THE OZONIZATION AND STRUCTURE OF LIGNIN IN RELATION TO SOLUBILITY IN BISULPHITE SOLUTIONS

A Thesis

Submitted to the Faculty of Graduate
Studies and Research of McGill University
in Partial Fulfilment of the Requirements
for the Degree of Doctor of Philosophy

by

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CLAIM TO ORIGINAL RESEARCH

Isolated lignins, obtained by extraction with formic and acetic acids, which are normally insoluble in aqueous bisulphite solution, have been shown to yield water-soluble lignin sulphonic acids after a prior treatment of the lignin with ozone. The alkaline cleavage of these lignin sulphonic acids has given rise to vanillin and acetovanillone, both of which have hitherto evaded isolation from these lignins.

The use of formic acid as an ozonization solvent has been developed, and its characteristics in this capacity demonstrated by experiments on organic compounds of known structure.

For the first time direct chemical evidence has been demonstrated for the presence of a carbonyl group in the solubilization of lignin by aqueous bisulphites. A study of various lignins has produced evidence to show that the carbonyl group is associated with an hydroxyl group and that the presence of both these groups is necessary for the formation of lignin sulphonic acids. A new interpretation of the process of delignification by means of aqueous bisulphite solution has been postulated in view of these new experimental findings.

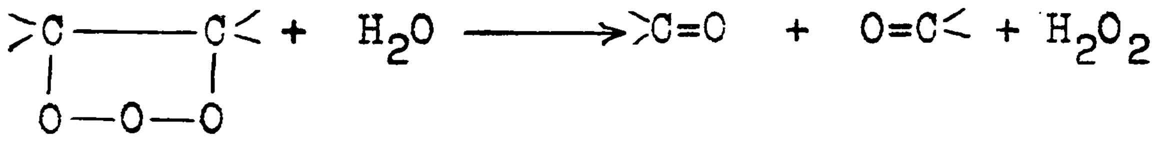
HISTORICAL REVIEW.

PART I. - THE ACTION OF OZONE.

It has been known for many years that ozone is capable of reacting with unsaturated hydrocarbons. Schoenbein (I) in 1855 noted the reaction of ozone with ethylene to form formaldehyde, formic acid and carbon dioxide. Houzeau and Renard (2) observed the reaction of ozone with benzene but they regarded the product as a peroxide rather than as an ozonide. Harries (17, 18), in his classical researches on the action of ozone, was the first to point out the true character of these so-called ozonides and he was able to show, by analysis, that the reaction products contained an $-O-O-O-$ grouping for each double bond present in the original substance.

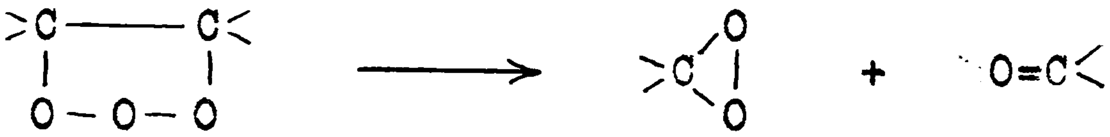
These ozonides which are formed by the action of ozone in the absence of any moisture may then break down in one of two ways:

(a) Reaction with water brings about complete rupture of the double bond with the formation of aldehydes or ketones, thus,



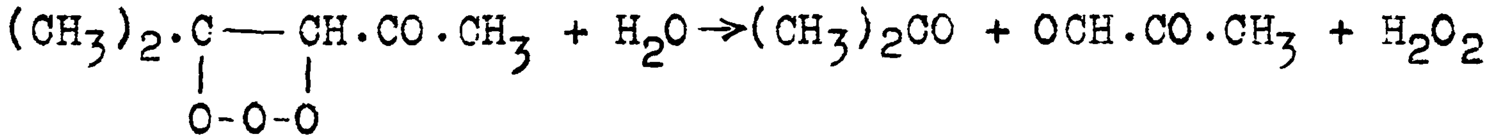
$$\underset{O-O-O}{>C-C<} + H_2O \longrightarrow >C=O + O=C< + H_2O_2$$

(b) In solvents such as absolute alcohol or glacial acetic acid, or sometimes merely on warming, decomposition will take place in the absence of water to form a ketone, or aldehyde, and a peroxide.

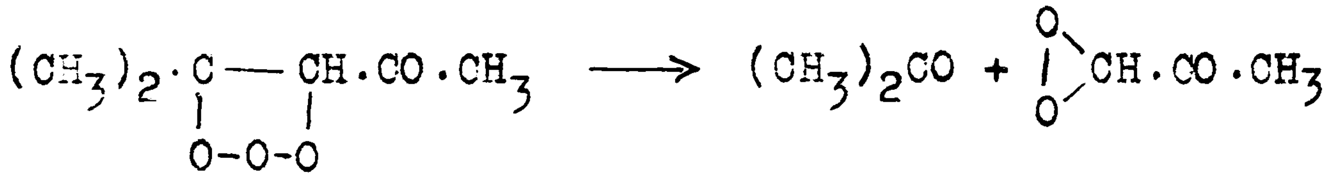


These peroxides so formed often then break down under the action of water to give carboxylic acids.

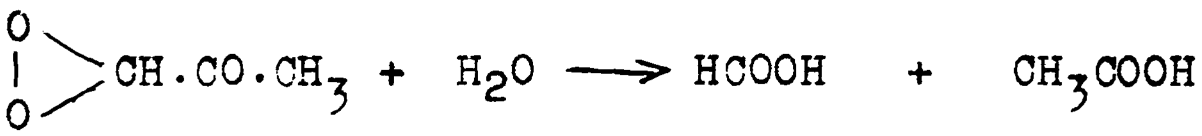
Thus mesityl oxide ozonide breaks down in two different ways and the larger amount of decomposition taking place according to the second scheme accounts for the relatively small yields of methyl glyoxal and the observed occurrence of formic and acetic acids.

(i) 

$$\underset{O-O-O}{(CH_3)_2\cdot C - CH}\cdot CO\cdot CH_3 + H_2O \rightarrow (CH_3)_2CO + OCH\cdot CO\cdot CH_3 + H_2O_2$$

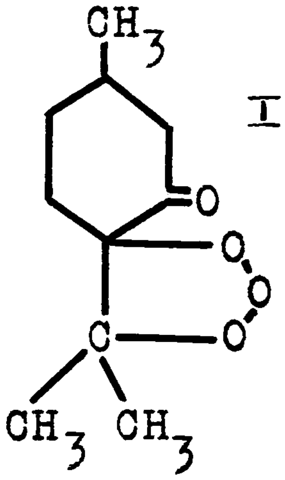
(ii) 

$$\underset{O-O-O}{(CH_3)_2\cdot C - CH}\cdot CO\cdot CH_3 \longrightarrow (CH_3)_2CO + \underset{O}{\overset{O}{|}}\!>CH\cdot CO\cdot CH_3$$



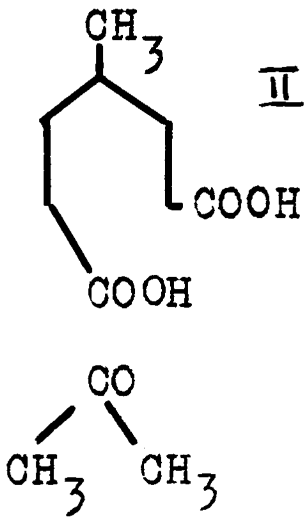
$$\underset{O}{\overset{O}{|}}\!>CH\cdot CO\cdot CH_3 + H_2O \longrightarrow HCOOH + CH_3COOH$$

The type of decomposition shown in (ii) accounts for a number of reactions which for some time were considered abnormal. Thus pulegone ozonide, I, yielded β-methyladipic acid, II, and acetone and not the substance which would be expected normally from the usual type of ozonide decomposition, namely, 1-methylcyclohexanedione-3,4,III, (3)



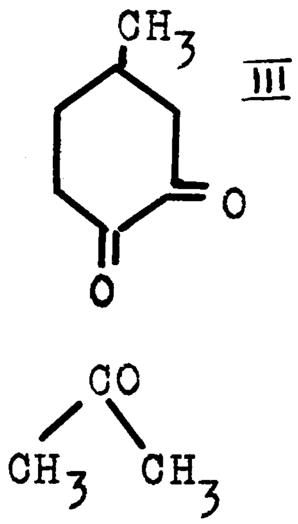
I

pulegone ozonide



II

β-methyladipic acid

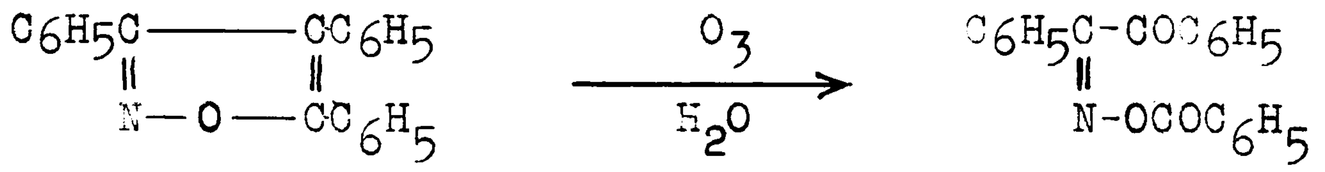


III

1-methylcyclohexanedione-3,4

The behaviour of ozone towards ethylene double bonds is substantially as general a reaction as is that of bromine. In fact many substances which are not regarded as having unsaturated bonds of the ethylenic type, such as benzene and naphthalene, also react with ozone. Noller and co-workers (4) have studied the relative rates of ozonization of unsaturated compounds. They obtained curves showing the relative rates of ozonization of a wide variety of double bonded systems. It was found that whereas a double bond, unaffected by the presence of other groups, adds ozone extremely rapidly, the rate is markedly decreased when the double bond is conjugated with carbonyl groups. Three or more phenyl groups or two chlorine atoms attached to the doubly bound carbon atoms also decrease the rate of addition. Where two or three double bonds are conjugated with each other, one bond adds ozone rapidly while the others add only slowly. In the case of cis-trans isomers where the rate of addition of ozone is decreased by other groups, the trans isomer was found to add more rapidly than the cis form. They point out that the procedure should prove valuable in the investigation of unsaturated compounds of unknown structure, and outline an efficient experimental procedure for obtaining the curves for the relative rates of ozonization.

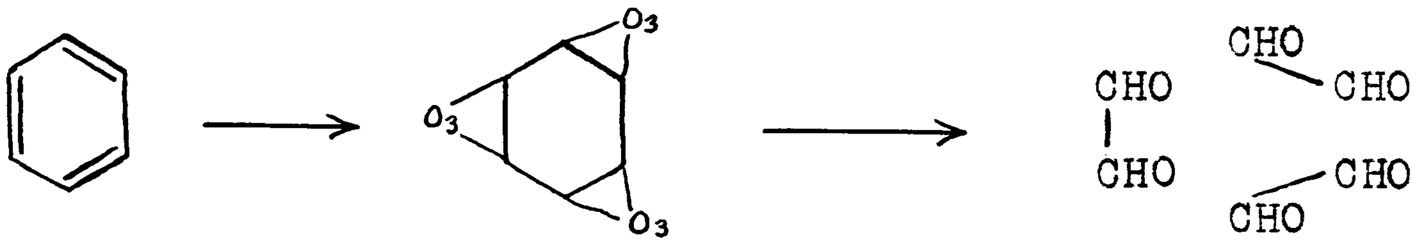
In addition ozone reacts much more rapidly with open chain ethylenic linkages than it does with those present in aromatic systems, and furthermore it reacts more readily with a carbon to carbon, than with a carbon to nitrogen double bond. Meisenheimer (5) found that the ozonization of triphenylisoxazole gave the benzoate of benzil monoxime.



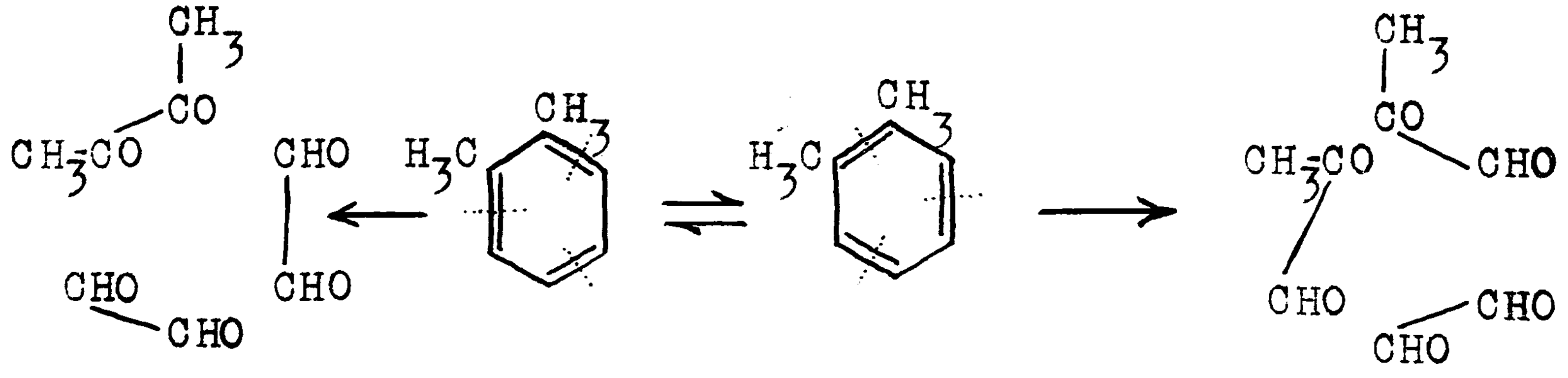
In many cases however the presence of carbon to nitrogen double bonds may lead to complications and render the results of the ozonization somewhat inconclusive.

Brus and Peyresblaugues (6) made a study of the rates of absorption of ozone and found evidence to indicate a two-stage reaction for ethylenic compounds, first the ozonide formation and then the so-called perozonide formation (Addition of O_4). Benzene and naphthalene were found to react in a single stage. Absorption curves for styrene and phenylcyclohexene revealed that the quantitative ozonization of the ethylenic linkage precedes ozonization of the aromatic mucleus. Acetylenic derivatives such as heptine reacted more slowly than ethylenic compounds but more readily than benzene. The three classes of compounds yielded distinctive absorption curves which permitted an easy differentiation.

Renard (7) by the action of ozone on benzene obtained a gelatinous, very explosive mass, which he termed "Ozobenzol" and to which he ascribed the formula $C_6H_6O_6$. Later Harries (8) showed that actually a triozonide was formed which underwent decomposition in the usual manner to yield three mols of glyoxal. Since the formation and degradation of an ozonide is accepted as excellent evidence for the presence of unsaturated carbon atoms these results were interpreted as being extremely favourable to the Kekule benzene ring formula.



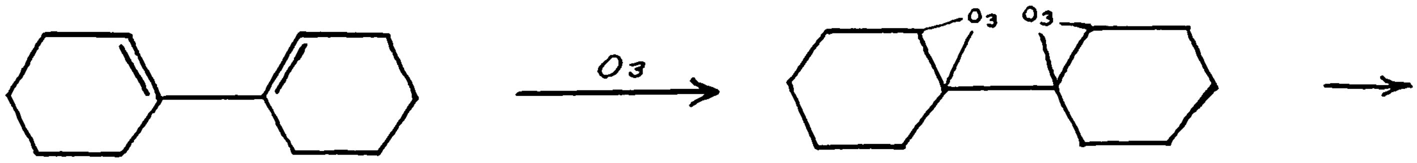
This type of investigation was extended by Levine and Cole (9) who obtained glyoxal, methyl glyoxal and diacetyl from the ozonization of o-xylene. Since neither form of the xylene could yield all three of the products observed it was concluded that the hydrocarbon must exist as an equilibrium mixture of the two Kekule forms, as is shown.

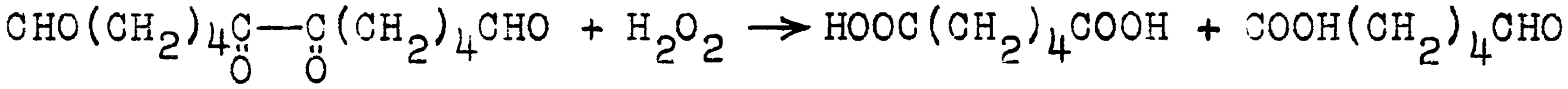


The ozonization of acetylenic compounds is of interest and has been investigated only in recent years. This type of reaction has been shown to give rise to 1,2 diketones, in other words the carbon to carbon link at the point of unsaturation is not broken. Further oxidation, however, may give rise to fission at this point with the production of the corresponding acids. Briner and Wunenburger report an 81% yield of glyoxal from the ozonization of acetylene, (10). Hurd and Christ studied the ozonization of a number of acetylenes and although they isolated no 1,2 diketones they postulated that the mechanism involved the preliminary formation of the diketone and then subsequent oxidation to the acids by the hydrogen peroxide formed (11). Thus phenyl propiolic acid gave rise to benzoic and oxalic acids and phenyl acetylene to benzoic and formic acids. Jacobs also studied this reaction and succeeded in isolating the 1,2 diketones from two acetylenic compounds (12). He isolated benzil and benzoic acid from the ozonization of diphenylacetylene, and benzylphenyldiketone and acids from benzylacetylene. Mention is made of the fact that the solvent used, and the temperature of the ozonization, as well as the method of splitting the ozonide are of great importance.

Smith (13) investigated the action of a number of reagents on ozone and found that there was negligible destruction on passing ozone through water, dilute or concentrated sulphuric acid, or water followed by concentrated sulphuric acid or acid permanganate. However 5% sodium hydroxide or commercial phosphorus pentoxide destroyed nearly all of the ozone. He also found that if the phosphorus pentoxide were resublimed in a current of oxygen then it had no deleterious effect on the ozone.

