Ph.D. Chemistry

Moh-Lian Kee

THE DEGRADATION OF LIGNIN WITH NITROUS ACID

ABSTRACT

Birch (I) and spruce (II) dioxane lignins, and related models were each reacted with nitrous acid in an aqueous medium. Both I and II underwent side-chain displacement, oxidation, dealkylation and fixation of nitrogen. From I, 4-nitroguaiacol (III), 4,6-dinitroguaiacol (IV) and 2,6-dimethoxy-1,4-benzoquinone (V) were formed; from II, only III and IV were isolated. Reactions of model compounds have yielded products which can help to explain the reaction of lignin. From 1-(4-hydroxy-3-methoxyphenyl)-1-propanol, treatment with HNO_2 yielded III, IV and 1-(4-hydroxy-3-methoxy-5-nitrophenyl)-1-propanone. From 1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane, only III and IV were isolated. Both 1-(3,4-dimethoxyphenyl)-1-propanol (VI) and 1-(3,4-dimethoxyphenyl)-1-methoxypropane yielded the same products on mild treatment: 1-(3,4-dimethoxyphenyl)-1-propanone and 1-(3,4-dimethoxyphenyl)-1-nitropropane. But when VI was treated under harsher conditions, it yielded 6-nitroveratric acid, 4-nitroveratrole, 4,5-dinitroveratrole and 1-(3,4-dimethoxy-6-nitrophenyl)-1-propanone. Syringyl alcohol, syringaldehyde and syringic acid all yielded V and 2,6-dimethoxy-4-nitrophenol.

Two mechanisms have been proposed: (1) for the cleavage of phenyl ether links in hardwood lignin; and (2) for the manner of degradation of the lignin polymer network.

THE DEGRADATION OF LIGNIN WITH NITROUS ACID

BY

MOH-LIAN KEE

THE DEGRADATION OF LIGNIN WITH NITROUS ACID

by

Moh-Lian Kee, B.Sc. (Nanyang Univ., 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.

March 1968

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to his research director, Dr. H.I. Bolker, for his considerate and inspiring supervision of this project, and his invaluable help in the preparation of the thesis.

Grateful acknowledgement is extended to:

The late Dr. C.B. Purves for his assistance and encouragement,

Fellow members of the Organic Chemistry Division of the Pulp and Paper Research Institute of Canada for their suggestions and co-operation at all times,

Mrs. T.W. Yim for her contribution of Russian translations,

McGill University for demonstratorships during 1964-65 and 1965-66,

Spruce Falls Power and Paper Company for a Fellowship during 1965-66,

The Pulp and Paper Research Institute of Canada for a summer grant in 1965,

The National Research Council of Canada for a Studentship during 1966-67 and 1967-68.

TABLE OF CONTENTS

TABLE OF CONTENTS (Cont'd.)

TABLE OF CONTENTS (Cont'd.)

LIST OF FIGURES

LIST OF FIGURES

LIST OF TABLES

GENERAL INTRODUCTION

Nitrous acid, generated in situ from $NaNO_2$ and HCl, has been found to be an effective delignifying agent in the bleaching of sulfite pulp (1). The suggestion that nitrous acid could be used instead of chlorine as the first stage in bleaching sequences for pulp (1) is of considerable industrial interest, technically and economically. Most of the problems of the chlorination process, such as uniform mixing, production of by-product hydrochloric acid, harmful to both pulp and equipment, and hazards of shipping and handling, are not encountered in the nitrous acid system. In addition, sodium nitrite, if used in the tonnage quantities required by the pulp and paper industry, should become a very inexpensive bleaching chemical.

The delignifying effect of nitrous acid is a result of several competing reactions occurring among the various structural components of the lignin, the latter being degraded into fragments which are soluble in water or dilute aqueous alkali. However, the mechanism of delignification has not been studied to any appreciable extent. The object of the present investigation was to discover how nitrous acid degrades lignin, because this information might be applicable to the practical problem of delignifying pulps. This purpose was approached in two ways: by studying reactions first of isolated lignins, and second, of related model compounds. Since the normal procedure for delignifying pulps requires a second stage, i.e., extraction with alkali, such extraction on the portions of lignins which had not dissolved in the aqueous nitrous acid were also performed, and the degradation products from this treatment were isolated and identified.

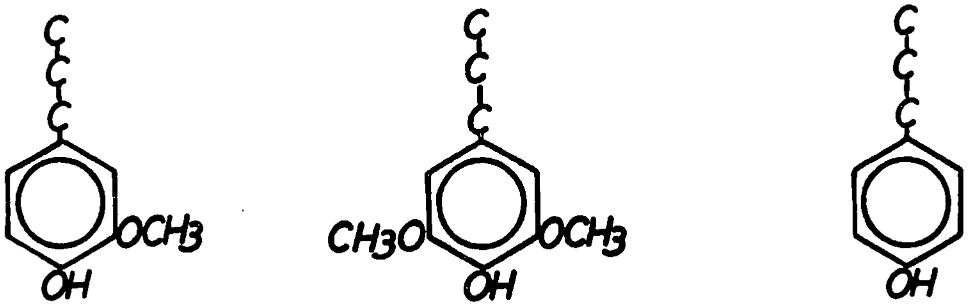
HISTORICAL INTRODUCTION

Chemical Constitution of Wood Lignin

Lignin occurs with cellulose and hemicelluloses as one of the major components of woody plant tissue. In mature wood tissue lignin is present in amounts ranging from about 18 to 38% (2). It deposits partly in the intercellular spaces, where it may constitute up to 70 percent of the solid present, and partly in the xylem cell walls.

Wood lignins can be divided into two broad classes depending upon whether they occur in softwoods (gymnosperms) or hardwoods (dicotyledonous angiosperms). In softwood lignin, the building units appear to be almost entirely guaiacylpropane monomers, whereas in hardwood lignin they appear to be made up of both guaiacylpropane and syringylpropane units. As a minor component, some wood lignins may also contain p-hydroxyphenylpropane monomers. For instance, Leopold has isolated a small amount (0.25 percent) of p-hydroxybenzaldehyde from the nitrobenzene oxidation of spruce wood lignin (3). However, in the lignins of grasses (monocotyledonous angiosperms) including bamboo, bagasse and straw, the p-hydroxyphenylpropane unit is said to be a major constituent (4,5,6,7).

In the lignin macromolecule, the monomeric phenylpropane units are generally believed to be linked with each other both by ether and by carbon-to-carbon linkages. Figure 2 summarizes current views on the nature of such linkages in lignin. These linkages were originally proposed to account for the chemical behaviour of lignin, and it should be emphasized that many of them have not yet been confirmed by rigorous



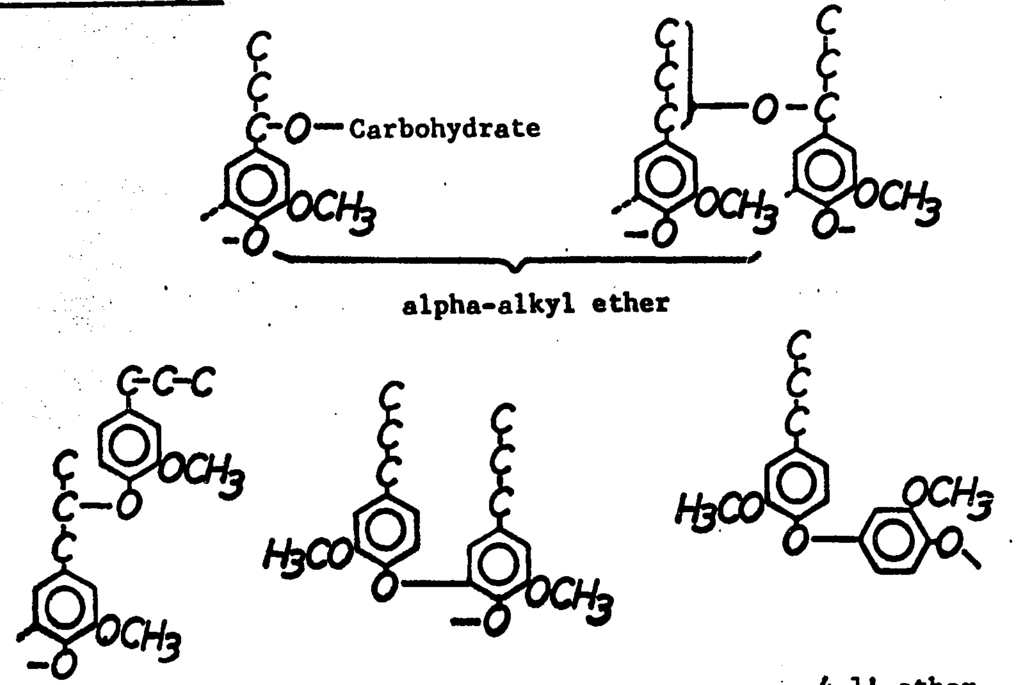
Guaiacylpropane Monomer

Syringylpropane Monomer

p-Hydroxyphenylpropane Monomer

FIGURE 1 Postulated Phenylpropane Monomers of Lignin

A Ether Linkages



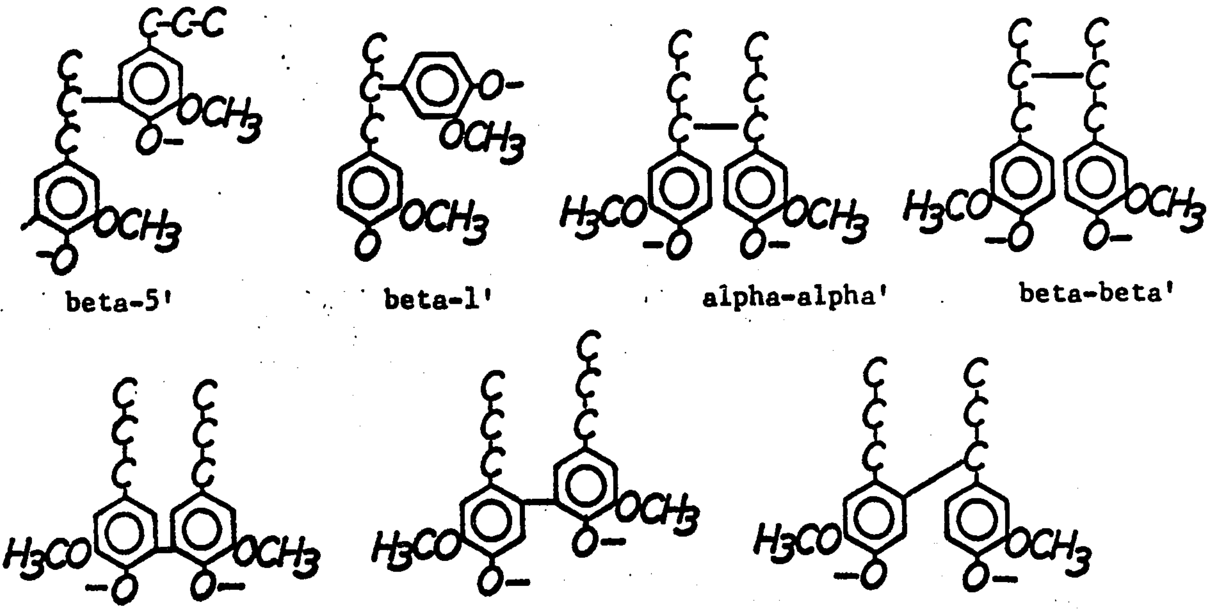
alpha-alkyl ether

beta-4' ether

4-5' ether

4-1' ether

B Carbon-carbon Linkages


beta-5'

beta-1'

alpha-alpha'

beta-beta'

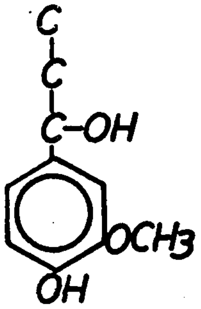
5-5'

2-5'

2-alpha'

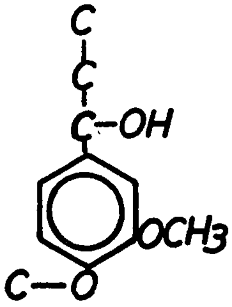
FIGURE 2 Postulated Intermonomeric Linkages in Lignin (2, 8) chemical proof. However, the recent biogenetic and biosynthetic studies using C^{14}-labelled precursors (9,10) has given them strong support. All the indecisive chemical evidence available and the biochemical proof as well have been reviewed by Sarkanen (2) and will not be reviewed here.

Lignin contains a high proportion of guaiacyl carbinol elements, with free or etherified phenolic or alcoholic hydroxyl groups. From experiments on the methylation with methanolic hydrochloric acid of spruce milled-wood lignin and of a great variety of model compounds, Adler (11) concluded that the total number of various types of guaiacyl carbinol units is 0.43 per methoxyl group. Of these, 0.05 were shown to be guaiacyl carbinol groups with both hydroxyls free, and 0.14 were non-phenolic benzyl alcoholic groups. The number of non-cyclic benzyl ether groups would therefore be 0.43-(0.05 + 0.14) = 0.24.



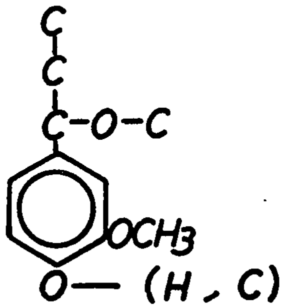
0.05

phenolic guaiacyl carbinol



0.14

etherified guaiacyl carbinol



0.24 per OCH_3

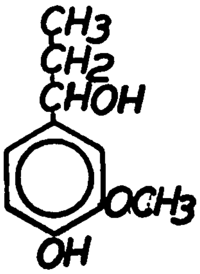
aryl carbinol ether

Based on the reactivities of these guaiacyl carbinol elements towards sulphonation, Erdtman (12), Lindgren (13,14), and Mikawa et al. (15,16) divided them into three types: 1. X groups - phenolic benzyl alcohol groups, or their corresponding ethers, were sulfonated rapidly in neutral solution (pH 5-6); 2. Z groups - guaiacyl carbinols etherified at the phenolic hydroxyl groups, were sulfonated slowly in neutral solution but rapidly in acidic sulfite solutions; 3. B groups - guaiacyl carbinols etherified at both the phenolic and benzylic hydroxyl groups, were sulfonated only in an acidic medium. In the present studies, four model compounds, 1-(4-hydroxy-3-methoxyphenyl)-1-propanol (I), 1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane (II), 1(3,4-dimethoxyphenyl)-1-propanol (III) and 1-(3,4-dimethoxyphenyl)-1-methoxypropane (IV) were synthesized and their reactions with nitrous acid were investigated. Compounds I and II represent X groups, compound III represents Z groups, and compound IV represents B groups.

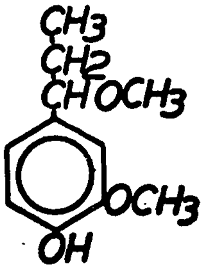
It should also be mentioned here that lignin is considered to have the following functional groups: (a) methoxyl, (b) hydroxyl (both phenolic, and primary and secondary alcoholic groups), (c) carboxyl (traces), (d) terminal methyl, (e) carbonyl, (f) ethylenic linkages and (g) coniferyl aldehyde groups. Among them, methoxyl is probably the most characteristic. The methoxyl content of softwood protolignin has been estimated to be 15 to 16%, while hardwood lignin contains 20.5 to 21.5% (17). The methoxyl group is expected to play an important role in the delignification process; for instance, its cleavage from lignin may be accompanied by the introduction of a new hydrophilic group, which would help to make the lignin soluble in alkali.

Some Relevant Reactions of Lignin

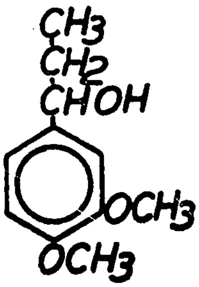
Lignin is a highly reactive substance. As mentioned above,



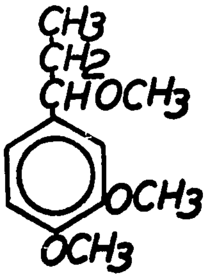
(I)



(II)



(III)

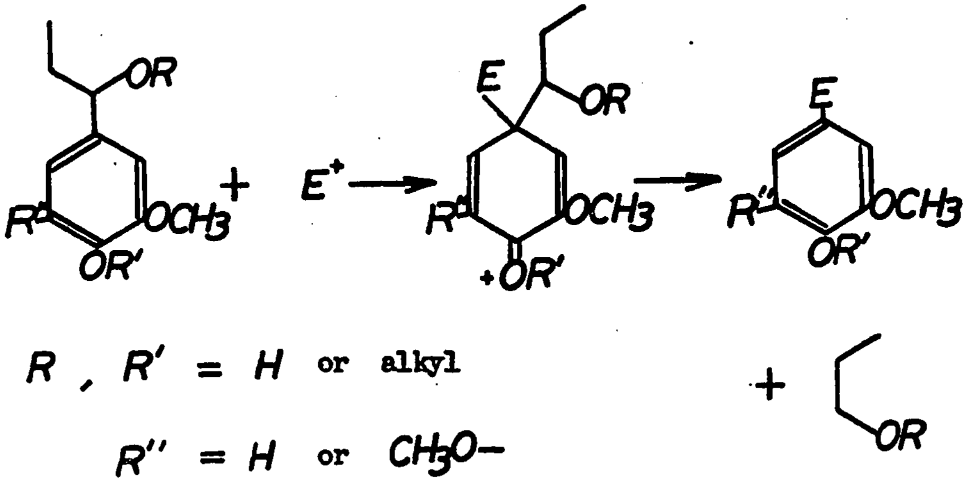


(IV)

its molecule contains certain functional groups, most of which are free to undergo their characteristic reactions. Functional groups, such as phenolic hydroxyl and methoxyl, are electron-releasing substituents which activate the aromatic nuclei and enhance their reactivities towards electrophilic displacement reactions.

Of all the reactions which lignin undergoes, electrophilic displacement, oxidation and demethylation are of particular importance because of their significance to pulping and bleaching processes. In the wood pulp industries, chlorine represents the most important bleaching chemical. Acidic chlorine water, which is used in the first stage of the commercial bleaching process, acts as a delignifying agent and converts most of the residual lignin in pulps to water- or alkali-soluble forms. Likewise, industrial pulps can be produced from wood and straw by the nitration method, although this process has not yet proven competitive with the more common pulping procedures. Both the chlorination and nitration of lignin are believed to involve electrophilic displacement, oxidation and demethylation.

Chlorination (18-21) and nitration (22-28) of lignin usually involve electrophilic substitution resulting in the displacement of either or both a hydrogen atom or the side chain from the aromatic ring. The displacement of the side chain is significant from a practical viewpoint because it is responsible for degrading and solubilizing the residual lignin during the delignification process. By analogy with aromatic substitution, the electrophilic displacement of the side chain may proceed via a transitory sigma complex according to the following scheme (20,29):

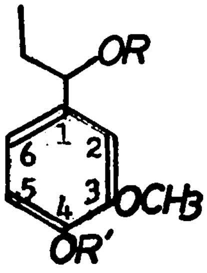


R, $R' = H$ or alkyl

$R'' = H$ or CH_3O-

where E^+ represents the reactive species in the displacement reactions of lignin, such as Cl_2 and Cl^+ in chlorination, NO_2^+ and N_2O_5 in nitration. The reactions have been shown to be particularly facilitated when the side chain contained a carbinol group adjacent to the ring (18,28). The replacement of the carbinol side chain by chlorine is a commonly observed reaction among model compounds such as vanillyl and veratryl alcohols, 1-(4-hydroxy-3-methoxyphenyl)-1-propanol (I), 1-(3,4-dimethoxyphenyl)-1-propanol (III) and the end units of spruce lignin as well (18,19,21). In nitration (23-28), the electrophilic side-chain displacement by a nitro group has been demonstrated by the isolation of 4,6-dinitroguaiacol (XI) from both lignins and model compounds.

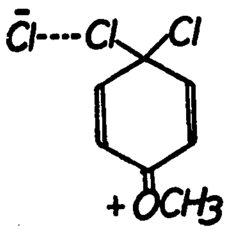
It is of interest to consider the relative reactivities of the aromatic sites in some lignin model compounds towards electrophiles. The site of attack of the electrophilic reagent is determined by whether or not the phenolic hydroxyl group of the guaiacyl nucleus is free. Chlorination of lignin model compounds suggested the sequence of reactivities of the aromatic sites as $k_6 > k_1 > k_5 > k_2$ for an etherified guaiacylpropane unit (20) where k is the rate constant. Very recent work by Van Buren and Dence (21) has suggested that during acidic chlorination the 5- and 6-positions of the free guaiacyl nucleus were activated to nearly the same degree, and 5- and 6-monochlorosubstituted products were found in about equal amounts. The sequence becomes $k_5 \approx k_6 > k_1 > k_2$ (21). This finding is in disagreement with the earlier reaction sequence proposed by Hibbert and co-workers (30) in which the 5-position of a guaiacyl unit was designated as the most reactive site. In the nitration of guaiacyl derivatives containing free phenolic hydroxyl groups (23-28), position 5 is also the most reactive site, and no 6-nitro derivative has been isolated. However, when the phenolic hydroxyl of the guaiacyl nucleus is blocked, substitution takes place mainly in the 6-position. Substitution of the 2-position has also been observed in the presence of a large amount of nitric acid (24).



R , R' = H or alkyl

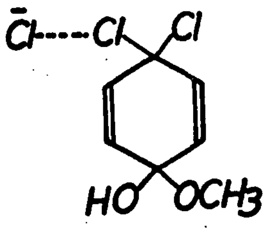
Lignin is very susceptible to oxidation, and even under mild oxidizing conditions, it is partly broken down into aromatic acids. Under more drastic conditions, lignin is oxidized to acids such as formic, acetic, oxalic and succinic (31). Under the relatively mild conditions used in the commercial bleaching process, it is converted into products which are soluble in either water or alkali. The oxidation reaction has been observed in the nitration of lignin model compounds; for instance, the formation of 2-hydroxy-5-methyl-3-nitro-1,4-benzoquinone (XLIII) from 4-methylguaiacol (32), 1-(3,4-dimethoxyphenyl)-2-propanone from 1-(3,4-dimethoxyphenyl)-2-propanol (25), and an orthoquinone from 3-(4-hydroxy-3-methoxyphenyl)-1-propanol (26). In the reaction with nitrous acid, the formation of 2,6-dimethoxy-1,4-benzoquinone (XVII) from a hardwood model, 1,2,3-trimethoxybenzene (XVI), is another example of oxidation (33). In the present studies, products from the oxidation by nitrous acid of both the aliphatic and aromatic portions of lignin have been isolated: 2,6-dimethoxy-1,4-benzoquinone (XVII) from birch dioxane lignin and from its model compounds, syringyl alcohol (XXXIX), syringaldehyde (XXI) and syringic acid (XXIV); 6-nitroveratric acid (XXXII) and 1-(3,4-dimethoxyphenyl)-1-propanone (XXX) from 1-(3,4-dimethoxyphenyl)-1-propanol (III); 1-(4-hydroxy-3-methoxy-5-nitrophenyl)-1-propanone (XXIX) from 1-(4-hydroxy-3-methoxyphenyl)-1-propanol (I). In the chlorination of lignin, the nature of the oxidation has been the subject of considerable speculation, much of which is unsupported by experimental results. In all probability, the chlorination involves oxidation of the pyrocatechols formed as the result of demethylation, and their conversion to the corresponding ortho-benzoquinones. These compounds are stabilized either by undergoing further oxidation or by polymerization (21).

Demethylation is another important competing reaction which has been encountered in the nitration and chlorination of lignin. Hibbert and co-workers (30) found that demethylation by chlorine occurred most easily in acid solution, less easily in neutral solution and only to a negligible extent in alkali. Dence and Sarkanen (18,19) have proposed that in the acidic chlorination of softwood lignin the formation of catechol derivatives is a result of demethylation. In their early publication (19) two alternative mechanisms were considered for the chlorine-catalyzed demethylation. The first consisted of an attack of the polarized chlorine molecule at the position <u>para</u> to the methoxyl group, and resulted in the formation of intermediates, V and VI.



(V)

and



(VI)

Either of these species may be converted to the free phenol with simultaneous generation of methanol and regeneration of the chlorine molecule. The second mechanism involved an attack of a molecular chlorine on the oxygen atom of the methoxyl group, and of a water molecule on its carbon atom, fellowed by removal of a molecule of methanol (Figure 3).

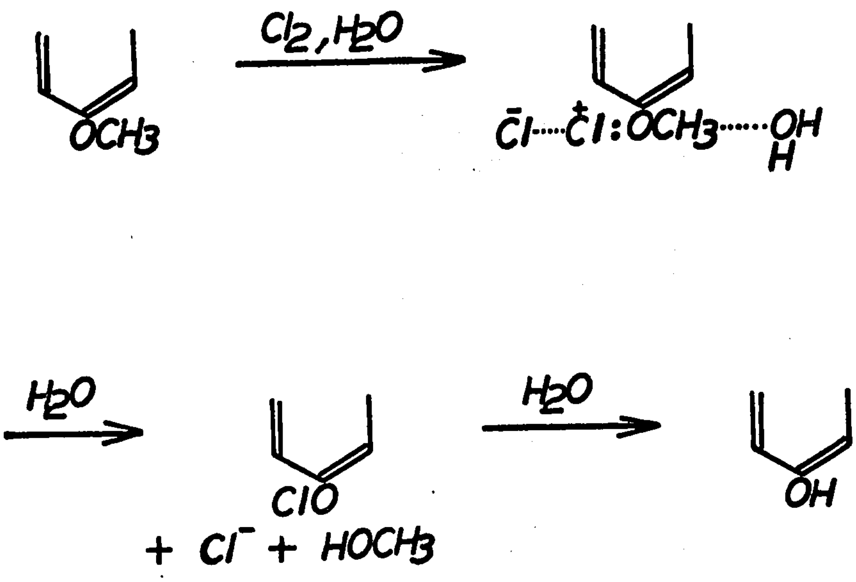


FIGURE 3 Mechanism of Chlorine-catalyzed Demethylation of Phenolic Methyl Ethers as proposed by Sarkanen and Dence (19)

Later results from model compound studies showed the first mechanism to be less probable (34): whether the group _para_ to a methoxyl was chlorine or an aldehyde group had no major effect on the demethylation. Furthermore, _ortho_-substituents showed a strong inhibitive effect which was not easily compatible with the first mechanism, and might be due to inhibition either of the formation of the transition complex of the second mechanism or of the proper solvation of this complex (34). Recently, Van Buren and Dence (21) investigated the demethylation behaviour of 1-(4-hydroxy-3-methoxyphenyl)-1-propanol (I) and 1-(3,4-dimethoxyphenyl)-1-propanol (III), and reported that the application of 1 mole of chlorine resulted in the loss of 30% of the methoxyl content in the first compound and 10-15% in the second. However, the nature of the aromatic products resulting from the demethylation was not determined. In the nitration of lignin, a loss of methoxyl content, as well as the formation of methanol, has been observed (35). The occurrence of demethylation has also been observed in the nitration of aromatic methyl ethers (32,36,37). Bunton and co-workers (36) have suggested that these reactions have the nature of a nitrosonium ion-catalyzed solvolytic cleavage (Figure 18). Later, when Sobolev (32) studied nitration of a lignin model compound, 4-methylguaiacol, he proposed that 5-methyl-3-nitropyrocatechol (XLII) was formed from 4-methyl-6-nitroguaiacol (XLI) via termolecular displacement catalyzed by nitrosonium ion (Figure 20). Recently, Sergeeva and co-workers (26) reported the formation of a red crystalline substance from the nitration of 3-(4-hydroxy-3-methoxyphenyl)-1-propanol. Spectroscopic and chemical analyses indicated that the substance was an orthoquinone containing a nitro group in the 5-position with respect to the unaffected side chain. They suggested that demethylation of lignin during nitration proceeded in the same manner. The mechanism of nitrosonium ion-catalyzed demethylation will be discussed in detail in a later chapter.

Review of the Delignification of Wood and Pulp with Nitrous Acid

The potential utility of nitrous acid as a delignifying agent was first suggested in a patent by Clemo (38) as early as 1860; it claimed the use of "nitric or nitrous acid, or the aqua fortis of commerce for converting straw and grasses into pulp suitable for the manufacture of paper". Clemo treated straw and grasses with a dilute acid solution for 4 hours at 200°F, and then digested the washed residue with a dilute alkaline solution at boiling temperature for 2 hours. He thus obtained a pulp which he deemed readily bleachable by the methods then in common use. Young and Pettigrew, in 1884 and 1885, received British, Canadian and U.S. patents (39) for a process for treating vegetable substances to produce fibers suitable for making paper. The first step was reaction with nitric or nitrous acid. The treated products were subsequently extracted with alkali and thus reduced to a pulp which could be washed and bleached, and afterward dealt with as ordinary pulp. In 1910, Schwalbe (40) patented the use not only of nitric and nitrous acids as pulping chemicals but also of oxides of nitrogen and several other oxidizing agents. Darling (41), in 1931, was granted a patent for the use of a nitrogen acid as pulping agent. In his specification, nitrogen acid was referred to as nitric or nitrous acid, or equivalents thereof, such as, for example, a mixture of sodium nitrate and sulphuric acid; sodium nitrite and hydrochloric acid or sulphuric acid, or a solution of fuming nitric acid which consists of a mixture of nitrous and nitric acids, etc. He claimed that when straw was treated with a nitrogen acid of a strength substantially equivalent to 2% HNO_3, under conditions avoiding the liberation of nitrogen oxides and physical disintegration of the straw, followed by alkaline digestion of the treated straw, the resulting cellulose was high in alpha-cellulose content. Dreyfus (42), in 1938 and 1940, received patents which described more clearly the use of nitrous acid as a pulping agent for lignin-containing cellulosic materials such as wood, straw and the like. The nitrous acid was generated <u>in situ</u> from a suitable nitrite, such as sodium or potassium nitrite, and an organic or a suitable mineral acid, present in amount sufficient or more than sufficient to decompose the nitrite. The nitrous acid treatment was again followed by a treatment with alkali. Further, Dreyfus mentioned the possibility, although without examples, of using the method for the purification of pulps which still contain residual lignin.

