from benzene-petroleum ether (b.p. 60-110°), and gave colourless crystals. Yield, 97 g. (75%), m.p. 103-4° (lit.m.p. 102.5-3° (51)).

(C) ω-(2-Methoxyphenoxy)-Acetovanillone Benzyl Ether (XVI, R=OCH₃,
R¹=OCH₂C₆H₅)

A mixture of ω-bromoacetovanillone benzyl ether (55 g.; 0.17 moles), guaiacol (27.5 g.; 0.22 moles) and finely powdered anhydrous potassium carbonate (44 g.) in dry acetone (550 ml.) was stirred vigorously for 1.5 hours at 60°. The large amount of acetone was distilled off and the residual mixture was poured into water and extracted with chloroform. The chloroform extract was washed with aqueous sodium hydroxide (2 N) and water, dried over anhydrous sodium sulphate, and evaporated to give a yellowish orange oil. By the addition of a small amount of methanol and scratching the inner surface of the flask with a spatula, the oil was caused to solidify slowly. Repeated recrystallization from ethanol gave colourless prisms. Yield, 47.5 g. (76%), m.p. 98° (lit. m.p. 86.5-7.5° (51); 103-4° (4)).
(D) 3-Hydroxy-2-(2-Methoxyphenoxy)-Propiovanillone Benzyl Ether
(XVII, \(\text{R}=\text{OCH}_3\), \(\text{R}^1=\text{OCH}_2\text{C}_6\text{H}_5\))

A suspension of \(\text{\(\Delta\)}\)-(2-methoxyphenoxy)-acetovanillone benzyl ether (XVI, 15 g.; 0.04 moles) in ethanol (25 ml) was prepared. Anhydrous potassium carbonate (0.75 g.; 5.4 \times 10^{-3} \text{ moles}) and formalin (6.3 ml. of 38%; 0.08 moles) were introduced and stirred at 38° for two hours. Despite of the fact that the reaction proceeded normally, the solid materials appeared to remain undissolved. The solid was collected by filtration, washed with dilute hydrochloric acid (1:3) and water until free of acid. It was recrystallized from ethanol-water, and yielded colourless prisms, m.p. 82-3.5° (lit.m.p. 72-8° (4), 115° in sealed tube (10)).

Anal. Calcd. for \(\text{C}_{24}\text{H}_{26}\text{O}_7\) (M.W., 426); C, 67.6; H, 6.10; found: C, 67.8; H, 6.31. The empirical formula contains a mole of water in excess of the expected formula, \(\text{C}_{24}\text{H}_{24}\text{O}_6\) (M.W., 408), which suggests that the product contains a mole of water of crystallization. This suggestion was supported by the infrared spectrum of this compound (Figure III-6) in which three bands at 3502, 3320 and 1638 cm\(^{-1}\) were due to water of crystallization (9). Similar analytical results were reported by Kratzl et al. (4).

(E) 3-Acetoxy-2-(2-Methoxyphenoxy)-1-(4-Benzyl oxy-3-Methoxyphenyl)-1-Propanone (XVIII, \(\text{R}=\text{OCH}_3\), \(\text{R}^1=\text{OCH}_2\text{C}_6\text{H}_5\))

A mixture of 3-hydroxy-2-(2-methoxyphenoxy)-2-propiovanillone
benzyl ether (XVII, 50g.; 0.12 moles) and pyridine-acetic anhydride (1:1, 360 ml) was prepared, and was stirred at room temperature for 24 hours. The excess of reagents was distilled off under reduced pressure, at temperatures not higher than 40°. The residue was dissolved in ethanol and cooled in a freezer at -10°. Fine colourless crystals precipitated after several hours. Recrystallization from ethanol gave pure material. Yield 45 g. (85%), m.p. 84-7° (lit.m.p. 84-6° (4)). The infrared spectrum showed the introduction of ester carbonyl at 1740 cm⁻¹ with the disappearance of hydroxyl absorption in the 3500 cm⁻¹ region.

(F) 3-Acetoxy-2-(2-Methoxyphenoxy)-1-(4-Benzyloxy-3-Methoxyphenyl)-l-Propanol (XIX, R=OCH₃, R₁=OCH₂C₆H₅)

At room temperature, a solution of sodium borohydride (2 g.; 0.053 moles) in dioxane (140 ml) was added dropwise over two hours to a stirred solution of 3-acetoxy-2-(2-methoxyphenoxy)-1-(4-benzyloxy-3-methoxyphenyl)-l-propanone (XVIII, 42 g.; 0.93 moles) in dioxane (240 ml). The dioxane was distilled off under reduced pressure at 40° yielding an oily residue, which was dissolved in chloroform and washed with water to remove inorganic materials. After drying over anhydrous sodium sulphate, the chloroform was distilled to leave a colourless syrup (39 g., 92%). Its infrared spectrum showed the disappearance of carbonyl absorption at 1670 cm⁻¹ and the appearance of hydroxyl absorption at 3480 cm⁻¹.
(G) 1,3-Diacetoxy-2-(2-Methoxyphenoxy)-1-(4-Benzylxoy-3-Methoxyphenyl)-Propane (XX, R=OCH₃, R¹=OCH₂C₆H₅)