Noller and Kaneka (14) ozonized a hydrogenated biphenyl and found the following reaction took place,

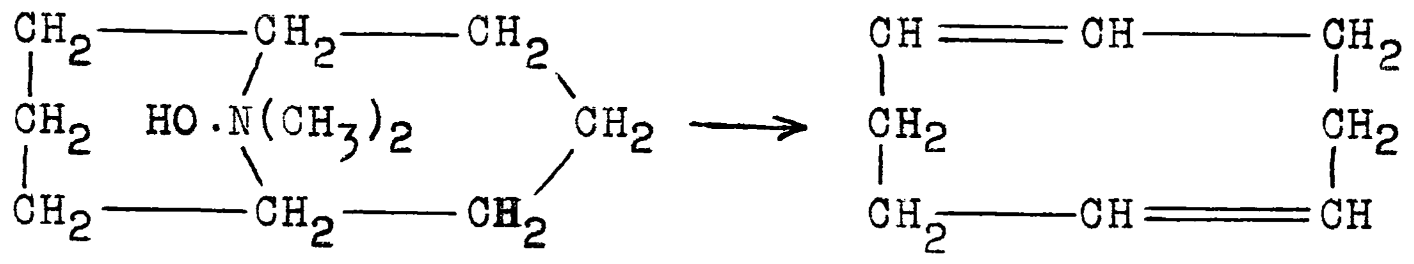




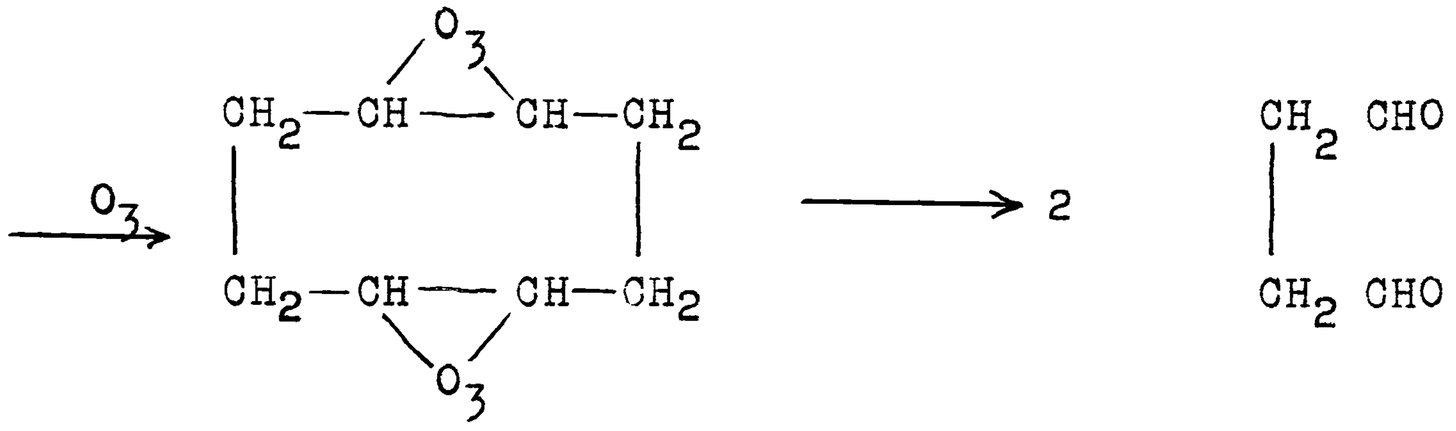
$$CHO(CH_2)_4\underset{\overset{\|}{O}}{C}-\underset{\overset{\|}{O}}{C}(CH_2)_4CHO + H_2O_2 \rightarrow HOOC(CH_2)_4COOH + COOH(CH_2)_4CHO$$

From these results they were led to conclude that steric hindrance is not the factor involved in the failure of diphenyl itself to form a <u>hexa-</u>ozonide.

When the alkaloid, pseudo-pelletierin, is decomposed by the exhaustive methylation method, the removal of the basic nitrogen atom results in the formation of a cyclo-octadiene which Willstätter and Verguth (15) believed to contain a pair of conjugated double bonds. This cyclo-octadiene polymerized very easily. By the ozonization of this compound Harries (16) was able to show that the double bonds were not conjugated but were in the 1,5 position because it formed a diozonide the hydrolysis of which yielded succinic dialdehyde and succinic acid



cyclo-octadiene, 1,5



Carl Harries did a great deal of the pioneer work on the investigation of the action of ozone on almost every possible variety of organic compound and has summarized his researches in four general articles (17), and in one bound volume, (18). Although Harries work has been very valuable the following statement by Brooks (19) is significant: "Critical examination of Harries' work is apt to elicit the fact that he frequently paid little attention to the history or purity of his original material and also that more definite results might often have been obtained, in the terpene series, in the hands of other well-known specialists in this field."

The successful application of the ozonization technique depends upon a number of different factors. One important consideration is the nature of the solvent employed. Needless to state, the solvent must be of an absolutely anhydrous nature if the ozonide is to be isolated as such. Chloroform and carbon tetrachloride have the disadvantage that they are themselves attacked by the ozone. Thus chloroform gives rise to phosgene and hydrogen chloride and when sensitive aldehydes are expected from the splitting of the ozonide, solvents from which no hydrogen chloride can originate must be used. Since a great many ozonides are of a very unstable nature the use of increased temperatures in the removal of the solvent by distillation is a decided disadvantage. Ethyl, and particularly methyl chloride are attacked only very slightly by ozone and on this basis, and also since they are easily removed by evaporation, are very useful. Unfortunately their high price hinders a more frequent use. Glacial acetic acid is also very slightly attacked; however most ozonides are soluble in it and do not separate out. Their isolation by evaporation of the glacial acetic acid may entail certain difficulties because of the relatively high boiling point of the acid. Many ozonides easily decompose at this temperature or distil with the solvent. However glacial acetic acid as a solvent is capable of very wide application. Acetone is very little changed by ozone, while, on the other hand, methylethylketone is greatly affected. Acetone, while an excellent solvent, is not used widely and of course cannot be used when acetone is expected as a product of the decomposition of the ozonide.

Petroleum ether is slowly attacked by ozone and adipic acid has been found to be formed from the action of ozone on hexane. Ethyl acetate which is frequently used is slowly split into acetaldehyde and acetic acid.

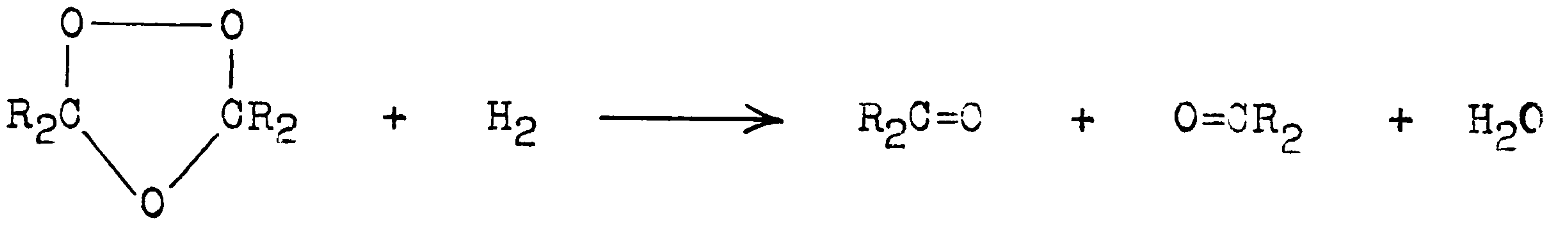
The temperature at which the solvent is held during the course of the ozonization is of importance. Not only is the degradation to the solvent lessened at the lower temperature but the optimum conditions for the formation of the ozonide of various compounds depend to a large extent upon the temperature. Thus Briner and co-workers (20) ozonized maleic anhydride in both chloroform and ethyl chloride at -60°C. and found that a very unstable ozonide was formed. At the other extreme Bogert and Isham (21) carry out their ozonizations in 75% acetic acid at a temperature of 50°C.

The method of decomposition of the ozonide is another very important factor in determining the yield of the resulting products. Perhaps the most serious difficulty is the production of hydrogen peroxide when the ozonide is split, and its subsequent reaction with the products formed. Prior to the researches of Harries, vanillin had been made by the action of ozone on isoeugenol (22). It was found that better yields could be obtained by using a crude ozonizing apparatus giving dilute ozone of about 1% concentration than when using more concentrated ozone made by improved apparatus. Later Harries (23) showed that 70% yields of the vanillin could be obtained by treating the ozonide with zinc dust and acetic acid. Semmler and Bartlett have shown that the side chain on safrol also reacts readily with the production of homopiperonylic aldehyde (24).

McCubbin (25), in an investigation of the oxidation of tri-isobutylene by ozone, carried out the ozonization in glacial acetic acid containing 2% acetic anhydride. The hydrolysis of the ozonide formed in this manner was effected by slowly adding water and zinc dust to the reaction mixture. Considerable heat was evolved and cooling was necessary. When the addition of the water and zinc dust was completed, the mixture was first refluxed and then the resulting products separated by distillation. McCubbin and Adkins (26) used this same method in their investigation of di-isobutylene.

Whitmore and Church (27) studied the isomers of di-isobutylene which had been separated by fractional distillation. The ozonide was isolated as an explosive sirup and was decomposed by pouring it very slowly into a well stirred boiling mixture of 500 cc. of water and an amount of zinc dust, equivalent to the ozonide, together with traces of hydroquinone and silver nitrate. This was found to be a rather violent reaction. By means of a rather complex system of condensers the volatile products which immediately distilled out of the boiling mixture were caught in suitable receivers.

Much attention has been given the question of increasing the yields of aldehydes from the splitting of ozonides and the catalytic hydrogenation of these ozonides is perhaps the newest and most efficient method that has been devised. Thus Harries and Tank (28) in 1908 obtained only a 5% yield of glutaric dialdehyde by treating cyclopenteneozonide with water. Later Menzies and Robinson (29) by means of reduction of cyclopenteneozonide and cyclohexeneozonide with titanium trichloride obtained 20% yields of glutaric and adipic dialdehyde respectively. F. Fischer and co-workers have made a study of the catalytic hydrogenation of various ozonides (30) and have reported yields of from 50 to 75% of glutaric and adipic dialdehydes. The catalytic hydrogenation of the ozonide obtained from methyl heptenone was found to give a 70% yield of levulinic aldehyde. They postulate the following mechanism for the reaction of hydrogen with the ozonide:



The ozonization technique has also invaded the realm of micro-chemistry. Kuhn and Roth (31) have devised a method for the determination of isopropylidene groups by means of ozone (32) The isopropylidene groups attached to carbon are split off as acetone by the ozone. Any aldehydes which are formed are decomposed by permanganate, the acetone is then distilled over and an excess of standardized iodine is added to the alkaline solution. Titration with thiosulphate gives a measure of the iodine not used in iodoform formation. It was established that the acetone is stable towards ozone and also towards permanganate under the selected conditions of the experiment. This is a micro method and 2 to 3 milligrams of acetone may be determined with an absolute error of less than 0.5%.

While isopropylidine groups attached to oxygen (as in the acetone derivatives of sugars) may be determined with great accuracy by means of acid hydrolysis, the theoretical amount of acetone is only obtained in a few cases after ozonization of compounds in which these groups are attached to carbon. With all terpenes in which "acetone groups" are assumed to be present the acetone values are below those expected. Because the deficit is frequently associated with the production of formic acid and formaldehyde, V. Grignard (33) came to the conclusion that there are present equilibrium mixtures of compounds containing CH_2 groups, such as,

$$CH_2{=}C(CH_2)\cdot CH_2{-} \qquad \alpha \text{ form}$$

and isomers containing the isopropylidine radical,

$$(CH_3)_2C{=}CH{-} \qquad \beta \text{ form.}$$

This conception is shared by J. L. Simonsen, who is one of the best authorities on terpene chemistry. He considers that even in beautifully crystallized compounds, which to all appearance are uniform, equal amounts of the α and β forms are present.

It should also be noted that acetone is formed in considerable amounts from compounds which contain no isopropylidine groups such as thymol, terpin hydrate, and isopropyl alcohol. Isopropyl groups in the neighborhood of which hydroxyl and double bonds occur tend especially to form acetone.

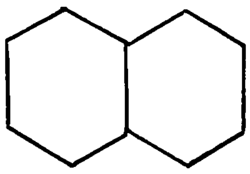
Of recent interest is the work of Durland and Adkins (34) in which they attempted to determine with ozone the position of unsaturation in a hydrophenanthrene containing one double bond. Their experiments were based on what they later termed "the naive assumption that ozone was a rather specific reagent for alkene linkages", for indeed, they found that the chief product of the ozonization of the unsaturated hydrophenanthrene was an unsaturated ketone, produced by the oxidation of a methylene group, the alkene linkage remaining intact.

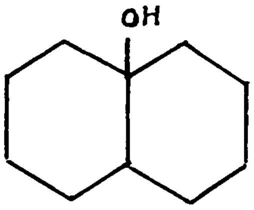
In view of their rather anomolous findings Durland and Adkins (35) undertook a further study of this type of reaction and ozonized a number of known compounds. They found that ozone reacted at 0°C. with saturated as well as unsaturated linkages oxidizing CH and CH_2 groups to tertiary alcohol and ketones respectively. They isolated a number of tertiary alcohols as the result of the ozonization of alicyclic hydrocarbons and showed that unsaturated hydrocarbons or ketones may be formed by the dehydration of these alcohols under rather mild conditions. Relatively high yields of acids were found as the result of the ozonization of saturated hydrocarbons.

Thus the following interesting results were found in the case of the ozonization of decahydronaphthalene, I, (decalin).

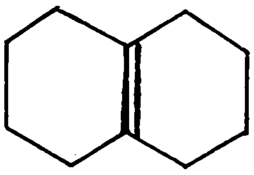
The cis-decalin form oxidized more rapidly than the trans isomer so that if a mixture of the isomers was ozonized the products were chiefly those from the cis compound. Subsequent studies were therefore made with pure cis and trans compounds.

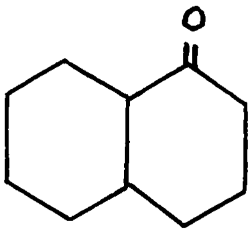
The fractionation of the products from the ozonization of 26 grams of cis-decalin for 147 hours at 0°C. gave 7.5 grams of cis-decahydronaphthol-9, II; 2 grams of Δ9,10 octahydronaphthalene, III; and small amounts of cis-αdecalone, IV. About 10 grams of a mixture of unidentified acids was also obtained. Trans-decalin gave in 28% yield the trans-decahydronaphthol-9, II; and the trans-αdecalone, IV; but unless special precautions were taken the chief product was the octalin, III, in 21% yield. A mixture of acids similar in amount to that from the cis-isomer was obtained. Among these was identified trans-cyclohexane 1,2 diacetic acid, V.


I

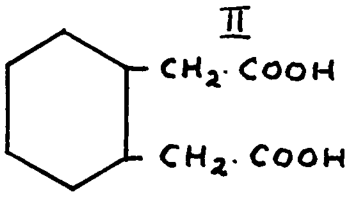


II

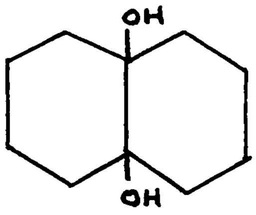

III



IV



V



VI

The ozonization of the cis-isomer of II gave the ditertiary glycol VI, as well as a mixture of acids. The cis and trans isomeric alcohols, II, differed considerably with respect to the ease of dehydration to the octalin III, the trans isomer being much more easily dehydrated.

PART II. - THE ACTION OF OZONE ON LIGNIN.

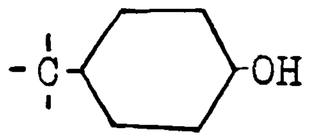
A number of workers have studied the action of ozone on various lignin preparations but in general the fragments split off by this treatment have been too small to be of any real value in the elucidation of its structure. In 1906 König (36) noted the production of formic and oxalic acids by the action of ozone on Willstätter lignin. Dorée and Hall have observed the formation of these same two acids on the ozonization of lignin sulphonic acids (37). Erdmann subjected Willstätter lignin to the action of a cold stream of oxygen containing 2% ozone and found that the lignin was slowly destroyed (38).

Pauly and Feuerstein (39) in a patent application treated woodmeal suspended in glacial acetic acid with ozone and hydrolysed the resulting product claiming to obtain vanillin. No yields were reported and this work requires confirmation.

Freudenberg, Sohns and Janson (40) treated methylated lignin in acetic acid solution with ozone. The acetic acid was then removed by evaporation and the residual material taken up in water and again subjected to the action of ozone. They reported the production of a 20% yield of oxalic acid and other unidentified water-soluble products.

Phillips and Goss studied the oxidation of alkali lignin obtained from corn cobs (41). When the lignin in glacial acetic acid solution was treated with a dry stream of oxygen containing

6% ozone for 25 hours, oxalic acid was obtained as a product of the reaction. When the lignin was methylated with dimethyl sulphate and then subjected to the same treatment a small quantity of anisic acid, $CH_3O.C_6H_4.COOH$, was identified in the ether extract of the resulting aqueous solution. When the lignin was ethylated with diethyl sulphate and then oxidized with nitric acid p-ethoxy benzoic acid was found, $CH_3CH_2O.C_6H_4.COOH$. No yields were reported in either case. In view of these results Phillips was led to conclude that the penolic hydroxyl was free in the orignal lignin and that furthermore, the following grouping must be present in the lignin,



Bell, in these laboratories (42), isolated acetone by the action of ozone on formic acid lignin. Fully methylated birch formic acid lignin gave acetone, but no acetone was found using fully methylated spruce formic acid lignin. Various fractions of acetic acid birch lignin gave no trace of acetone whatsoever. The yields from different fractions of the formic acid birch lignin varied from 1.4 to 6.6%. It was assumed that the dehydrating effect of the formic acid caused the formation of an isopropylidine group.

Thus, it will be observed that the ozonization of lignin gives rise to fragments of such a small nature that they throw very little light on the general structure of lignin. Another difficulty frequently encountered in this work is the great insolubility of the majority of lignin preparations in the usual anhydrous ozonization solvents. In general, it may be said that when a lignin is subjected to the action of ozone there results, when the resulting product is treated with water, a water-insoluble lignin and an aqueous solution. This latter solution contains mainly a water soluble lignin-like material and oxalic acid. The more prolonged the treatment with ozone, the smaller is the resulting water-insoluble and the greater the water-soluble fraction. This action is accompanied by a decrease in the methoxyl content of the lignin.

PART III - THE CHEMICAL ASPECTS OF THE SULPHONATION OF LIGNIN BY MEANS OF AQUEOUS BISULPHITES.