Clarke (43), in 1944, investigated the use of the nitrous acid system described by Schwalbe (40), i.e., a combination of equivalent weights of sodium nitrite and hydrochloric acid, and found that it was moderately effective as a delignification agent for pulps even with only 3% of sodium nitrite. He also observed that temperature was an important factor in the reduction of the lignin content of pulp, but he did not report the viscosities of the pulps so delignified. It should be noted that none of the previous authors appears to have recognized or suggested that acidity of the reacting solution might be a key factor in controlling the degree of delignification and viscosity of the pulps.

Quantitative results on the physical properties of pulps delignified with nitrous acid were recently reported by Bolker (1). He found that unbleached pulps, particularly sulfite pulp, could be partially delignified by consecutive treatment with nitrous acid, generated in situ from sodium nitrite and hydrochloric acid, and alkali, without degradation of the cellulose. Analysis of a statistically designed series of experiments on a commercial sulphite pulp of Kappa number 11.7 and viscosity 33.1 revealed that the removal of lignin and degradation of cellulose could be independently controlled, as shown by equations (2) and (3), which were derived by regression analysis:

$$K = -13.5 - 0.69\,P + 7.75\,\log_e t + 0.69\,T - 1.04\,\log_e C$$
$$- 0.125\,PT + 0.12\,T\log_e C \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$V = 30.4 - 3.99\,P + 0.51\,\log_e t + 0.23\,T - 2.08\,P^2$$
$$- 0.0034\,T^2 + 1.27\,P\log_e t - 0.16\,PT - 0.067\,T\log_e t \quad \ldots\ldots \quad (3)$$

where K = Percent reduction of Kappa Number

V = Viscosity (cp., 0.5% C.E.D.)

T = Temperature (°C)

t = Time (minutes)

P = pH

C = Concentration (%) of sodium nitrite on pulp

It is clear from these equations that the extent of delignification was a function of pH, temperature, time and concentration of $NaNO_2$, while the reduction in viscosity depended only on pH, temperature and time.

In contrast with nitric acid or nitrogen dioxide, nitrous acid can be generated in solution at various values of pH. Thus pH becomes one of the variables in the search for optimum conditions of delignification.

Review of Reactions of Nitrous Acid with Isolated Lignins and Model Compounds

As a delignification process, treatment with nitrous acid is unique in that, under conditions far milder than those employed in the conventional pulping operations, lignin is more or less readily degraded into fragments soluble in water or alkali. Although there is a large body of literature on the reaction of nitric acid with lignin and its model compounds, the reaction of nitrous acid does not appear to have been investigated in any detail. However, an early contribution to this study came from the field of soil chemistry when an effort was made to determine, by reaction with nitrous acid, how much of the nitrogen in humic acid occurs as free amino groups. Since a lignin-like fraction is believed to be a component of humic acid (44), Bremner (45), who recognized that lignin itself could react with nitrous acid, studied this latter reaction so that he might determine how to distinguish it from the reaction of amino groups. Two-gram samples of soluble native spruce lignin, Meadol, Indulin A, Tomlinite, alkali wheat lignin prepared according to Waksman and Jyer (46a), alkali oat straw lignin prepared according to Norman and Jenkins (46b), acid oat straw lignin prepared according to Lancaster (40c) were each treated with 20 ml of 3N hydrochloric acid and 8 ml of 50% sodium nitrite solution at room temperature for various periods of time; all yielded nitrogen-containing lignins. It was found that lignins, like humic acids, fixed nitrogen and lost methoxyl groups in their reaction with nitrous acid, and the amount of nitrogen fixed increased with the time of reaction. The behaviour of the nitrogen in modified lignin in the Van Slyke method for determining amino groups was also investigated. It was found that lignin interfered with the estimation of amino groups, and was responsible for the high apparent amino-nitrogen values obtained with humic acid preparations.

Sarkar (47) has reported that treating lignin with nitrous acid, as with nitric acid, led to the formation of oxalic acid in considerable quantity. The nature of gases produced by the reaction has been investigated by several workers. Bremner (45) observed the evolution of hydrogen cyanide and what appeared to be nitrous oxide or nitrogen during the reaction. Stevenson and co-workers (48,49) demonstrated the presence of methyl nitrite among the reaction products of the lignin-HNO_2 reaction. Since other experiments showed that only compounds containing the syringyl group produced methyl nitrite, they concluded that the syringyl group was the structural unit in lignin responsible for forming this gaseous substance. The mechanism of this type of demethylation, however, has not been defined.

All the previous results seem to indicate that reaction of lignin with nitrous acid is similar in many respects to its reaction with nitric acid. For example, fixation of nitrogen, destruction of methoxyl groups, formation of oxalic acid, and evolution of gaseous products such as hydrogen cyanide, methyl nitrite, nitrous oxide and nitrogen, have all been observed in the reaction of lignin with nitric acid (31).

Recently, Bolker _et_ _al_.(33) studied the reactions of nitrous acid with compounds such as guaiacol (VII), veratrole (XII), 2,6-dimethoxyphenol (XIV), and 1,2,3-trimethoxybenzene (XVI), all models for various structures found in lignin (Figure 4). At pH 2.0 and 70°, treatment of guaiacol (VII) with nitrous acid gave mainly the 4-monoxime of 2-methoxy-1,4-benzoquinone (VIII), and traces of 6-nitro- (IX), 4-nitro- (X) and 4,6-dinitroguaiacol (XI); veratrole (XII) reacted only slightly, producing traces of XI and 4-nitroveratrole (XIII); 2,6-dimethoxyphenol (XIV) yielded cerulignone (XV); 1,2,3-trimethoxybenzene (XVI) did not react. At pH 1.0 and 100°, VII yielded IX and X, both in greater yields than under milder conditions; and XVI yielded 2,6-dimethoxy-1,4-benzoquinone (XVII) and 5-nitro-1,2,3-trimethoxybenzene (XVIII). Thus, only XVI underwent extensive demethylation resulting in the formation of XVII. The mechanism of demethylation was further studied and shown to follow the sequence of intermediates (50): 5-nitroso-1,2,3-trimethoxybenzene ⟶ 4-nitroso-2,6-dimethoxyphenol ⟶ 2,6-dimethoxy-4-oximino-2,5-cyclohexadienone-1 (Figure 21).

Results from these model compound experiments (33,50) provide some important information for the study of the mechanism whereby nitrous acid degraded lignin in the delignification process. For instance, it gave a possible mechanism for the cleavage of the phenyl ether linkage in hardwood lignin. However, since electrophilic side-chain displacement is one of the most important competing reactions in the bleaching process, in order to study the mechanism of the degradation, it seems necessary to include a study on reactions of some other model compounds with a side chain <u>para</u> to a hydroxyl or etherified hydroxyl group in the aromatic nuclei. In part of the present studies, this line of attack has been pursued.

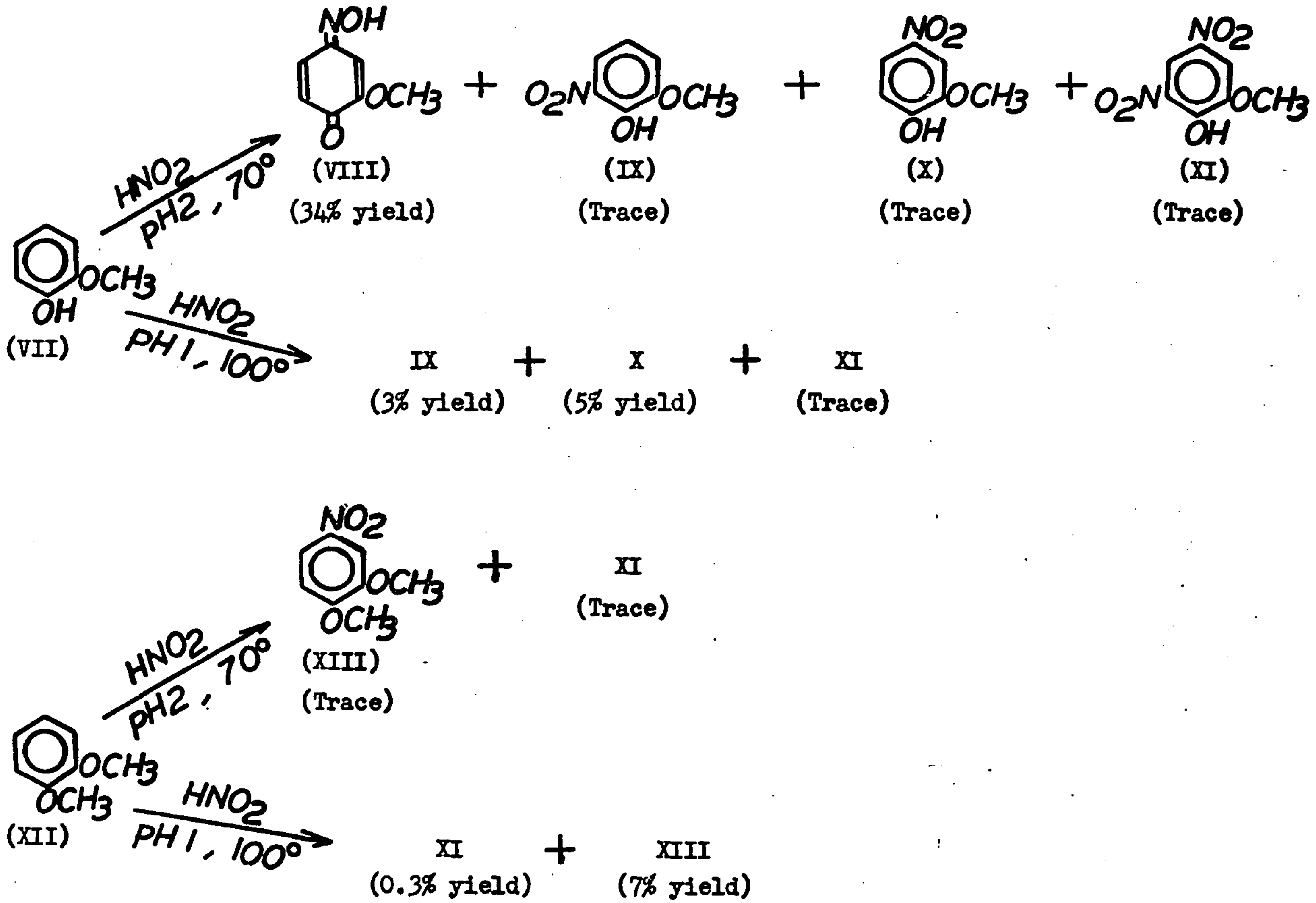


FIGURE 4 Products from Reactions of Nitrous Acid with Guaiacol (VII), Veratrole (XII), 2,6-Dimethoxyphenol (XIV), and 1,2,3-Trimethoxybenzene (XVI) (33)

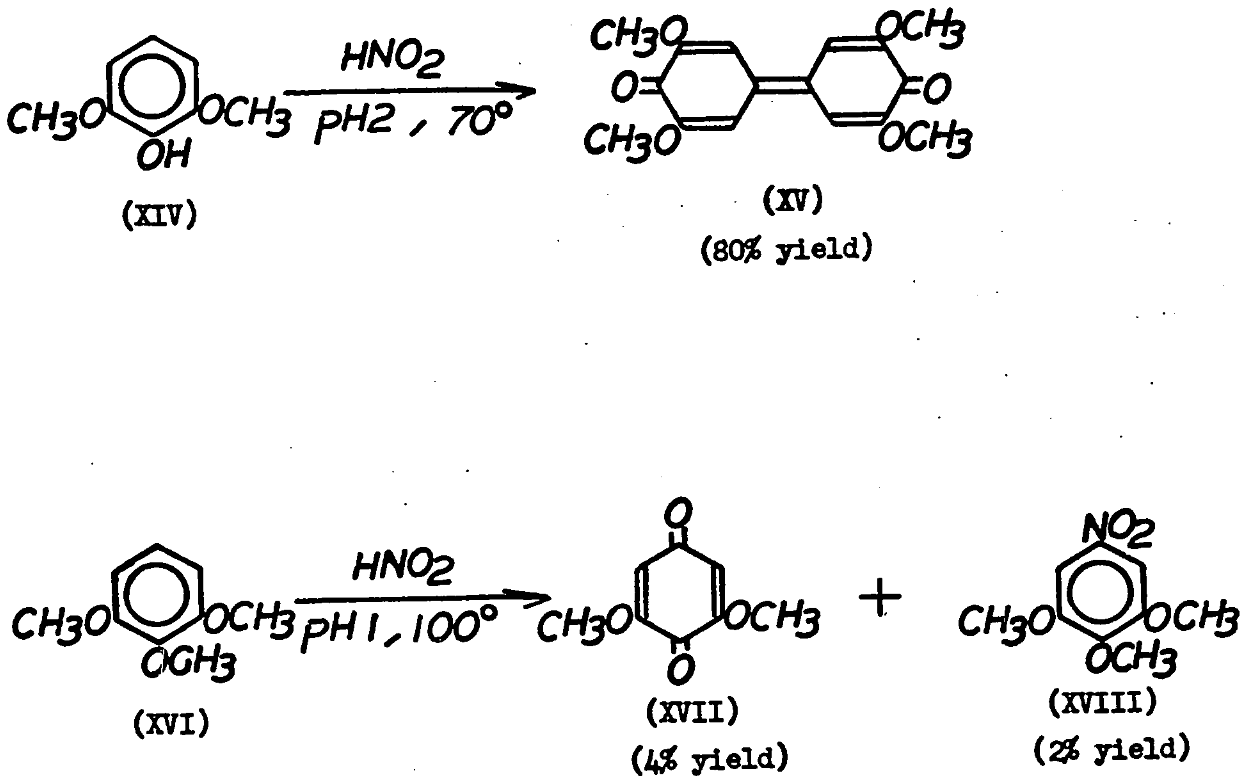


FIGURE 4 (cont'd)

RESULTS AND DISCUSSION

Degradation of Lignin with Nitrous Acid

When birch dioxane lignin was treated with aqueous nitrous acid at pH 2 and 70° for 1 hour, an insoluble residue (90% yield*) remained in the acid liquor. From the latter 4-nitroguaiacol (X), 4,6-dinitroguaiacol (XI), 2,6-dimethoxy-1,4-benzoquinone (XVII) and oxalic acid were isolated in yields of 1.06%, 2.64%, 1.06% and 1.80%, respectively. Spruce lignin, subjected to the same treatment, yielded only the two nitro compounds (X, 4.81%; XI, 0.47%), oxalic acid (1.09%) and 95 percent of insoluble residue. The two nitro compounds, X and XI are products of the electrophilic displacement of aliphatic side chains of phenylpropane units, while 2,6-dimethoxy-1,4-benzoquinone (XVII) is product of electrophilic displacement and oxidation as well.

The electronic influences operative in the guaiacyl nucleus of lignin during the action of nitrous acid are such that the electrophile is directed strongly to the position <u>para</u> or <u>ortho</u> to the phenolic hydroxyl . Substitution of the nitroso group <u>para</u> to the phenolic hydroxyl causes the displacement of the propyl side chain, by which the end units of lignin are cleaved from the remainder of lignin macromolecule. This displacement gives rise to the expected product, 4-nitroguaiacol (X). When both nitroso substitution at the <u>ortho</u>-position and side-chain displacement at the <u>para</u>-position are applied to the same guaiacyl unit, a water-soluble compound,

---

* Yields are expressed here as a percentage of the starting material.

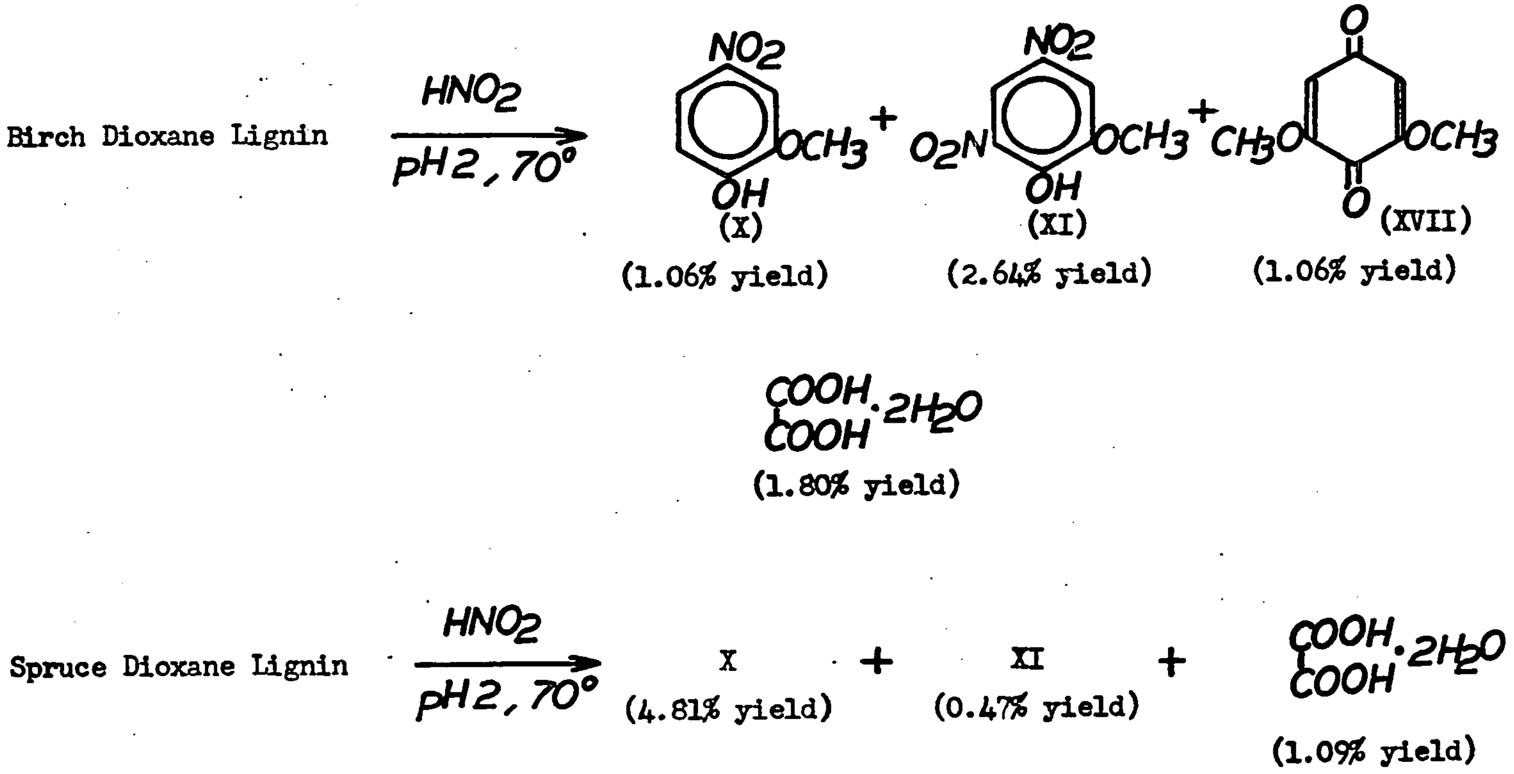


FIGURE 5 Water-soluble Products from Reactions of Nitrous Acid with Lignins

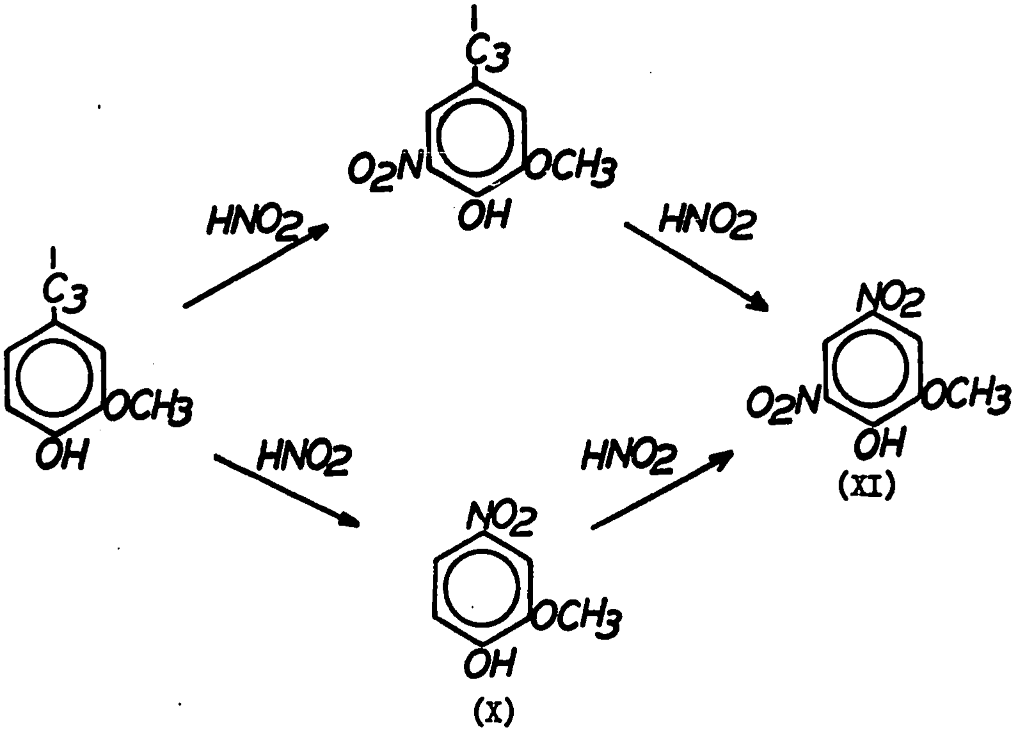
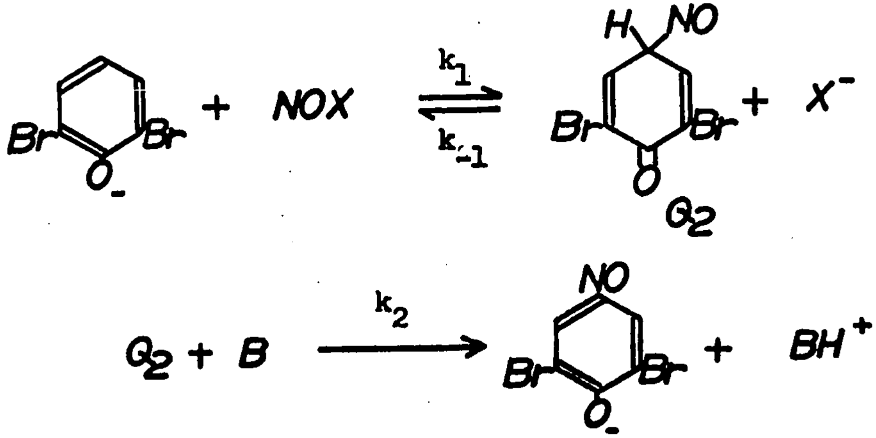


FIGURE 6 Electrophilic Displacement Reaction of HNO_2 on the Guaiacylpropane Units of Lignin

4,6-dinitroguaiacol (XI) is formed. In the absence of side-chain displacement by the nitroso group, nitrosation of the aromatic rings occurs in the vacant position ortho to phenolic hydroxyls, as shown by the isolation of a series of 5-nitro phenolic compounds from the alkaline digestion of nitrolignin. This reaction will be discussed in a separate section.

Aromatic substitution by nitrosating species has been investigated and summarized by several authors (36,51,52). Bunton et al. (36) concluded that the reaction of nitrosonium ion with the aromatic nucleus, when no point of attachment for the ion is present in any side chain, involves a rate-determining addition of the nitrosonium ion, succeeded by a fast ejection of the aromatic proton, and a fast oxidation of the nitroso compound to a nitro compound. However, more recently, the existence of a significant kinetic hydrogen-isotope effect(${}^{k}H/k_{D} = 3.6$) was interpreted by Ibne-Rasa (53) in terms of a two-step mechanism involving the reversible formation of a quinonoid intermediate, Q_2, which then undergoes loss of the proton to give the nitrosated product :



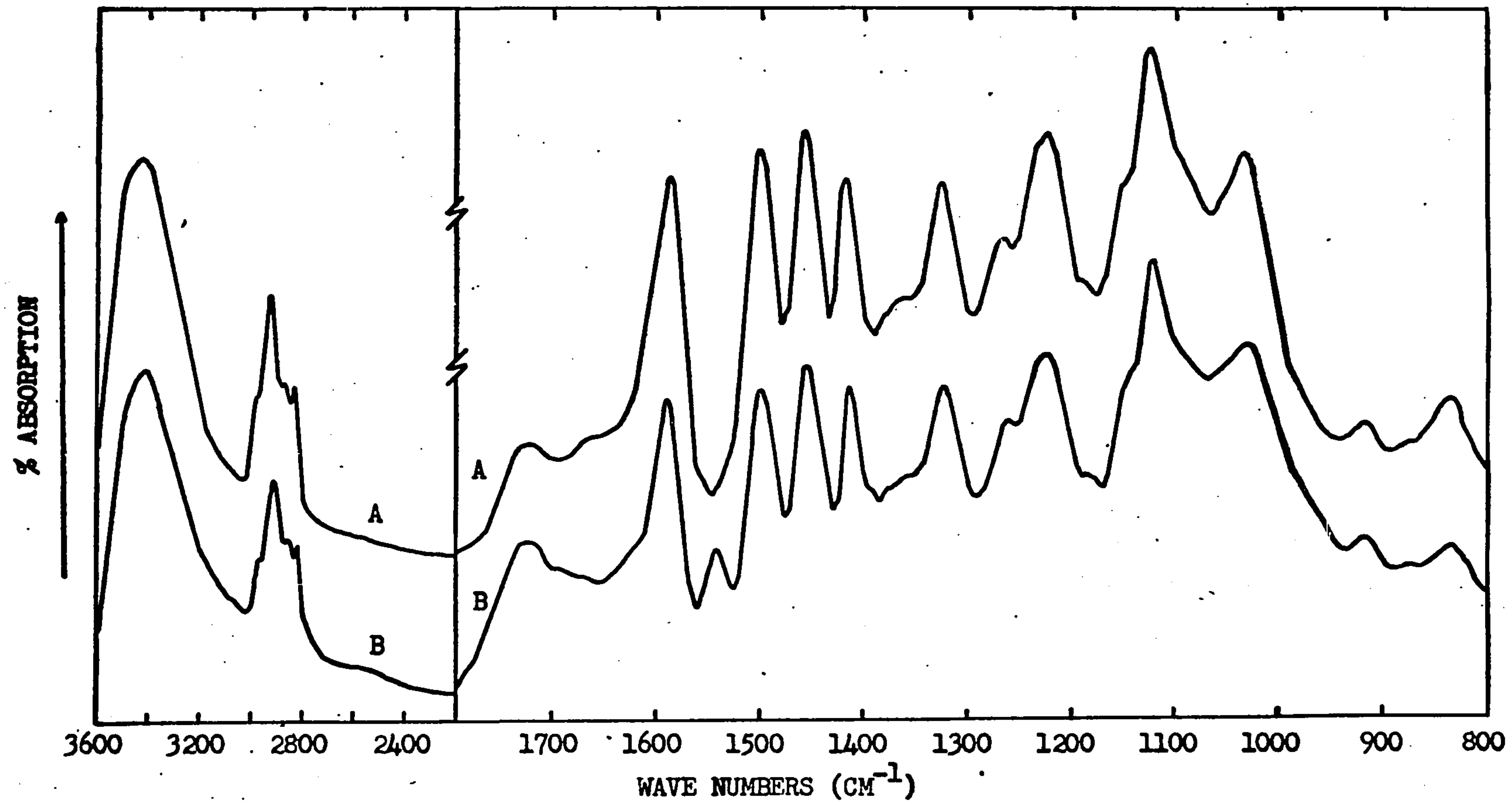
where B is the solvent or an added base, and NOX is the nitrosation carrier. Since the kinetic hydrogen-isotope effect is pronounced and since this reaction is base-catalyzed, the second step must be rate-determining. The view is in accord with the views of other authors. For example, reactions such as the iodination of phenol where the ring deuterated phenol reacts at only one-fourth the rate of the unlabelled phenol (54), and aromatic sulfonation where H^3 is substituted considerably more slowly than H^1 (55), are believed to follow the two-step mechanism, the second step being rate-controlling. More recently, Schmitt (56) proposed a mechanism of nitrosodeprotonation of phenol which is essentially the same as the above. By analogy with aromatic substitution, the electrophilic displacement reaction may also proceed via a transitory sigma complex as shown in equation (1) (20,29).

It has previously been reported that only the analogue of a fully etherified hardwood lignin, such as 1,2,3-trimethoxybenzene showed evidence of any appreciable degree of demethylation (Figure 4, XVI→XVII) (33). This evidence, together with the formation of 2,6-dimethoxy-1,4-benzoquinone (XVII) from birch dioxane lignin, suggested that the phenyl ether links in hardwood lignins might be more readily cleaved than in softwood lignins. This reactivity may be responsible for the previous results that hardwoods are more readily pulped than softwoods by mixtures of nitric and nitrous acids (57). This suggestion was further substantiated by the observation that, under the same degradation conditions, the yield of insoluble residue from hardwood lignin is lower than that from softwood lignin, and, on digestion with alkali, the hardwood residue dissolved completely while the softwood did not.