The syrup of 3-acetoxy-2-(2-methoxyphenoxy)-1-(4-benzyloxy-3-methoxyphenyl)-1-propanol (30 g.; 0.066 moles) was dissolved in pyridine-acetic anhydride (1:1, 200 ml), and was stirred at room temperature for 24 hours. After removal of the excess of reagents under reduced pressure at 40°, the residue was poured into ice-water to decompose the unreacted acetic anhydride. The acetate was extracted with ether and the ether solution was washed with water, and dried over anhydrous sodium sulphate. Evaporation of the ether gave the diacetate as a light yellowish oil (32 g., 99%). This was used for the next step without further purification.

(H) 1,3-Diacetoxy-2-(2-Methoxyphenoxy)-1-(4-Hydroxy-3-Methoxyphenyl)-Propane (XXI, R=OCH₃, R¹=OH)

The yellowish oil (12 g.; 0.027 moles) obtained from the previous step was dissolved in methanol (80 ml) and a catalyst, palladium on barium sulphate (10 g. containing 5% Pd (52)), was added. Catalytic hydrogenation was done at room temperature in a Paar apparatus. After about two hours, hydrogen consumption had nearly ceased. The catalyst was removed by filtration, and the methanol was distilled off under reduced pressure to leave behind a light yellowish oil. Yield, 8.8 g. (90%). This was used for the next step without further purification.

(I) 1,3-Diacetoxy-2-(2-Methoxyphenoxy)-1-(4-Acetoxy-3-Methoxyphenyl)-Propane (XXII, R=OCH₃)
The oil (8.8 g.) from the previous step was acetylated with pyridine-acetic anhydride (1:1) by the procedure described above. The oily triacetate product was dissolved in ethanol and placed in a freezer (-10°) for a few days. White crystals were obtained, and gave a pure product after recrystallization from ethanol. Yield, 7.1 g. (74%), m.p. 101-103.5° (lit. m.p. 106-8° (4)).

Anal. Calcd. for C_{25}H_{26}O_9 (M.W., 444.5): C, 61.9; H, 5.87; found: C, 61.6; H, 5.82. The infrared spectrum showed ester bands at 1780 and 1760 cm⁻¹. N.m.r. signals (CDCl₃) at δ, 1.96 (OCOCH₃), 2.05 (OCOCH₃), 2.24 (OCOCH₃), 3.77 (OCH₃), 3.81 (OCH₃), 4.70 (CH, quartet), 6.10 (CH, doublet) and 6.90, 7.10 (phenyl H).

(J) 1-(4-Hydroxy-3-Methoxyphenyl)-2-(2-Methoxyphenoxy)-Propan-1,3-Diol (X)

The triacetate (2 g.) was dissolved in dry chloroform (50 ml), and the solution was cooled to -40° and mixed with a solution of sodium (0.5 g.) in absolute methanol (20 ml). After half an hour, the mixture was warmed to 0°, water (50 ml) was added and then dilute sulphuric acid (1:4, 3.3 ml). The organic phase was extracted with chloroform, and dried over anhydrous sodium sulphate. Evaporation of the chloroform left a yellow oil, yield, 1.8 g. It was decolourized by active carbon in methanol, and was finally obtained as a thick glassy material.

Anal. Calcd. for C_{17}H_{20}O_6 (M.W., 320): C, 63.8; H, 6.25; OCH₃, 19.4; found: C, 64.4; H, 6.11; OCH₃, 19.5. The infrared spectrum is shown in Figure III-19.
FIGURE III-19

Infrared Spectrum of 1-(4-Hydroxy-3-Methoxyphenyl)-
2-(2-Methoxyphenoxy)-Propan-1,3-Diol
6) Preparation of 1-Ethoxy-1-(4-Hydroxy-3-Methoxyphenyl)-2-(2-Methoxyphenox)-Propanol-3 (XII)

The compound 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-propan-1,3-diol (X, 500 mg) was treated with dry ethanol containing 0.5% hydrogen chloride at room temperature for 24 hours. The work-up procedure was the same as in the preparation of 1-ethoxy-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-3-propanol (XI). A pale yellowish oil was obtained. Yield, 392 mg. (72%).