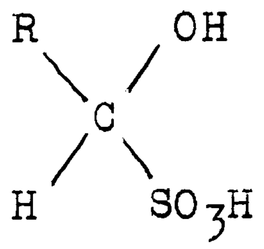
The method of producing pulp by heating wood in aqueous solutions containing calcium or magnesium bisulphite and free sulphurous acid has been an important industrial process for many years. In this reaction the lignin in the wood is removed as water-soluble lignin sulphonic acids. The two main purposes in this sulphite process are, on the one hand, the removal of the maximum amount of the lignin from the wood and, on the other, the production of as large a quantity of cellulose as possible with a minimum of degradation of the latter.
The mechanism of the reactions involved in this delignification has been the object of a great deal of investigation and many theories have been proposed to explain the formation of these water-soluble lignin sulphonic acids from the native lignin.
Despite the extensive research[#], and the many theories which have been proposed both from the chemical as well as the physico-chemical standpoint, no completely satisfactory mechanism exists, as yet, and furthermore it seems likely that such an explanation must in all probability await a further elucidation of the structure of "protolignin".

[#] Peter Klason was actively engaged in lignin sulphonic acid investigations for some 38 years.

A number of workers have studied the action of bisulphites and of sulphurous acid on known organic compounds in an attempt to gain some insight into the particular chemical groupings in the lignin at which the sulphonation occurs.

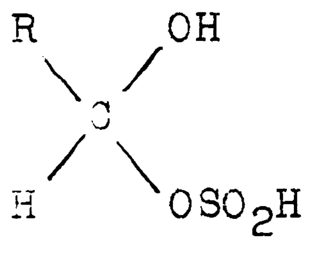
The Action of Bisulphites and Sulphurous Acid on Organic Compounds.

It is well known that sodium bisulphite forms addition compounds with many carbonyl compounds, in particular most aldehydes, methyl ketones and cyclic ketones. For some time it was not known whether the structure of the resulting compounds was one of an hydroxy sulphite ester, (II), or that of an hydroxy sulphonic acid (I), in other words whether or not the carbon was linked to sulphur directly or through oxygen to the sulphur. One of the chief characteristics of these bisulphite addition compounds is the ease with which acid or alkali will reverse the reaction.



(I)

α hydroxy sulphonic acid.



(II)

α hydroxy sulphite ester.

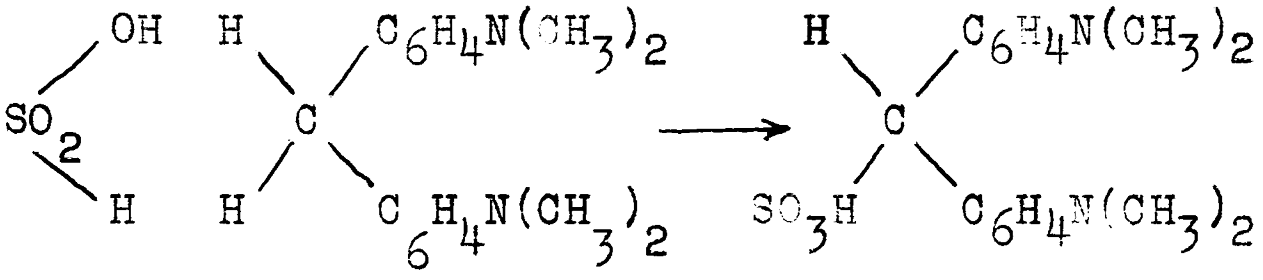
It is now generally agreed that the structure of these addition compounds is that of the true sulphonic acid (I), (43,44). It still remains necessary however to explain the marked unstability of these compounds to acid as compared with the stability of normal alkyl sulphonic acids to both acid and alkali. This is assumed to be due to the fact that in the hydroxyl sulphonic acid structure the C-S linkage is weakened by the presence of the OH group on the same carbon atom. Thus an hydroxyl and a sulphonic acid group on the same carbon atom form an unstable arrangement similar to that formed when two hydroxyl groups are attached to one carbon.

Aldehydes can also unite with sulphurous acid itself. This is the basis for Schiff's test to distinguish aldehydes from ketones. A solution of the dye fuchsin is just decolorized with sulphurous acid; addition of an aldehyde removes the sulphurous acid and the pink color reappears.

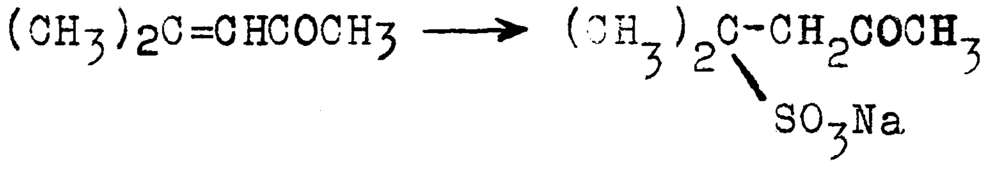
Now bisulphites also react with an ethylenic double bond. Thus allyl alcohol, $CH_2=CHCH_2OH$, reacts with a concentrated solution of potassium bisulphite to give $CH_3CH(SO_3K)CH_2OH$. This compound is the salt of a true sulphonic acid. Amylene, cyclohexene, pinene and dipentene combine with the elements of sulphurous acid on shaking with bisulphites to give sulphonic acids (45).

Unsaturated acids, aldehydes and ketones give rise to beta sulphonic acids. In the case of the unsaturated aldehyde there are two competing reactions, one reversible to give the unstable bisulphite addition compound with the aldehyde group, and one, slower and irreversible, to give the stable beta sulphonic acid.

Free sulphurous acid is known to sulphonate by dehydration in certain cases (46), e.g.



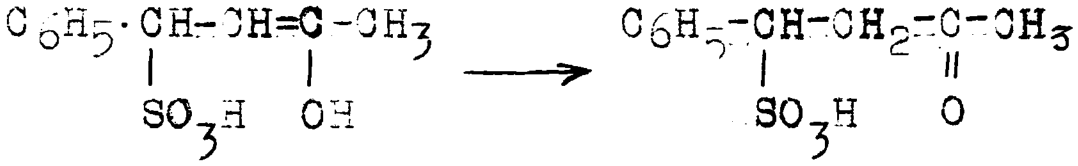
Mesityl oxide gives an addition product on long standing,



$$(CH_3)_2C{=}CHCOCH_3 \longrightarrow (CH_3)_2\underset{\displaystyle SO_3Na}{C}{-}CH_2COCH_3$$

Hibbert and Sankey (47) carried out an investigation from the physico-chemical standpoint on the reaction between sulphurous acid and organic substances of known structure. They found that, when a saturated aldehyde or ketone was heated in the presence of sulphurous acid, the specific conductivity increased with rise in temperature due to the greater conductivity at higher temperatures of the resulting sulphonic acid. At 60-80° the specific conductivity dropped because of the dissociation of of the sulphonic acid into the original aldehyde, and the weakly ionized sulphurous acid. However, if an unsaturated aldehyde such as cinnamic aldehyde were used, the specific conductivity increased with rise in temperature, decreased slightly at 80° due to the effect previously mentioned, and then increased at higher temperatures due to the formation of the stable sulphonic acid by addition to the ethylene bond.

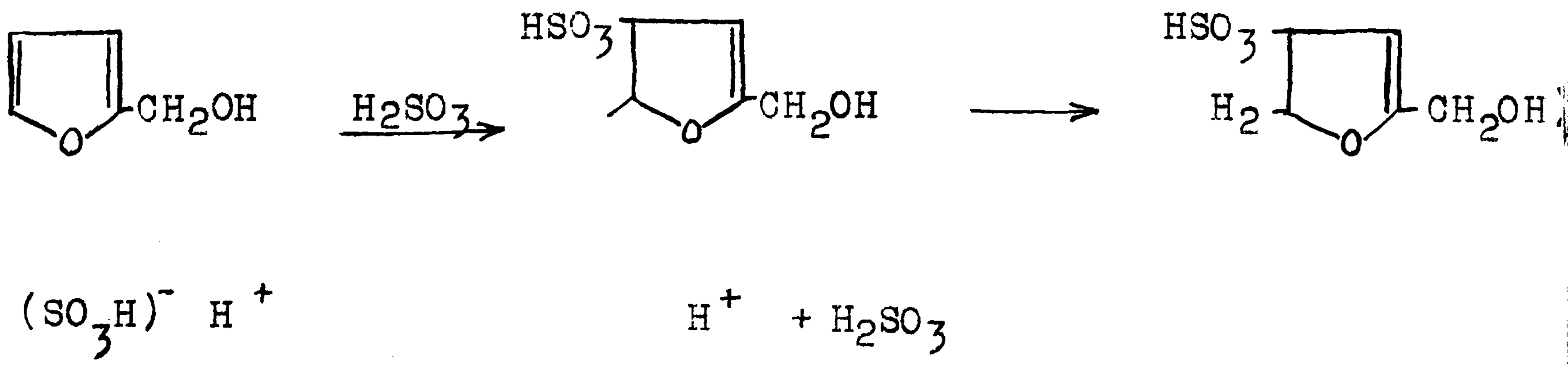
Thus methyl ethyl ketone, $CH_3COCH_2CH_3$, reacted very slowly. But $C_6H_5-CH=CHCOCH_3$ reacted at room temperature and since the addition of sulphurous acid to an ethylene double bond is slow when it is the only reactive group and higher temperatures are necessary Hibbert and Sankey proposed a 1,4 addition of the sulphurous acid and a subsequent shift of the hydrogen,



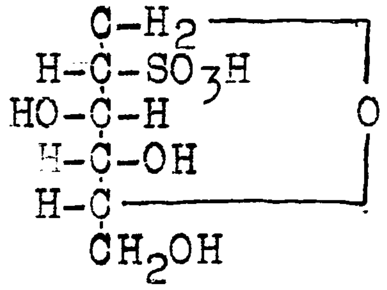
$$C_6H_5\cdot\underset{SO_3H}{\underset{|}{CH}}-CH=\underset{OH}{\underset{|}{C}}-CH_3 \longrightarrow C_6H_5-\underset{SO_3H}{\underset{|}{CH}}-CH_2-\underset{O}{\underset{\|}{C}}-CH_3$$

Dibenzyl ketone, $C_6H_5-CH_2-CO-CH_2-C_6H_5$, did not react to form a sulphonic acid. Dibenzoyl methane, $C_6H_5-CO-CH_2-CO-C_6H_5$, did not react although one might expect enolization and 1,4 addition to the resulting conjugate system. Quinone was rapidly reduced to hydroquinone with the formation of sulphuric acid. With resorcinol and phloroclucinol there was little reaction up to 145°C. Cinnamyl alcohol, $C_6H_5-CH=CH-CH_2OH$, which has an isolated double bond reacted very slowly even at 145°C.

Tetrahydrobenzene gave a slight reaction at high temperatures and the resulting sulphonic acid was stable. Furfural gave addition only to the aldehyde group. Furfuryl alcohol at 100-110°C. gave addition forming a stable sulphonic acid. With this substance Hibbert and Sankey postulated the preliminary formation of an oxonium addition product with the sulphurous acid followed by entry of the sulphurous acid into the furane ring, thus,

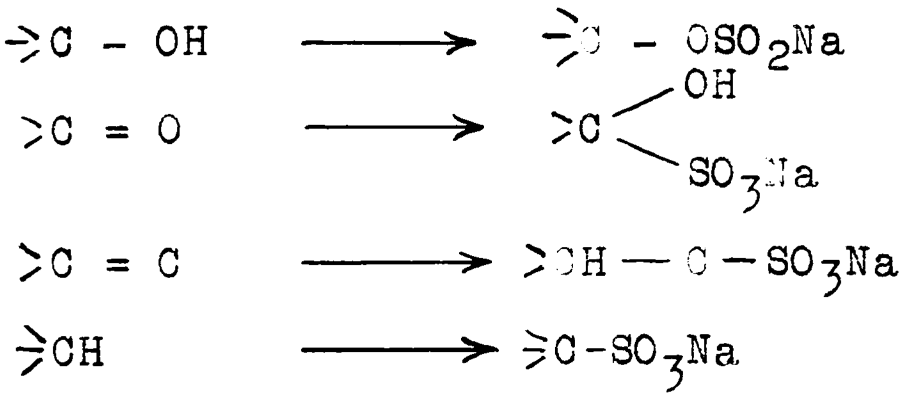


With glucal reaction took place at 100°C. to give the acid with the sulphonic acid grouping at the position indicated. This was believed to go through an intermediate oxonium compound.



They also showed from conductivity experiments that lignin sulphonic acids are very strong acids comparable in strength to a mineral acid.

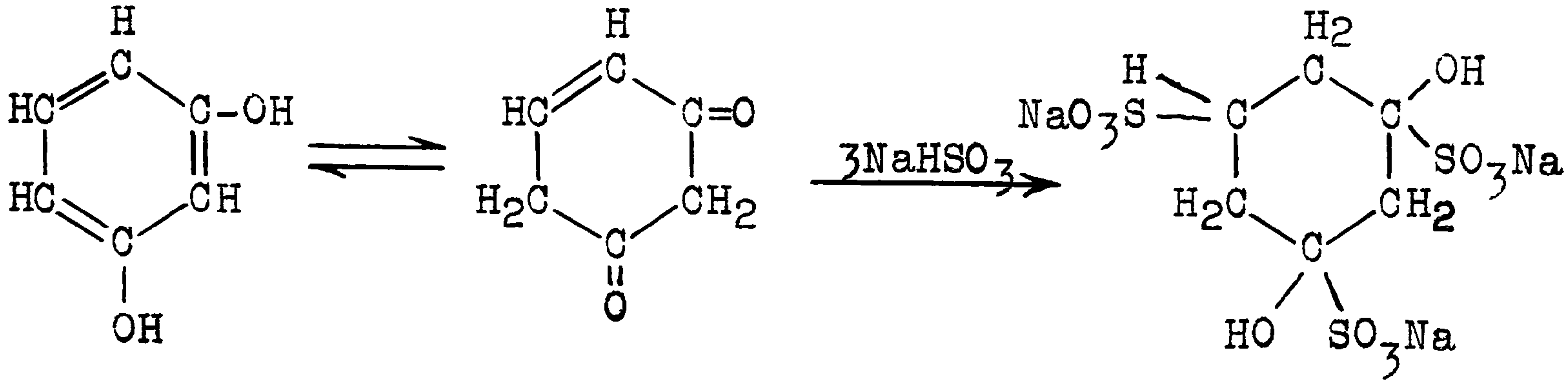
Bucherer (48), after an extensive investigation of the reactions of sulphurous acid and its salts with various organic groupings, summed up these reactions in the following manner.



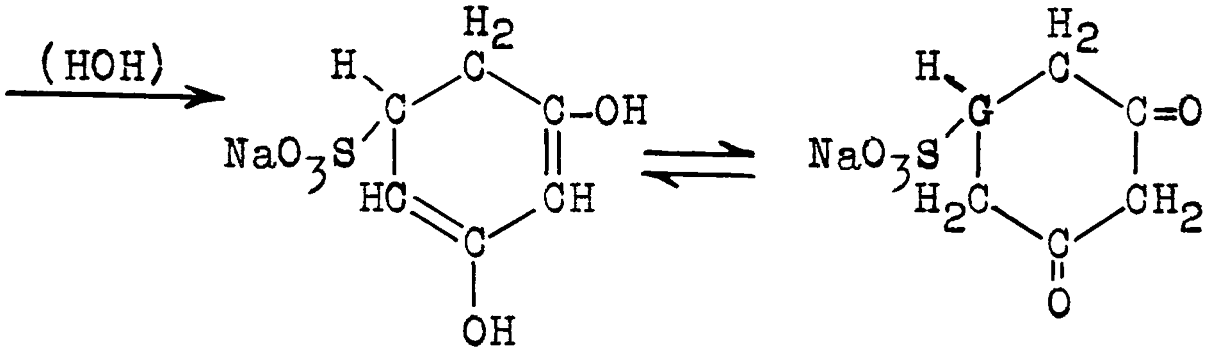
$$\geq C-OH \longrightarrow \geq C-OSO_2Na$$
$$>C=O \longrightarrow >C\begin{matrix}OH\\SO_3Na\end{matrix}$$
$$>C=C \longrightarrow >CH-C-SO_3Na$$
$$\geq CH \longrightarrow \geq C-SO_3Na$$

Bucherer found that phenol gave only a trace of sulphonic acid derivative when treated with sulphurous acid under the conditions of the sulphite cook but that resorcinol formed a sulphonic acid quantitatively.

Fuchs and Elsner (49) in 1920 suggested that in the formation of the sulphonic acid of resorcinol one molecule of the dihydroxy phenol in the keto form reacts with three molecules of sodium bisulphite,

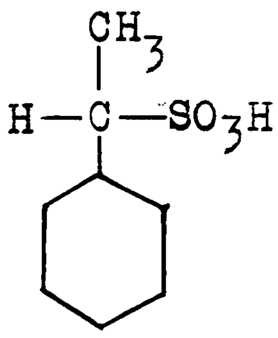


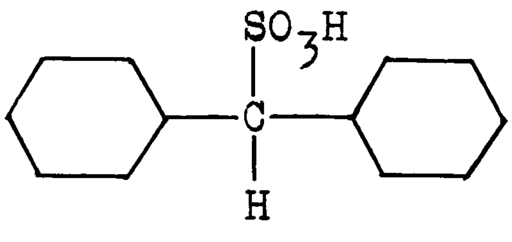
and that this resulting complex, on hydrolysis with either acid or alkali yields 3,5 diketo hexamethylene 1-sulphonic acid.



This appears reasonable since one would expect the unstable keto-bisulphite addition complex to be removed by the acid and the sulphonic grouping formed by 1,4 addition to the conjugated system to remain stable. However Bucherer (50) has pointed out that pyridine will add bisulphite to form a similar addition compound, $C_6H_5N \cdot 3NaHSO_3 . 2H_2O$, and in this case no carbonyl group would be involved.

Holmberg (51) has conducted a series of model experiments in which he showed that methylphenylcarbinol and diphenylcarbinol formed bisulphite reaction products as follows:





Physico-chemical Aspects of the Reactions of Lignin with Aqueous Bisulphites.

The rate of extraction of lignin from wood in the Sulphite Process is controlled by three distinct factors: (1) the rate of penetration of the sulphite liquor into the wood chip, (2) the rate of sulphonation of the lignin to form an insoluble sulphonic acid, and, (3) the rate of solution of the latter. With the use of a finely divided wood-meal the penetration factor becomes negligible and it is thus possible to study the second and third stages, and to determine which is the controlling factor in the rate of solution of the lignin from the wood.