Fixation of Nitrogen and Demethylation

The infrared spectra of insoluble products obtained from the reaction of nitrous acid with birch and spruce lignins revealed a band at about 1550 cm^{-1}, not present in the spectra of the original lignins (Figures 7 and 8). It thus appeared likely that nitroso or nitro compounds were formed. Spot tests (58), using phenol and sulfuric acid, and sodium pentacyanoamine ferroate, respectively, failed to indicate the presence of nitroso groups. Quantitative determination by titanous chloride, based on the method proposed by Kneckt et al. (59), and modified by Ma et al. (60), showed that nitrogen in the modified lignin was present mainly as nitro groups and only traces as nitroso groups; some nitrogen was present in another form not yet determined. For instance, the product from the birch lignin — HNO_2 reaction at pH 2, 70° and 1 hour contained 76.4% of the total nitrogen present as nitro groups and only 2.1% present as nitroso groups; the product from spruce lignin contained 80.1% of the total nitrogen present as nitro groups and 1.4% as nitroso groups. However, an iodometric method (61) indicated the absence of nitroso group in both insoluble products. The absence of the nitroso group can be attributed to its instability towards oxidizing agents such as NO^+ and $H_2O\cdot NO^+$ in the aqueous HNO_2 solution. It is reasonable to believe that the lignin-HNO_2 reaction involves nitrosation followed by oxidation of the nitroso compound, as does the nitrous acid-catalyzed nitration of aromatic compounds (36). For convenience, the insoluble lignins are commonly referred to as "nitrolignins".

The nitrolignins from treatment of the originally buff-coloured lignin with nitrous acid were light brown in colour. It was



<u>FIGURE 7</u> Infrared Spectra of (A) Birch Dioxane Lignin and (B) Birch Nitrolignin

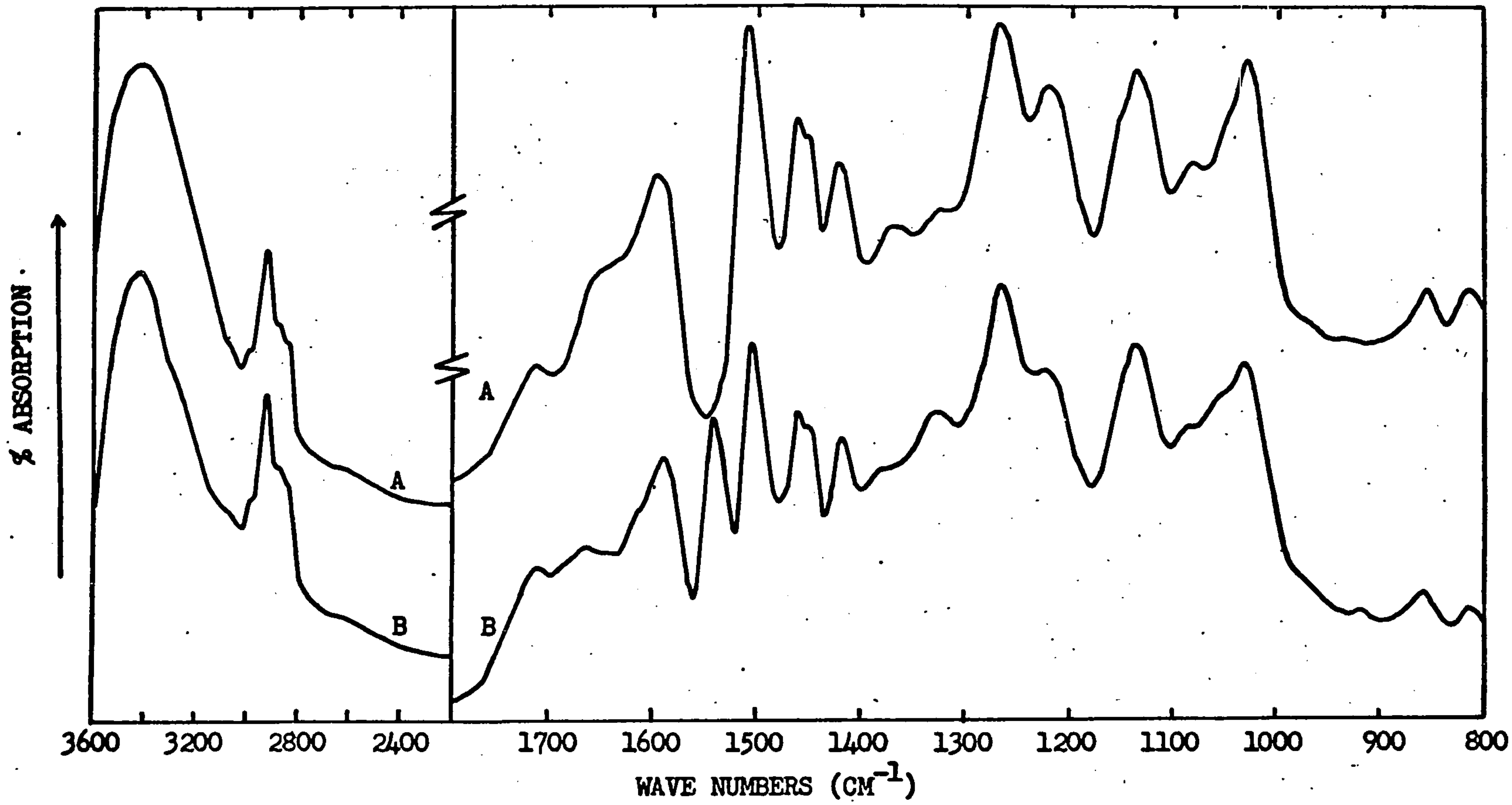


FIGURE 8 Infrared Spectra of (A) Spruce Dioxane Lignin and (B) Spruce Nitrolignin

TABLE I

Analysis of Lignins before and after Treatment with Nitrous Acid (pH 2, 70°, 1 hour)

Sample	N Content (%)	CH_3O- Content (%)
Birch dioxane lignin	0	20.7
Product from birch lignin	0.93	18.2
Spruce dioxane lignin	0	15.4
Product from spruce lignin	2.06	13.6

found that the product obtained from birch dioxane lignin (pH 2, 70°, 1 hour) contained 0.93% of nitrogen and 18.2% of methoxyl, while that from spruce lignin contained 2.06% of nitrogen and 13.6% of methoxyl. Clearly, treatment of nitrous acid led to the fixation of nitrogen and destruction of methoxyl groups (Table I).

It was found that the amount of nitrogen fixed and the amount of methoxyl lost increased with the initial pH of the mixture, reaction time and temperature. The nitrogen and methoxyl contents, and the yields of the insoluble products from experiments done with varying acidity, reaction time and temperature are given in Tables II, III and IV. The methoxyl group determinations were done by the method (slightly modified) developed by Cobler et al.(62). It was found that nitrosation of lignin, or more correctly, the action of nitrous acid on lignin, occurred more readily with increasing acidity, temperature and reaction time, as shown by the increase in nitrogen content. However, reactions at temperatures above 90° gave irregular results, for example, birch and spruce nitrolignins obtained from reactions at 95° contained 1.07% and 0.93% of nitrogen, respectively, which were lower than the values obtained from reactions at 90°. At temperatures above 90°, the powdered lignin resinified into an adhesive and elastic mass, and hence impaired the vigorous stirring of the reaction system. This thermal softening and adhesive behaviour has been investigated by Goring et al. (63,64); it is due to a glass transition in the lignin polymer and the temperature at which it occurs is lower in the presence of water than in dry lignin.

TABLE II

Effect of pH on the Yield and Composition of Nitrolignin (70°, 1 hour)

Starting Lignin	Initial pH of Reaction Mixture	Analysis of Nitrolignin		Yield, %
		N, %	CH_3O-, %	
Birch dioxane lignin	3	0.47	18.6	92
Birch dioxane lignin	2	0.93	18.2	90
Birch dioxane lignin	1	1.15	18.0	78
Birch dioxane lignin	0.5	1.29	17.8	78
Spruce dioxane lignin	3	1.63	14.6	96
Spruce dioxane lignin	2	2.06	13.6	95
Spruce dioxane lignin	1	2.09	12.7	90
Spruce dioxane lignin	0.5	2.30	11.1	88

TABLE III

Effect of Reaction Time on the Yield and Composition of Nitrolignin (pH 2, 70°)

Starting Lignin	Time of Reaction (hours)	Analysis of Nitrolignin		Yield, %
		N, %	CH_3O-, %	
Birch dioxane lignin	1	0.93	18.2	90
Birch dioxane lignin	3	1.10	17.1	87
Birch dioxane lignin	5	1.16	16.8	80
Birch dioxane lignin	7	1.23	16.5	78
Spruce dioxane lignin	1	2.06	13.6	95
Spruce dioxane lignin	3	2.06	13.0	93
Spruce dioxane lignin	5	2.07	12.4	90
Spruce dioxane lignin	7	2.08	12.1	89

TABLE IV

Effect of Reaction Temperature on the Yield and Composition of Nitrolignin (pH 2, 1 hour)

Starting Lignin	Temperature of Reaction (C°)	Analysis of Nitrolignin		Yield,%
		N, %	CH_3O-, %	
Birch dioxane lignin	30	0.66	20.1	93
Birch dioxane lignin	50	0.86	18.6	91
Birch dioxane lignin	70	0.93	18.2	90
Birch dioxane lignin	90	1.18	18.0	79
Birch dioxane lignin	95	1.07	18.9	77
Spruce dioxane lignin	30	0.71	13.8	99
Spruce dioxane lignin	50	1.15	13.8	97
Spruce dioxane lignin	70	2.06	13.6	95
Spruce dioxane lignin	90	2.25	12.1	90
Spruce dioxane lignin	95	0.93	13.8	89

The yields of the insoluble nitrolignins under different conditions also revealed that the degree of degradation of lignin was a function of pH, temperature and time. This result was consistent with the equation for reduction of Kappa number of pulp as found by Bolker (1).

It should be noted that softwood lignin, although less readily degraded, could have been "nitrated" or "nitrosated" to a greater extent than hardwood lignin as shown by its greater nitrogen content. This nitrogen-rich nitrolignin, however, was less readily soluble in alkali. At this point, we can assume that delignification using nitrous acid consists of nitrosation and oxidation, the latter rather than the former determining the solubility of the modified lignin. Since the normal procedure for delignification of pulp requires a second stage, i.e., extraction with alkali, both the birch and spruce nitrolignins were, accordingly, digested in NaOH solution and the compounds thus solubilized were isolated and identified. The results will be discussed in the next section.

Alkaline Hydrolysis of Nitrolignin

Nitrolignins recovered from the reaction of lignins with nitrous acid were subjected to alkaline hydrolysis with N sodium hydroxide solution at 80° for 24 hours. The aqueous solution was filtered and acidified with dilute hydrochloric acid. A brown precipitate was produced; it was filtered off while hot. In the alkaline treatment about 24% of birch and 21% of spruce nitrolignins were degraded into substances which could not be precipitated by acidification. The acidic solution was first extracted with chloroform and then ether. By thin-layer chromatography using a solvent system consisting of benzene-methanol (1:5.5), the chloroform

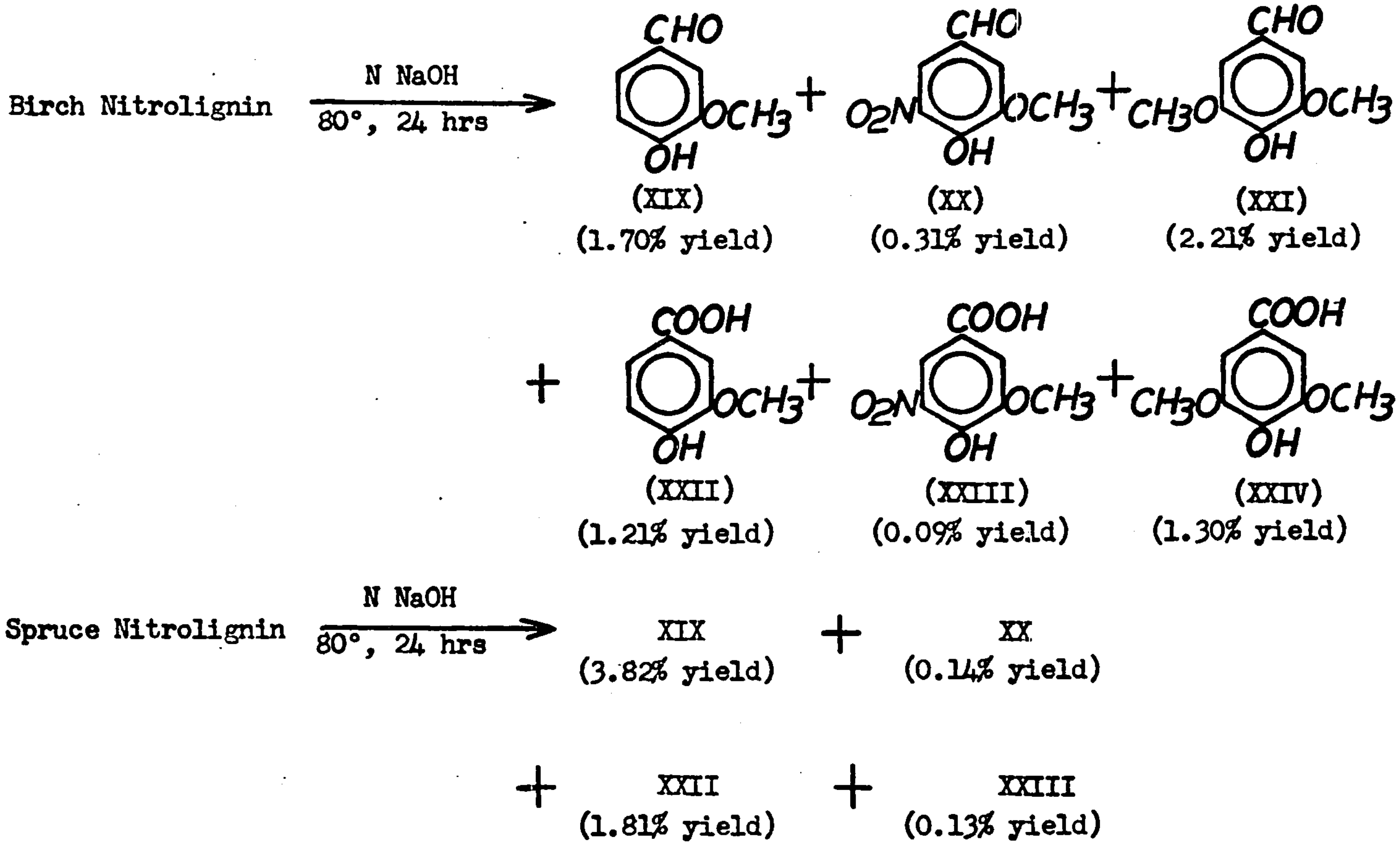


FIGURE 9 Products from the Hydrolysis of Nitrolignins by Alkali

extract of birch nitrolignin gave vanillin (XIX), 5-nitrovanillin (XX) and syringaldehyde (XXI) (with R_f 0.44, 0.12 and 0.30, respectively). The ether extract, on thin-layer chromatography using a solvent mixture of n-butyl ether (saturated with water) - glacial acetic acid (10:1), showed three zones at R_f 0.43, 0.34 and 0.25. These zones were scraped from the plates and the eluates were identified as vanillic (XXII), 5-nitrovanillic (XXIII) and syringic (XXIV) acids, respectively. Similar treatment of spruce nitrolignin gave rise to vanillin (XIX), vanillic acid (XXII), 5-nitrovanillin (XX) and 5-nitrovanillic acid (XXIII).

The formation of 5-nitrovanillin and 5-nitrovanillic acid was not surprising, because of the known presence of free phenolic hydroxyl groups in lignin; these groups have a high activating strength and an <u>ortho</u>-<u>para</u> directing influence, and should induce 5-substitution during nitrosation. The presence of free phenolic hydroxyl groups in nitrolignins was also indicated by their infrared spectra, which had a strong band at 3450 cm^{-1} (Figures 7 and 8). Chromatographic evidence indicated the absence of 6-nitrovanillin or 6-nitrovanillic acid among the products of hydrolysis. The result was in contrast with those from the hydrolysis or alkaline oxidation of other lignin derivatives. Pearl (65) isolated 6-chlorovanillin from the ether extract obtained from the chlorite liquor in which black spruce wood holocellulose had been digested. Lautsch and Piazolo (66) obtained 6-bromovanillin by treating cuoxam spruce bromolignin with 10% potassium hydroxide and oxygen in the presence of cobaltic hydroxide. However they did not isolate any detectable amount of 5-bromovanillin, although the free phenolic groups in lignin have a strong orienting influence towards substitution in the 5-position. Jayne (67), also reported the isolation of 6-chlorovanillin and the absence of 5-chlorovanillin from the oxidation of chlorite lignin with alkaline nitrobenzene. He suggested that the guaiacyl units of lignin containing free phenolic hydroxyl groups might possibly undergo chlorination in the 5-position during the chloriting reaction, and were cleaved or degraded from the more resistant position of the lignin molecule which was later isolated as chlorite lignin. In fact, Barton (68) found the phenolic group to be absent from chlorite lignin. Recently, Dence and Sarkanen (18) indicated, by spectrophotometric techniques, that veratryl and vanillyl alcohols and spruce lignosulfonic acid alike underwent rapid substitution by chlorine into the 6-position of the aromatic nucleus. However, experiments with model compounds containing phenolic hydroxyl groups para to their aliphatic side chains have demonstrated that chlorine substitutes in the 5-position of these nuclei as well (18, 21).

In the present studies, the isolation of 5-nitro phenolic compounds leads to the conclusion that during the nitrous acid treatment, in the absence of side-chain displacement by nitroso groups, nitrosation of aromatic rings in lignin occurs in the vacant position ortho to phenolic hydroxyls. As later seen in the studies of model compounds, the formation of 1-(4-hydroxy-3-methoxy-5-nitrophenyl)-1-propanone (XXIX) among other products from the action of nitrous acid on 1-(4-hydroxy-3-methoxyphenyl)-1-propanol (I) seemed to substantiate this point.

Nitrolignin recovered by acidifying the alkaline hydrolysis mixture was obtained in 76.1% yield from birch, and 79.0% from spruce. The former contained 0.20% of nitrogen and 17.9% of methoxyl, and the latter 0.45% and 11.91%, respectively. Thus, about 70 to 80% of the nitrogen in

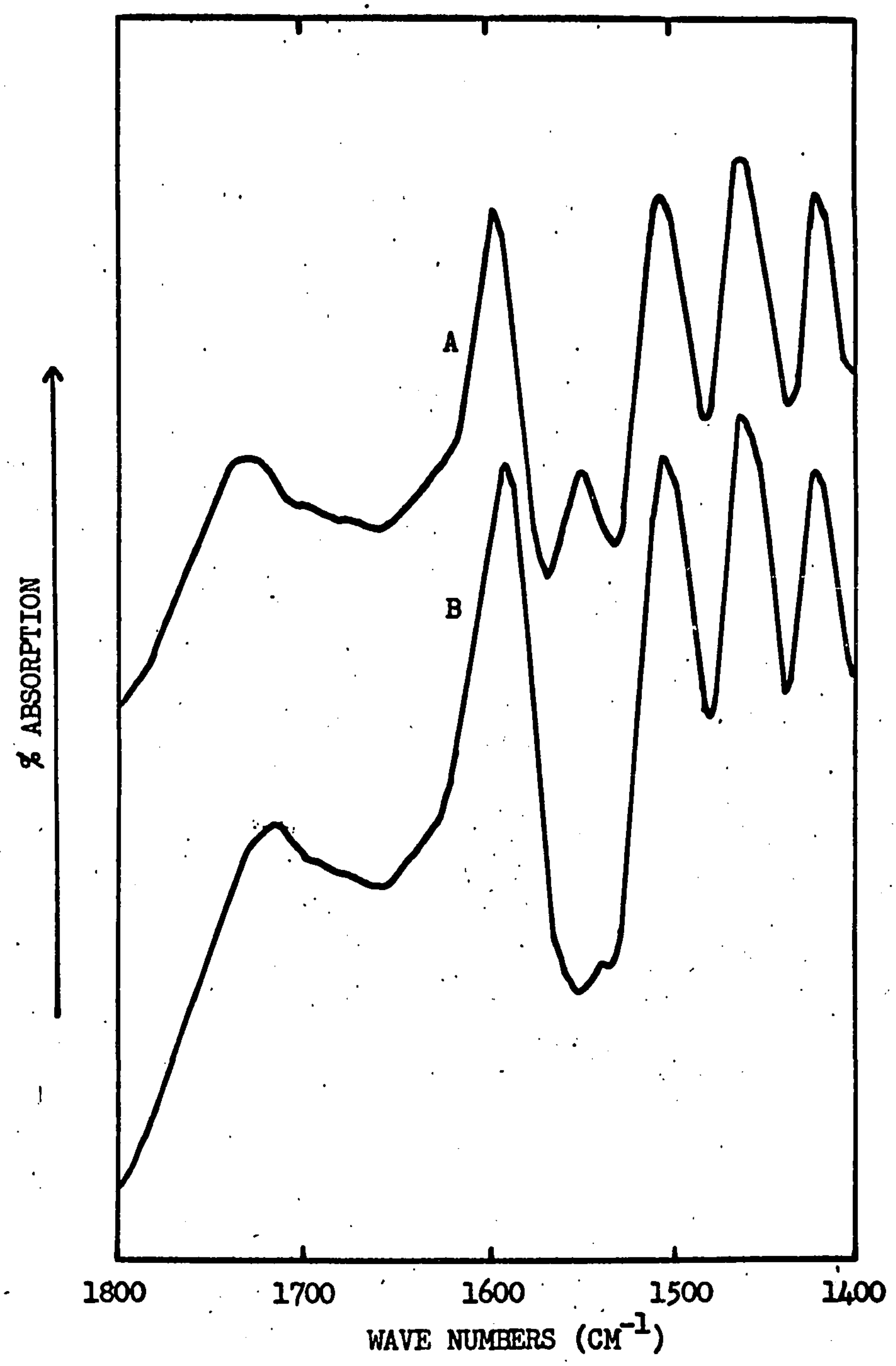


FIGURE 10 Infrared Spectra of (A) Birch Nitrolignin and (B) Acidified Residue from Alkaline Hydrolysis of Birch Nitrolignin in the Region of 1400-1800 cm^{-1}

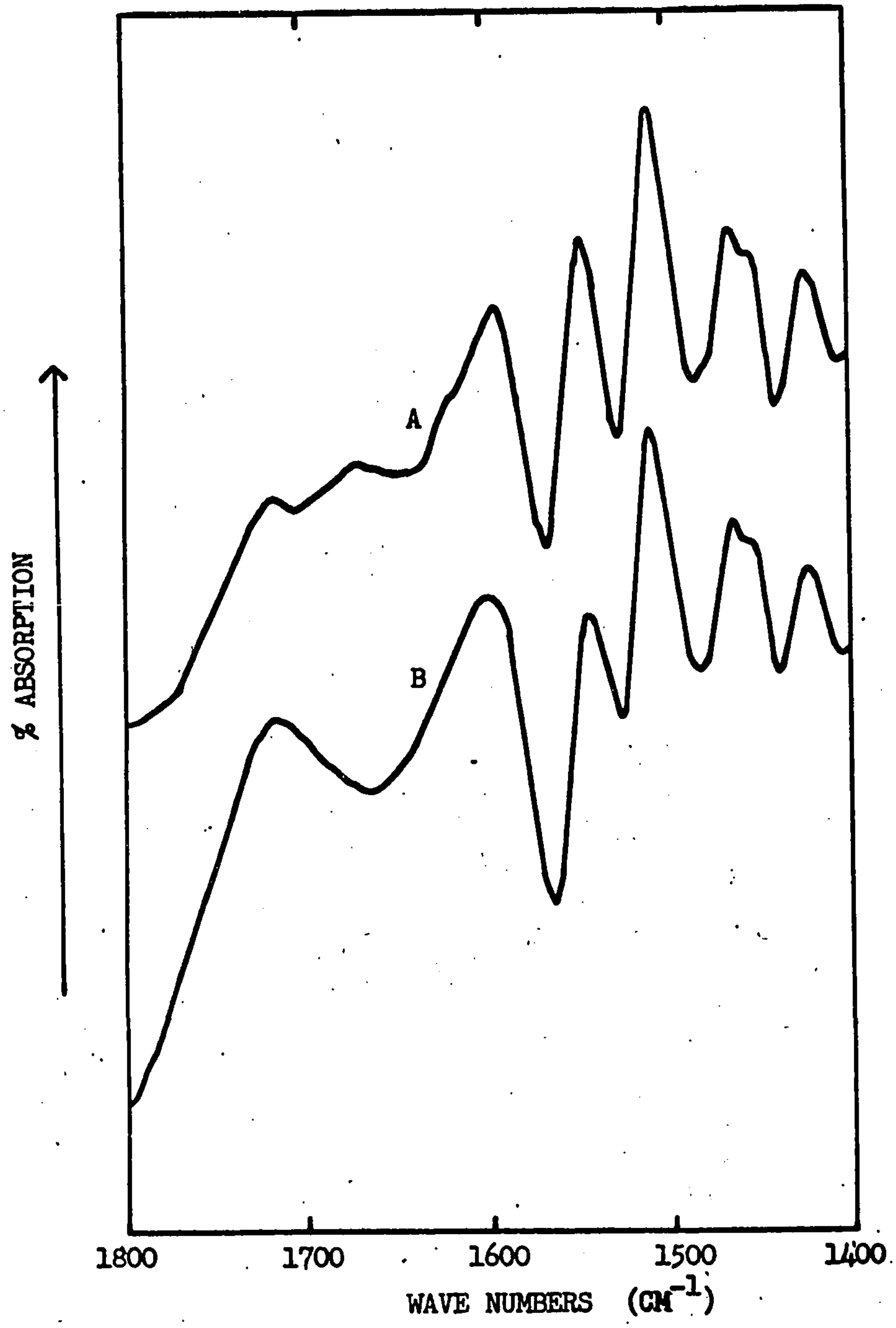


FIGURE 11 Infrared Spectra of (A) Spruce Nitrolignin and (B) Acidified Residue from Alkaline Hydrolysis of Spruce Nitrolignin in the Region of 1400-1800 cm^{-1}

nitrolignins had been removed from the macromolecules in the hydrolysis process. Infrared spectra also showed a remarkable reduction in the intensity of the band at 1550 cm^{-1} (Figures 10 and 11). Demethylation appeared to be another important aspect in the alkaline hydrolysis process, as indicated by the reduced methoxyl content of the recovered nitrolignins.

Repetition of the hydrolysis of these recovered nitrolignins yielded additional amounts of the same degradation products. Oxidation with nitrobenzene yielded only the products previously obtained from the liquor of the alkaline digestion as shown by comparing the thin-layer chromatograms.

Permanganate Oxidation of Methylated Spruce Nitrolignin

The permanganate method has been widely used for elucidating the structure of lignin. This method, developed by Freudenberg (69,70), consists of the hydrolysis of phenolic ether linkages by alkali, followed by methylation to protect the phenolic groups thus liberated and by permanganate oxidation that degrades the side chains to carboxylic groups. A significant part of the information concerning the occurrence of phenylpropane units in lignin substituted or unsubstituted in the 5-position has been obtained by this method (2). When spruce nitrolignin was subjected to alkaline hydrolysis, then methylation and subsequent oxidation, it yielded 5-nitroveratric (XXV), veratric (XXVI), isohemipinic (XXVII) and dehydrodiveratric (XXVIII) acids.