Anal. Calcd. for C\textsubscript{19}H\textsubscript{24}O\textsubscript{6} (M.W., 348): C, 65.5; H, 6.90; OCH\textsubscript{3}, 17.8; OC\textsubscript{2}H\textsubscript{5}, 12.9; found: C, 65.3; H, 6.81; OCH\textsubscript{3}, 17.5; OC\textsubscript{2}H\textsubscript{5}, 12.8.

N.m.r. signals (CDCl\textsubscript{3}) at δ, 1.15 (triplet, CH\textsubscript{3}), 3.45 (area 3, after D\textsubscript{2}O exchange, area 2, CH\textsubscript{2} and OH overlap), 3.74 and 3.79 (singlet, 2 OCH\textsubscript{3}), a group of multiplets at 3.88-4.58 (area 4, overlapping of CH\textsubscript{2} and 2 CH), 6.90 (area 7, multiplet, phenyl H), 7.34 (phenolic OH). Mass spectrometric analysis gave molecular weight 348, isotopic analysis gave

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Rate Determination

(1) Reaction of 1-Phenyl-2-Phenoxypropan-1,3-Diol (VIII) with Dimethoxy-Propane

The rates of reaction were determined at 90° under anhydrous
FIGURE III+20 Mass Spectrum of 1-Ethoxy-1-(4-Hydroxy-3-Methoxyphenyl)-2-(2-Methoxyphenoxy)-Propanol-3
conditions and in an atmosphere of nitrogen. To 500 mg. of each sample, 65 ml. of purified anhydrous dioxane containing 0.2 N dry HCl was added. The mixture was brought to temperature as quickly as possible, and 10 ml. of redistilled dimethoxypropane was introduced into the mixture. At each predetermined period about 10 ml. of the mixture was withdrawn, stirred with finely powdered sodium carbonate (0.2 mg.) and centrifuged. The supernatant solution was evaporated at 50° in vacuo and the C-methyl contents of the residual oils were analyzed. The reactions of the other four models (IX-XII) were performed similarly. The results are shown in Figures III-9 and III-11.

(2) Reaction of 1-Phenyl-2-Phenoxypropan-1,3-Diol (VIII) with Ethanol

The experiment was done under conditions similar to those of the dimethoxypropane experiments except that 9.5 ml. of anhydrous ethanol was used instead of dimethoxypropane. The total amount of solution remained 75 ml. Ethoxyl contents were determined on the residual oil at each predetermined interval. The other models (IX-XII) were treated in similar way. Results are shown in Figures III-10 and III-11.

Isolation of Reaction Products

As found in the rate determination experiment, the reaction of 1-phenyl-2-phenoxypropan-1,3-diol with dimethoxypropane in the presence of acid was almost complete within two hours of reaction time. Therefore the conditions used in the experiment aimed at isolating the product were
the same as in the rate experiment, and the reaction time was two hours.

The volatile components in the final reaction mixture were evaporated immediately after the reaction, leaving a dark residue which was purified by column chromatography (silica gel, benzene). The second fraction was collected and the solvent was evaporated, leaving a light yellow oil (yield, 83% of theoretical). It solidified after a few days. This solid was washed with small amount of ether, and white prisms (XXV) were obtained, m.p. 82-3°.

Anal. Calcd. for $C_{18}H_{20}O_3$ (M.W., 284): C, 76.0; H, 7.09; found: C, 76.2; H, 7.23. Spectroscopic evidence showed the compound to be 5-phenoxy-4-phenyl-2,2-dimethyl-1,3-dioxane (Figures III-7 and III-8), and the structure was supported by the following hydrolysis experiment.

**Acid Hydrolysis of XXV**

Compound XXV (100 mg.) was dissolved in 50% aqueous dioxane-HCl equivalent to 0.5 N. The mixture was warmed in a water bath for five minutes at 65°. When the solution was evaporated, it left a residue, whose infrared spectrum was identical to that of 1-phenyl-2-phenoxypropan-1,3-diol (VIII).
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CHAPTER IV

GENERAL CONCLUSIONS
The work described here began on the vague premise that the mechanisms of some reactions which extract lignin from wood could be determined by applying new techniques. The importance of discovering these mechanisms was that they might reveal whether lignin and carbohydrate, in their natural state, were joined by covalent bonds, and, if they were, what kind of bonds they might be.

The evidence obtained from the reactions of model compounds (Chapter III), and the high C-methyl content of lignins extracted with 2,2-dialkoxypropanes indicate strongly that the side chains of lignin form 1,3-dioxanes on extraction with that reagent. In addition, it is clear that the formation of the dioxane is generally much more rapid and complete than ethanolation, except in units where the phenolic hydroxyl is unetherified. From these results, and from those previously obtained by Bolker and Terashima (Chapter III, reference 48), it seems likely that the only possible interpretation for the rapid and extensive solubilization of lignin by dialkoxypropanes is that 1,3-dioxane groupings are formed.