If a sulphite cook is stopped before all the lignin has passed into solution, the residual lignin in the pulp that is obtained is found to be sulphonated, but it is not in a form in which it is soluble in water. However on boiling this pulp with dilute hydrochloric acid the lignin sulphonic acid passes into solution, and Hägglund (52) has concluded that this is the result of a hydrolytic reaction, possibly involving a glucosidic linkage between the lignin sulphonic acid and carbohydrates.

When wood is treated with sulphite cooking liquor having a low free sulphurous acid content, such as sodium bisulphite (without the addition of the excess sulphur dioxide), the lignin sulphonates readily, but the hydrolysis is greatly delayed due to the low hydrogen ion concentration. The pulp so obtained is characterized by high lignin, sulphur and methoxyl content. Hägglund (53) treated such a pulp for 1 hour at 135°C. with buffered solutions of $H_3PO_4.K_3PO_4$. At a pH of 1.85, over 90% of the lignin passed into solution in 1 hour, while at a pH of 3.53, only 50% of the lignin had dissolved, and Hägglund concluded that the rate of solution of the lignin at the end of the cook is dependent on the pH of the cooking liquor. Stangeland (54) considers the removal of the "encrustants" from the wood by the sulphite process to proceed as a first order reaction, in which the hydrolysis reaction determines the total rate of the process.

The work of Hägglund has shown that the rate of delignification is slow at the very beginning of the cook, due to the time required for the sulphonation. By commencing the cook at the maximum temperature, rather than taking one or two hours to bring the temperature to 110°C., Yorston (55) has confirmed this observation. From a kinetic study of the reaction he finds that the rate of sulphonation at any time is proportional to the unsulphonated lignin remaining in the pulp while the rate of removal of sulphur from the pulp, which is an index of the sulphonated lignin, is proportional to the quantity of sulphonated lignin present.

Kullgren (56) also considers that the delignification of wood depends upon the initial formation of an insoluble lignin sulphonic acid complex. Now this would exist largely as the calcium salt, but there would be a certain equilibrium between the salt and the free acid. Kullgren considers that only the portion which existed in the form of the free acid would pass into the soluble lignin sulphonic acid. Then immediately a new equilibrium would be formed between the calcium and hydrogen ions, resulting in further solution.

An undercooked sulphite pulp will of course contain the insoluble calcium lignin sulphonate complex and if this is washed with dilute hydrochloric acid, cation exchange takes place between the calcium and hydrogen ions in the pulp. A pulp so treated was washed with water and then in three experiments Kullgren refluxed it at 100°C. with (i) distilled water, (ii) 0.004 N HCl and (iii) 0.004 N HCl solution which was also 0.04 N with respect to NaCl.

In the first two experimetns in which the insoluble lignin sulphonic acid complex existed as such rather than as the sodium salt, its solution proceeded very rapidly and at the same rate in both cases. However, he found that in the presence of the sodium chloride practically no extraction took place in spite of the acidity of the medium. From this extremely interesting observation he concluded that the rate of solution depended on the ratio of the free undissolved sulphonic acid to its salt which is not necessarily dependent on the pH of the extraction medium. If the solution resulted from the normal hydrolysis reaction, the rate would be directly dependent on the pH. The more rapid extraction of the calcium salt at low pH as observed by Hägglund might result from this change in the cation equilibrium.

Kullgren, however, overlooks the fact that the free sulphonic acid when in solution shows a lower solubility and a much greater tendency to polymerization than do its salts. It hardly seems likely that the solution of the lignin can be due to a greater solubility of the free acid. When wood is heated with sulphurous acid without the addition of lime, dark colored pulps and liquors are obtained.

Perhaps these apparent discrepancies may best be reconciled if the assumption is made that the solid lignin sulphonic acid complex undergoes some chemical change before passing into solution, and that this change is dependent on the sulphonic acid radical in the molecule. Such a change might be the hydrolysis of a lignin-carbohydrate complex, or a depolymerization of the lignin itself.

It has been shown by Campbell and Maass (57) that a normal cooking liquor of 5.12% total and 1.28% combined sulphur dioxide at a temperature of 100°C. has a hydrogen ion content of 0.002 molar and these authors suggest that the reason sulphite solutions are effective in promoting lignin removal is not because the sulphite addition compounds are soluble but that the solution is so slightly acid under the conditions employed that resinification of the lignin is minimized.

Hägglund (58) agrees with Hibbert (59) that sulphonation takes place in two stages and it is now generally accepted (60) that in the first phase a water insoluble lignin sulphonic acid in the solid state is formed and that this in the second phase is split into carbohydrate and water soluble lignin sulphonic acid. Hägglund has examined these two phases very thoroughly and has found that the speed with which the first phase takes place is dependent upon the speed with which the cooking liquor penetrates the chips, and on the ion concentration. The second phase is mainly dependent on the temperature and pH of the liquor. The question of the homogeneity of native lignin must be considered before proceeding to the various chemical mechanisms that have been proposed for the sulphite cook. Of course very little is known of the exact nature of the lignin as it actually occurs in the plant but the sulphite cooking process gives rise to a product which may be fractionated into two components, namely α and β lignin sulphonic acids. Klason (61) first showed that the α lignin sulphonic acid may be obtained by precipitation with β-naphthylamine hydrochloride as an insoluble addition complex of the lignin sulphonic acid and the base. Klason (62) pointed out that only aldehydes and not ketones will react with this reagent and that therefore this reaction of the α lignin sulphonic acid is typical of an aldehyde group. The second fraction, the β lignin sulphonic acid, is not precipitated from its acid solution by β naphthylamine hydrochloride (63), forms no insoluble barium salt, but may be precipitated with lead acetate as a white solid.

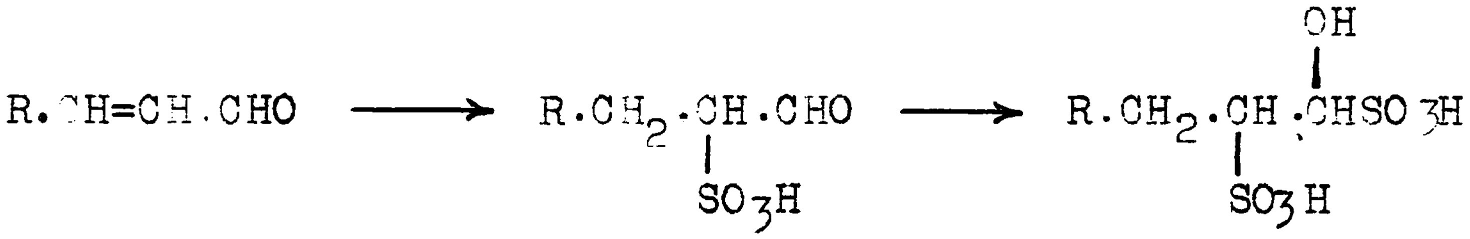
Klason believed that the α-lignin sulphonic acid was an oxidized form of the β-lignin sulphonic acid, because he found (64) that the latter when oxidized with hydrogen peroxide in the presence of ferric chloride, could then be precipitated with β-naphthylamine hydrochloride. The resulting product closely resembled the addition complex formed from the α-lignin sulphonic acid and the base.

It has been shown by Tomlinson and Hibbert (65) that the various fractions of lignin sulphonic acid from spruce wood yield on alkaline cleavage, varying amounts of vanillin, pointing to a constitutional difference between these fractions. However, Hägglund (66) claims that the lignin is homogeneous in the plant but that lignin sulphonic acids of varying particle size are formed depending upon the conditions of the cook. He believes that the fractionation is principally a question of salting out and that sodium chloride, salts of aromatic amines, alkaloids, etc., salt out the less finely dispersed lignin sulphonic acids which are designated as α-lignin sulphonic acid. The portion which cannot be precipitated in this manner he believes to be in a finely dispersed condition as shown by the ease with which it passed through a parchment membrane. This β lignin sulphonic acid is further characterized by its ability to reduce Fehling's solution and its high degree of sulphonation. This may be due to the fact that the β lignin sulphonic acid is less highly polymerized and that there are consequently more free aldehyde groups present in the aliphatic portion. In accord with this it has a lower methoxyl content and holds more sulphur dioxide in loose combination than does the αlignin sulphonic acid.

Organo-chemical Aspects of the Reactions of Lignin with Aqueous Bisulphites.

A great number of theories have been advanced with regard to the actual groupings in the lignin to which the SO_3H radicle becomes attached. For some time it has been known that a portion of the sulphur consumed in the formation of lignin sulphonic acids is firmly and irreversibly bound, while another portion is held loosely and may be removed by acid or alkali. Hägglund and Carrick (67) found that, of the total sulphur consumed, the loosely bound sulphur dioxide represented 20-30%, the firmly bound 50-60%, and that about 20% was oxidized, presumably by atmospheric oxygen to sulphuric acid. Sulphur contents have been reported in the literature varying from 4% (68) to 12% (69), although the average value is about 6%. For this reason it seems very probable that the calcium bisulphite reacts with more than one type of group in the lignin. At least one of these groups is necessary for the subsequent solution of the lignin and reacts quite readily. A second and possibly a third group react with relative ease but beyond that stage high temperature and prolonged heating are necessary. Hägglund suggested (70) that the loosely bound sulphur was attached to an aliphatic group or to a pyrocatechol residue with which it was bound through the keto form.

Klason (71) in 1893 suggested that lignin sulphonic acids were bisulphite addition products of a tautomerized phenol, but later, in view of his work on the precipitation of lignin sulphonic acids with βnaphthylamine hydrochloride, (72) considered that the sulphurous acid added to an acrolein group in the lignin,

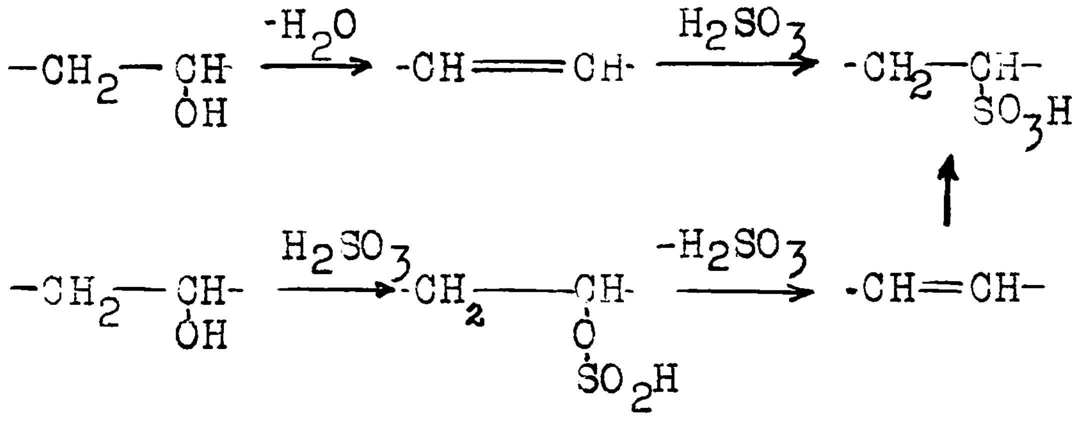


$$\mathrm{R.CH{=}CH.CHO} \longrightarrow \mathrm{R.CH_2.\underset{SO_3H}{CH}.CHO} \longrightarrow \mathrm{R.CH_2.\underset{SO_3H}{CH}.\overset{OH}{C}HSO_3H}$$

As has been pointed out there is very little evidence indicating a difference in chemical structure between the two fractions which would account for the precipitation of one and not the other when βnaphthylamine hydrochloride is added. Klason's orignal idea was that the formation of an inner cyclic salt was necessary to explain the insolubility of the product, and that the βlignin sulphonic acid contained an acrylic acid rather than an acrloein group. Reference has already been made to the fact that in 1928 (64) he found that the βacid, after treatment with hydrogen peroxide, could then be precipitated with β-naphthylamine hydrochloride. Klason explained this by assuming that the original βlignin sulphonic acid contained an acrylic alcohol, rather than the acrylic acid group which had previously been considered present. Oxidation with the peroxide would then convert the acrylic alcohol into an acrolein derivative.

capable of forming a cyclic salt with the β naphthylamine hydrochloride. However, as has been mentioned, Hägglund considers the difference is not so much a chemical one as a question of different states of molecular aggregation.

Freudenberg (73), in 1932 proposed, instead of his original view of addition to the aromatic nucleus, the direct addition of sulphurous acid to ethylenic double bonds in the lignin complex. Since lignin is known to contain an insufficient number of double bonds to account for this theory, Freudenberg assumed that water is first eliminated between a methylene group and an adjacent secondary hydroxyl, and that the sulphurous acid then added to the resulting double bond. He considered that the reaction might be a direct elimination of water or else that it might proceed through the initial formation of a sulphite ester, from which sulphurous acid could be eliminated to form the double bond.

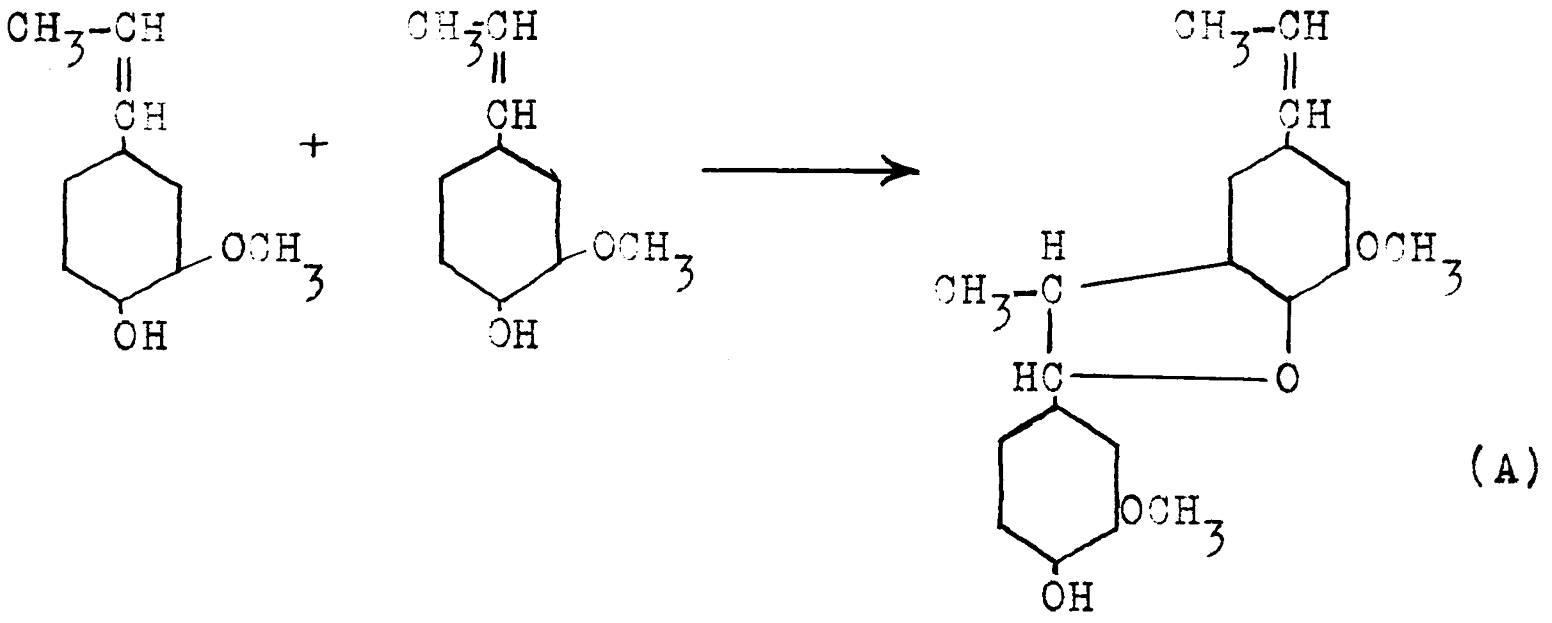


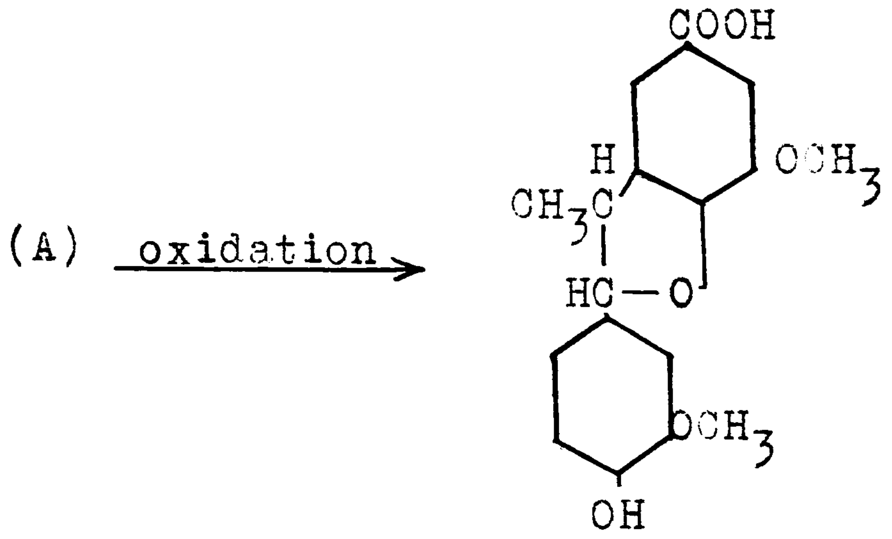
$$-\mathrm{CH_2}-\underset{\mathrm{OH}}{\mathrm{CH}}\xrightarrow{-\mathrm{H_2O}}-\mathrm{CH}=\mathrm{CH}-\xrightarrow{\mathrm{H_2SO_3}}-\mathrm{CH_2}-\underset{\mathrm{SO_3H}}{\mathrm{CH}}-$$

$$-\mathrm{CH_2}-\underset{\mathrm{OH}}{\mathrm{CH}}-\xrightarrow{\mathrm{H_2SO_3}}-\mathrm{CH_2}-\underset{\underset{\mathrm{SO_2H}}{\mathrm{O}}}{\mathrm{CH}}-\xrightarrow{-\mathrm{H_2SO_3}}-\mathrm{CH}=\mathrm{CH}-$$

As will be observed from these reactions one hydroxyl group is lost for each sulphonic **acid** group which is introduced into the lignin complex. Hägglund and Carlsson (74) showed that this is not the case since both the lignin sulphonic acid and the original lignin on methylation gave the same methoxyl value, and from these results postulated that for every sulphonic acid group which entered there was also formed an hydroxyl group which could be methylated. They interpreted their results on the hypothesis that an ethylenic oxide ring was opened during the sulphonation with the formation of the sulphonic acid group on one carbon and the new hydroxyl group on the adjacent carbon atom. In view of the fact that there was no experimental proof that the oxygen ring which was opened was of the ethylene oxide type Hägglund at a later date (75) changed his hypothesis to include any oxygen ring.