It is said that substituted guaiacylpropane units may constitute up to 50% of softwood lignin macromolecules. The groups are of two different

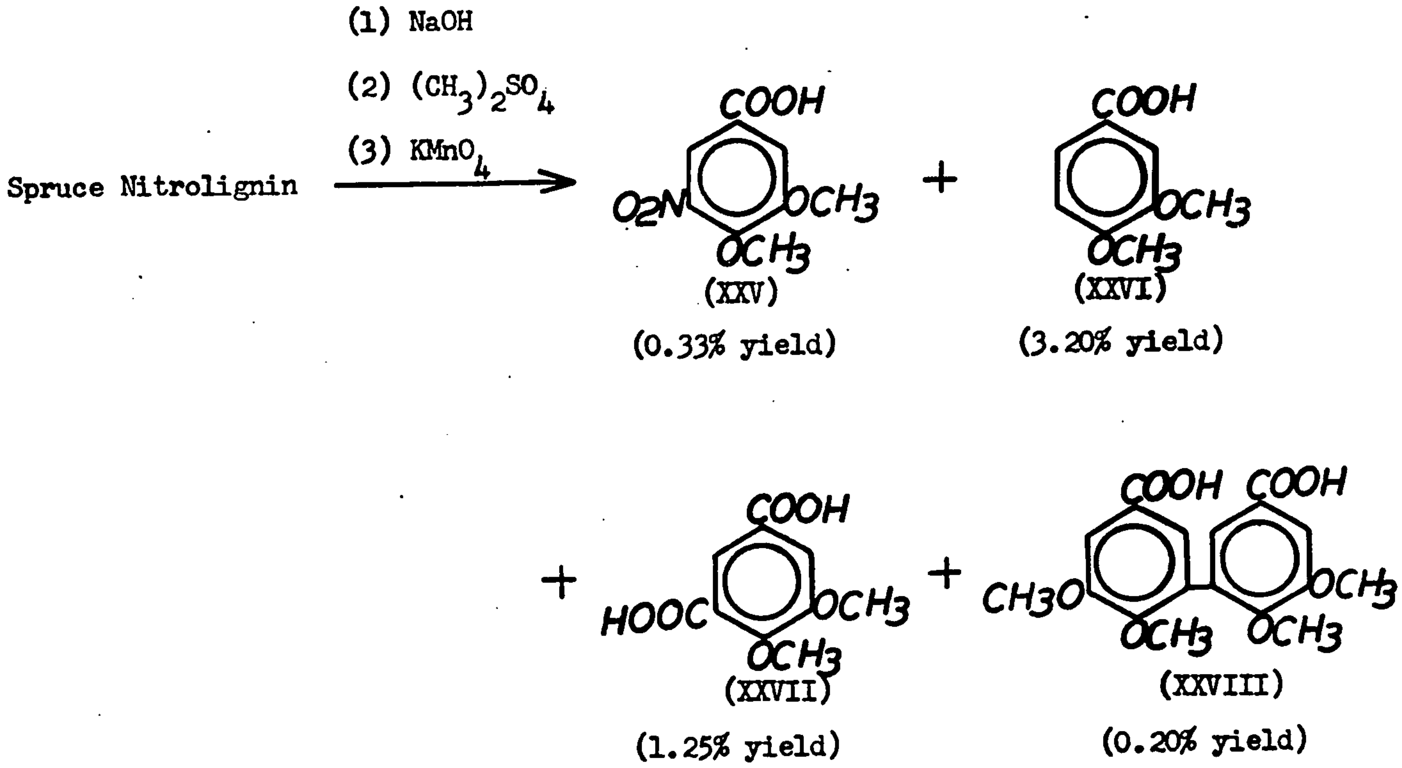
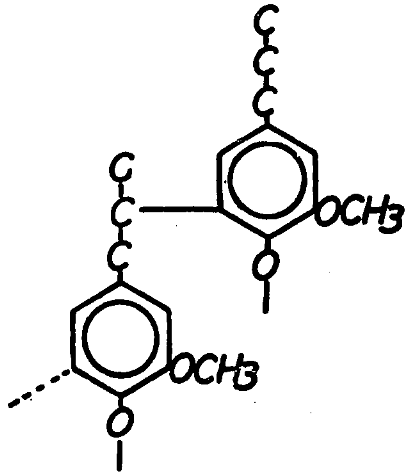


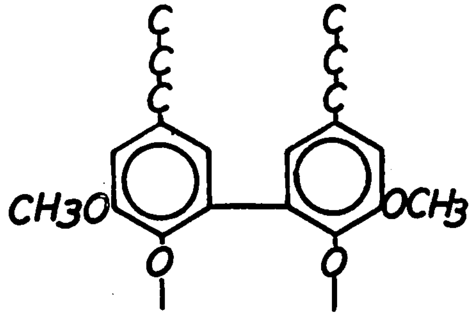
FIGURE 12 Products from the Oxidation of Spruce Nitrolignin with Permanganate (after Alkaline Hydrolysis and Methylation)

types (2). In the majority of substituted units, the 5-position is linked to a carbon atom of the side chain of the other unit.



β-5' intermonomeric linkage

These substituted units are partially converted to isohemipinic acid (XXVII) by the hydrolysis-methylation-oxidation treatments (69,70). The isolation of isohemipinic acid (XXVII) from nitrolignin indicated that some of β-5' C-C bonds had survived nitration. The small yield of dehydrodiveratric acid (XXVIII) showed that some biaryl links had also survived.



5-5' intermonomeric linkage

From spectrographic studies, Aulin-Erdtman (71) has estimated that about 5% of such units containing free phenolic hydroxyl groups were present in Brauns' native lignin from conifers and less in other preparations of coniferous lignins.

The veratric acid (XXVI) isolated was derived from unsubstituted guaiacyl units in the lignin macromolecule, while 5-nitroveratric acid (XXV) was derived from "nitrosated" units. Since it has been previously reported that part of the veratric (XXVI) and isohemipinic (XXVII) acids was destroyed during the oxidation (69), the amount of aromatic acids actually formed should have been larger than that found. As a matter of fact, pure 5-nitroveratraldehyde, when oxidized by $KMnO_4$ in alkali yielded only 61.9% of 5-nitroveratric acid (XXV), with no trace of residual 5-nitroveratraldehyde. This indicated that 5-nitroveratric acid (XXV) was also very sensitive to $KMnO_4$. The formation of XXV from nitrolignin substantiated the earlier conclusion that during the treatment with nitrous acid (at pH 2, 70°), in the absence of side-chain displacement by nitroso groups, nitrosation of the aromatic rings in lignin occurred in the vacant position *ortho* to the phenolic hydroxyls.

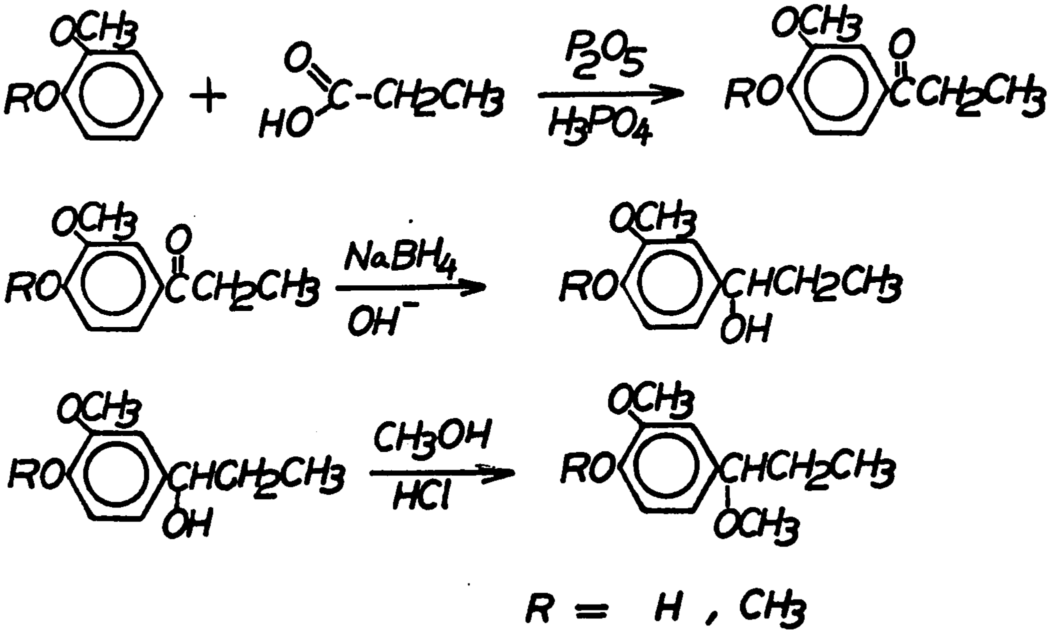
Reaction of Nitrous Acid with Model Compounds representing Units of Lignin

In the present studies, since the complexity of the products from the reaction of nitrous acid and lignin itself made structural evaluation extremely difficult, the subject was approached by using model compounds related to lignin. Although the compounds used here may have been simpler than the actual structures which occur in lignin, some of the products were the same as those obtained from isolated lignins treated with nitrous acid. The results from the model compound experiments have helped to explain the reaction of lignin under similar conditions.

1) Model Compounds for Softwood Lignin

Of the model compounds used, 1-(4-hydroxy-3-methoxyphenyl)-1-propanol (I), 1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane (II), 1-(3,4-dimethoxyphenyl)-1-propanol (III) and 1-(3,4-dimethoxyphenyl)-1-methoxypropane (IV) represented non-condensed, etherified and non-etherified softwood lignin units.

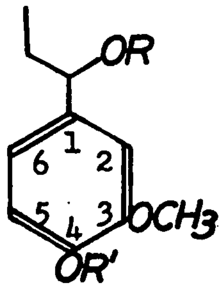
Compounds I and III were prepared by reducing 1-(4-hydroxy-3-methoxyphenyl)-1-propanone and 1-(3,4-dimethoxyphenyl)-1-propanone with sodium borohydride. The ketones had been obtained by means of a Friedel-Crafts type of synthesis, using polyphosphoric acid as the Lewis acid catalyst according to the procedure developed by Nakazawa (72) and by Gardner (73), respectively.



The completeness of reduction of the carbonyl functions was determined from infrared spectra. Compounds II and IV were prepared from their corresponding alcohols I and III by methylation with methanolic hydrochloric acid as described by Sergeeva and Shorygina (27). Elemental analysis of the methylated products gave results which agreed with the structures II and IV, and thus indicated that I, II, III and IV had all been the desired compounds.

When 1-(4-hydroxy-3-methoxyphenyl)-1-propanol (I) was treated with aqueous nitrous acid at pH 2, 70° for 1 hour, 4-nitroguaiacol (X), 4,6-dinitroguaiacol (XI) and 1-(4-hydroxy-3-methoxy-5-nitrophenyl)-1-propanone (XXIX) were isolated from among the reaction products. The yields* were X, 1.1%; XI, 13.8%; and XXIX, 1.6%. Similar treatment of 1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane (II) gave rise to 1.3% of X and 11.9% of XI, and a black gummy mixture whose nature could not be determined.

From the results it seemed apparent that the free guaiacyl units in lignin reacted readily under mild conditions, and that the nitroso



$R, R' = H$ or alkyl

---

* Yields are expressed here as a percentage of the starting material.

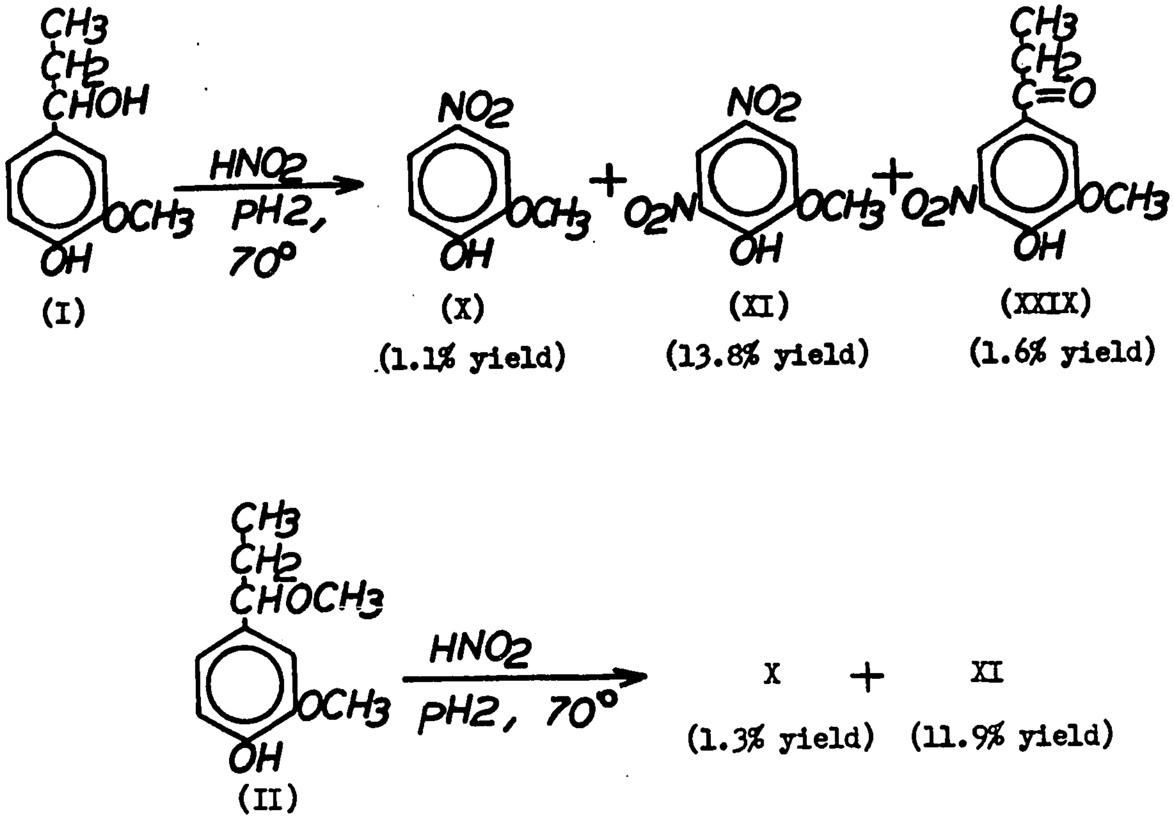
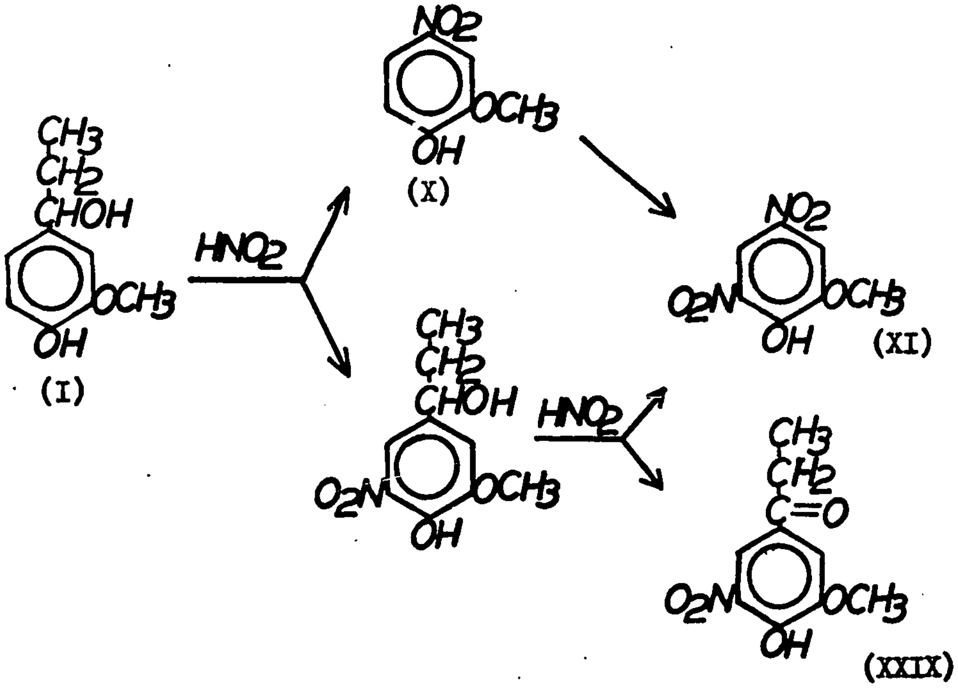


FIGURE 13 Products from Reactions of Nitrous Acid with 1-(4-Hydroxy-3-Methoxyphenyl)-1-Propanol (I) and 1-(4-Hydroxy-3-Methoxyphenyl)-1-Methoxypropane (II)

group substituted in the 5-position and displaced the side chain of these aromatic nuclei as well. From the isolation of the oxidation product, 1-(4-hydroxy-3-methoxy-5-nitrophenyl)-1-propanone (XXIX), it might be concluded that similar oxidation of benzyl alcoholic OH occurred in lignin. Moreover, it seemed that etherification of the benzyl alcohol group of guaiacyl units did not retard the side-chain displacement to a great extent, but it did lead to failure to isolate the benzyl ketone. Comparing the yields of X and XI from I and II, it seemed that oxidation of the side chain was not a necessary step preceding electrophilic substitution. Treatment of I (representing X groups in lignin) with nitrous acid might follow the sequence of reactions:



The first reactions were displacement of the side chain or nitration at C-5. The second reactions were displacement of the side chain or oxidation of the benzyl alcohol group. Compound II probably followed a similar reaction sequence except that no oxidation of the side chain occurred.

Etherification of the 4-hydroxyl group of guaiacyl units, however did have a strong retarding effect on the displacement of the side chain. This was demonstrated from the studies of the veratryl model compounds. The compound 1-(3,4-dimethoxyphenyl)-1-propanol (III), reacted with aqueous nitrous acid at pH 2 and 70° for one hour, formed 44.0% of 1-(3,4-dimethoxyphenyl)-1-propanone (XXX) and 11.8% of 1-(3,4-dimethoxyphenyl)-1-nitropropane (XXXI). From its reaction with nitrous acid at pH 2 and 70°, 1-(3,4-dimethoxyphenyl)-1-methoxypropane (IV) was recovered unchanged in a yield of 67.0%, and 1-(3,4-dimethoxyphenyl)-1-propanone (XXX) was found in 6.0% yield with a small amount (0.6%) of 1-(3,4-dimethoxyphenyl)-1-nitropropane (XXXI).

Failure to isolate 4-nitroveratrole (XXXIV) or 4,5-dinitroveratrole (XXXV) demonstrated that electrophilic substitution in compounds III and VI either did not occur at all, or was substantially retarded. When the results obtained were applied to consideration of lignin itself, they indicated that etherified guaiacyl units in lignin, under the same reaction conditions, probably were less prone to undergo electrophilic substitution, either the displacement of an aromatic hydrogen or a side-chain displacement than when they were free. More drastic treatment, such as increasing acidity, temperature, and HNO_2

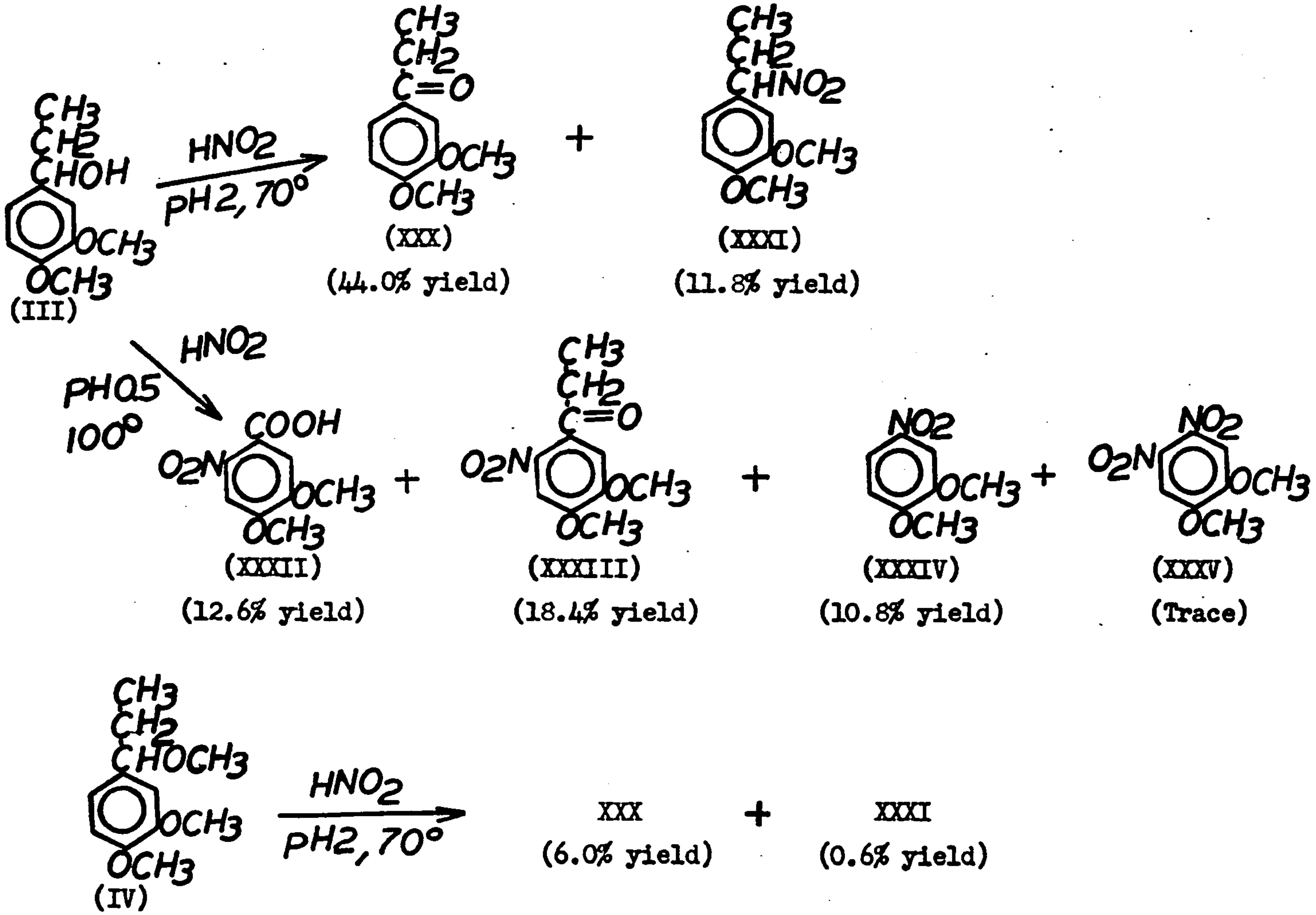


FIGURE 14 Products from Reactions of Nitrous Acid with 1-(3,4-Dimethoxyphenyl)-1-Propanol(III), 1-(3,4-Dimethoxyphenyl)-1-Methoxypropane (IV) and Veratryl Alcohol (XXXVI)

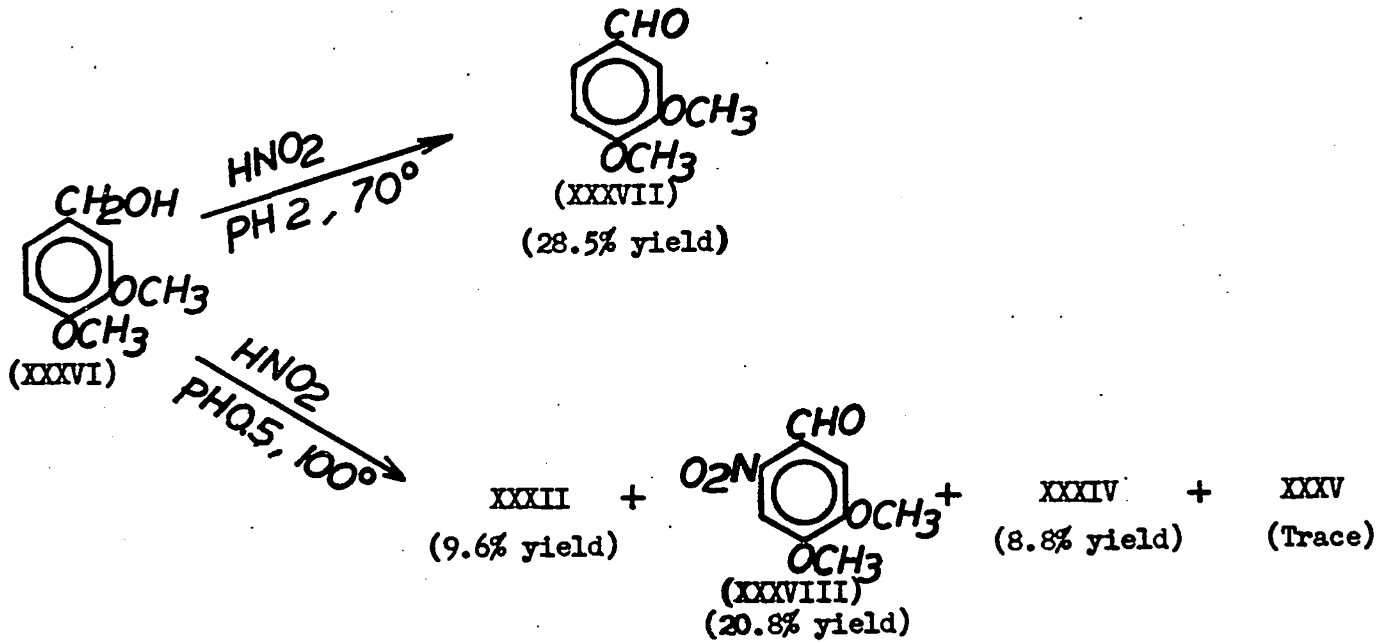
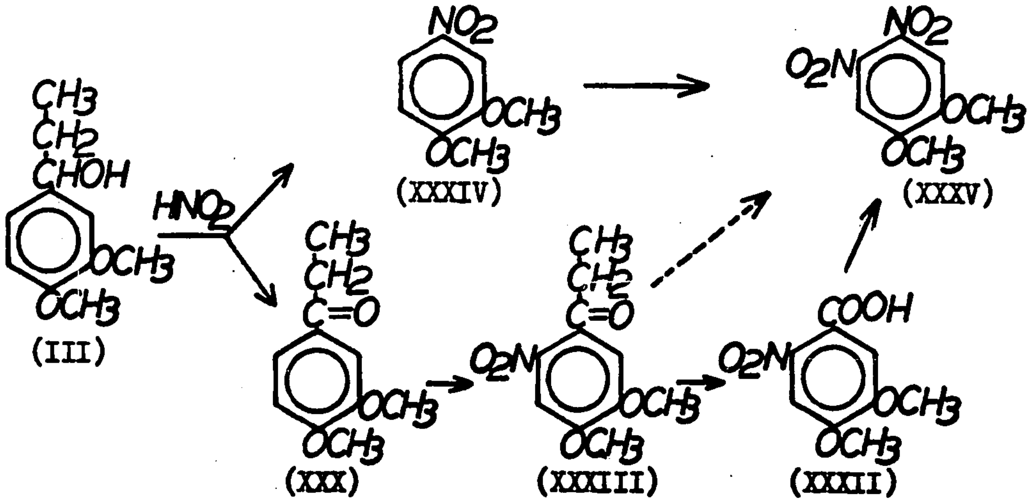


FIGURE 14 (cont'd.)

concentration is required.

In fact, III, when treated with HNO_2 at pH 0.5 and 100° for 1 hour (III: $NaNO_2$, 1:10), gave 6-nitroveratric acid (XXXII), 1-(3,4-dimethoxy-6-nitrophenyl)-1-propanone (XXXIII), 4-nitroveratrole (XXXIV) and 4,5-dinitroveratrole (XXXV). The yields were 12.6%, 18.4%, 10.8% and traces, respectively. By the same treatment, veratryl alcohol (XXXVI), which had yielded only veratraldehyde (XXXVII) at pH 2 and 70°, was transformed to 6-nitroveratric acid (XXXII), 6-nitroveratraldehyde (XXXVIII), 4-nitroveratrole (XXXIV) and 4,5-dinitroveratrole (XXXV). The yields were 9.6%, 20.8%, 8.8% and traces, respectively.

This evidence indicated that nitrosation of etherified guaiacyl units, under the conditions used in these experiments, resulted in the introduction of a nitro group into the ring on carbon 6, and electrophilic displacement and oxidation of the side chain as well. The sequence of reactions of III (representing the Z groups in lignin) may be generalized as follows :



The first reactions were oxidation of the benzylic hydroxyl group (main reaction) or displacement of the side chain. The α-keto group could have a retarding effect on further reactions. Only on treatment under drastic conditions was the NO_2 group introduced into the 6-position of the aromatic nuclei, and finally, the side chain oxidized again to give a carboxyl group. The nitro group at C-6 withdraws electrons from C-1, by both mesomeric and inductive effects, and induces stability of the side chain towards electrophilic displacement. Hence, only to a very small extent, the oxidized side chain of the nitro compound might be displaced by an NO_2 group to give 4,5-dinitroveratrole (XXXV). This finding was different from that reported with respect to chlorination of model compounds, in which the mesomeric effect of the substituted chlorine at C-6 would activate C-1 and counterbalance part of the inductive effect; in the chlorination of III, 4,5-dichloroveratrole was found in appreciable amount (21).

It should be noted that, in the present series of experiments on model compounds, although cleavage of the side chain, including oxidation and electrophilic displacement, took place only to the extent of 13-24%, such cleavage, especially that in Z groups, would effectively reduce the size of the lignin macromolecule. Such a reduction might increase the solubility of lignin even if cleavage had occurred only in a small proportion of the phenylpropane units.

The identify of a new compound 1-(3,4-dimethoxyphenyl)-1-nitropropane (XXXI) was determined by permanganate oxidation and by use of infrared, proton magnetic resonance and mass spectrometry.