In other words, whatever happens to solubilize the lignin occurs on the side chains of monomeric units. There are two possibilities. The first is that the cleavage of ether bonds need not be invoked: it may be sufficient to assume that masking the hydroxyl groups is itself sufficient to solubilize the lignin in dioxane. If this is so, then there can be no covalent bonds between lignin and carbohydrate.
The other possibility is that if, as is generally believed, carbohydrate is joined to lignin by a benzyl ether bond, then this bond is rapidly cleaved in the reaction with dialkoxypropanes. This possibility is not excluded by the present evidence: there was no sufficiently clearcut difference between reactions of benzyl ether and benzyl alcohol groupings.

However, the results of experiments on the alcoholysis itself (Chapter II) permit making a tentative choice. In this reaction, as in hydrolysis, the mechanism for separating lignin from carbohydrate results in the formation of keto groups on the beta-carbons of the side chains of phenylpropane building blocks. Ethoxyl groups are introduced into the lignin mainly at the sites of pre-existing free benzyl alcohol and benzyl ether groups, but also on the terminal (γ) hydroxyls and γ-carboxyl groups. If present notions of the distribution of functional groups of lignin are correct, then the quantitative results obtained on the proportion of ethoxyl groups introduced accord better with the hypothesis that lignin and carbohydrate are joined in their natural state through benzyl ether bonds, than with any other of the hypotheses considered.
1) Solvolytic extraction of lignin from wood by ethanol-hydrogen chloride has been investigated by infrared spectroscopic studies and quantitative analysis of the lignins isolated.

2) Infrared studies on ethanolysis of wood and isolated lignins indicated that alkylation occurs with the formation of unconjugated carbonyl groups, including some carboxyl groups which absorb at 1720 cm\(^{-1}\).

3) Evidence obtained from spectrometry and alkoxy determination revealed that a decrease in intensity of alpha-carbonyl group absorption at 1660 cm\(^{-1}\) does not necessarily mean that alkylation takes place at the carbonyl function, but that the conjugation is lost as a result of keto-enol tautomerization, such as proposed by Hibbert in his ethanolysis studies.

4) Average empirical formulae and ratios of ethoxyl to methoxyl of various lignin derivatives were calculated. Results seem to indicate that the dissolution of lignin from wood by ethanol-hydrogen chloride requires cleavage of a covalent bond, which might be in the form of a benzyl ether link between lignin and carbohydrate.

5) Examination of the residual ethoxyl groups in acid-hydrolyzed ethanol lignins indicated that the ethoxyl groups introduced into the lignins might differ in reactivity towards acid. This difference in reactivity may arise from the nature of substituents at the beta-carbon of the phenylpropane side chain.
6) Lignin model compounds, 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-ethanol-1 (V) and its ethyl (VI) and methyl (VII) ethers, 1-phenyl-2-phenoxypropan-1,3-diol (VIII), 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-propan-1,3-diol (IX), 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-propan-1,3-diol (X), 1-ethoxy-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-propanol-3 (XI) and 1-ethoxy-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-propanol-3 (XII) were synthesized by appropriate modification of methods already used by some authors (2, 3, 4, 10).

7) The following new compounds were prepared and characterized:
   (a) α-Phenoxy-β-hydroxypropiophenone (XVII, R=R'=H),
   (b) 2-Phenoxy-2,2-dihydroxymethyl-1-phenyl-ethanone-1 (XXIII)
   (c) 5-Phenoxy-4-phenyl-2,2-dimethyl-1,3-dioxane (XXV),

8) The rates of reactions of model compounds VIII-XII with 2,2-dimethoxypropane in acidic dioxane at 90° in an atmosphere of nitrogen were faster than with ethanol under the same conditions; similar to the solvolytic extraction of lignin from wood by the same reagents.

9) Chemical nature of substituents on benzene ring greatly affects the rates of ethanolysis. The order of rates decreases as X > XII > IX > XI > VIII.

10) Chemical nature of substituents on benzene ring has little influence on the rates of ketalysis. The order of rates decreases as VIII > X > XII ≤ IX > XI.
11) Reaction mechanisms of ethanolysis and ketalysis were discussed. The formation of a benzylic carbonium ion seems to be the rate-determining step in the ethanolysis reaction; whereas in the ketalysis reaction, the formation of a tertiary carbonium ion \((\text{CH}_3)_2\text{OCH}_3\) seems to be the rate-determining step.

12) Relationship between the reactions of model compounds and of lignin were investigated. It was suggested that the dissolution of lignin from wood by both reagents required covalent bond cleavage. The experimental results appear to favour the cleavage of benzyl ether bonds between lignin and carbohydrates. However, based only on the model compound studied, the possibility of no covalent bonds between lignin and carbohydrates cannot be excluded.