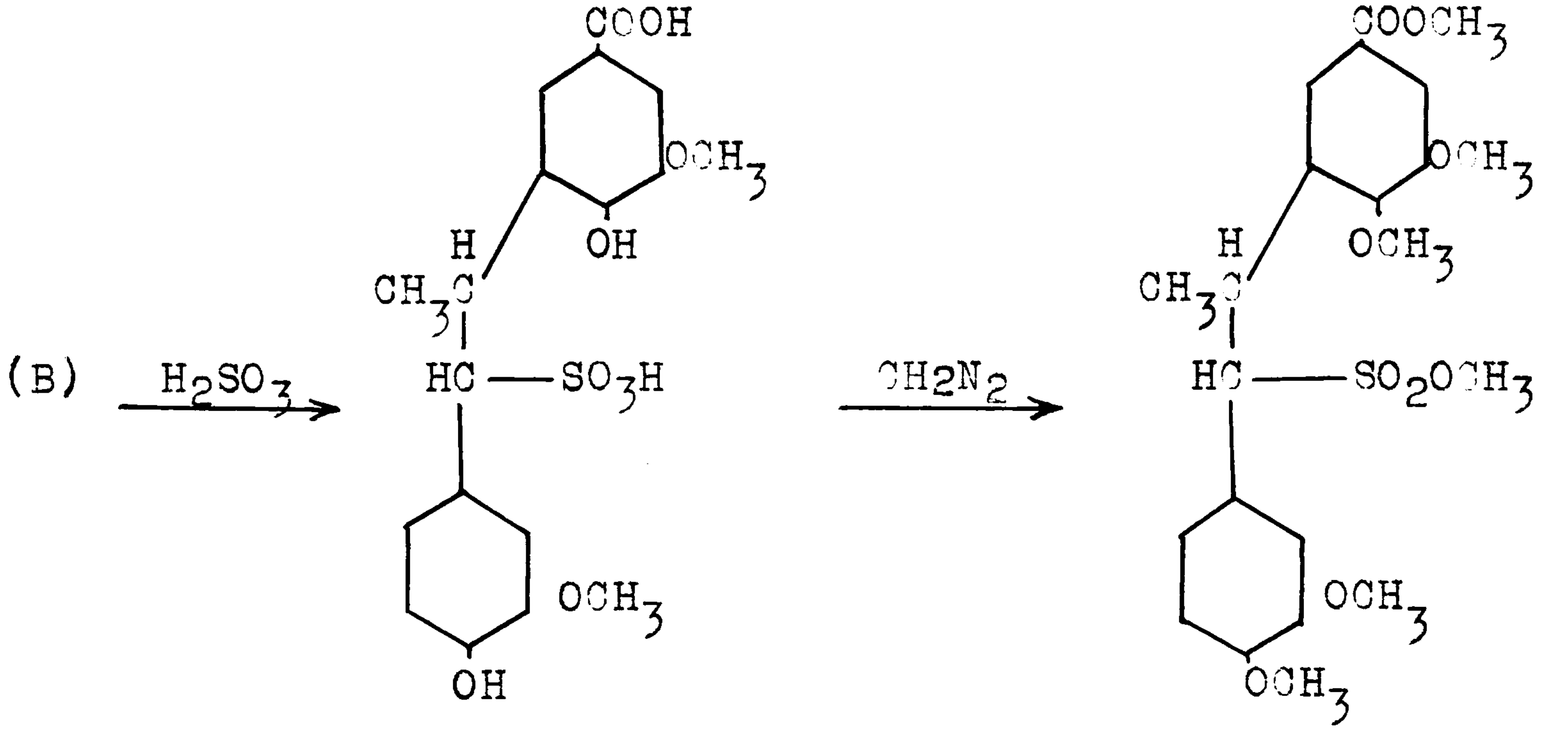
Freudenberg (76) has recently supported this theory and has produced evidence to show that this new hydroxyl group is phenolic and is methylatable with diazomethane. Freudenberg conducted a series of experiments using Erdtman's acid, which is formed by the oxidation of two molecules of isoeugenol. The mechanism is assumed to be as follows:





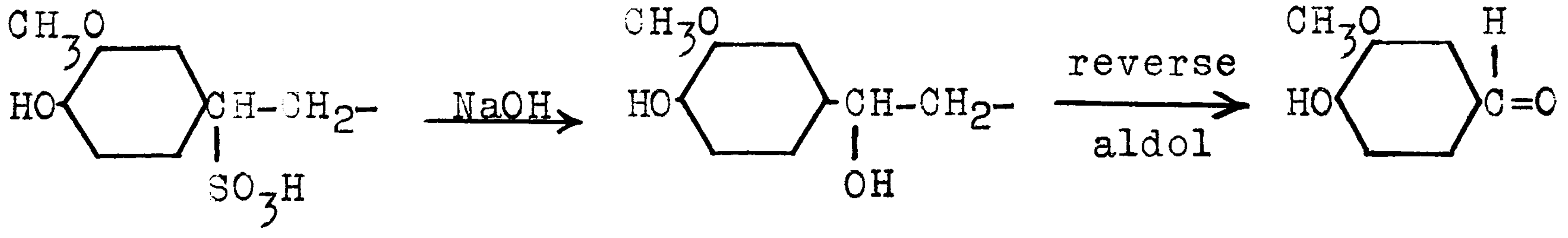
(B)

This acid was treated with sulphurous acid under the conditions of the sulphite cook and the sulphonation product as well as the methylated derivatives were assigned formulae, although there was no complete proof of structure.

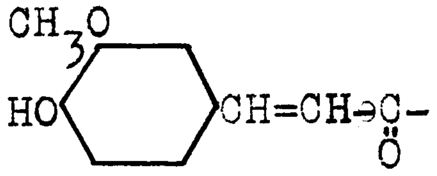


Oxidation gave 17% veratric acid and 4% isohemipinic acid out of a calculated 39 and 48% respectively. The formation of these acids was in accord with the results obtained when lignin itself was treated in the same manner. The low yields were explained as being due to the fact that the sulphonated product had polymerized and hence only the terminal groups were liberated. It should be noted that these model experiments account for the formation of a new hydroxyl group for each sulphonic acid group formed, as was shown by Hägglund and Carlsson (74).

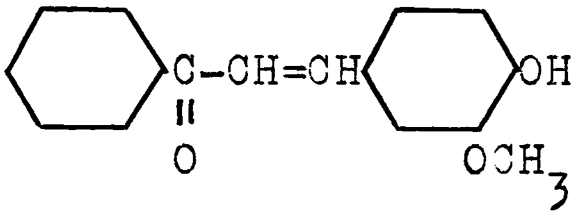
Freudenberg upholds Hibbert's view that in the lignin sulphonic acid the sulphonic acid group is adjacent to the aromatic nucleus. Tomlinson and Hibbert (77) found that vanillin was obtained by boiling lignin sulphonic acids from spruce wood with alkali and furthermore that methylated lignin sulphonic acids yielded veratric aldehyde. This afforded conclusive proof that the original lignin sulphonic acid contained a free phenolic hydroxyl group. The fact that only sulphonated lignins yield vanillin on boiling with alkali indicated that the presence of the sulphonic radicle is essential, and indicated further that it is probably adjacent to the guaiacol nucleus. The Tomlinson-Hibbert explanation was as follows,



If the following grouping were present in the native lignin,



sulphurous acid would add readily to the conjugated system to give a sulphonic acid. However Tomlinson and Hibbert found that the chalcone, 3 hydroxy, 4 methoxy benzalacetophenone



gave rise to an 80% of the theoretical yield of vanillin on boiling in alkaline solution. Therefore if the 1,4 conjugated system existed in the native lignin then a high yield of vanillin should be obtained by boiling the woodmeal with alkali <u>without the previous sulphonation</u>. As this is not the case it is therefore doubtful that the above group exists in native lignin.

Holmberg (78) has compared the reaction between thioglycollic acid and lignin with that of sulphurous acid and lignin and has concluded that the two mechanisms are identical. As a result of his intensive studies he believes that a hydroxyl group of the lignin molecule reacts with the mercapto group of the thioglycollic acid through the elimination of water, and he suggests the possibility that in the sulphite cooking process the bisulphite reacts with an alcoholic hydroxyl group according to the equation:

$$\geq C \cdot OH + HSO_3H \longrightarrow C \cdot SO_3H + H_2O$$

Recently Ahlm and Brauns (79), in an investigation of the reaction of thioglycollic acid with spruce lignin, have obtained results which indicate that with the entrance of each new thioglycollic acid group no new hydroxyl group is formed nor is a hydroxyl group split off.

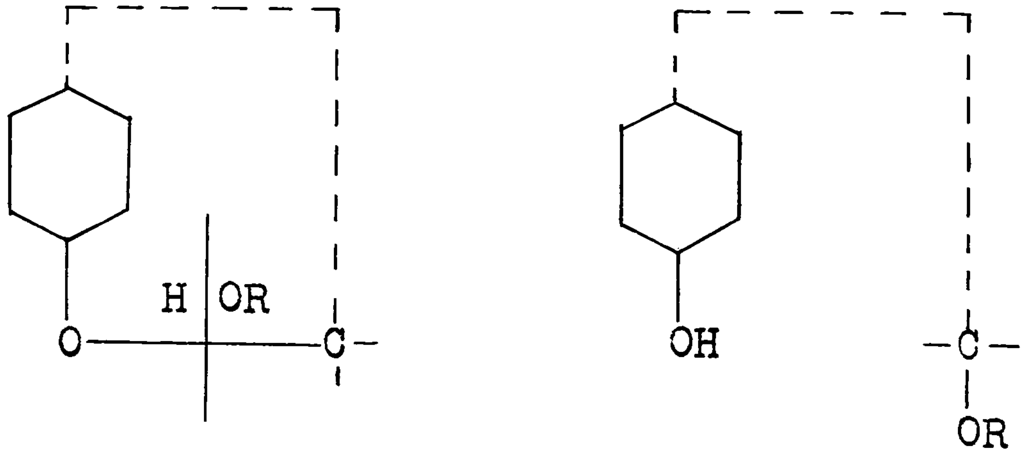
Brown and Brauns (80) made an extensive investigation into "The Effect of Pretreatment of Spruce Wood on the Reaction in the Sulphite Cooking Process". These authors ran parallel sulphite cooks under the same conditions on a sample of native spruce woodmeal and also on a sample of the same woodmeal which had been methylated with diazomethane. They found that the unmethylated woodmeal gave a normal pulp containing about 3% lignin, but that the methylated woodmeal gave a pulp which had the same lignin content as the starting material.

In view of the fact that the methylations were carried out in neutral solution and at low temperature the authors decided that an hydroxyl group present in the original lignin played the decisive role in the sulphite cook. More drastic pretreatment of the original wood meal such as the action of acids or alkalies, was found to greatly hinder the process of the delignification with the sulphurous acid. They suggested that these reagents might bring about a condensation or a polymerization of the lignin.

Brown and Brauns are of the belief that the sulphite cook takes place in two stages. The first stage represents the addition of sulphurous acid to the native lignin to form a solid lignin sulphonic acid. In the second stage they believe a rearrangement takes place, dependent on an hydroxyl group, this rearrangement giving rise to the soluble lignin sulphonic acid. In the methylated wood meal the process is thought to proceed as far as the first step, since analysis shows that the lignin after methylation is still capable of binding sulphurous acid, but the amount held is considerably less than that held by the untreated wood. The reaction stops at this point, they believe, since the blocking of the hydroxyl group prevents the rearrangement from taking place. It seems to the writer that this view of Brown and Brauns is open to some criticism since there is no indication that the small amount of sulphurous acid held by the methylated wood meal is that which is actually involved in the rearrangement. It seems more reasonable to consider this sulphurous acid as NOT being involved in the actual delignification but rather as the loosely bound sulphur which is known to exist; and that the introduction of the sulphurous acid necessary for the solubilization of the lignin is prevented because of the fact that the hydroxyl group present in the lignin has been blocked by the methylation.

For some time it has been an established fact that, with few exceptions, all lignins isolated by such extractants as glacial acetic acid, formic acid, sulphuric and hydrochloric acids, alcoholysis (alcohols plus mineral acid), etc. are insoluble in sulphite liquor. In other words these isolated lignins do not form lignin sulphonic acids, a behaviour in marked contrast to that of "Protolignin".

Hägglund (81) has suggested the following mechanism for the alcoholysis reaction which gives a lignin which is alkali solüble but does not react with sulphite liquor.



He attributes the failure of these lignin preparations to dissolve in sulphite liquor to the blocking of the resulting aliphatic hydroxyl group by an alkyl rest.

However oak lignin, isolated by an acetylation technique (82) has been shown by Steeves and Hibbert (83) to yield a lignin sulphonic acid on treatment with sulphite liquor under the conditions of the sulphite cook and this lignin sulphonic acid has been shown to yield equal quantities of vanillin and syringic aldehyde by alkaline cleavage. The same treatment of the lignin sulphonic acid obtained from oak wood meal (i.e. from the true native oak lignin) also gives rise to the same yields of these compounds. Hawkins and Hibbert showed further (84) that treatment of the isolated oak lignin with glacial acetic acid at the reflux temperature or methylation with either dimethyl sulphate or diazomethane rendered the lignin insoluble in the sulphite liquor. They produced evidence to show that tertiary hydroxyl groups were involved in the mechanism of the delignification of the wood, by means of aqueous bisulphites.

Cuproxam lignin has been shown by Freudenberg (85) to be soluble in sulphite liquor. Recently Brauns has obtained a lignin (86) which he considers to be very similar to the actual native lignin. This "native lignin" is characterized by being soluble in sulphite liquor, but no information is given as to its mode of isolation or assumed structure.

DISCUSSION OF RESULTS

Introduction

The properties of birch formic acid lignin have been thoroughly studied by Lieff (92) and those of birch acetic acid lignin by Bell (42) in these laboratories. Reference has already been made to the fact that Bell studied the action of ozone on birch formic acid- and acetic acid lignins and found that acetone in small yield was a product of the degradation of the former but not of the latter. The work of Bell was concentrated on the yield of acetone and no reference was made to the water soluble and water insoluble fractions arising from the ozonization. It was the purpose of this investigation to characterize these fractions. Unlike the non-ozonized lignins, they are soluble in aqueous bisulphite solution and this afforded the opportunity, by their further study, of throwing additional light on the mechanism of lignin sulphonic acid formation.

Formic Acid as a Solvent for Ozonization

The ozonization technique requires that the ozone-oxygen mixture be passed through a solution of the substance under investigation. However the marked insolubility of the larger fractions of birch formic acid lignin in the usual anhydrous organic solvents, such as chloroform and ethyl acetate, renders these useless for this purpose. Glacial acetic acid was also found to be unsatisfactory in this respect. The similarity of formic to acetic acid, and the fact that the lignin was actually obtained by extraction with the former, suggested its use as an ozonization solvent. In so far as the writer can determine its behaviour has not been previously studied in this respect. Consequently it was deemed advisable first to compare its characteristics with those of a typical solvent such as ethyl acetate, by preliminary ozonization experiments on a number of compounds of known structure.

Maleic acid was chosen as a typical unsaturated compound. This would yield glyoxylic acid, and any further oxidation of the latter would result in the formation of oxalic acid.
A sample of maleic acid in formic acid solution was subjected to ozonization and, after removal of the solvent by distillation under reduced pressure, the ozonide was decomposed with water. Aliquot samples of the resulting aqueous solution were then examined. The absence of oxalic acid was confirmed by the

lack of a precipitate on the addition of calcium hydroxide solution. This test was shown to be sensitive to as small an amount as 0.0005 g. of oxalic acid. The glyoxylic acid was determined as its 2,4 dinitrophenylhydrazone and was obtained in a yield of 98%.

Maleic acid was then subjected to a similar ozonization in anhydrous ethyl acetate. In this case the yield of glyoxylic acid, determined as the 2,4 dinitrophenylhydrazone, was found to be only 14.5%. The yield of oxalic acid, determined as calcium oxalate, was 80%.

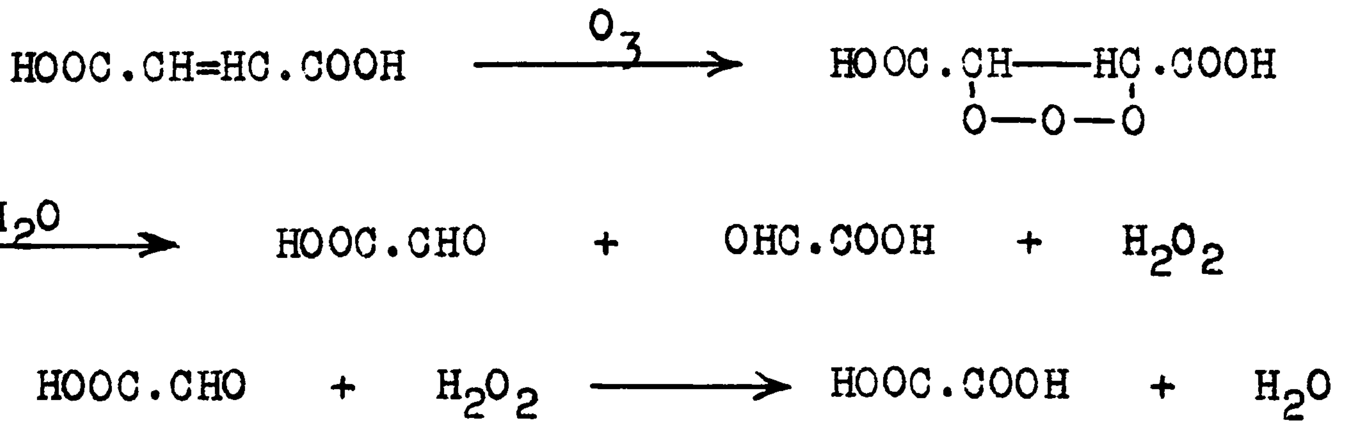
Since it was hoped that aromatic compounds might be isolated by the action of ozone on lignin it was of interest to determine the stability of certain typical aromatic compounds towards ozone. This was done in the two solvents under comparison, namely, formic acid and ethyl acetate.