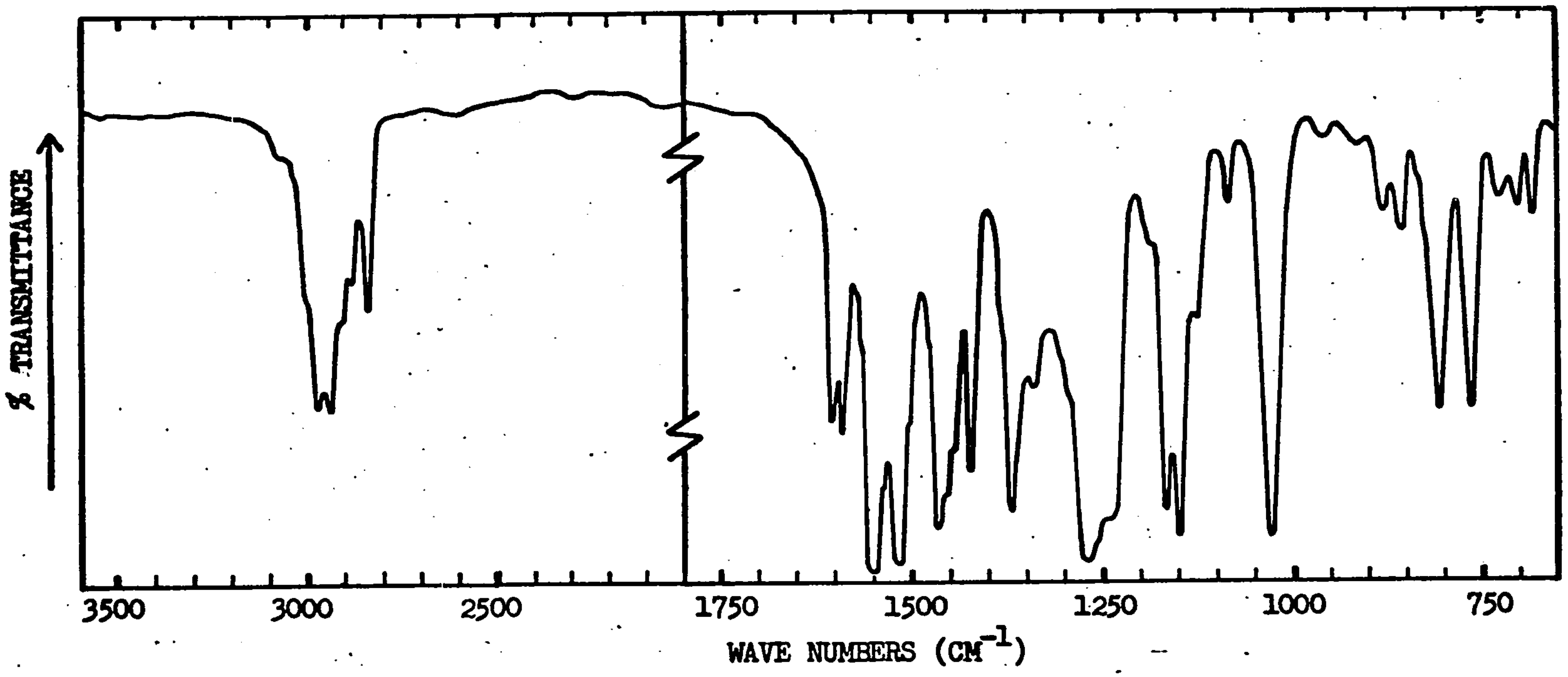


FIGURE 15 Infrared Spectrum of 1-(3,4-Dimethoxyphenyl)-1-Nitropropane (XXXI)

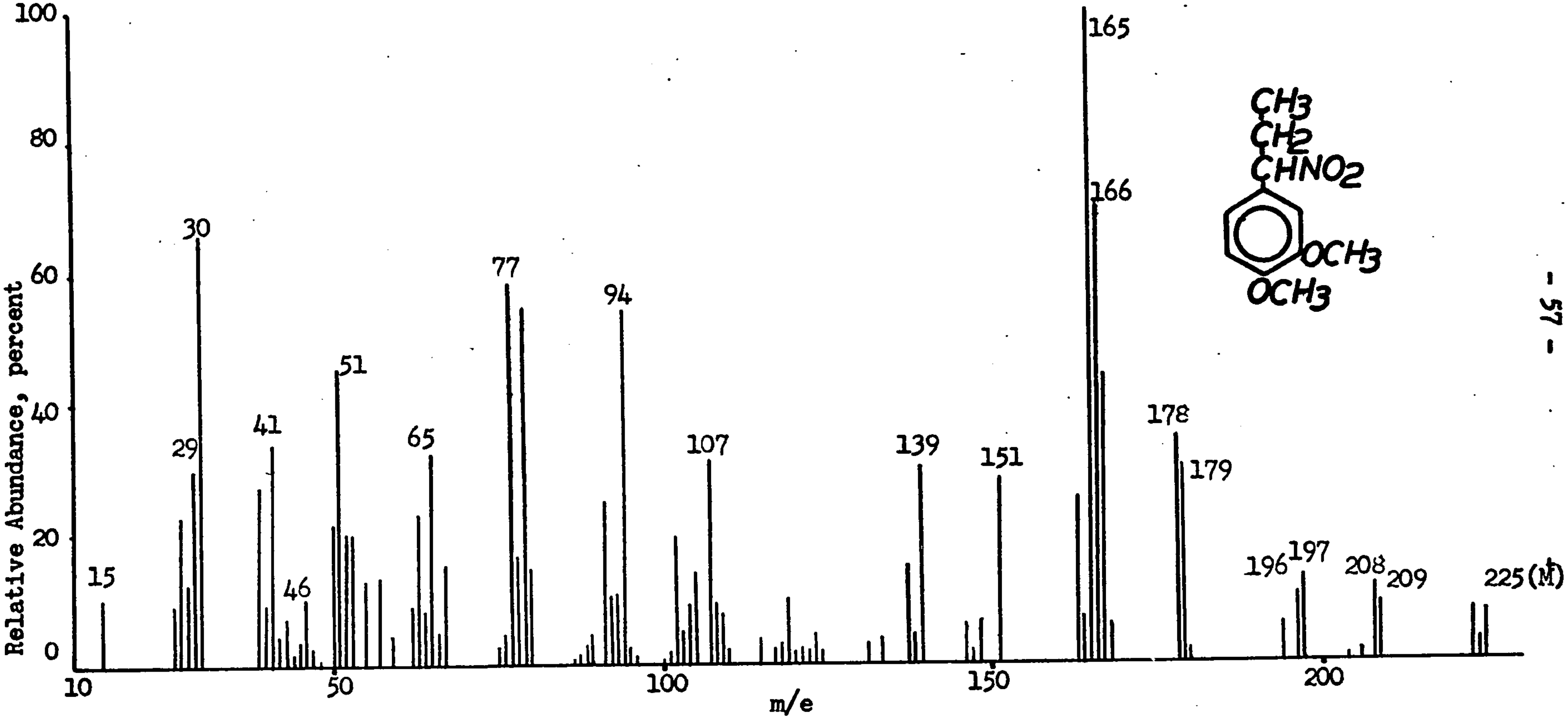
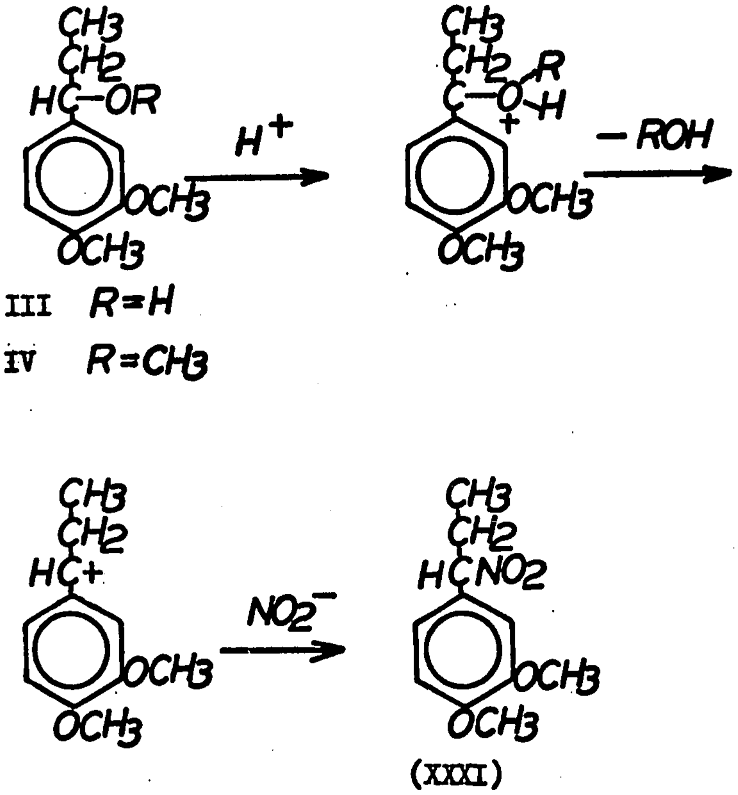


FIGURE 16 Mass Spectrum of 1-(3,4-Dimethoxyphenyl)-1-Nitropropane (XXXI)

The infrared spectrum of this compound (Figure 15) exhibited the asymmetric and symmetric stretchings of a nitro group at 1550 cm^{-1} and 1350 cm^{-1}, respectively. The p.m.r. spectrum ($CDCl_3$) had a singlet at δ 3.85 (area 6) corresponding to 2 methoxyls, and a multiplet between δ 6.60 to 6.92 corresponding to 3 phenyl ring protons. On oxidation with permanganate, the compound formed veratric acid (XXVI) in 68% yield, indicating that the nitro group must have been on the side chain located on carbon 4 of 1,2-dimethoxybenzene. The mass spectrum (Figure 16) showed a parent peak at $^{m}/_{e}$ 225, an intense peak at $^{m}/_{e}$ 179 corresponding to the loss of a nitro radical, and a peak at $^{m}/_{e}$ 46 due to the nitronium ion as well. There was also a peak at $^{m}/_{e}$ 196 due to loss of an ethyl radical and a peak at $^{m}/_{e}$ 29 due to the corresponding ion ($C_2H_5^+$). The result indicated the presence of a 1-nitropropyl side chain attached to the benzene ring.

The formation of such a compound from the action of nitrous acid on 1-(3,4-dimethoxyphenyl)-1-propanol (III) and its methyl ether (IV) has pointed to the possibility that nitro groups might be introduced into the aliphatic side chain and occupy a position α with respect to the aromatic ring when lignin itself is subjected to the same treatment. The formation of XXXI might occur through protonation of the hydroxyl group of III (or methoxyl group of IV), and formation of an intermediate carbonium ion, by analogy with the sulfonation of 1-(4-hydroxy-3-methoxyphenyl)-1-propanol (I) (74). Very recently, Shorygina and co-workers (75), on the basis of model compounds and lignin, reported that nitration of lignin in CCl_4 involved the introduction of nitrate groups into the α position of the aliphatic side chain. It is reasonable to assume that



III $R=H$

IV $R=CH_3$

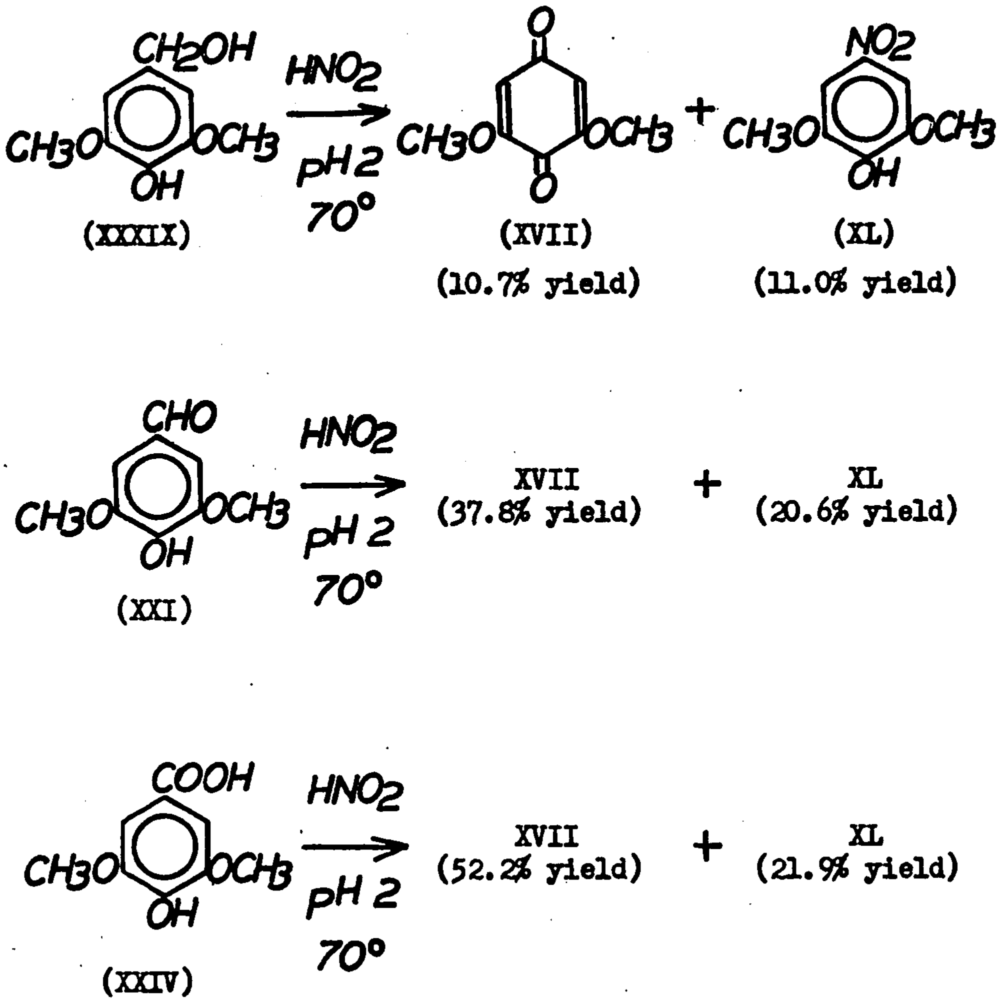
(XXXI)

formation of the nitrate might also pass through a benzyl carbonium ion, with attack by nitrate ion as a subsequent step.

2) Model Compounds for Hardwood Lignin

In complement to the work done by Bolker et al. (33) which indicated that a model for fully etherified hardwood lignin, such as 1,2,3-trimethoxybenzene (XVI) showed an appreciable degree of cleavage of the phenyl ether linkage and was converted to 2,6-dimethoxy-1,4-benzoquinone (XVII), reactions of nitrous acid with some related model compounds with a free phenolic hydroxyl group para to the aliphatic side chain were studied.

Syringyl alcohol (XXXIX), syringaldehyde (XXI) and syringic acid (XXIV), treated with nitrous acid at pH 2 and 70° for 20 minutes, produced (among other coloured products which have not been identified) 2,6-dimethoxy-1,4-benzoquinone (XVII) (yields: 10.7%, 37.8% and 52.2%, respectively) and 2,6-dimethoxy-4-nitrophenol (XL) (yields: 11.0%, 20.6% and 21.9%, respectively). Of these models for hardwood lignin, syringaldehyde (XXI) might be considered the least reactive towards nitrous acid, in the sense that from its reaction, about 5% of the unchanged starting material was recovered. Syringyl alcohol (XXXIX) and syringic acid (XXIV) both reacted vigorously and no starting material was detected among the products. Treatment of XXIV was accompanied by an immediate and rapid evolution of carbon dioxide, and the formation of a purple colour in the reaction mixture from which XVII and XL were isolated. The reaction of XXXIX was more complicated: although it reacted completely, it gave the lowest yields of XVII and XL. Other products were formed, but they

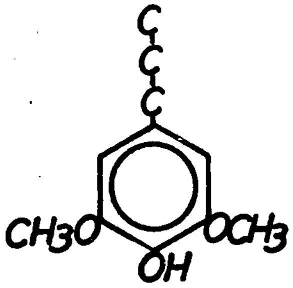


<u>FIGURE 17</u> Products from Reactions of Nitrous Acid with Syringyl Alcohol (XXXIX), Syringaldehyde (XXI) and Syringic Acid (XXIV)

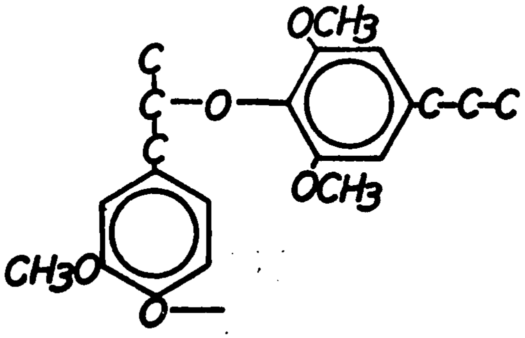
have not been identified.

According to Andersen (76), when phenolic compounds were treated with nitrous acid in ether or ether-acetone, the stability of substituents decreased in the order $CH_3 \gg CHO > CO_2H > CH_2OH$. The same order of stability appeared among the lignin model compounds studied by Gustafsson and Anderson (28). Under their conditions, it seemed that the release of substituents was the rate-determining step in the reaction process. Such a release would conceivably be facilitated by the stability of the departing species.

It has already been noted that Bolker et al. (33) found that 1,2,3-trimethoxybenzene (XVI) yielded 2,6-dimethoxy-1,4-benzoquinone (XVII), thus implying that XVII from the treatment of lignin had arisen from fully etherified syringyl units. Results from the present model compound studies have indicated the likelihood that the formation of XVII from hardwood lignin could also be attributed to oxidation of terminal syringyl units with free phenolic hydroxyl.



terminal syringyl unit



etherified syringyl unit

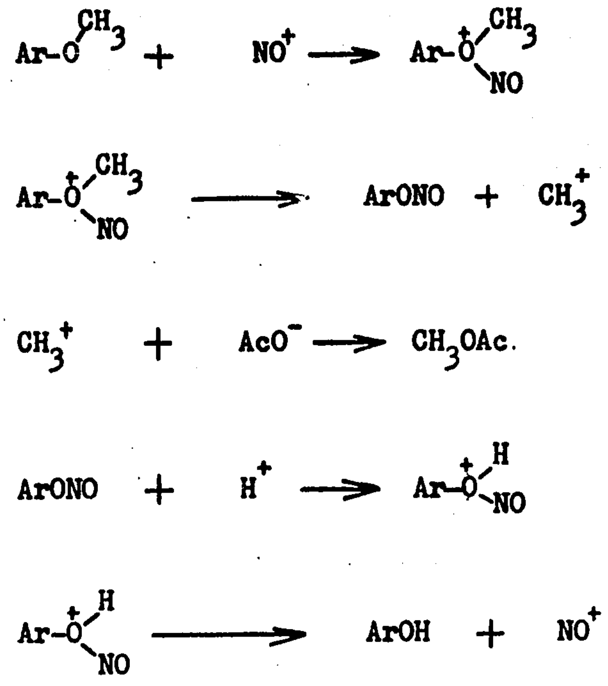
According to Levitt (77), the formation of this oxidation product from the above model compounds or terminal syringyl units in hardwood lignin should be initiated by the attack of a positive charged species such as NO^+, and should result in a nitroso intermediate. As a matter of fact, 2,6-dibromo-4-nitrosophenol has been obtained from 3,5-dibromo-4-hydroxybenzoic acid through electrophilic displacement of side chain by the action of $NaNO_2$ (53). The tautomeric behaviour of nitrosophenols (quinone oximes) is well known (78, 79). Bolker and Kung (50) have investigated the tautomerization of 2,6-dimethoxy-4-nitrosophenol (XLV) to 2,6-dimethoxy-1,4-benzoquinone-4-oxime (XLVI) which was then converted by nitrous acid to XVII (Figure 21). Based on their mechanism, a reaction scheme is here proposed for the cleavage of a syringyl unit from hardwood lignin by the action of nitrous acid (Figure 22). It is also possible that after nitrosation the nitroso intermediate may be oxidized to a nitro compound, as indicated by the formation of 2,6-dimethoxy-4-nitrophenol (XL).

The formation of XVII from an etherified syringyl unit involves cleavage of the ether group which lies between the two methoxyl groups in the syringyl unit. The mechanism of such a cleavage will be discussed in the next section.

Mechanism of Dealkylation and Subsequent Reaction

Dealkylation of phenolic ethers during nitration or nitrosation has been studied by several investigators. Bunton and co-workers (36) have suggested that dealkylation during nitration involved the addition of nitrosonium ion to the oxygen atom of a phenolic ether, followed by departure of the alkyl group as a carbonium ion. The aryl nitrite then suffered rapid acidolysis to yield a phenol (Figure 18). A second possible mechanism for dealkylation, which was suggested to Bunton et al. by Bunnett and Cook (36), involved a slow stage of nitrosation (or nitration) which led to an almost fully quinonoid oxonium ion, and then the loss of a methyl cation (Figure 19). Bunton et al. offered three pieces of evidence to support either of these mechanisms. First, the proportion of dealkylation under a single nitration mechanism was independent of the absolute rate of nitration; this indicated that the processes of dealkylation and of normal nitration had kinetics of the same form. Second, unlike anisole and p-chloroanisole, 2,6-dichloroanisole and 2,6-dimethylanisole were not dealkylated during nitration; the lack of dealkylation was attributed to steric hindrance in the formation, either of a branched side chain as in the first mechanism, or of the fully quinonoid structure as in the second. Third, the methyl group eliminated during the nitration of p-chloroanisole in acetic acid as solvent was recovered as methyl acetate.

Sobolev (32) has studied the oxidation of 4-methylguaiacol with nitric acid and has proposed that 5-methyl-3-nitropyrocatechol (XLII) was formed from 4-methyl-6-nitroguaiacol (XLI) via a termolecular displacement catalyzed by nitrosonium ion (Figure 20). The demethylation mechanism involved attack of a nitrosonium ion on the oxygen atom of the phenolic ether, and of a water molecule on the carbon atom of the methoxyl, followed by the loss of a proton and a methanol molecule, and, finally, the removal of the nitrosonium ion in consequence of the uptake of a proton to give XLII. Compound XLII could undergo further oxidation to



$$Ar{-}O{-}CH_3 + NO^+ \longrightarrow Ar{-}\overset{+}{O}(CH_3)(NO)$$

$$Ar{-}\overset{+}{O}(CH_3)(NO) \longrightarrow ArONO + CH_3^+$$

$$CH_3^+ + AcO^- \longrightarrow CH_3OAc.$$

$$ArONO + H^+ \longrightarrow Ar{-}\overset{+}{O}(H)(NO)$$

$$Ar{-}\overset{+}{O}(H)(NO) \longrightarrow ArOH + NO^+$$

FIGURE 18 Mechanism of Nitrous Acid-catalyzed Demethylation of Phenolic Methyl Ethers as proposed by Bunton *et al.* (36)

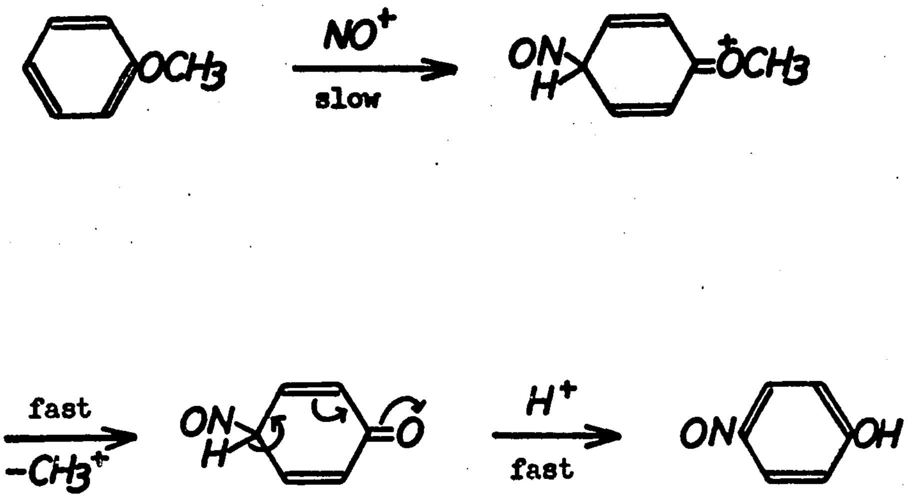


FIGURE 19 Mechanism of Nitrous Acid-catalyzed Demethylation of Phenolic Methyl Ethers as proposed by Bunnett and Cook(36)

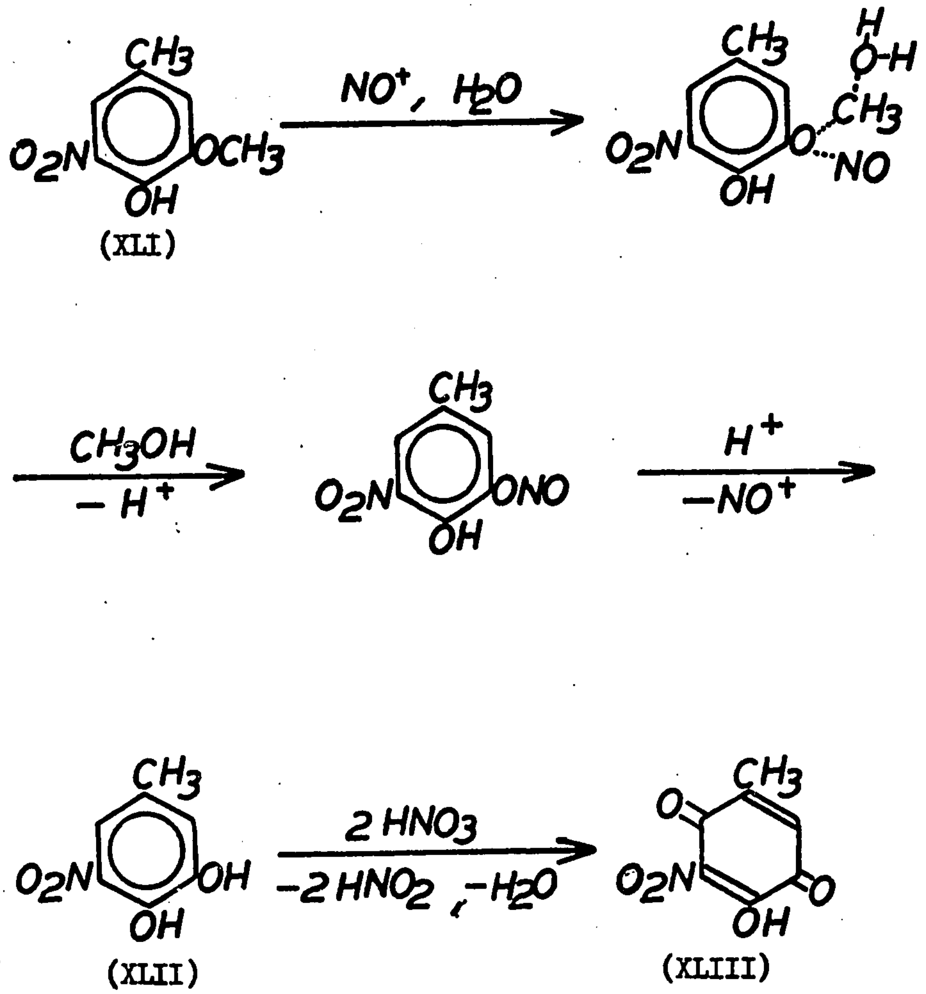


FIGURE 20 Mechanism of Nitrous Acid Oxidation of 4-Methyl-6-Nitroguaiacol (XLI) as proposed by Sobolev(32)

give 2-hydroxy-5-methyl-3-nitro-1,4-benzoquinone (XLIII). Sobolev concluded that formation of a "free" methyl carbonium ion in an S_N1 reaction as originally proposed by Bunton et al. was unlikely, since he had detected no methyl acetate, when the reaction had been run in nearly anhydrous acetic acid.

More recent work has been done by Bolker and Kung (50), in this laboratory, on the mechanism of formation of 2,6-dimethoxy-1,4-benzoquinone (XVII) from 1,2,3-trimethoxybenzene (XVI) by nitrous acid in an aqueous medium. The mechanism of demethylation was formulated as comprising the following steps (Figure 21): nitrosodeprotonation of XVI to give 5-nitroso-1,2,3-trimethoxybenzene (XLIV); approach of a proton to the nitroso group leading to an electron deficiency on carbon 2 which then attracted a water molecule; loss of a proton and methanol to give nitrosophenol (XLV), which tautomerized to its mono-oxime (XLVI); nitrous acid-catalyzed hydrolysis of XLVI to give the end product XVII. This mechanism stands in direct contradiction to that of Bunton et al. (36). In support of their mechanism, Bolker and Kung have offered two observations as evidence. First, they performed a control experiment for the one from which Bunton et al. had obtained 2.8 g of methyl acetate from 36.5 g of p-chloroanisole. In Bolker and Kung's experiment, without p-chloroanisole, but with a molar equivalent (8.17 g) of methanol in the reaction mixture, 2.5 g of methyl acetate was isolated, indicating that the methyl carbonium ion postulated by Bunton et al. could have been a secondary product of the reaction, while methanol could have been the primary one. Secondly, Bolker and Kung did, in fact, isolate a dealkylated product from 1,2,3-trimethoxybenzene (XVI). This evidence

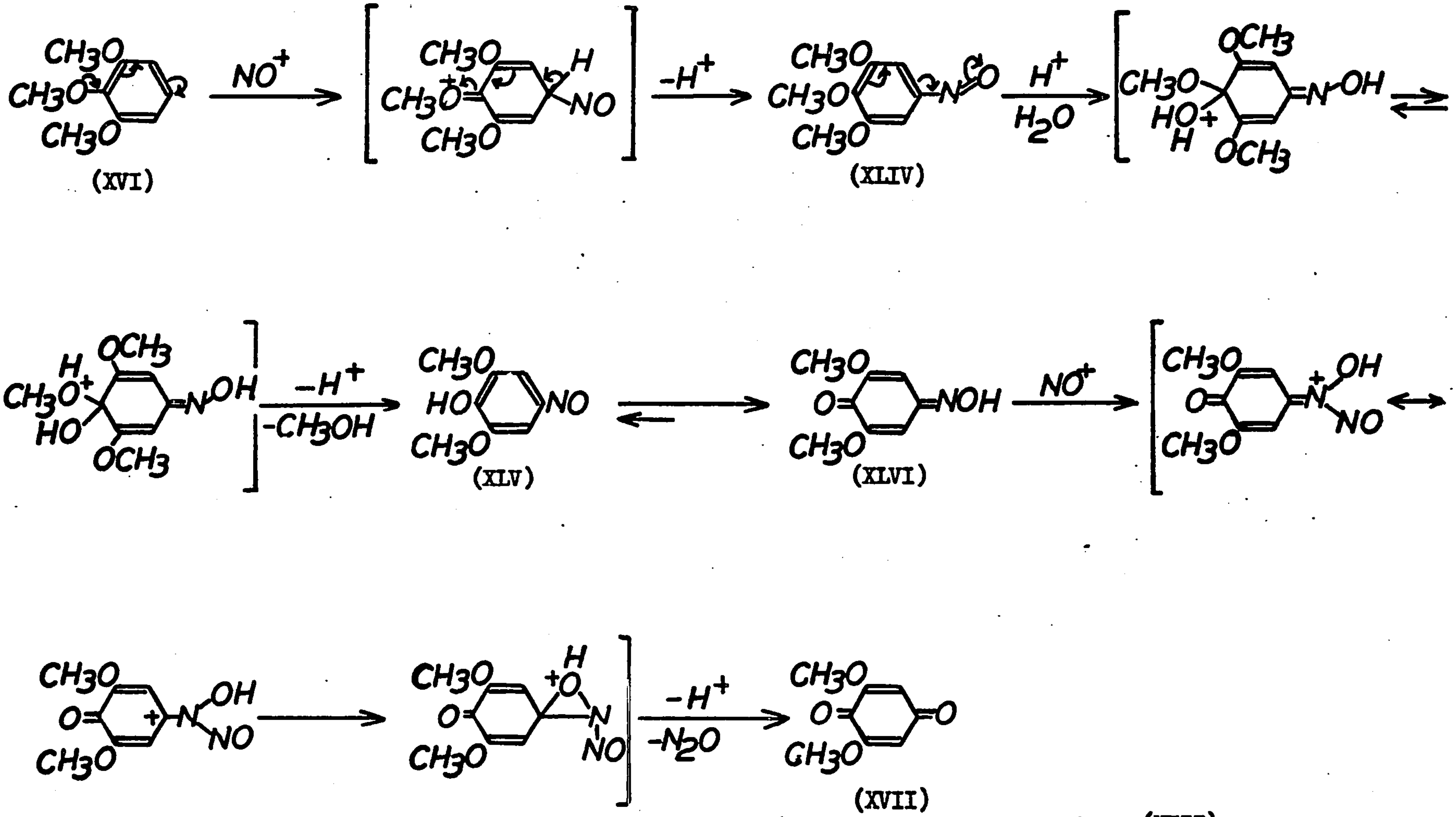
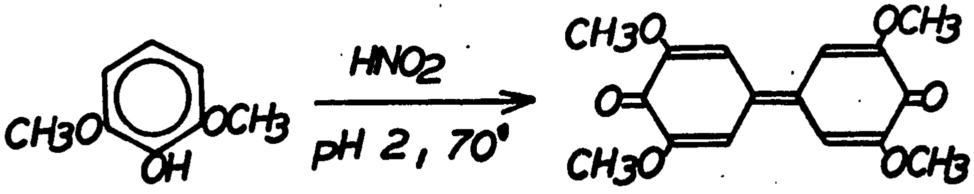


FIGURE 21 Mechanism of Formation of 2,6-Dimethoxy-1,4-Benzoquinone(XVII) by the Action of Nitrous Acid on 1,2,3-Trimethoxybenzene(XVI) as proposed by Bolker and Kung(44)

has made untenable Bunton's argument of inhibition of demethylation by steric hindrance.

The proposal that nitrosation was the first reaction in the course of demethylation was strengthened by the experimental evidence (33) that 2,6-dimethoxyphenol (XIV), treated with nitrous acid, produced 80% yield of 3,3′,5,5′-tetramethoxydiphenoquinone (XV). Hence



(XIV)

(XV)

(80% yield)

in the reaction of 1,2,3-trimethoxybenzene (XVI), nitrosation should occur before demethylation otherwise the dimer quinone (XV) would be found among the products.

In the present studies, part of the 2,6-dimethoxy-1,4-benzoquinone (XVII) which was isolated from hardwood lignin after treatment with nitrous acid, was presumed to have been derived from an etherified syringyl unit. It was reasonable to assume that its formation from lignin followed the mechanism proposed for its formation from 1,2,3-trimethoxybenzene (XVI). Figure 22 shows this mechanism as applied to the present problem. Since ionic organic oxidations are always considered to be initiated by the attack of cationic species derived from the oxidant (77), we may assume that oxidation of the syringyl unit is initiated by electrophilic displacement of the side chain by a nitroso group. The powerful activating effect of the nitroso group is enhanced by protonation leading to an electron deficiency of the ring at the _para_ position which is then attacked by a water molecule to give an activated protonated hemiketal. From this hemiketal 4-nitroso-2,6-dimethoxyphenol is then split and tautomerizes to an oxime which finally undergoes a nitrous acid-catalyzed hydrolysis to give 2,6-dimethoxy-1,4-benzoquinone (XVII) by the mechanism proposed by Wieland and Grimm (80).

Though the mechanism proposed by Sobolev (32) cannot be applied to the formation of 2,6-dimethoxy-1,4-benzoquinone (XVII) from 1,2,3-trimethoxybenzene (XVI) and hardwood lignin, it might account for the demethylation which was observed in both softwood and hardwood lignins. It was mentioned earlier that nitration or nitrosation on guaiacyl derivatives would only induce substitution on the position _ortho_ or _para_ to the phenolic hydroxyl group; it seems difficult to introduce a nitroso group to the 5-position of 4-methylguaiacol and even more difficult to do so in 4-methyl-6-nitroguaiacol (XLI) in order for the demethylation to proceed by the mechanism proposed by Bolker and Kung. Furthermore, Sobolev (32) did isolate the demethylated product, 3-nitropyrocatechol (XLII) from 4-methyl-6-nitroguaiacol (XLI) and thus gave strong support to the mechanism which he proposed.

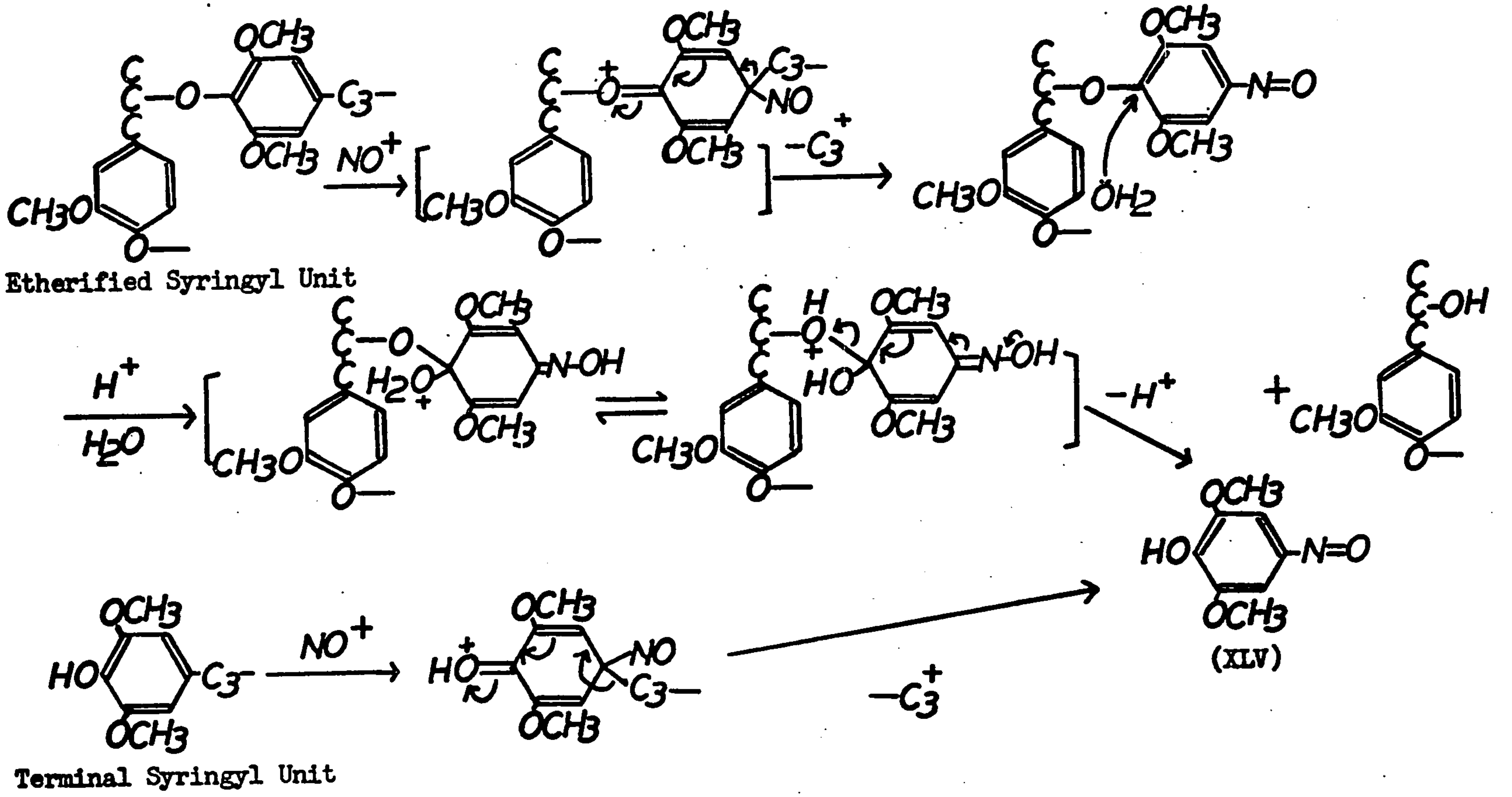


FIGURE 22 Proposed Mechanism for the Formation of 2,6-Dimethoxy-1,4-Benzoquinone (XVII) by the Action of Nitrous Acid on Hardwood Lignin

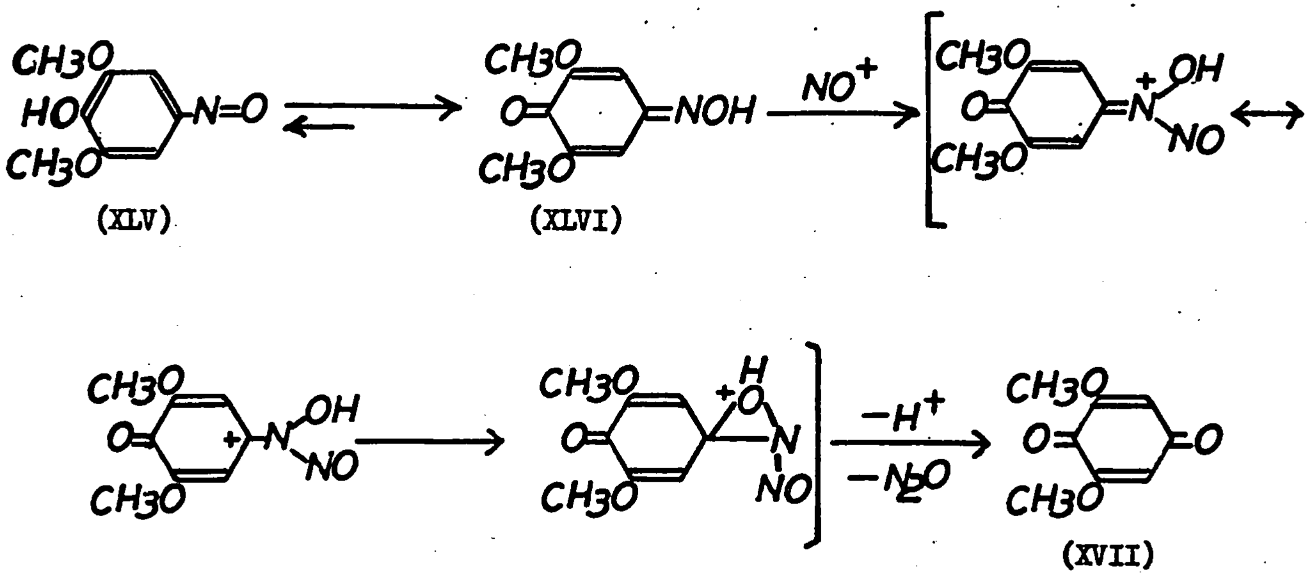


FIGURE 22 (cont'd.)

GENERAL CONCLUSIONS

There is one striking difference between the results obtained in experiments with spruce lignin and those with model compounds. The low-molecular weight nitrated products from lignin, whenever they were nitrated at all in any position other than at C-1, were invariably nitrated at C-5. This seems an indication that all such products were derived from guaiacyl units ("X groups") in the lignin. Figure 23 shows how these compounds were formed, and how the formation of 4-nitroguaiacol (X) and 4,6-dinitroguaiacol (XI) does not break the polymer chain. The chain is broken only when 5-nitrovanillin (XX) and 5-nitrovanillic acid (XXIII) are split out by the action of alkali.

On the other hand, from veratryl model compounds (III and XXXVI) products substituted with nitro groups at C-6 were obtained. Since there are believed to be nearly two-and-a-half times as many etherified phenolic groups as free groups in lignin, some answer must be offered as to why 6-nitro compounds were never isolated.

The explanation lies in the further observation that no evidence was found of demethylation (of aromatic methoxyl groups) among the model compounds III, IV and XXXVI. Although it may be argued that demethylated compounds might have been present among the unidentifiable products, considerable weight must be given to the fact that not even a trace of such a compound was detected. Thus, if, as seems probable, nitrous acid does not break aromatic ether links in veratryl compounds, those units with the 6-nitro group, derived from "Z groups", must remain

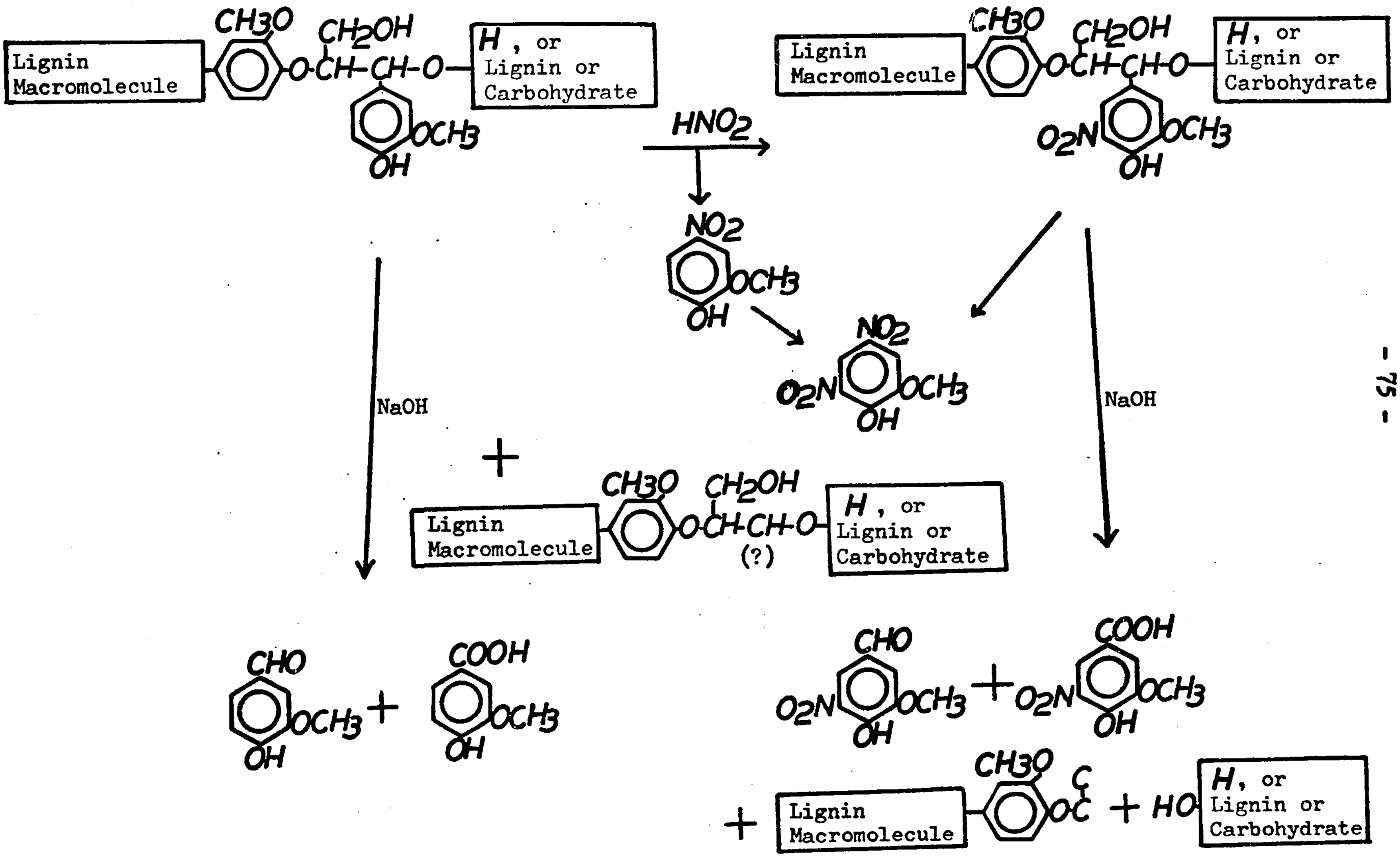


FIGURE 23 Reactions of "X Groups" in Lignin

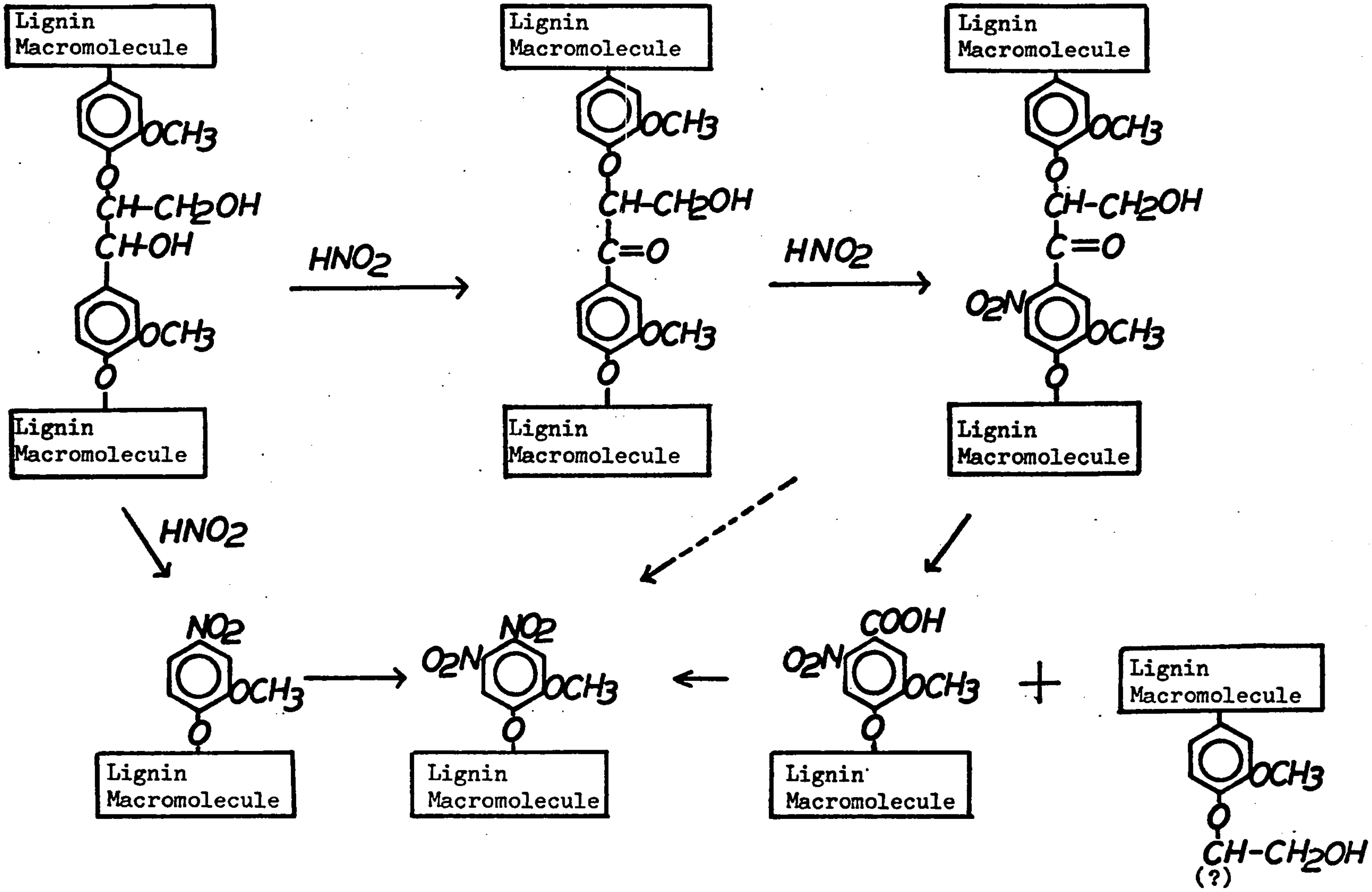


FIGURE 24 Reactions of "Z Groups" in Lignin

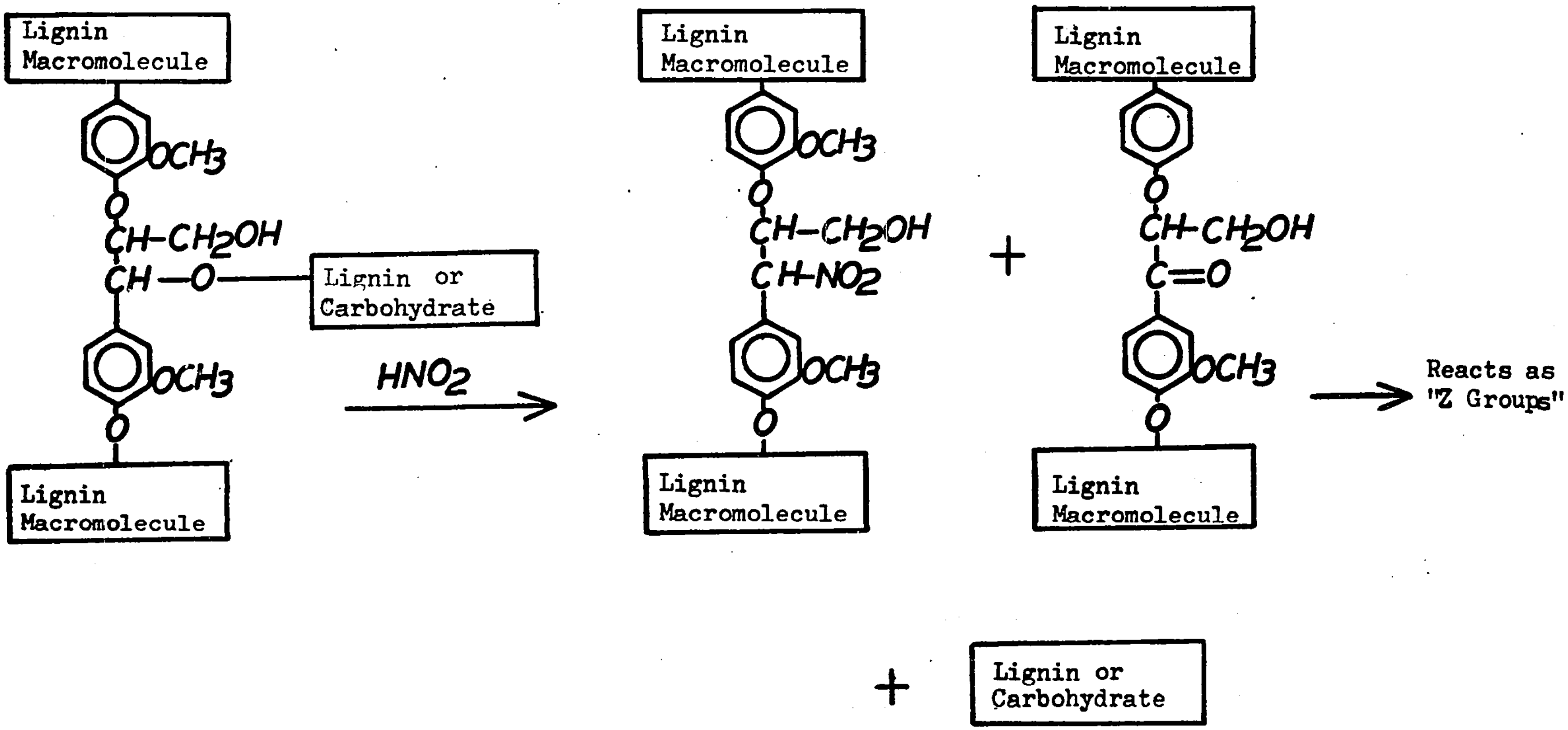


FIGURE 25 Reactions of "B Groups" in Lignin

attached to macromolecular fragments of lignin, as shown in Figure 24. This conclusion holds even when the reaction conditions are sufficiently harsh to break the polymer chain at C-1 or between the α- and β-carbons of the side chains.

The "B groups" of lignin are of special significance, for they represent trifunctional units, and as such are sites of cross-linking within lignin itself or with carbohydrate residues. It is reasonable to assume that the cleavage of such cross-links constitutes as important a contribution towards the solubilization of lignin as does simple reduction in molecular weight. Indeed, it may be more important, since reduction in molecular weight is difficult to achieve unless an appreciable proportion of cross-links are broken.

Figure 25 illustrates the fate of "B groups" under normal conditions of degradation of lignin with nitrous acid. The benzyl ether groups are cleaved, and the trifunctionality of the groups is reduced to difunctionality. If, as is thought, linkages with carbohydrate occur at the benzyl carbon, then this reaction has a special meaning in terms of releasing lignin into solution. However, the fact that the yields of nitro and keto products from compound IV were not much more than six percent may explain why delignification with nitrous acid has not been found to be as efficient as might be desired.

Figures 24 and 25 illustrate yet another point: they show why the spectra and analyses of "nitrolignins" indicate the presence of nitro groups (presumably some alkyl nitro, but mainly 6-nitro) and a higher content of carbonyl groups, both conjugated and unconjugated, than in the untreated lignins.

The model compounds employed in the present series of experiments were actually inadequate to represent the complex structure found in lignin itself. In evaluating the significance of the products, the influence of carbon-carbon bonds, and of more fully oxygenated side chains could not be estimated.

Nevertheless, the conclusions so far described lead to another line of thought that may have even wider significance in the general field of delignification.

The picture published by Freudenberg in 1965 of an "average" fragment of lignin (Figure 26) (81) may still be incomplete, but it is useful to consider it in the terms just discussed, and to examine the effects of cleavage of its X, Z and B groups.

Freudenberg's model contains 7 1/2 trifunctional units among eighteen phenylpropane monomer units. Of these, two are attached at one link to end-groups, and cannot be considered cross-links. Nevertheless, the model may be described as having only about 3.3 linear units per cross-link. This is surely a ratio which would render any polymer completely insoluble. The idea that natural lignin is insoluble is quite in accord with the views of Pew (82) and others. At least 85% of the cross-links must be broken (to achieve, say, a ratio of about 20 linear units per cross-link) before one might expect such a molecule to become slightly soluble.

Cleavage of "X groups" is not efficient in this respect. Some breakage of chains occurs (Figure 23) so that new end-groups are formed to render cross-links ineffective, but the molecule probably retains a

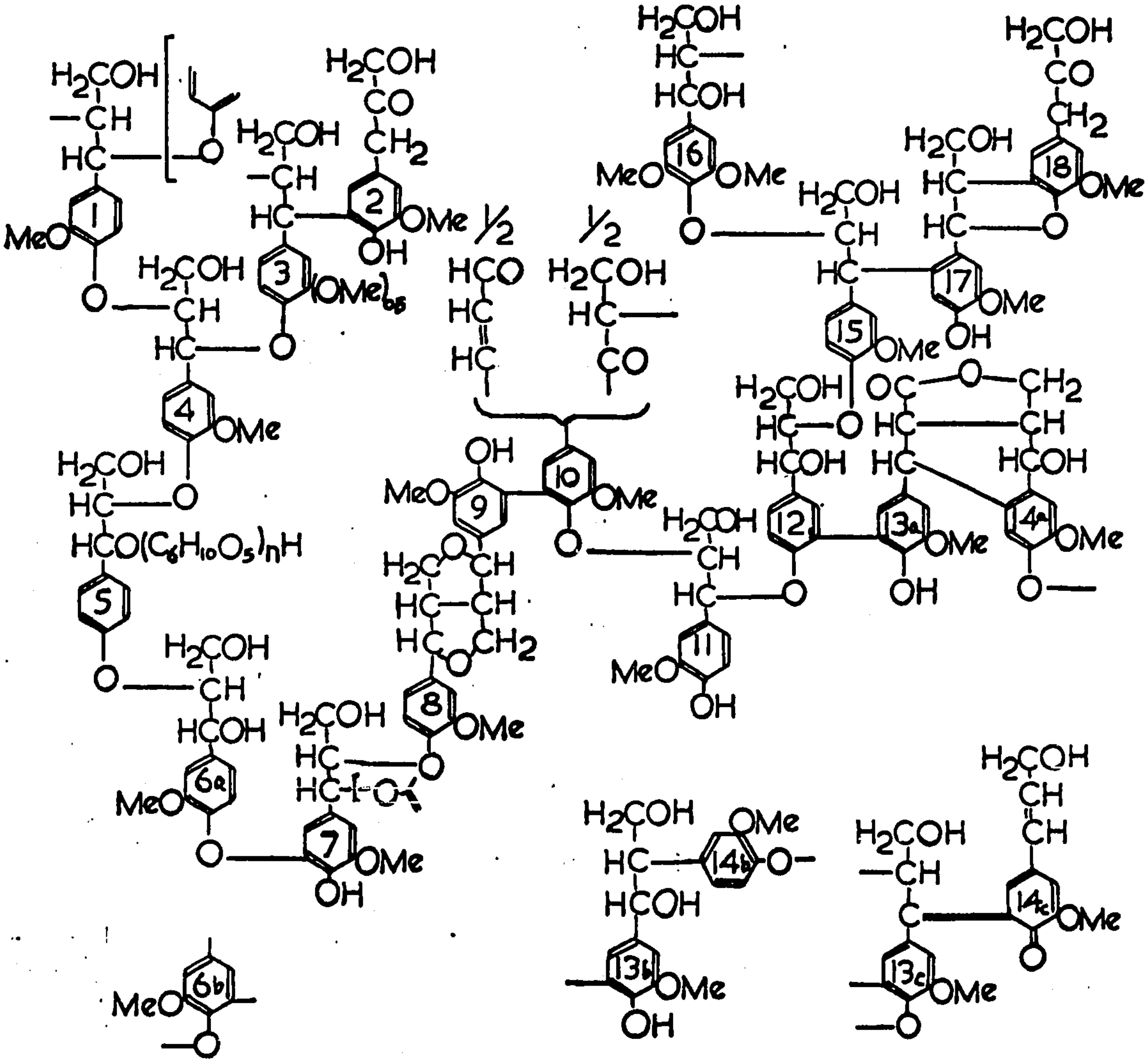


FIGURE 26 Constitutional Model of Spruce Lignin (81)

ratio of about 4.5 linear units per effective cross-link.