The first aromatic compound chosen was vanillin. A formic acid solution of this compound was subjected to the action of ozone for two hours. The vanillin remaining in an aliquot of the aqueous solution of the reaction mixture was determined as the meta-nitrobenzoylhydrazone, and was found to be present in a yield of 42% based on the original weight of vanillin. Another aliquot of the reaction solution was extracted with ether and the vanillin isolated and purified by sublimation. This method gave a yield of 48%. No oxalic acid was formed in this reaction.

The ozonization was then repeated using anhydrous ethyl acetate as the solvent. Neither oxalic acid nor vanillin was found in the reaction mixture. The ethereal extract of an aliquot of the reaction mixture gave, on sublimation, vanillic acid in a 21% yield.

Veratric aldehyde was then ozonized in both formic acid and ethyl acetate solution. The reaction mixture from the ozonization in formic acid was found to contain 62% of unchanged veratric aldehyde, determined as the meta-nitrobenzoylhydrazone. No formation of oxalic acid took place. With ethyl acetate as solvent for the ozonization no unchanged veratric aldehyde was left but veratric acid was found in a 45% yield. The results of this study are summarized in Table I, page 47.

Thus it is seen that formic acid, in comparison with ethyl acetate, possesses some rather unique properties in regard to its use as a solvent for ozonization. A very definite protection of the aldehyde group from oxidation is evident and furthermore, the degradation of the aromatic nucleus is lessened by its use. In the case of the maleic acid the formation of the oxalic acid is undoubtedly due in part to oxidation of the glyoxylic acid, formed on decomposition of the ozonide, by the hydrogen peroxide formed simultaneously.



$$\mathrm{HOOC.CH{=}HC.COOH} \xrightarrow{O_3} \begin{array}{c}\mathrm{HOOC.CH{-}{-}HC.COOH}\\ \mathrm{O{-}O{-}O}\end{array}$$

$$\xrightarrow{H_2O} \mathrm{HOOC.CHO} + \mathrm{OHC.COOH} + H_2O_2$$

$$\mathrm{HOOC.CHO} + H_2O_2 \longrightarrow \mathrm{HOOC.COOH} + H_2O$$

TABLE I

FORMIC ACID AND ETHYL ACETATE AS OZONIZATION SOLVENTS

Original Compound	Formic Acid Solvent		Ethyl Acetate Solvent	
	Substance	Yield	Substance	Yield
Maleic acid	Glyoxylic acid	98%	Glyoxylic acid Oxalic acid	15% 80%
Vanillin	Vanillin	45%	Vanillic acid	21%
Veratric aldehyde	Veratric aldehyde	62%	Veratric acid	45%

It should be noted that this explanation, in view of the yield of hydrogen peroxide formed, accounts for the formation of only a 50% yield of oxalic acid. It now becomes necessary to explain the lack of oxalic acid formation when formic acid is used as the solvent. This is in all probability due to the fact that the formic acid used was not of an anhydrous nature but was of 95% strength and thus the ozonide first formed was immediately decomposed by the water present to form glyoxylic acid. It is known that hydrogen peroxide reacts with formic acid to produce carbon dioxide. This fact would also explain the large percentage recovery of vanillin and veratric aldehyde when formic acid solvents are used, the oxidizing effect of the ozone being exerted on the formic acid rather than the compounds in question.

These facts seemed to indicate that in any further investigation of this problem formic acid might serve as a valuable and unique ozonization solvent. This was not investigated further however, since the primary object has been to ascertain its applicability as a solvent for the ozonization of lignin.

It will be noted that vanillin and veratric aldehyde undergo considerable decomposition on ozonization but it should be borne in mind that these compounds are subjected to the action of all the ozone that is passed into solution, i.e. there is present no ethylenic double bond to which the ozone can add and thereby be absorbed. In view of this fact vanillin and veratric aldehyde must be regarded as being comparatively stable under the conditions employed. The degradation using formic acid as the solvent was found to be somewhat less than when ethyl acetate was used in this capacity. Of considerable importance is the fact that no oxalic acid was formed due to the decomposition of these aromatic nuclei by the ozone. This will be discussed in a later section.

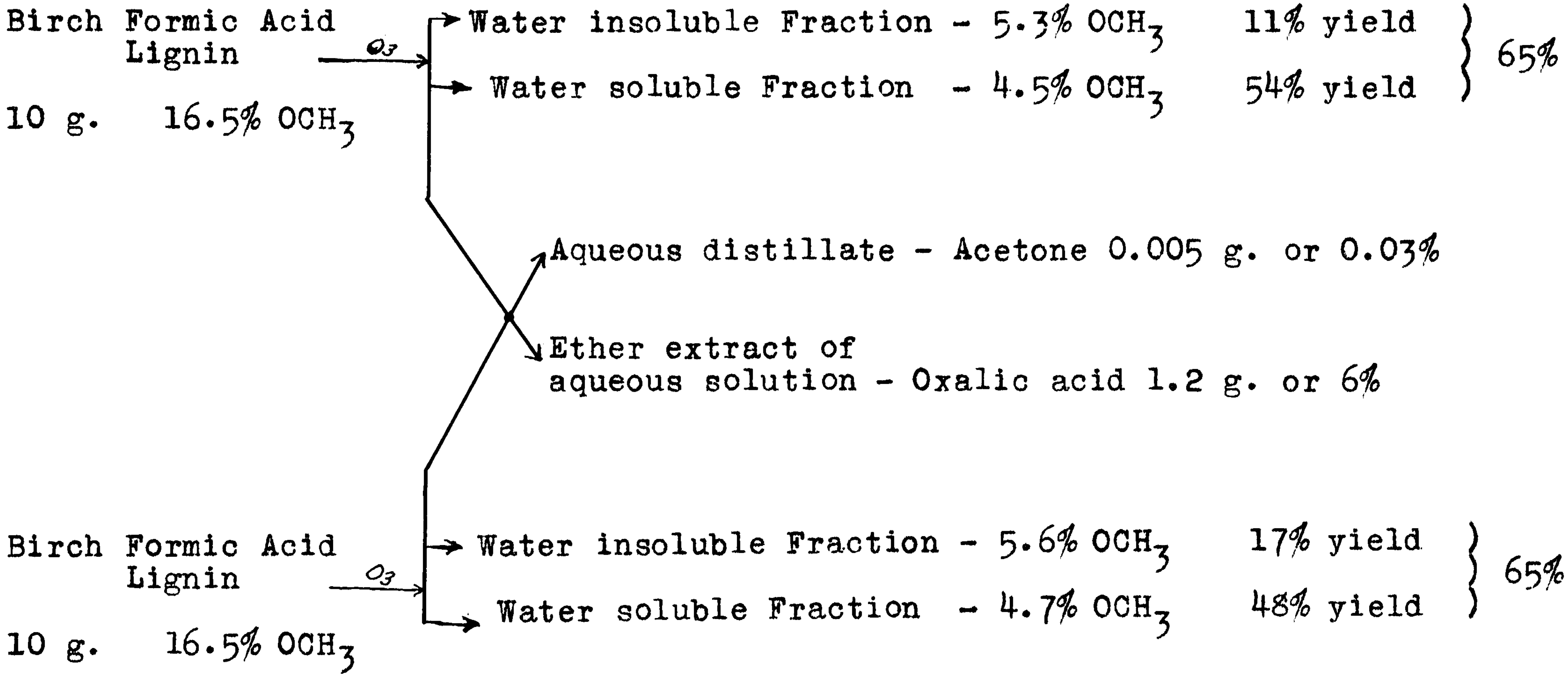
The Ozonization of Lignin

In two separate experiments 10 grams of the dioxane soluble fraction of birch formic acid lignin were dissolved in 250 cc. of formic acid and subjected to the action of 6.65 grams of ozone. After removal of the solvent under reduced pressure the residue was distilled with water and acetone was identified in this distillate as the 2,4 dinitrophenylhydrazone. The water insoluble lignin fraction was obtained by filtration of the aqueous solution. The filtrate, after continuous extraction with ether, was evaporated to dryness and the water soluble lignin fraction obtained by precipitation of this residue from acetone into ether. Oxalic acid (1.2 grams) was identified in the ether extract.

The average yield of the water insoluble fraction was 1.4 grams or, in other words, the ozonization had converted 86% of the lignin into water soluble products. Of this 86% an average of 51% was recovered as the water soluble lignin fraction. The remaining 35% was accounted for as ether soluble material, of which 6% was identified as oxalic acid. There was observed a very marked decrease in the methoxyl content of the lignin. Thus the original lignin had a methoxyl content of 16.5% which dropped on ozonization to an average of 5.4% for the water insoluble fraction, and to 4.6% for the water soluble fraction. These results are summarized in Table II, page 51.

TABLE II

THE OZONIZATION OF BIRCH FORMIC ACID LIGNIN



The yield of acetone was extremely small, namely 0.03%, as compared with a value of 1.35% obtained by Bell (42) on the ozonization of a chloroform soluble fraction of birch formic acid lignin. This is explained by the fact that the ozonization by Bell had been carried out in anhydrous chloroform, whereas the work under discussion had been done in 95% formic acid. The presence of this 5% of water in the reaction mixture would cause the immediate splitting of any ozonide formed and thus the larger amount of the acetone, if formed, would be expected to be swept out of the reaction mixture by the stream of oxygen-ozone mixture passing through it. It should also be pointed out that all of Bell's work was done on chloroform soluble lignin fractions in contrast to the chloroform insoluble fractions used in the present work, and there does not exist any evidence that the grouping responsible for the acetone production exists to the same extent in the two different fractions. In this connection it might be well to note that Kuhn and Roth (31) have found that, in addition to isopropylidine groups, isopropyl groups also tend to form acetone under the action of ozone, especially when the latter are in close proximity to hydroxyl groups or double bonds.

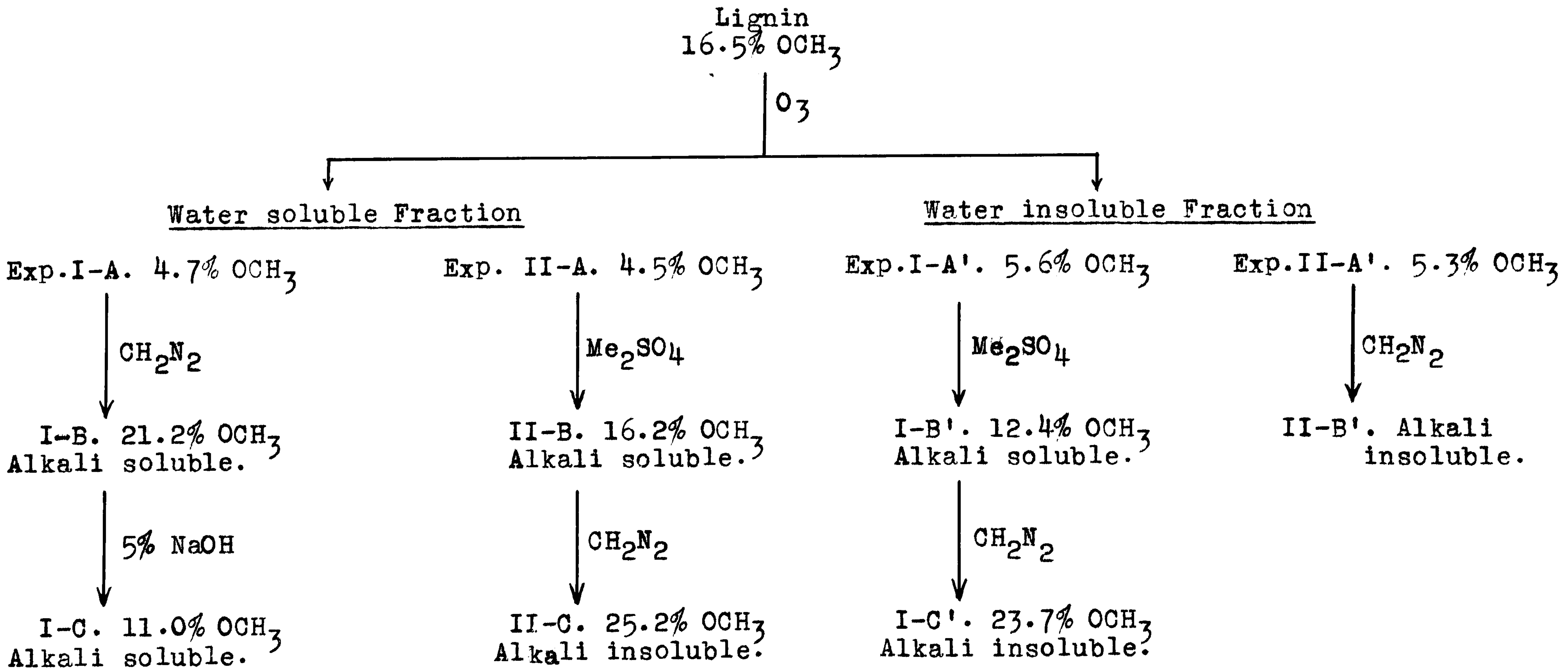
The yield of oxalic acid was found to be 6% and it seems very probable that this compound arose from degradation of the aliphatic portion of the lignin complex since, as has previously been pointed out, no oxalic acid is formed by the action of ozone on typical aromatic compounds such as vanillin and veratric aldehyde.

The water soluble, and water insoluble ozonization products were then subjected to a methylation study, using both diazomethane and dimethylsulphate as well as following the dimethylsulphate treatment by one with diazomethane. The results of this study are summarized in Table III, page 54.

It will be observed that neither dimethylsulphate nor diazomethane alone (with the exception of sample II-B') is capable of fully methylating the ozonized lignins and that they still retain their alkali solubility. Methylation with both reagents, however, gives a product which is insoluble in alkali. The methylation with diazomethane following the dimethylsulphate treatment adds approximately 10% methoxyl to the sample and this is also the amount of methoxyl lost when the diazomethane methylated sample (I-B) is treated in the cold with alkali. The original lignin on ozonization was found to have lost nearly this same value of 10%

TABLE III

SUMMARY OF THE METHYLATION STUDY OF THE PRODUCTS FROM THE OZONIZATION OF BIRCH FORMIC ACID LIGNIN*



* Chloroform insoluble fraction.

With regard to the observed loss in methoxyl on ozonization of lignin it was of interest to determine if there existed in the lignin a type of what might be termed 'unstable methoxyl', and if methoxyl which was added to the lignin would remain stable to the ozone treatment. For this purpose three samples of an acetone soluble fraction of birch formic acid lignin were employed; one was ozonized directly, the second was fully methylated with diazomethane and ozonized under the same conditions and the third fully methylated with dimethylsulphate and also ozonized under the same conditions. Yields of the water soluble, the water insoluble and the ether soluble fractions were determined and methoxyl analyses carried out on the various products. These results are summarized in Table IV, page 56.

There arose the possibility that the loss in methoxyl was due to the splitting off of a fragment having a high methoxyl content and which was present in the ether soluble fraction. That this was not the case, however, was shown by the methoxyl analysis of the ether soluble fraction after removal of the oxalic acid. This fraction also showed a decrease in methoxyl.

Examination of the results in Table IV reveals a number of interesting facts. Whereas the yield of the ether soluble fraction remains constant to a remarkable degree the yield of the water insoluble, and consequently of the water soluble fraction as well, appears to depend directly upon the methoxyl content. The yield of oxalic acid also remains quite constant,

TABLE IV

THE DISAPPEARANCE OF METHOXYL ON THE OZONIZATION OF BIRCH FORMIC ACID LIGNIN

Fraction	Wt.	Wt. of ozone used.	Water insoluble Fraction		Water soluble Fraction		Ether soluble Fraction		
			Yield	OCH_3	Yield	OCH_3	Yield	Oxalic acid	Residue OCH_3
Acetone soluble (17.3% OCH_3)	2.5 g.	1.07 g.	31%	7.05%	40%	6.25%	30%	4.8%	-
Acetone soluble methylated with CH_2N_2 (24.05% OCH_3)	2.5 g.	1.15 g.	56%	14.2%	14%	10.8%	28%	4.4%	10.1%
Acetone soluble Methylated with Me_2SO_4 (31.55% OCH_3)	2.5 g.	0.97 g.	66%	19.6%	5%	12.75%	30%	4.8%	15.1%

and it is suggested that the greater part of this ether soluble fraction arises from degradation of the aliphatic portion of the lignin complex. It has already been pointed out that no oxalic acid is found to arise from the action of ozone on such aromatic compounds as vanillin and veratric aldehyde.

Another observation is the fact that there does appear to be evidence for a type of 'unstable methoxyl' in the lignin. Thus when the methoxyl content of the lignin is increased from 17 to 24% with diazomethane, the methoxyl content of the resulting water insoluble fraction is also found to be increased by the same amount, i.e. from 7 to 14%, an increase of 7. Similarly the dimethylsulphate treatment increased the methoxyl content from 17 to 31% (an increase of 14) and the resulting water insoluble fraction was found to have a methoxyl content of almost 20% as compared to 7% for the unmethylated water insoluble fraction, which is an increase of 13 as compared to the 14 originally added. This unstable methoxyl may exist as such by virtue of its mode of linkage or more probably by juxtaposition to a point in the lignin molecule at which fission takes place under the action of ozone.

The Structure of Lignin and its Relation to Solubility in Aqueous Bisulphite Solution

The alkaline cleavage of the lignin sulphonic acids from a number of different woods has been rather thoroughly investigated. The isolation of vanillin from spruce lignin sulphonic acid by Kürschner (93) has been more recently improved upon by Tomlinson and Hibbert (65), who have shown that vanillin may be obtained in yields of 6% based on the lignin content of the liquor. Still more recently another guaiacyl derivative, namely acetovanillone, has been isolated by Buckland, Tomlinson and Hibbert (94). Leger and Hibbert (95) have also obtained guaiacol itself from the alkaline cleavage of spruce waste sulphite liquor.

In view of the distinctly higher methoxyl content of hard woods as compared to soft woods, Hawkins, Wright and Hibbert (96) undertook a study of the alkaline cleavage of the lignin sulphonic acids prepared from a number of representative hard woods such as birch, oak and maple. They found that equal quantities of vanillin and syringic aldehyde were obtained, the combined yield being of the order of 6%. Further investigation by Leger and Hibbert revealed the presence of the syringyl analogues of acetovanillone and guaiacol in the alkaline cleavage product from birch waste sulphite liquor. Thus acetosyringone, but no acetovanillone, was found to be present (97); and the analogue of guaiacol, namely pyrogallol 1,3 dimethyl ether, was also isolated in small yield (98).

Since it was found by the author that the water insoluble ozonization product from formic acid lignin was soluble in aqueous bisulphite solution, presumably with formation of a lignin sulphonic acid, it became of interest to determine the effect of alkaline cleavage on this newly formed product.

Before this investigation could be undertaken, however, it was necessary to determine the minimum amount of ozonization of the lignin which would render it completely soluble in aqueous bisulphite solution. For this purpose numerous runs were carried out on identical samples of birch formic acid lignin with varying percentages of ozone. The methoxyl content and percentage solubility in standard sulphite liquor under standardized conditions was determined on the water insoluble fraction resulting from the ozonization. In addition it was found that birch acetic acid lignin and maple ethanol lignin also gave rise to bisulphite soluble fractions on ozonization. The results of this study are incorporated in Table V, page 60.

It was also observed that a continued refluxing of the ozonized lignin with water after removal of the formic acid solvent somewhat increased the solubility in sulphite liquor. The relation of loss in methoxyl to increased solubility in sulphite cooking liquor, and the increase of the latter with increasing ozonization, is shown in the graphs on page 61.

TABLE V

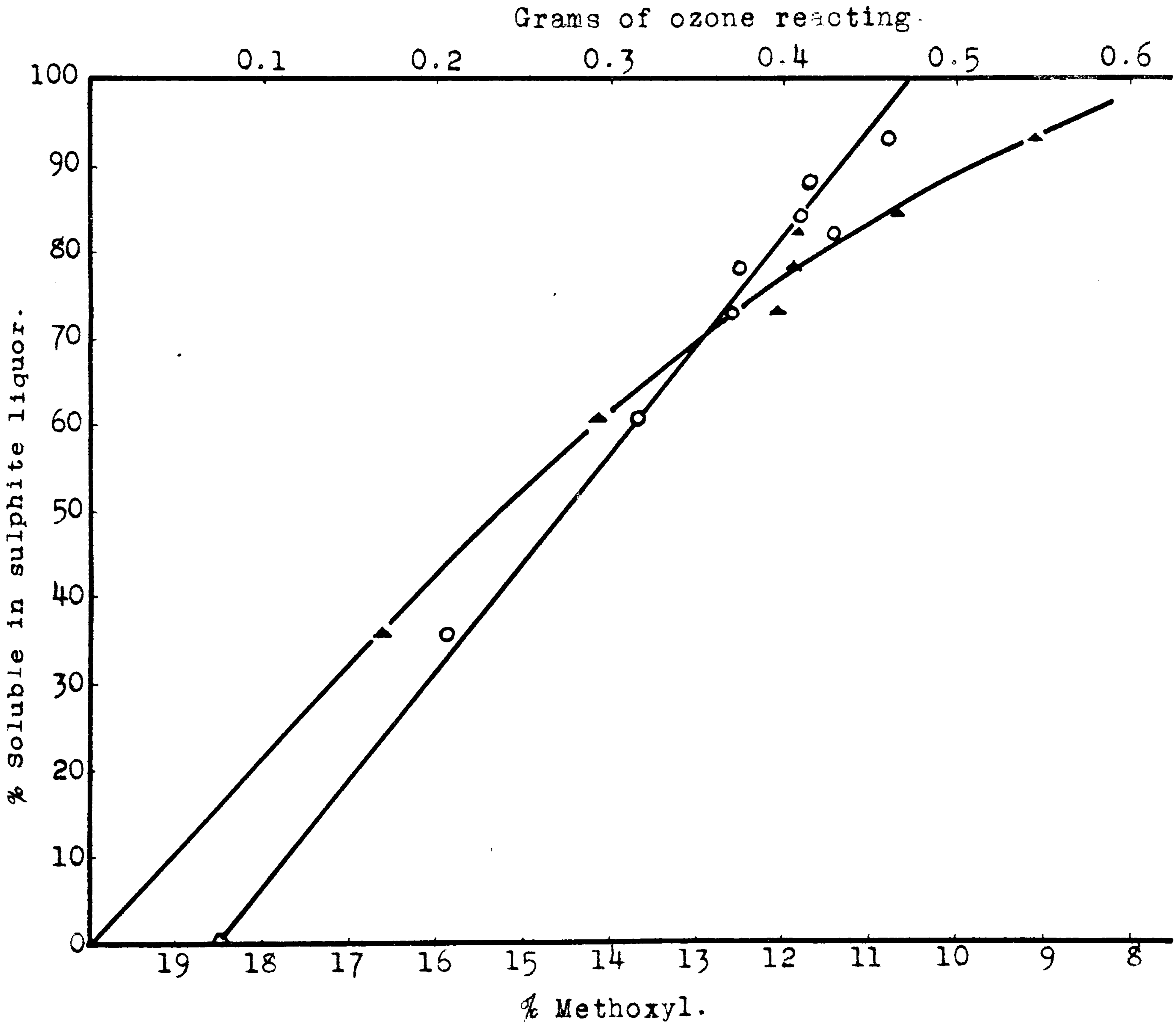
RELATION OF DECREASE IN METHOXYL AND INCREASE IN SOLUBILITY IN SULPHITE LIQUOR FOLLOWING OZONIZATION OF LIGNIN

Run	Wt.	% OCH_3	g. of O_3 added	g. of O_3 reacting	Water insoluble Fraction		Loss in OCH_3	% Solubility in Sulphite Liquor
					% yield	% OCH_3		
Formic acid Lignin - Acetone soluble Fraction -								
1.	2 g.	18.55	0.189	0.167	95%	15.9	2.65	36%
2.	2 g.	18.55	0.341	0.290	88%	13.85	4.7	61%
3.	2 g.	18.55	0.426	0.368	68%	11.65	6.9	88%
3b.	Water Treatment					11.5	0.15	100%
4.	2 g.	18.55	0.485	0.404	72%	12.5	6.05	78%
4b.	Water Treatment					11.75	0.75	85%
6.	2 g.	18.55	0.502	0.396	73%	12.6	5.95	73%
7.	2 g.	18.55	0.505	0.405	65%	11.45	7.1	82%
7b.	Water Treatment							98%
8.	2 g.	18.55	0.590	0.463	60%	11.8	6.75	82%
9.	2 g.	18.55	0.705	0.544	53%	10.85	7.7	93%
Formic Acid Lignin - 90% acetone-water soluble Fraction-								
10.	2 g.	15.5	0.606	0.462	74%	9.5	6.0	88%
5.	2 g.	15.5	0.488	0.394	82%	10.4	5.1	76%
14.	2 g.	15.5	0.538	0.432	75%	9.6	5.9	87%
Acetic Acid Lignin - Acetone soluble Fraction -								
11.	2 g.	18.7	0.280	0.223	66%	14.5	4.2	62%
11b.	Water Treatment							65%
12.	2 g.	18.7	0.498	0.371	53%	12.85	5.85	86%
13.	2 g.	18.7	0.350	0.278	60%	14.2	4.5	75%
Ethanol Lignin -								
15.	2 g.	26.0	0.580	0.434	62%	17.5	8.5	72%

THE ACTION OF OZONE ON LIGNIN

▲ Increase in solubility of birch formic acid lignin in sulphite liquor with increasing ozonization.

○ Increase in solubility of birch formic acid lignin in sulphite liquor with loss in methoxyl.



From these results the amount of ozone necessary to bring about 100% solubility in sulphite cooking liquor of formic acid lignin was calculated. The ozonization was carried out and the resulting water insoluble lignin fraction was then cooked with sulphite liquor in the usual manner. A portion of the resulting liquor was freed from inorganic material by dialysis and the purified lignin sulphonic acid recovered and analysed for sulphur and methoxyl. The remaining liquor was subjected to alkaline cleavage in 24% sodium hydroxide and vanillin and acetovanillone were identified as products of this degradation. These compounds were found to be present in approximately equal yields of 1% based on the lignin content of the liquor.

A similar procedure was next carried out on birch <u>acetic</u> acid lignin, and again vanillin and acetovanillone were formed, this time in somewhat higher yield, (2.7% in each case).

The similarity of these results with those obtained in the isolation of vanillin, acetovanillone, etc. from lignin sulphonic acids prepared from the protolignin pointed to the fact that these newly formed lignin sulphonic acids from formic and acetic acid lignins were indeed comparable to the usual type of lignin sulphonic acid; and further, that any evidence on the mechanism of the formation of these sulphonic acids might be justifiably compared to that of the reaction of protolignin itself with sulphurous acid.

For this reason the solubility or non-solubility in sulphite cooking liquor of a large number of lignin samples, both before and after ozonization, before and after methylation with dimethylsulphate and/or diazomethane, after treatment with the Grignard reagent, etc. was determined. Some of the results are summarized in Table VI, page 64 and Table VII, page 65; others will be described subsequently.

The next section will be devoted to a discussion of the theoretical implications of the experimental results and the additional experimental data will be presented as the various points under discussion are developed.

TABLE VI

SOLUBILITY IN SULPHITE LIQUOR OF VARIOUS LIGNINS BEFORE AND AFTER OZONIZATION

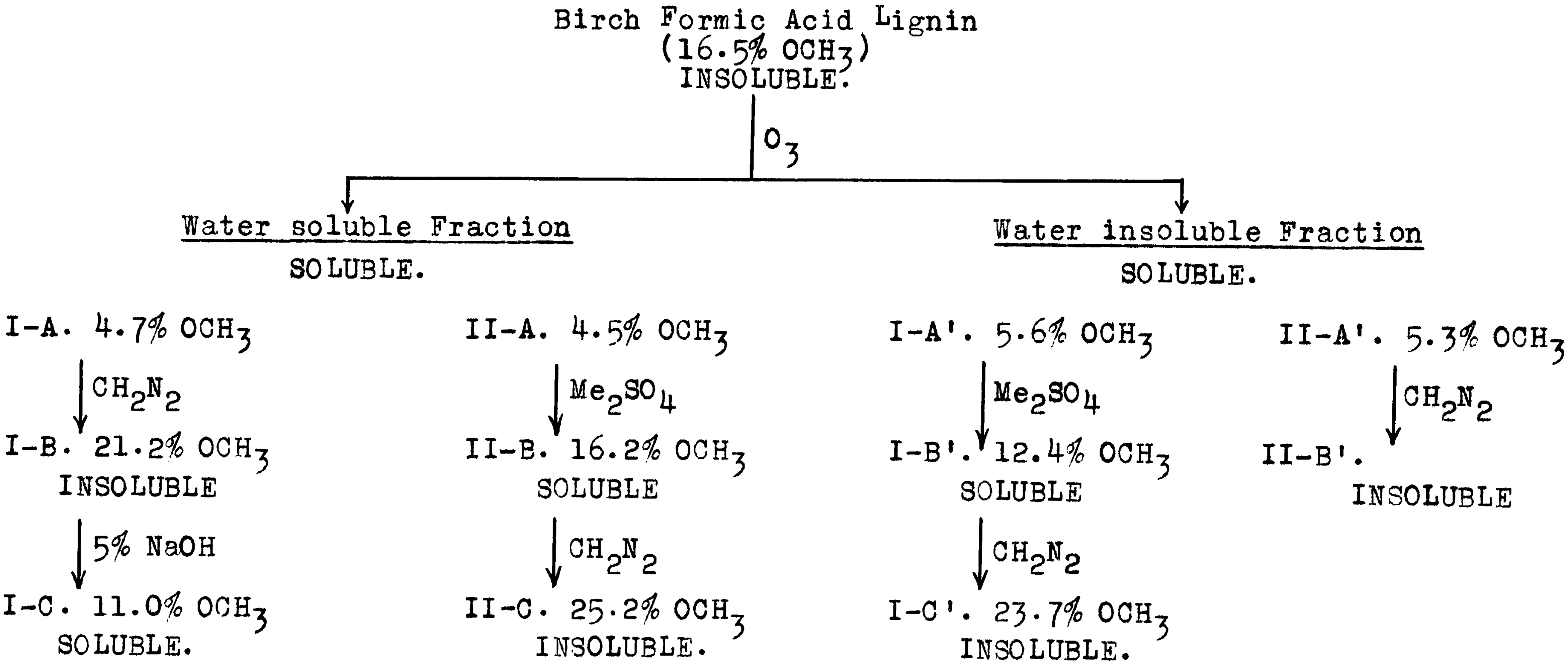
(1) Formic Acid Lignin (17.3% OCH_3) INSOLUBLE.	O_3	→Water insoluble Fraction (7.05% OCH_3)	Completely SOLUBLE.
		→Water soluble Fraction (6.25% OCH_3)	Completely SOLUBLE.
(2) 1. above treated with CH_2N_2 (24.05% OCH_3) INSOLUBLE.	O_3	→Water insoluble Fraction (14.2% OCH_3)	Completely SOLUBLE.
		→Water soluble Fraction (10.8% OCH_3)	Completely SOLUBLE.
(3) 1. above treated with Me_2SO_4 (31.55% OCH_3) INSOLUBLE.	O_3	→Water insoluble Fraction (19.6% OCH_3)	Completely SOLUBLE.
		→Water soluble Fraction (12.75% OCH_3)	Completely SOLUBLE.

INSOLUBLE - Insoluble in Standard Sulphite Liquor at 110° for 12 hrs

SOLUBLE - Soluble in Standard Sulphite Liquor at 110° for 12 hrs.

TABLE VII

SOLUBILITY IN SULPHITE LIQUOR OF VARIOUS PRODUCTS FROM THE OZONIZATION OF FORMIC ACID LIGNIN *



* Chloroform insoluble fraction.

Theoretical Discussion of Experimental Results

(a) The Action of Formic and Acetic Acids on Protolignin

For some time it has been recognized that the action of the extractant in the isolation of a lignin must be considered as the first in a series of reactions on the protolignin. Wright and Hibbert (99) in a study of spruce formic acid lignin showed that the more soluble, and therefore simpler fractions, had higher hydroxyl and lower methoxyl values than the more complex, insoluble analogues. This decrease in hydroxyl value with decreasing solubility suggested that increasing aggregation of the lignin complex is associated with loss of hydroxyl. Wright and Hibbert also produced evidence for the presence of what they termed 'hidden hydroxyl groups'. Thus from an active hydrogen content of four hydroxyl groups per kilo of native formic acid lignin it was calculated that complete methylation should give a product having a methoxyl content of 24.6%. However it was found that repeated methylations with dimethylsulphate and alkali gave a product having 30% methoxyl and still containing 2 - 3 hydroxyls per kilo. They interpreted these results as evidence that new hydroxyl groups appear on methylation with alkali and supported this view by showing an increase from 4 to 12.5 hydroxyls per kilo and a decrease in methoxyl from 12.9 to 12.0% on allowing spruce formic acid lignin to stand in the presence of 1% alkali for forty-five days.

Thus formic acid may be pictured as increasing the polymerization of the protolignin by causing a loss of water between hydroxyl groups, this reaction being reversed by the action of alkali.

A method of isolating lignin by means of glacial acetic acid was developed by A. Bell (42) and was applied to birch wood. In general, the properties of the acetic acid lignin were found to be similar to those of formic acid lignin. It was demonstrated that the more soluble fractions contained no 'hidden hydroxyl groups' but that the more complex and insoluble fractions did appear to contain 'hidden hydroxyls' similar to those found in the formic acid lignin.

From the point of view of the present research the most significant change which the protolignin undergoes after isolation with formic or acetic acids is its inability to react with sulphurous acid to form the water soluble lignin sulphonic acids. Due to the action of these organic acids the groups present in the lignin complex which are responsible for the sulphonation lose this ability. If this action may be assumed to be due to dehydration the importance of the hydroxyl groups in the sulphonation of lignin is at once apparent.

A native oak lignin, readily soluble in bisulphite solution, is obtained by the acetolysis of oak wood meal at temperatures not above 30°, followed by a subsequent deacetylation of the isolated lignin (82). This isolation for the first time of a completely bisulphite soluble extracted lignin is undoubtedly connected with the protection of the hydroxyl groups in the protolignin by their acetylation. Hawkins (84) has shown that refluxing this native oak lignin (in which the hydroxyl groups are now free) with glacial acetic acid causes complete insolubility of the reaction product in bisulphite solution. In addition, treatment of the oak lignin with methanol-hydrogen chloride, a reaction which would be expected to block certain hydroxyl groups or possibly result in their disappearance, also caused the lignin to become insoluble in bisulphite solution.

Ozonized birch formic acid lignin, which is completely soluble in bisulphite solution, becomes insoluble upon treatment of the lignin with formic acid at the reflux temperature. It has also been shown that lignins isolated by alcoholysis with ethanol, propanol and butanol are bisulphite insoluble. This type of isolation is one in which certain hydroxyl groups would be blocked by ether formation. The importance of the hydroxyl group in the sulphonation reaction will be shown further in the next section.

(b) The Action of Ozone on Isolated Lignins

From the point of view of the solubility of ozonized lignins in bisulphite solution the action of the ozone is the reverse of that brought about by the action of formic and acetic acids on the protolignin in that with the former reaction bisulphite soluble products are formed. As has been pointed out the further action of formic acid on the ozonized lignin reverses this reaction to give a product which is insoluble in bisulphite solution. Thus the ozone must in some manner create or liberate the grouping involved in sulphonation. It has been shown by the author that hydrogen peroxide, acting upon formic acid lignin, gives a product which is bisulphite soluble; also that continued refluxing with water of ozonized lignin results in a somewhat greater bisulphite solubility, thus indicating that a type of hydrolysis is in all probability involved.

The facts in Table VI, page 64, show that complete methylation of the lignin, either with dimethylsulphate or diazomethane, prior to the ozonization has no effect on the bisulphite solubility of the ozonized products and thus the groupings involved in sulphonation must have been formed as a result of the ozone treatment. Perhaps the most natural assumption to make with regard to the effect of ozone on lignin, especially in view of the usual change involved in the ozone reaction, is that carbonyl groups are created, these latter being of either an aldehydic or a ketonic nature. To test this theory Grignard analyses of a lignin before and after ozonization, (i.e. of a lignin completely insoluble and of one completely soluble in bisulphite solution), were made. Insoluble birch formic acid lignin prior to treatment with ozone on analysis showed an addition of 1.9 mols per kilogram and active hydrogen of 6.25 mols per kilogram. The bisulphite soluble ozonized lignin showed an increase in addition of 0.6 mols to 2.5 mols per kilogram, and a slight decrease in the active hydrogen to 5.5 mols per kilogram. These results would tend to favour the assumption that sulphonation is due at least in part to the formation or liberation of carbonyl groups during ozonization. This assumption was later shown to be incorrect, (page 73), and is discussed in detail in the next section.