When "Z groups" are cleaved (Figure 24), the ratio of linear units to cross-links is again about 7.2 to 1.

However, when the benzyl ether groups are completely broken, the net effect on this model (Figure 25) is that all cross-links are lost..

This reasoning may seem fanciful, but it leads to a conclusion which may be subjected to experimental test: that any reagent which cleaves the benzyl ether links of models of the "B" type faster and more extensively than another reagent, should be the more effective and efficient agent for delignifying wood or pulp. This prediction has already received a partial test in the recent work of C.H. Chew (83) who found that dialkoxypropanes were somewhat more efficient than alcohols in reacting with the benzyl ether links of models for the "B groups". This result accorded with the previous finding of Bolker and Terashima (84) that dialkoxypropanes extracted lignin from wood far more rapidly and in greater yields than when alcohols were used as agents of delignification.

If the converse of this proposition also proves to be true then certain of the puzzling features of commercial delignification may become explicable.

Models for hardwood (birch) lignin have not been extensively investigated in the present research. An earlier study had pointed to the possibility that fully etherified syringyl compounds might readily undergo de-etherification when treated with nitrous acid. This was taken as the reason for the readier degradability of hardwood lignins. From the present work it was seen (Figure 17) that syringyl alcohol (XXXIX) and syringaldehyde (XXI) gave the same products as had been obtained from 1,2,3-trimethoxybenzene (XVI), but, in this instance, yields were considerably higher. These reactions must contribute to depolymerization, but, as the structure of hardwood lignin is even less well-known than that of softwood lignin, the significance of these new observations is not readily apparent.

EXPERIMENTAL

Analytical Methods

Infrared spectra were measured on Unicam SP-100 and SP-200 prism-grating spectrometers. P.m.r. spectra were recorded on a Varian A-60 spectrometer, with tetramethylsilane as an internal standard. Mass spectra were determined by the Morgan Schaffer Corp., Montreal, on a Hitachi Perkin-Elmer RMU60 mass spectrometer operating at 70 ev. All the melting points were determined with a calibrated Fisher-Johns apparatus. Acidity of the solutions was measured with a Beckman glass electrode pH meter, Model H2. Microanalyses (C,H,N) were performed by Beller Mikroanalytisches Laboratorium of Göttingen, West Germany. Determination of Klason lignin was done by the general procedure outlined in Tappi Standards (85).

Methoxyls were determined using the method (slightly modified) developed by Cobler, Samsel and Beaver (82), for determining alkoxyl groups in alkyl cellulose ethers. A quantity of 20-30 mg lignin was suspended in a solution of 2 grams of phenol and 6 ml of hydroiodic acid, and the mixture was heated at 150° for 1 hour in an atmosphere of nitrogen. Liberated methyl iodides were distilled through a washing device, containing water, to a trap containing 2 ml of 2,2,5-trimethylhexane cooled by dry ice in acetone. Of this solution, 50 µl were used for analysis on the gas chromatograph at 75°. A Perkin-Elmer vapor fractometer model 154, with a column of diisodecylphthalate (Perkin-Elmer column A), was used for this purpose. Curves for quantitatively measuring methyl iodides had been prepared previously using p-methoxyphenol and vanillin as standards.

Preparation of Wood Meal

Birch wood

Canadian white birch wood meal, 60 mesh, was extracted continuously with a mixture of ethanol-benzene (1:1) for 48 hours, washed with hot water, air-dried, and finally dried to constant weight in a vacuum desiccator over phosphorus pentoxide. Analysis: Klason lignin,(a) 22.46%.

Spruce wood

Black spruce wood meal, 60 mesh, was extracted with ethanol-benzene (1:1) for 48 hours, washed with hot water, air-dried, and finally dried to constant weight over phosphorus pentoxide. Analysis: Klason lignin,(a) 28.53%.

(a) Ash and moisture-free basis

Preparation of Dioxane Lignins

Dioxane lignins were isolated from wood meals by modifying the procedure of Pepper (86), and using 0.2N HCl dioxane-water (9:1) as extracting solvent.

Birch dioxane lignin

Two hundred grams of pre-extracted, vacuum-dried birch wood meal was placed in a 4-liter, three-necked, round bottom pyrex flask, fitted with reflux condenser, nitrogen inlet, a two-necked adapter with a dropping funnel and a three-way stopcock. The stopcock was connected to a water pump. The flask was first evacuated by the water pump for 15-20 minutes. Then the stopcock leading to the pump was closed and 2 liters of solvent was added from the dropping funnel slowly so that no air entered the flask. The pressure in the flask was restored by slowly introducing nitrogen. Another 2 liters of solvent was added. The flask was gradually heated to 87±2° in an oil bath. This temperature was maintained for one hour. A slow stream of nitrogen was maintained throughout the period of heating and subsequent cooling. The reaction mixture was cooled down to about 35° and filtered. The wood residue was washed with neutral dioxane-water (9:1) until the washings became colourless.

The red-brown filtrate and washings were combined, and reduced to a volume of about 300 ml by vacuum evaporation. The concentrate was pipetted slowly into 16 liters of vigorously stirred water. After the whole concentrate had been added, the precipitated lignin was recovered by centrifugation. It was then washed several times with a large excess of oxygen-free water until neutral. Finally, it was freeze-dried and weighed. The yield was 26.46 grams (i.e., 58.9%, based on Klason lignin). Analysis: carbon, 58.6%; hydrogen, 5.97%; nitrogen, 0.00%; methoxyl, 20.7%.

Spruce dioxane lignin

Pre-extracted wood meal, 200 grams, was first extracted with 0.2N HCl dioxane-water (9:1) and the lignin was recovered according to the procedure described above. The yield was 10.80 grams (i.e., 19.5%, based on Klason lignin). Analysis: carbon, 63.9%; hydrogen, 6.13%; nitrogen, 0.00%; methoxyl, 15.4%.

General Procedure for the Reaction of Lignins with Nitrous Acid

Solutions of nitrous acid were prepared by gradually adding hydrochloric acid to a stirred solution of 2 grams of $NaNO_2$ in 150 ml of distilled water until the desired pH was reached, as read on a pH meter. To prepare solutions at pH 1, 2 and 3, about 56.0 ml, 28.0 ml and 14.5 ml of 1.0N HCl were used respectively.

Two grams of dioxane lignin was introduced to the above freshly prepared nitrous acid solution in a three-necked 300 ml flask, equipped with a water-cooled reflux condenser. By means of an oil bath placed over a magnetic stirring hot plate, the flask was rapidly heated to the desired temperature. The reaction mixture, under constant stirring, was kept at this temperature for the desired reaction time. A slow stream of nitrogen was maintained throughout the reaction period. The mixture was then cooled to room temperature and filtered. The brown residue was washed with distilled water until the washings were neutral to pH paper. It was freeze-dried and then analyzed.

Following this general procedure, lignins were treated at the different initial acid concentrations (pH 0.5, 1, 2 and 3), different reaction temperatures (30°, 50°, 70°, 90°) and different reaction times (1, 3, 5 hours). The effects of the reaction conditions on the composition of the insoluble products can be seen from the analytical results given in Table II, III and IV.

The filtrate and the washings were combined. Analysis of this aqueous solution is described in the following sections. Yields of the products are expressed as a percentage of the starting material.

Products from Birch Dioxane Lignin treated at pH 2 and 70° for 1 hr.

Treating birch dioxane lignin with nitrous acid at pH 2 and 70° for 1 hour yielded a brown insoluble fraction. The yield was 1.8 g (90%).

Analysis showed carbon, 56.3%; hydrogen, 5.86%; nitrogen, 0.93%; and methoxyl, 18.2%.

The filtrate and washings were combined and extracted with ether (4 times, 50 ml each). The aqueous layer was retained for later work. The ether solution was extracted with saturated aqueous $NaHCO_3$ solution (4 times, 40 ml each), the $NaHCO_3$ extract was acidified and back-extracted with ether (4 times, 30 ml each). This last ether extract was dried over anhydrous sodium sulfate, and evaporated to yield 52 mg of a yellow residue, m.p. 103-118°, corresponding to 2.64% of the starting material. Recrystallization from water gave 10 mg of yellow needles, m.p. 119.5-120°, identical with 4,6-dinitroguaiacol (XI), prepared according to Klemenc (88).

The $NaHCO_3$-extracted ether solution was further extracted with 1 N NaOH (4 times, 40 ml each). Acidifying the NaOH extract with dilute hydrochloric acid, extracting with ether, drying with anhydrous sodium sulfate and evaporating the ether extract gave 18 mg of a pale yellow residue (m.p. 90-98°); 1.06% yield, which could be recrystallized from ether-petroleum ether to yield yellow needle-like crystals (approximately 3 mg, m.p. 99-100°), found, by infrared spectrum and mixed m.p., to be identical with authentic 4-nitroguaiacol (X) prepared according to Pollecoff and Robinson (89).

The original ether-extracted aqueous layer was extracted with chloroform (5 times, 50 ml each), and the combined chloroform extracts after drying with sodium sulfate and evaporation gave a pale yellow compound (m.p. 249-252°); the yield was 21.2 mg (1.06%). This ether-insoluble product was completely soluble in chloroform and benzene. Crystallization from acetic acid gave yellow crystals of m.p. 251-252° (sealed tube), identical with authentic 2,6-dimethoxy-1,4-benzoquinone (XVII) (90) by mixed m.p. and infrared spectrum.

The reaction of nitrous acid with birch dioxane lignin was repeated. The acidic liquor was neutralized by ammonia, and 60 ml of saturated calcium acetate solution was added. The precipitated calcium salt was separated, washed with water and then dissolved in 40 ml of dil. HCl; the resulting solution was extracted with ether (3 times, 20 ml each) and ether solution evaporated to give 36 mg of a white crystalline substance, m.p. 101.0-101.4°. Its melting point and infrared spectrum were identical with those of oxalic acid. The yield was 1.80%.

Products from Spruce Dioxane Lignin treated at pH 2 and 70° for 1 hr.

Spruce dioxane lignin was treated by the same procedure as the birch. The yield of insoluble residue was 95%. Analysis: carbon, 58.3%; hydrogen, 5.53%; nitrogen, 2.06%; and methoxyl, 13.5%. Extraction of the water-soluble fraction yielded 96.2 mg of 4-nitroguaiacol (X) (4.81%) and 9.4 mg of 4,6-dinitroguaiacol (XI) (0.47%). No 2,6-dimethoxy-1,4-benzoquinone was found. Oxalic acid was isolated in 1.09% yield.

Alkaline Hydrolysis of Birch Nitrolignin

Ten grams of birch nitrolignin obtained from treatment at pH 2, 70° for 1 hour, was immersed in 100 ml of 1.0N sodium hydroxide solution and was stirred for 24 hours at 80°. The reaction mixture was then filtered and the filtrate was acidified with dilute hydrochloric acid to about pH 3. A brown precipitate was produced. The mixture was heated to boiling and filtered while hot, and the residue was washed with boiling water until it was neutral. This brown powdered residue was freeze-dried and weighed. The yield was 7.61 grams (76.1%). Analysis: carbon, 53.3%; hydrogen, 5.70%; nitrogen, 0.20%; and methoxyl, 17.1%

Isolation of Products of Hydrolysis of Birch Nitrolignin

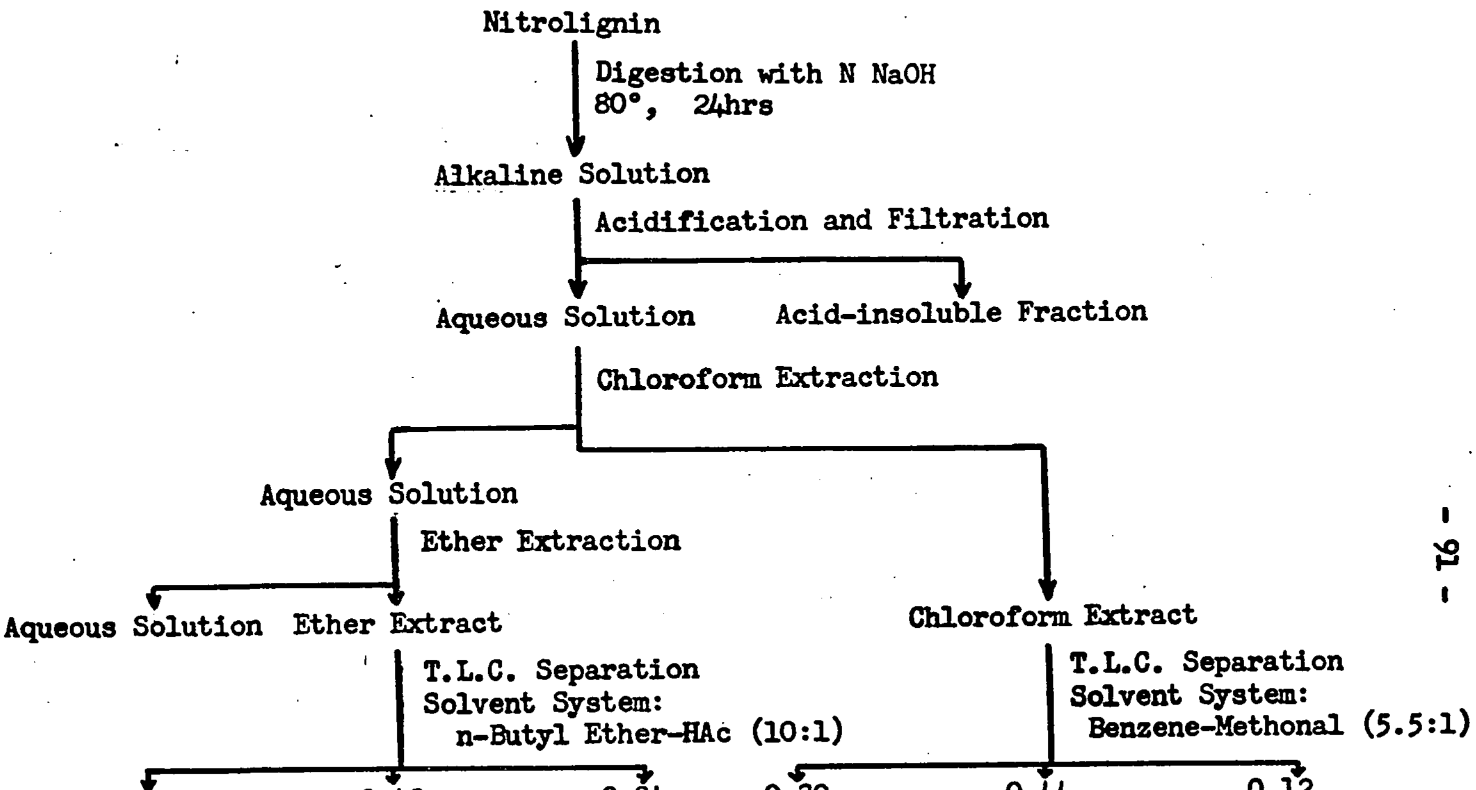
The filtrate from the above treatment was extracted with chloroform (3 times, 40 ml each). These chloroform extracts were combined, dried with anhydrous Na_2SO_4, and then evaporated. The aqueous layer from chloroform extraction was then extracted with ether (4 times, 50 ml each). These ether extracts were combined, dried over anhydrous Na_2SO_4, and evaporated. The ether and chloroform extracts were each used for analyses by thin-layer chromatography.

The chloroform extract was chromatographed on a thin layer of silica gel, with a solvent mixture of methanol-benzene (1:5.5). Its chromatogram revealed the presence of more than six separate compounds, which could not all be identified. Preparative TLC was used to isolate the materials of R_f 0.56, 0.44, 0.30 and 0.12. The zones at these R_f values were scraped from the plates and each was extracted with methanol. The methanol extracts were evaporated and then dissolved in chloroform, and the $CHCl_3$ solutions, after being washed several times with dilute HCl solution, were dried with anhydrous Na_2SO_4 overnight. Evaporating the dried chloroform solutions yielded residues of 0.083 g (R_f 0.56), 0.170 (R_f 0.44), 0.221 g (R_f 0.30) and 0.031 g (R_f 0.12), respectively. On recrystallization from water, the material at R_f 0.12 gave a yellow compound of m.p. 175-177°; the material at R_f 0.44 gave colourless needle-like crystals of m.p. 81.5-83°; the material at R_f 0.30 gave a white powder of m.p. 110-113°. Infrared spectra and mixed melting points revealed that these compounds were 5-nitrovanillin (XX) (R_f 0.12; Yield: 0.31%), vanillin (XIX) (R_f 0.44; Yield: 1.70%) and syringaldehyde (XXI) (R_f 0.30; Yield: 2.21%). The material with R_f 0.56 has not been identified.

The ether extract, on thin-layer chromatography with n-butyl ether (saturated with water)-glacial acetic acid (10:1), showed three spots at R_f 0.43, 0.25 and 0.34. The zone at R_f 0.34 was scraped from the plates, and extracted with methanol. When the methanol was evaporated, 9 mg of a light yellow solid was obtained (Yield: .09%). It was recrystallized from glacial acetic acid. The product was 4 mg of yellow needle-like crystals of m.p. 213-214°. This yellow compound proved, by its infrared spectrum and mixed melting points, to be identical to 5-nitrovanillic acid (XXIII) prepared according to Bentley (91). Chromatography of known compounds showed that the other two unknowns were probably vanillic acid (R_f 0.43) and syringic acid (R_f 0.25). Extracting the zones at R_f 0.43 and 0.25 with methanol and evaporating gave residues of 0.121 g (1.21%) and 0.130 g (1.30%), respectively. They were acidified with dilute HCl and extracted with chloroform. The chloroform extracts were dried with anhydrous Na_2SO_4 and evaporated. Infrared spectra of the residues were exactly identical to those of vanillic acid (XXII) and syringic acid (XXIV).

Alkaline Hydrolysis of Spruce Nitrolignin

Ten grams of spruce nitrolignin was hydrolyzed in alkali by a similar procedure. It gave 0.28% of alkali-insoluble residue. Acidification of the aqueous portion yielded 79% of brown powdered residue. Analysis: carbon, 56.2%; hydrogen, 5.20%; nitrogen, 0.45%;



	Syringic Acid	Vanillic Acid	5-Nitrovanillic Acid	Syring-aldehyde	Vanillin	5-Nitrovanillin
R_f	0.25	0.43	0.34	0.30	0.44	0.12
Birch Nitrolignin	1.30%	1.21%	0.09%	2.21%	1.70%	0.31%
Spruce Nitrolignin	--	1.81%	0.13%	-	3.82%	0.14%

FIGURE 27 Isolation of Products of Alkaline Hydrolysis of Nitrolignin

and methoxyl, 11.9%.

Isolation of Products of Hydrolysis of Spruce Nitrolignin

By the same procedure used above, chloroform extraction of the water-soluble fraction yielded 382 mg of vanillin (XIX) (R_f 0.44; Yield: 3.82%) and 14 mg of 5-nitrovanillin (XX) (R_f 0.12; Yield: 0.14%); ether extraction yielded 181 mg of vanillic acid (XXII) (R_f 0.43; Yield: 1.81%) and 13 mg of 5-nitrovanillic acid (XXIII) (R_f 0.34; Yield: 0.13%). Neither syringaldehyde nor syringic acid was found.

Nitrobenzene Oxidation of Acid-insoluble Residue from Alkaline Hydrolysis of Birch Nitrolignin

One gram of recovered birch nitrolignin was immersed in a solution of 2 ml of nitrobenzene and 10 ml of 6N sodium hydroxide solution. The mixture was heated to 150° for 24 hours in a stainless steel bomb (18 mm inside diameter and 15 cm in length). The total product was filtered and extracted for 24 hours with ether, and the ether extract was discarded. The aqueous layer was acidified, and extracted first with chloroform and then with ether.

The chloroform extract was chromatographed on a thin layer of silica gel with a methanol-benzene mixture (1:5.5) as solvent. Its chromatogram was identical to that of the aldehyde fraction from alkaline hydrolysis of birch nitrolignin, and revealed the presence of vanillin (XIX), 5-nitrovanillin (XX) and syringaldehyde (XXI). Similarly, the ether extract gave a chromatogram (solvent: n-butyl ether-glacial acetic acid, 10:1) which indicated the presence of vanillic (XXII), 5-nitrovanillic (XXIII) and syringic (XXIV) acids.

Nitrobenzene Oxidation of Acid-insoluble Residue from Alkaline Hydrolysis of Spruce Nitrolignin

Recovered spruce nitrolignin was oxidized by the same procedure described above. Thin layer chromatography showed that vanillin (XIX), vanillic acid (XXII), 5-nitrovanillin (XX) and 5-nitrovanillic acid (XXIII) had been formed.

Permanganate Oxidation of Methylated Spruce Nitrolignin

Four grams of spruce nitrolignin was added to 100 ml of 3N NaOH solution in a three-necked flask equipped with a separating funnel, a reflux condenser and a mercury-sealed mechanical stirrer. The suspension was refluxed for two hours, then cooled to about 10° by immersing the flask in an ice bath. Twenty four ml of dimethyl sulfate was added dropwise through a separatory funnel to the alkaline mixture whilst stirring the mixture vigorously. It was refluxed for two hours to complete the methylation, and was then allowed to cool.

To the resulting suspension, potassium permanganate was added in portions of one gram with continuous stirring. The pH of the solution was kept at 7-8 by adding dil. H_2SO_4 solution, and the temperature was kept at 90°. About 35 grams of $KMnO_4$ was needed so that the $KMnO_4$ colour was steady for 5 minutes. The MnO_2 was filtered while hot, and washed with hot water. The filtrate and washings were combined and acidified with dil. H_2SO_4 until precipitation occurred. The precipitate was filtered off and the filtrate was extracted with ether (4 times, 50 ml each). Drying (with Na_2SO_4) and evaporating the ether extract yielded a pale yellow residue (50 mg, i.e., 1.25% yield). Two recrystallizations from ether gave white crystals, m.p. 245.0-246.5°, which had previously been shown to be isohemipinic acid (XXVII) (92). The remaining ether solution was evaporated, and the residue was combined with the above precipitate and extracted exhaustively with methylene chloride. The CH_2Cl_2 was extracted with saturated aqueous $NaHCO_3$ solution (4 times, 40 ml each), and the $NaHCO_3$ extract was acidified and back-extracted with CH_2Cl_2 (4 times, 30 ml each). This last CH_2Cl_2 extract was dried over anhydrous Na_2SO_4, and evaporated to yield 1.1 grams of a residue. The residue was extracted with boiling ether and filtered. The ether insoluble portion was dissolved in boiling nitrobenzene which was then cooled to give 8 mg (0.2% yield) of a white precipitate, m.p. 299-301°. Its infrared spectrum was identical to that of authentic dehydrodiveratric acid (XXVIII) which was obtained by the methylation with dimethyl sulfate and subsequent oxidation with potassium permanganate of dehydrodivanillin, prepared by the method of Elbs and Lerch (93). The ether soluble portion was chromatographed on a thin layer of silica gel with a solvent mixture of n-butyl ether (saturated with H_2O)-glacial acetic acid (10:1). The chromatogram revealed the presence of more than four separate compounds, which could not all be identified. Chromatography of known compounds indicated that two of the unknowns were probably veratric acid (XXVI) (R_f 0.36) and 5-nitroveratric acid (XXV) (R_f 0.44). Preparative thin-layer chromatography was used to isolate the materials at R_f 0.36 and 0.44. Extracting the zone at R_f 0.36 with methanol and evaporating gave 128 mg of a white solid material. It was recrystallized twice from ethanol to yield small white crystals, m.p. 176-178°; no depression of m.p. was found when mixed with commercial authentic veratric acid. Extracting the zone at R_f 0.44 with methanol and evaporating gave 13 mg of a yellow residue, i.e., 0.33% yield.

It was purified by pouring on the top of an alumina column and eluting with carbon tetrachloride to give yellow crystals, m.p. 195-196°. It showed no depression in melting point when mixed with authentic 5-nitroveratric acid (94).

Permanganate Oxidation of 5-Nitroveratraldehyde

The compound 5-nitroveratraldehyde, 1.5 g, was added to 100 ml of a saturated solution of $KMnO_4$. After being stirred at room temperature for half an hour, the mixture was heated to boiling and filtered while hot. Acidifying and cooling the filtrate gave a pale yellow precipitate (1.0 g; 61.9% of theoretical), m.p. 195-196°. Its infrared spectrum showed the presence of a carboxyl group. Reported m.p. for 5-nitroveratric acid was 195-196° (94). The solution from which the yellow precipitate was separated was extracted with chloroform and examined by thin-layer chromatography (on silica gel with methanol-benzene, 1:5.5), and no unreacted 5-nitroveratraldehyde was detected.

Preparation of 1-(4-Hydroxy-3-Methoxyphenyl)-1-Propanone

This compound was prepared by a Friedel-Crafts type of synthesis, using polyphosphoric acid as the Lewis acid according to the method developed by Nakazawa (72).

To 12.4 grams of guaiacol was added 120 grams of polyphosphoric acid. The mixture was stirred vigorously, and eight grams of propionic acid was added dropwise over a period of 10 minutes from a separatory funnel. After completion of the addition, the mixture was heated at 70-75° for 30 minutes. The solution rapidly turned dark red. After cooling to room temperature, cold water was added, whereupon a heavy red oil separated. The mixture was extracted with ether (4 times, 100 ml each). The ether extract was dried with anhydrous sodium sulfate then evaporated in vacuo. The remaining oil was distilled under vacuum. Oily material was collected at 148-150° at 4.0 mm pressure. The yield was 12.1 grams (66.5% of theoretical).

Preparation of 1-(4-Hydroxyl-3-Methoxyphenyl)-1-Propanol (I)

Twelve grams of 1-(4-hydroxy-3-methoxyphenyl)-1-propanone was dissolved in 20 ml of 95% ethanol and 150 ml of 10% sodium hydroxide solution added. To the solution 1.5 grams of sodium borohydride was added in small portions over a period of 24 hours while the mixture was kept at 60°. When the reduction was completed, the solution was neutralized with dry ice and extracted with ether (4 times, 100 ml each). The ether extract was dried with anhydrous sodium sulfate, and then evaporated in vacuo. The remaining oil was distilled under vacuum and the desired product collected at 175-177° at 4.0 mm. An infrared spectrum of the product indicated complete reduction of the carbonyl function. The yield was 10.89 grams (90.8% of theoretical). The same reduction procedure has been reported by Schubert (74).

Preparation of 1(4-Hydroxy-3-Methoxyphenyl)-1-Methoxypropane (II)

Compounds 1(4-hydroxy-3-methoxyphenyl)-1-methoxypropane (II) and 1-(3,4-dimethoxyphenyl)-1-methoxypropane (IV) were prepared for their corresponding alcohols I and III by methylation with methanolic hydrochloric acid as described by Sergeeva and Shorygina (27).

Eight grams of 1-(4-hydroxy-3-methoxyphenyl)-1- propanol was dissolved in 100 ml of methanol containing 0.60% hydrochloric acid. The solution was kept at room temperature for 24 hours, and the reaction mixture was then neutralized by dilute alcoholic KOH solution. The methanol was evaporated in vacuo and the remaining oily material was dissolved in ether. This ether solution was washed with water and dried over anhydrous magnesium sulfate and then evaporated in vacuo. The oily residue was distilled under vacuum, and the desired product was collected at 134-135° at 4.0 mm. The yield was 4.15 grams (48.5% of theoretical). Analysis: Calcd. for $C_{11}H_{16}O_3$: carbon, 67.4%; hydrogen, 8.16%. Found: carbon, 67.4%; hydrogen, 8.13%. The analysis indicates that both II and its corresponding alcohol I, from which it was derived, were the desired compounds.

Preparation of 1-(3,4-Dimethoxyphenyl)-1-Propanone

This compound was prepared by the procedure of Gardner (73).

Veratrole, 33.5 grams, was mixed with 200 grams of polyphosphoric acid. To the mixture, 15 grams of propionic acid was added. It was heated at 60° for 2.5 hours with vigorous stirring. The red, viscous mixture was poured into 300 ml of water and extracted with ether (2 times, 100 ml each). The ether solution was washed with water, then dried over anhydrous Na_2SO_4, and evaporated in vacuo. After cooling, the oily residue turned to orange crystals which, after recrystallization from ether, had a m.p. 58-59°. The m.p. for 1-(3,4-dimethoxyphenyl)-1-propanone reported by Fuson et al. was 58-59° (87). The yield was 31.5 gm (80.3% of theoretical).

Preparation of 1-(3,4-Dimethoxyphenyl)-1-Propanol (III)

The compound 1-(3,4-dimethoxyphenyl)-1-propanone was reduced in the same way as described in the preparation of 1-(4-hydroxy-3-methoxyphenyl)-1-propanol (I). The yield was 93.5% of theoretical, b.p. 145-147° (5.0 mm).

Preparation of 1-(3,4-Dimethoxyphenyl)-1-Methoxypropane (IV)

Methylation of 1-(3,4-dimethoxyphenyl)-1-propanol (III) was performed as described in the preparation of 1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane (II). The yield was 7.40 gm (86.5%), b.p. 114-115° (5.0 mm). Analysis: Calcd. for $C_{12}H_{18}O_3$: carbon, 68.6%; hydrogen, 8.47%. Found: carbon, 68.6%, hydrogen, 8.58%

General Procedure for the Reaction of Model Compounds with Nitrous Acid

Solutions of nitrous acid were prepared by gradually adding HCl to a stirred solution of 2 grams of $NaNO_2$ in 150 ml of distilled water until the desired pH was reached, as read on a pH meter. The weight ratio of model compound to $NaNO_2$ was kept at 1:1 except in two experiments where the ratio was 1:10.

Two grams of the model compound being studied was added to the freshly prepared nitrous acid solution in a three-necked 300 ml flask, equipped with a thermometer, nitrogen inlet tube, and a water-cooled reflux condenser. The flask was rapidly heated to the desired temperature, by means of an oil bath placed on the magnetic stirring hot plate. The reaction mixture, under constant stirring, was kept at that temperature for the desired reaction time while the flow of nitrogen was maintained. It was then cooled to room temperature. Analysis of the mixture is described in the following sections. Yields of the products from the reactions are expressed here as a percentage of the starting material.

Products from 1-(4-Hydroxy-3-Methoxyphenyl)-1-Propanol (I) treated at pH 2 and 70° for 1 hr.

The reaction mixture was filtered to remove a black gummy material which was then washed twice with water. The filtrate and washings were combined and extracted with chloroform. The chloroform solution was dried over anhydrous Na_2SO_4 and chromatographed on a thin layer of silica gel, with a solvent mixture of methanol-benzene (1:5.5). The chromatogram revealed the presence of two separable compounds at R_f 0.50 and 0.23. The zones at these R_f values were scraped from the plates and each was extracted with methanol. The methanol extracts were evaporated and the residues were dissolved in chloroform. The $CHCl_3$ solutions, after being washed several times with dilute HCl solution, were dried overnight over anhydrous Na_2SO_4. Evaporating the dried chloroform solutions yielded yellow crystals, 22 mg (R_f 0.50) and 276 mg (R_f 0.23). Infrared spectra and mixed m.p. showed that they were 4-nitroguaiacol (X) (R_f 0.50) and 4,6-dinitroguaiacol (XI) (R_f 0.23). Yields: X, 1.1%; XI, 13.8%.

The black gummy material was chromatographed on an Al_2O_3 column (diameter, 2 cm; height, 60 cm) with elution by a mixture of benzene and methanol (8:1). It gave two fractions. The second fraction was evaporated to give a yellow crystalline compound with m.p. 160.5-161.5°. Analysis: carbon, 53.3%; hydrogen, 4.89%; and nitrogen, 6.22%. It gave an empirical formula of $C_{10}H_{11}NO_5$. The compound was identified, by means of infrared and p.m.r. spectra, as 1-(4-hydroxy-3-methoxy-5-nitrophenyl)-1-propanone (XXIX). Reported m.p. of this compound by Sergeeva et al (14) was 160-161°. The yield was 32 mg (1.6%). The first fraction from column chromatography, after evaporation, remained as a reddish gummy mixture.

It has not been identified.

Products from 1-(4-Hydroxy-3-Methoxyphenyl)-1-Methoxypropane (II) treated at pH 2 and 70° for 1 hr.

A black gummy material was filtered off from the reaction mixture, and was washed with water. The filtrate and washings were combined and extracted with chloroform. The chloroform extract was analyzed as described above. It gave 11.9% of 4,6-dinitroguaiacol (XI) and 1.3% of 4-nitroguaiacol (X).

Attempts to fractionate the black, gummy mixture by column chromatography were not successful.

Products from 1-(3,4-Dimethoxyphenyl)-1-Propanol (III) treated at pH 2 and 70° for 1 hr.

The reaction mixture was extracted with chloroform (4 times, 50 ml each), and the chloroform solution was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. Column chromatography on silica gel (Merck: 0.05-0.20 mm) with benzene as eluant was used to separate the residual mixture. It gave two fractions. The second fraction was evaporated to give a pale yellow crystalline compound with m.p. 58-59°. It was identified, by means of its infrared spectrum and by a mixed m.p., as 1-(3,4-dimethoxyphenyl)-1-propanone (XXX). The yield was 880 mg (44%).

The first fraction was passed twice more over a silica gel column, both times with benzene. The product was dried in vacuum to give a liquid substance (236 mg; Yield: 11.8%). Oxidation of 50 mg of this substance with 10 ml of a saturated potassium permanganate solution yielded 34 mg of veratric acid (XXVI) (Yield: 68%). Its identity was established by infrared, p.m.r. and mass spectrometry studies, which showed that it was

1-(3,4-dimethoxyphenyl)-1-nitropropane (XXXI). Analysis (found): carbon, 57.9%; hydrogen, 6.57%; and nitrogen, 6.17%. Calculated for $C_{11}H_{15}NO_4$: carbon, 58.5%; hydrogen, 6.67%; and nitrogen, 6.22%.

Products from 1-(3,4-Dimethoxyphenyl)-1-Methoxypropane (IV) treated at pH 2 and 70° for 1 hr.

The chloroform extract of the reaction mixture was chromatographed on a column of silica gel (Merck; 0.05-0.20 mm) with benzene as eluant. It gave three fractions. The first and third fractions were found, by comparing the infrared spectra, to be identical to 1-(3,4-dimethoxyphenyl)-1-nitropropane (XXXI) and 1-(3,4-dimethoxyphenyl)-1-propanone (XXX). The yields were 0.6% and 6.0% respectively. The second fraction gave an infrared spectrum identical to that of the starting material (Yield: 67.0%).

Product from Veratryl Alcohol (XXXVI) treated at pH 2 and 70° for 1 hr.

The reaction mixture was extracted with chloroform (4 times, 50 ml each). The chloroform was dried with anhydrous Na_2SO_4 and evaporated *in vacuo*. The remaining material, chromatographed over an alumina column (diameter, 2 cm; height, 60 cm) and eluated with carbon tetrachloride, gave two fractions. The first fraction gave an infrared spectrum identical to that of veratraldehyde (XXXVII). The yield was 28.5%. The second fraction gave 53.0% yield of starting material.

Products from Veratryl Alcohol (XXXVI) treated at pH 0.5 and 100° for 1 hr.

Veratryl alcohol (XXXVI), 0.5 gram, was added to a solution of 5 grams of $NaNO_2$ in 30 ml of water. Hydrochloric acid (1N) was added until the pH reached 0.5. The mixture was treated to 100°, and kept at that temperature for one hour.

The reaction mixture was extracted with chloroform (3 times, 20 ml each). The chloroform solution was extracted twice with 1N NaOH solution, (50 ml each time). Acidification of the alkaline solution with dilute HCl gave 48 mg of a yellow precipitate, which could be recrystallized from ethanol to yield yellow needle-like crystals with m.p. 191.0-192.5°. Its infrared spectrum indicated the presence of a carboxyl group (1710 cm^{-1}, 3450 cm^{-1}) and a nitro group (1540 cm^{-1}). Comparison with an authentic sample, which was obtained from permanganate oxidation of 6-nitroveratraldehyde (XXXVIII), showed this compound to be 6-nitroveratric acid (XXXII) (by mixed m.p. and infrared spectrum).

The chloroform layer was dried over anhydrous Na_2SO_4 and evaporated _in vacuo_ to give a viscous residue. This residue was chromatographed on an alumina column (diameter, 2 cm; height, 60 cm) and eluated with carbon tetrachloride. The first fraction (Yield: 8.8%) gave a yellow crystalline compound with m.p. 97.0-97.5°. Its infrared spectrum was identical to that of 4-nitroveratrole (XXXIV) (93). The second fraction gave only a trace of 4,5-dinitroveratrole (XXXV) (95) as detected by thin-layer chromatography and identified by its infrared spectrum. The third material eluted was a yellow solid with m.p. 129.5-131.0°. By mixed m.p. and infrared spectroscopy it proved to be 6-nitroveratraldehyde (XXXVI) (96). Its yield was 20.8%.

Products from 1-(3,4-Dimethoxyphenyl)-1-Propanol (III) treated at pH 0.5 and 100° for 1 hr.

The compound 1-(3,4-dimethoxyphenyl)-1-propanol (III), 0.5 grams, was added to a solution containing 5 grams of $NaNO_2$ in 30 ml of water. Hydrochloric acid (1N) was added until the pH of the solution reached 0.5, and the mixture was kept at 100° for one hour.

The reaction mixture was extracted with chloroform (3 times, 20 ml each), and the chloroform solution was extracted with 1N NaOH solution (2 times, 50 ml each). Acidification of the alkaline solution yielded 63 mg of a yellow precipitate, which could be recrystallized from ethanol to yield yellow needle-like crystals with m.p. 191.0-192.5°. Comparison with an authentic sample, by means of mixed m.p. and infrared spectroscopy, showed this compound to be 6-nitroveratric acid (XXXII).

The chloroform layer was dried with anhydrous Na_2SO_4 and then evaporated <u>in vacuo.</u> The resulting residue was chromatographed over an alumina column (diameter, 2 cm; height, 60 cm) and eluted with carbon tetrachloride. A yellow material was first eluted in a yield of 54 mg, and, after recrystallization from ethanol, it melted at 97.0-97.5°. By mixed m.p. and infrared spectroscopy, it proved to be 4-nitroveratrole (XXXIV) (95). The second fraction eluted was a bright yellow solid, m.p. 129.5-130.0° (Yield: 18.4%). Its infrared spectrum indicated the presence of a nitro group (1530 cm^{-1}) and a carbonyl group (1700 cm^{-1}). Comparison with an authentic sample, synthesized by nitration of 1-(3,4-dimethoxyphenyl)-1-propanone with nitric acid (Sp. gr. 1.4) at 10°, showed this compound to be 1-(3,4-dimethoxy-6-nitrophenyl)-1-propanone (XXXI). Reported m.p. of this compound, obtained from nitration of 1-(3,4-dimethoxyphenyl)-1-propanol (III), followed by oxidation with $KMnO_4$ in alkaline medium, was 130-131°(26).

<u>Products from Syringyl Alcohol (XXXIX) treated at pH 2 and 70° for 20 min.</u>

Addition of 2 grams of XXXIX to a freshly prepared solution of nitrous acid (pH 2) immediately gave a purple colour. Heating for 20 minutes at 70° caused no change. The reaction was extracted with chloroform

(3 times, 50 ml each). (A thin-layer chromatogram of this chloroform extract (on silica gel with benzene-methanol, 5:1) revealed the presence of at least 5 compounds, but no starting material was detected.) After removal of the chloroform, washing the residue with ether divided the mixture into two fractions: ether-soluble and ether-insoluble. A yellow material (220 mg), m.p. 135.5-137.0°, was separated from the ether. Recrystallization from ethanol gave 170 mg of yellow needles, m.p. 136.2-137.0°, which proved, from their infrared and p.m.r. spectra, to be 2,6-dimethoxy-4-nitrophenol (XL) (97) (lit. 136-137°).

Column chromatography on silica gel (Merck; 0.5-0.20 mm) was used to separate the ether-insoluble mixture. The column (diameter, 2 cm; height, 60 cm.) was prepared by wet packing technique and benzene-methanol (9:1) was used as eluting solvent. A yellow solid was first eluted in a yield of 214 mg, and after one recrystallization from glacial acetic acid the yellow needle-like solid, 150 mg, melted at 251-252° (sealed tube). By mixed melting point and infrared spectroscopy, the solid was shown to be 2,6-dimethoxy-1,4-benzoquinone (XVII) (90). A purple mixture (680 mg) was next eluted, but its nature has not been determined.

Products from Syringaldehyde (XXI) treated at pH 2 and 70° for 20 min.

When syringaldehyde (XXI) was treated with nitrous acid at pH 2 and 70°, a yellow precipitate separated from the resulting purple solution. The precipitate was collected by filtration and was washed with cold water. The filtrate was allowed to stand until no more yellow precipitate settled out. After the second filtration the filtrate was kept for further studies.

TLC (on silica gel with benzene-methanol, 5:1) revealed that the yellow precipitate was a mixture of two compounds with R_f 0.60 and 0.50, the same R_f values as 2,6-dimethoxy-1,4-benzoquinone (XVII) and 2,6-dimethoxy-4-nitrophenol (XL), respectively. Since XVII is insoluble in ether, extraction with ether was used to separate the yellow mixture. The ether-insoluble solid was separated by filtration and repeatedly washed with ether, yielding 730 mg, m.p. 251-252°, which proved to be XVII. On removal of the ether, the ether-soluble fraction yielded 212 mg of yellow crystals, m.p. 135.5-136.6°, identified as XL.

Additional yields of XVII and XL could be obtained from the filtrate described above. A chloroform extract (3 times, 50 ml each) of this filtrate was evaporated in vacuo, and then subjected to column chromatography on silica gel (Merck; 0.05-0.20 mm) using a benzene-methanol mixture (9:1) as eluting solvent. A yellow solid (26 mg) was first eluted. It melted at 250.5-252°, and was identified as XVII. The second eluted product was a yellow solid (200 mg), m.p. 135.5-136.5°, identified as XL. The third fraction was a purple mixture whose nature has not been determined. A white compound (99 mg) was next eluted. It melted at 110-113°, and proved to be the unreacted starting material (XXI).

Products from Syringic Acid (XXIV) treated at pH 2 and 70° for 20 min.

Addition of XXIV to an aqueous nitrous acid solution (pH 2) caused an immediate and rapid evolution of carbon dioxide. The resulting purple solution was extracted with chloroform (3 times, 50 ml each). A thin-layer chromatogram of the chloroform extract (on silica gel with benzene-methanol, 5:1) revealed the presence of three products at R_f 0.60 (yellow), 0.50 (yellow) and 0.45 (purple), respectively. The R_f values of the first two products were identical to those of 2,6-dimethoxy-1,4-benzoquinone (XVII) and 2,6-dimethoxy-4-nitrophenol (XL). Isolation of these two compounds was achieved by the procedure described in the treatment of syringyl alcohol (XXXIX). The yields were: XVII, 1.044 g (52.2%); and XL, 438 mg (21.9%)

CLAIMS TO ORIGINAL RESEARCH

1. An experimental survey of the reactions of birch and spruce dioxane lignins with nitrous acid in aqueous solution has revealed that the former was more susceptible to degradation and gave a lower yield of the insoluble nitrolignin.

2. Simple degradation products, 4-nitroguaiacol (X), 4,6-dinitroguaiacol (XI), 2,6-dimethoxy-1,4-benzoquinone (XVII) and oxalic acid were isolated from the reaction of birch dioxane lignin with nitrous acid at pH 2 and 70° ; while only the two nitro compounds, X and XI, and oxalic acid were isolated from spruce dioxane lignin on the same treatment.

3. Both birch and spruce dioxane lignins, on treatment with nitrous acid, underwent demethylation and fixation of nitrogen. The degree of demethylation and the amount of nitrogen fixed increased with acidity, reaction temperature (below 95°) and reaction time.

4. Alkaline hydrolysis of birch nitrolignin gave rise to vanillin (XIX), 5-nitrovanillin (XX), syringaldehyde (XXI), vanillic acid (XXII), 5-nitrovanillic acid (XXIII) and syringic acid (XXIV), which were isolated by the thin-layer chromatographic technique. Spruce nitrolignin, by the same treatment, yielded XIX, XX, XXII and XXIII.

5. Alkaline hydrolysis with sodium hydroxide, then methylation with dimethyl sulfate, followed by permanganate oxidation of spruce nitrolignin gave rive to 5-nitroveratric (XXV), veratric (XXVI), isohemipinic

(XXVII) and dehydrodiveratric (XXVIII) acids.

6. Reactions of model compounds for softwood lignin, 1-(4-hydroxy-3-methoxyphenyl)-1-propanol (I), 1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane (II), 1-(3,4-dimethoxyphenyl)-1-propanol (III) and 1-(3,4-dimethoxyphenyl)-1-methoxypropane (IV), with nitrous acid have yielded products which can help to explain the reaction of lignin under similar conditions. From I, at pH 2 and 70°, 4-nitroguaiacol (X) and 4,6-dinitroguaiacol (XI) were formed by electrophilic displacement of the side chain, and 1-(4-hydroxy-3-methoxy-5-nitrophenyl)-1-propanone (XXIX) by oxidation. With II, only displacement of the side chain occurred, and X and XI were isolated. Both III and IV yielded the same products on mild treatment (pH 2, and 70°): 1-(3,4-dimethoxyphenyl)-1-propanone (XXX) and 1-(3,4-dimethoxyphenyl)-1-nitropropane (XXXI). The results indicate that displacement of the side chain is facilitated by the presence of a free phenolic hydroxyl group. But when III was reacted under harsher conditions (pH 0.5 and 100°), it yielded 6-nitroveratric acid (XXXII), 4-nitroveratrole (XXXIV), 4,5-dinitroveratrole (XXXV) and 1-(3,4-dimethoxy-6-nitrophenyl)-1-propanone (XXXIII).

7. The compound 1-(3,4-dimethoxyphenyl)-1-nitropropane (XXXI) has been prepared for the first time. Its formation from III and IV by the action of nitrous acid points to the possibility that, when lignin is subjected to the same treatment, nitro groups can be introduced into the aliphatic side chain to occupy a position α with respect to the aromatic ring.

8. Model compounds for hardwood lignin, syringyl alcohol (XXXIX), syringaldehyde (XXI) and syringic acid (XXIV), on treatment with nitrous acid (pH 2 and 70°), yielded 2,6-dimethoxy-1,4-benzoquinone (XVII) and 2,6-dimethoxy-4-nitrophenol (XL). This result indicates that the formation of XVII from hardwood lignin can be attributed to oxidation of terminal syringyl units with free phenolic hydroxyl, as well as to oxidation of syringyl units with etherified phenolic hydroxyl.

9. Based on the evidence put forward in this thesis, a mechanism for the formation of 2,6-dimethoxy-1,4-benzoquinone (XVII) from hardwood lignin has been proposed for the first time. It involves electrophilic displacement of the aliphatic side chain, acid hydrolytic dealkylation and nitrous acid-catalyzed oxime cleavage.

10. Based on the studies of model compounds, mechanisms were proposed to account for various modes of degradation of the lignin polymer network.

BIBLIOGRAPHY

1. Bolker, H.I., Ind. Eng. Chem., Product Res. and Develop., 4, 74 (1965).

2. Sarkanen, K.V., "The Chemistry of Wood", Browning, B.L.,ed., Interscience, New York, 1963, p. 249.

3. Leopold, B., Acta Chem. Scand., 6, 38 (1952).

4. Creighton, B.H. and Hibbert, H., J. Am. Chem. Soc., 66, 37 (1944).

5. Hachihama, Y.,J. Chem. Soc., Japan, Ind. Chem. Section, 46, 132 (1943); 47, 763 (1944).

6. Aggarwala, J.C., Indian Pulp and Paper, 4, No. 1, 12 (1949).

7. Higuchi, T. and Kawamura, I., J. Japan Wood Res. Soc., 2, No. 6, 226 (1956).

8. Freudenberg, K., "Lignin Structure and Reactions ", Gould, R.F., ed., Am. Chem. Soc., Washington, D.C., 1966, p. 1.

9. Freudenberg, K., "Proceedings of the Fourth International Congress of Biochemistry-1958 ", Pergamon Press, Vienna, 1959, p. 132.

10. Kratzl, K. and Billek, G., Holzfurchung, 10, 161 (1956).

11. Adler, E., Paperi ja Puu, 11, 638 (1961).

12. Erdtman, H., Research 3, 63 (1950).

13. Lindgren, B.O., Acta Chem. Scand. 5, 603 (1951).

14. Lindgren, B.O., Svensk Papperstidn., 55, 178 (1952).

15. Mikawa, H.K., Takasaki, S.C. and Okada, T., J. Chem. Soc., Japan, Ind. Chem. Section, 54, 1299 (1951).

16. Mikawa, H.K., Ibid., 54, 671, 741, 761 (1951).

17. Brauns, F.E., "Wood Chemistry", Wise, L.E. and Jahn, E.C., eds., Vol. I, Reinhold, New York, 1952, p. 452.

18. Dence, C.W. and Sarkanen, K.V., Tappi, 43, No. 1, 87 (1960).

19. Sarkanen, K.V. and Dence, C.W., J. Org. Chem., 25, 715 (1960).

20. Sarkanen, K.V., Ericsson, B. and Suzuki, J., "Lignin Structure and Reactions", Gould, R.F., ed., Am. Chem. Soc., Washington, D.C., 1966, p. 38.

21. Van Buren, J.B. and Dence, C.W., Tappi, 50, No. 11, 553 (1967).

22. Chuksanova, A.A. and Shorygina, N.N., Izvest. Akad. Nauk SSSR, Otdel. Khim. Nauk, No. 8, 1511 (1960); ABIPC, 31, 657 (1961).

23. Sergeeva, L.L., Chuksanova, A.A. and Shorygina, N.N., Ibid., No. 5, 653 (1957); ABIPC, 29, 1004 (1959).

24. Chuksanova, A.A., Sergeeva, L.L. and Shorygina, N.N., Ibid., No. 12, 2219 (1959); ABIPC, 31, 347 (1960).

25. Sergeeva, L.L., Shorygina, N.N. and Lopatin, B.V., Ibid., No. 7, 1295 (1962); ABIPC, 33, 1260 (1963).

26. Sergeeva, L.L., Shorygina, N.N. and Lopatin, B.V., Ibid., No. 7, 1254 (1964); ABIPC, 35, 1257 (1965).

27. Sergeeva, L.L. and Shorygina, N.N., Izvest. Akad. Nauk SSSR, Ser. Khim., No. 9, 1630 (1965); ABIPC, 36, 6340 (1966).

28. Gustafsson, C. and Anderson, L., Paperi ja Puu, No. 1, 1 (1955).

29. Cowdrey, W.A., Hughes, E.D., Ingold, C.K., Masterman, S., and Scott, A.D., J. Chem. Soc., 1257 (1937).

30. White, F.V., Swartz, J.N., Peniston, Q.P., Schwartz, H., McCarthy, J.L. and Hibbert, H., Tech. Assoc. Papers, 24, No. 1, 179 (1941).

31. Brauns, F.E. and Brauns, D.A., "The Chemistry of Lignin-Supplement Volume", Academic Press, New York, 1960, p. 301.

32. Sobolev, I., J. Org. Chem., 26, 5080 (1961).

33. Bolker, H.I., Kung, F.L. and Kee, M.L., Tappi, 50, No. 4, 199 (1967).

34. Sarkanen, K.V. and Strauss, R.W., Tappi, 44, No. 7, 459 (1961).

35. Freudenberg, K. and Durr, W., Chem. Ber., 63, 2713 (1930).

36. Bunton, C.A., Hughes, E.D., Ingold, C.K., Jacobs, D.I.H., Jones, M.H., Minkoff, G.J. and Reed, R.I., J. Chem. Soc., 2628 (1950).

37. Schramm, R.M. and Westheimer, F.H., J. Am. Chem. Soc., 70, 1782 (1948).

38. Clemo, E., U.S. Patent , 29,059 (July 10, 1860).

39. Young, T.G., Pettigrew, J., British Patent, 14,735 (November 8, 1884), and 14,988 (November 14, 1884); Can. Patent, 21,563 (April 29, 1885); U.S. Patent, 332,882 (July 21, 1885).

40. Schwalbe, C.G., Ger. Patent, 220,645 (April 5, 1910); French Patent, 410,460 (May 21, 1910).

41. Darling, E.R., U.S. Patent, 1,829,852 (November 3, 1931).

42. Dreyfus, H., British Patent, 477,842 (January 3, 1938); U.S. Patent, 2,214,125 (September 10, 1940).

43. Clarke, G.L., Tappi, Tech. Assoc. Papers, Ser. 27, 631 (1944); Paper Trade J., 118, No. 3, 26 (February 24, 1944).

44. Flaig, W., Holzforschung, 9, 1 (1955).

45. Bremner, J.M., J. Agr. Sci., 48, 352 (1957).

46. Brauns, F.E., "The Chemistry of Lignin ", Academic Press, New York, 1952, (a) p. 605; (b) p. 135; (c) p. 600.

47. Sarkar, P.B., J. Indian Chem. Soc., 11, No. 6, 407 (1934).

48. Stevenson, F.J. and Swary, R.J., Nature, 199, 97 (1963).

49. Stevenson, F.J. and Kirkman, M.A., Nature, 200, 107 (1964).

50. Bolker, H.I. and Kung, F.L., " The Formation of 2,6-Dimethoxy-1,4-Benzoquinone by the Action of Nitrous Acid on 1,2,3-Trimethoxybenzene", paper in preparation.

51. Turney, T.A. and Wright, C.A., Chem. Rev., 59, 497 (1959).

52. Austin, A.T., Science Progr. (London), 49, 619 (1961).

53. Ibne-Rasa, K.M., J. Am. Chem. Soc., 84, 4962 (1962).

54. Grovenstein, E., Jr. and Kilby, J. Am. Chem. Soc., 79, 2972 (1957).

55. Melander, L., Arkiv Kemi, 2, 213 (1950).

56. Schmid, H., Muhr, G., and Riedl, P., Monatsh., 97, 781 (1966).

57. Bolker, H.I. and Singh, M.M., Pulp and Paper Mag. Can., 66, T-165 (1965).

58. Feigl, F., "Spot Tests", Vol. II, 4th ed., Elsevier Publishing Co., Amsterdam, 1954, p. 120.

59. Knecht, E. and Hibbert, E., "New Reduction Methods in Volumetric Analysis", 2nd ed., Longmans, London, 1925, p. 32.

60. Ma, T.S. and Earley, J.V., Mikrochim. Acta, 129 (1959).

61. Cheronis, D. and Ma, T.S., "Organic Functional Group Analysis by Micro and Semimicro Methods", Interscience, New York, 1964, p. 310.

62. Cobler, J.G., Samsel, E.P. and Beaver, G.H., Talanta, 9, 473 (1962).

63. Gupta, P.R., Rezanowich, A. and Goring, D.A.I., Pulp and Paper Mag. Can., 63, No. 1, T-21 (1962).

64. Goring, D.A.I., Pulp and Paper Mag. Can., 64, No. 12, T-517 (1963).

65. Pearl, I.A., J. Am. Chem. Soc., 68, 916 (1946).

66. Lautsch, W. and Piazolo, G., Chem. Ber., 73, 317 (1940).

67. Jayne, J.E., Tappi, 36, No. 12, 571 (1953).

68. Barton, J.S., Tappi, 33, No. 10, 496 (1950).

69. Freudenberg, K., Engler, K., Flickinger, E., Sobek, A. and Klink, F., Chem. Ber., 71, 1810 (1938).

70. Freudenberg, K., Fanson, A, Knopf, E. and Haag, A., Chem. Ber., 69, 1415 (1936).

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

72. Nakazawa, K., J. Pharm. Soc., Japan, 74, 836 (1954).

73. Gardner, P.G., J. Am. Chem. Soc., 76, 4550 (1954).

74. Schubert, S.W., Ph. D. Thesis, University of Washington, Seattle, Washington (1960).

75. Shorygina, N.N., Sergeeva, L.L., Lopatin, B.V., Izvest. Akad. Nauk SSSR, Ser. Khim., No. 2, 372 (1967); ABIPC, 38, 341 (1967).

76. Andersen, L., Soc. Sci. Fennica Commentationes Phys. Math., 19,No. 1, 51 (1956); C.A., 54, 22442d (1960).

77. Levitt, L.S., J. Org. Chem., 20, 1297 (1955).

78. Havinga, E. and Schors, A., Rec. Trav. Chem., 69, 457 (1950).

79. Schors, A., Kraaijeveld, A. and Havinga, E., Ibid., 74 , 1243 (1955).

80. Wieland, T. and Grimm, D., Chem. Ber., 96, 275 (1963).

81. Freudenberg, K., Science, 148, 595 (1965).

82. Pew, J. C. and Weyna, P., Tappi, 45, No. 3, 247 (1962).

83. Chew, C.H., Ph. D. Thesis, McGill University, (1968).

84. Bolker, H.I. and Terashima, N., "Lignin Structure and Reactions", Gould, R.F., ed., Am. Chem. Soc., Washington, D.C., 1966, p. 110.

85. Tappi Standards, T222, M-50.

86. Pepper, J.M. and Siddiqueullah, M., Can. J. Chem., 39, 1454 (1961).

87. Fuson, R.C., Gaertner, R. and Chadwich, D.H., J. Org. Chem., 13, 489 (1948).

88. Klemenc, A., Monatsh., 33, 701 (1912).

89. Pollecoff, F. and Robinson, R., J. Chem. Soc., 645 (1918).

90. Baker, W., J. Chem. Soc., 665 (1941).

91. Bentley, W.B., Am. Chem. J., 24, 178 (1892).

92. Richtzenhain, H., Svensk Papperstidn, 53, 644 (1950).

93. Elbs, K. and Lerch, H., J. Prakt. Chem., 93, 1 (1916).

94. Simonsen, J.L. and Rau, M.G., J. Chem. Soc., 113, 24 (1918).

95. Huntress, E.H. and Mulliken, S.P., "Identification of Pure Organic Compounds", John Wiley and Sons, New York, 1941, p.542.

96. Salway, A.H., J. Chem. Soc., 95, 1163 (1909).

97. Collins, R.F. and Davis, M., J. Chem. Soc., 1863 (1961).