The Grignard analyses cannot be considered as being of great value in this study for the following reasons. Greater or lesser degradation of the lignin occurs, depending upon the extent of the ozonization, the water insoluble fraction predominating when the ozonization is comparatively mild.
From the lignin-like nature of this fraction the essential aromatic nucleus is apparently still intact, and only non-nuclear fragments seem to have been affected. Grignard analyses of this water insoluble fraction indicate the total carbonyl and hydroxyl groups present, whereas the actual amount of these concerned in the sulphonation undoubtedly constitutes only a small fraction. In addition, there is no way in which the amount of active hydrogen and carbonyl lost due to degradation can be determined.

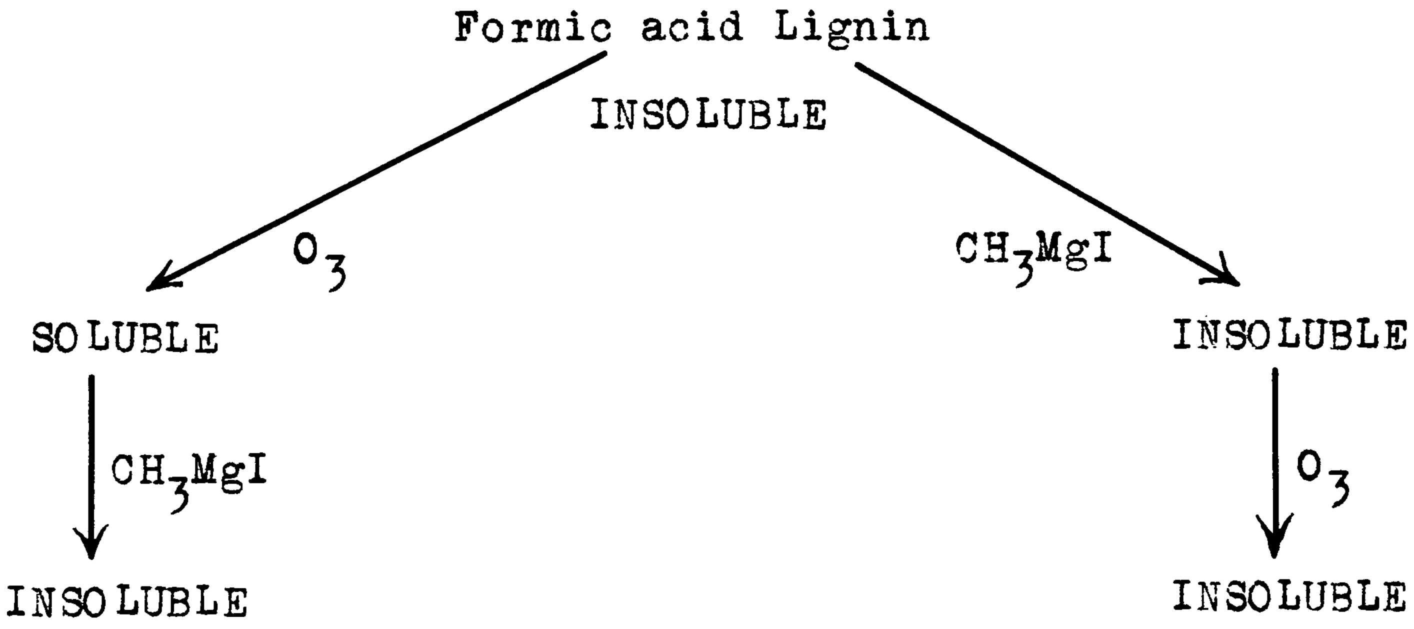
From the graphs on page 61 it will be noted that the relation between the loss in methoxyl and the increase in the solubility in bisulphite solution is practically linear. This loss in methoxyl, however, is to be regarded as entirely fortuitous and not directly concerned with the liberation of the group or groups involved in the sulphonation reaction. The relation between the amount of ozone used and the increase in bisulphite solubility is not quite linear and is in all probability due to the fact that increasing amounts of ozone are required as the concentration of reacting groups in the solution diminishes, since the ozone does not remain in solution unless it reacts but is constantly being bubbled through it. Further consideration of the action of ozone will be dealt with in the next section in connection with the reaction of lignins with sulphurous acid.

(c) The Reaction of Lignin Preparations with Sulphurous Acid

Perhaps the most important single piece of evidence in this investigation relating to the formation of lignin sulphonic acids is the fact that a carbonyl group is intimately associated with the ability of lignins to react with sulphurous acid. In 1920 Klason (72) postulated the participation of a carbonyl group in this reaction but this is apparently the first time that direct chemical evidence for this fact has been obtained. This has been demonstrated in a number of different instances which will be described. Furthermore it will be shown that this carbonyl group, either active or nascent, is present in the original lignin prior to ozonization and is not created by the action of the ozone.

The ozonized birch formic acid lignin (bisulphite soluble) was recovered from the Grignard analysis by precipitation into water, washed with water and dried. This sample, in which presumably there now existed no carbonyl groups, was subjected to a sulphite cook under the standard conditions of 110° for 12 hours employed throughout this investigation. The sample was found to be completely insoluble.

Now, in view of this result, if the solubility were due to a carbonyl group or groups created by the action of the ozone then treatment of the lignin with the Grignard reagent prior to the ozonization should have no effect on the subsequent solubility of the ozonized lignin. However this was found to be not the case. A sample of birch formic acid lignin was treated with methyl magnesium iodide and was found to be insoluble in sulphite liquor. The ozonized lignin was also found to be insoluble under the same conditions. Thus the carbonyl group involved in the sulphonation must have existed in the original formic acid lignin, as is indicated in the following scheme;



Native oak lignin is believed to closely resemble protolignin, especially in regard to its solubility in aqueous bisulphite solution. A sample of this lignin was next treated with methyl magnesium iodide and the product of this reaction was found to be insoluble in sulphite liquor. The ozonization of this product gave a material which was also insoluble.

Further proof of the important role played by the carbonyl group in the sulphonation reaction was obtained in the following manner. A sample of solvent extracted spruce wood meal (4.8% OCH_3) was 'grignardized' by prolonged treatment in the

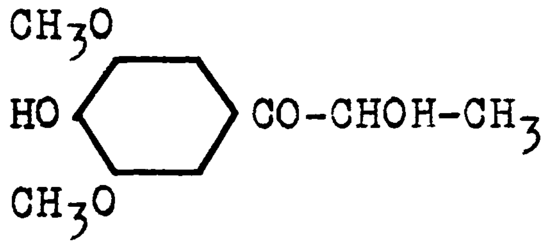
Grignard machine with methyl magnesium iodide. The reaction product was dried and subjected to a standard sulphite cook, upon the completion of which the pulp was filtered off, washed and dried. The methoxyl content of this pulp was 4.1%. Comparison of this value with the original value of 4.8% shows that very little delignification had occurred.

Bearing in mind these facts concerning the carbonyl group it is now necessary to return to a consideration of the hydroxyl group and its relation to the sulphonation. Evidence has already been presented to show that the insolubility in bisulphite solution of most isolated lignins is due to reactions concerned with the hydroxyl group. Furthermore the experiments with the Grignard reagent have shown that apparently the carbonyl group involved in the sulphonation is not affected by the action of the formic acid on the protolignin, since the group exists in the isolated formic acid lignin, the 'grignardization' of which destroys the solubility of the ozonized product.

Consideration of the facts in Table VI, page 64 and Table VII, page 65, shows the importance of the hydroxyl group in the sulphonation reaction. Thus methylation with diazomethane, but not with dimethylsulphate, in some manner blocks the solubility of the ozonized lignin in the sulphite liquor. It appears that about 10% methoxyl cannot be introduced by the dimethylsulphate and that it is this particular methoxyl that is involved in the sulphonation and consequent solubility. It will be noted that alkaline hydrolysis of an insoluble lignin (insoluble due to diazomethane treatment) restores the solubility of the lignin. This fact would favour the view that the methyl group was on an hydroxyl of a rather labile character, possibly of an enolic nature. It has previously been pointed out that the particular hydroxyl concerned must be created by the action of the ozone since complete methylation of formic acid lignin, either with dimethylsulphate or diazomethane, prior to ozonization has no effect on the resulting bisulphite solubility. Further confirmation of this fact was obtained in the following manner. Complete methylation of bisulphite soluble native oak lignin destroys this solubility. Upon ozonization of this methylated lignin the product was found to remain insoluble, thus rendering very unlikely the possibility that in this case the hydroxyl group concerned in sulphonation is liberated by the action of the ozone.

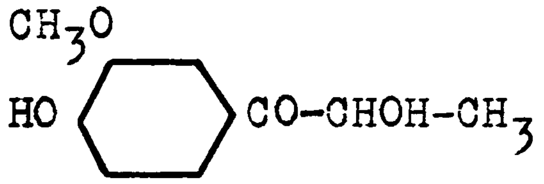
The dependence of the sulphonation reaction on an hydroxyl group was also demonstrated in the following manner. Extracted spruce wood meal (4.8% OCH_3) was cooked in standard sulphite liquor for 12 hours at 110°. The methoxyl content of the resulting pulp was 2.4% indicating the removal of a portion of the lignin. The low temperature of the cook is undoubtedly responsible for the more or less incomplete removal of the lignin. A sample of the same spruce wood which had been methylated with diazomethane to a value of 7.2% OCH_3 was then cooked under the same conditions. The methoxyl analysis of the resulting pulp was 5.8%. This shows that the great majority of the lignin had not been removed and that the blocking of a certain hydroxyl group in the protolignin with diazomethane had hindered the process of delignification. This result is in harmony with the results of Brown and Brauns, (80).

Recent work in these laboratories by Cramer, Hunter and Hibbert (90) on the ethanolysis of spruce and maple woods has given rise to the isolation of two new compounds, the ethoxy derivatives of I and II, and it is their belief that all extracted lignins are condensation polymers derived from building units such as I and II or their dismutation isomers.



I

∝-hydroxypropiosyringone



II

∝-hydroxypropiovanillone

In soft woods only II would be concerned, while in the hard woods both I and II are believed to take part in the formation of extracted lignins. It was thus of interest to determine the behaviour of these compounds to hot bisulphite solution. It was found however, that both these compounds were quite stable to sulphite liquor under the standard conditions of 110° for 12 hours. In addition, ∝-ethoxypropioveratrone was found to be stable under the same conditions. Benzoin was recovered quantitatively and unchanged after cooking for 12 hours. These results quite definitely point to the fact that addition of sulphurous acid does NOT take place at the double bond resulting from enolization between adjacent carbonyl and hydroxyl groups.

Cramer (90) has shown that, on warming with dilute sulphuric acid, α-hydroxypropiovanillone is converted to a light brown condensation product to which is attributed many of the properties of Freudenberg's 'Cuproxam' lignin. However, with regard to its action with bisulphite solution, the writer has found that, in contrast to Freudenberg's lignin, this material is <u>in</u>soluble in hot bisulphite solution. Furthermore it was found that a prior treatment with ozone of this material had no effect on the bisulphite solubility, a behaviour in marked contrast to the results observed in the cases of formic and acetic acid and ethanol lignins.

Formic acid 'lignin' was prepared by refluxing α-hydroxypropioveratrone with formic acid and pouring the reaction mixture into water. This gave rise to a lignin-like precipitate which was found to be completely insoluble in hot bisulphite solution. The action of ozone was found to have no effect on this solubility.

The sulphur analyses of the lignin sulphonic acids prepared from ozonized formic and acetic lignins were approximately 6%, which is of the same order as the isolated lignin sulphonic acids obtained directly from the wood. The methoxyl contents were, however, considerably lower, namely 3.3 and 6.4% for the lignin sulphonic acids from formic and acetic lignins respectively as compared to approximately 15% for an isolated birch lignin sulphonic acid. The ozonized lignins, before cooking with the sulphite liquor, had methoxyl values of 8.3 and 13.3% as compared to 15.5 and 18.8% for the formic and acetic lignins before ozonization.

(d) The Action of Alkali on Lignin Sulphonic Acids

The lignin sulphonic acid prepared by cooking yellow birch wood meal with sulphite liquor has been shown on alkaline cleavage to yield 2.7% vanillin and 2.8% syringaldehyde based on the lignin (96). Only acetosyringone, and no acetovanillone, was found in birch waste sulphite liquor and the yield was much smaller, being of the order of 0.8% (97). Pyrogallol 1,3 dimethyl ether was also found to be present in birch waste sulphite liquor in the small yield of 0.2% (98).

In contrast to these results the lignin sulphonic acid prepared from ozonized birch formic acid lignin gave rise, on alkaline cleavage, to only guaiacyl derivatives, namely vanillin and acetovanillone, and in approximately equal yields of 1%. The same results were observed when the lignin sulphonic acid from ozonized birch acetic acid lignin was subjected to alkaline treatment, although in this case the yields were of the order of 2.7% based on the lignin. Presumably this is the first time that acetovanillone has been obtained from a hard wood since, as previously mentioned, Leger and Hibbert found only acetosyringone in birch waste sulphite liquor. In Table VIII, page 80, is presented a summary of the various yields which have been obtained by various means from birch lignin sulphonic acids.

TABLE VIII

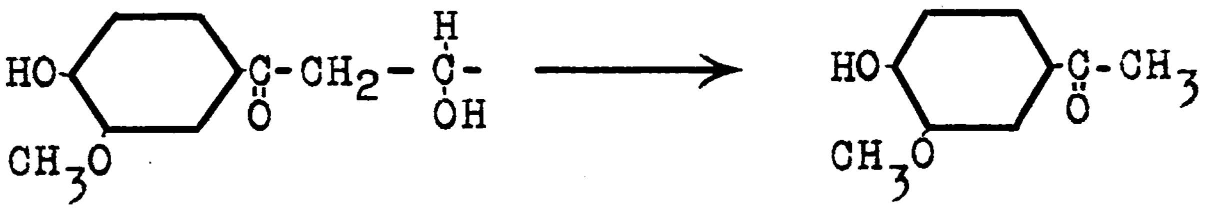
COMPOUNDS ISOLATED FROM VARIOUS BIRCH LIGNIN SULPHONIC ACIDS

Compound	Isolated birch lignin sulphonic acid	Birch waste sulphite liquor	Lignin sulphonic acid from	
			Ozonized formic acid lignin	Ozonized acetic acid lignin
Vanillin	2.7%	2.9%	1%	2.7%
Syringaldehyde	2.8%	2.9%		
Acetovanillone			1%	2.7%
Acetosyringone		0.8%		
Pyrogallol 1,3 dimethyl ether		0.2%		
Totals	5.5%	6.8%	2%	5.4%

There are two possible explanations as to why no syringyl analogues were found in the present investigation. In the first place the formic and acetic acid lignins which were used had previously been subjected to a solvent fractionation. Some workers have even termed those fractions having the higher methoxyl content, the syringyl fractions, and those of the lower methoxyl content the guaiacyl fractions (92). In both cases the vanillin and acetovanillone was obtained from fractions of the crude lignin having the lowest methoxyl content, and the results obtained thus support the correctness of the term 'guaiacyl fraction'. It must also be noted that the formic and acetic acid lignins, previous to the preparation of the lignin sulphonic acids, were subjected to a treatment with ozone. Thus there is the possibility that if syringyl radicals did exist in these fractions they might be split off as part of the water soluble fraction by the ozone. It is difficult to believe that the observed demethoxylation upon ozonization could be the conversion of syringyl to guaiacyl nuclei.

Tomlinson and Hibbert (100) in accounting for the production of vanillin from spruce lignin sulphonic acid postulated a reverse aldol condensation as shown, this aldol being an intermediate in the action of the alkali on the lignin sulphonic acid.

In this investigation the production of acetovanillone in a yield equal to that of the vanillin might be explained by a somewhat similar mechanism,

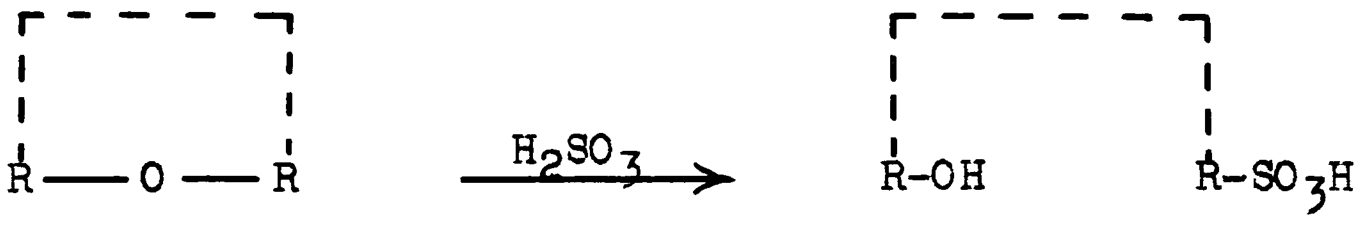


Conclusions

A consideration of the facts which have been discussed brings to light some important conclusions regarding the mechanism of lignin sulphonic acid formation.

That the sulphonation does not take place at an aromatic hydroxyl group has already been demonstrated quite conclusively by Tomlinson and Hibbert (100) who showed that a methylated lignin sulphonic acid gave rise, on alkaline cleavage, to veratric aldehyde in place of vanillin. Thus the phenolic hydroxyl must be considered as free in the lignin sulphonic acid.

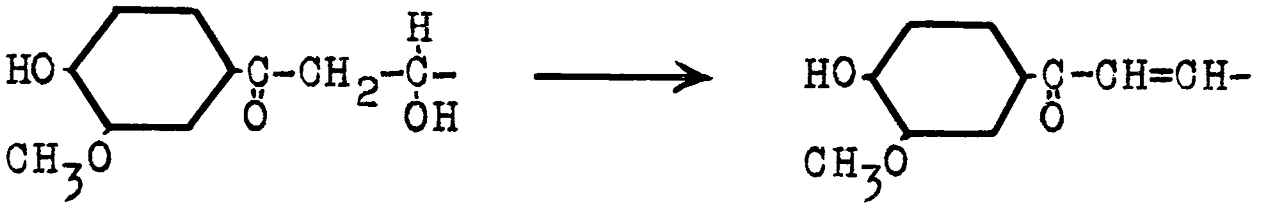
Perhaps the most commonly accepted theory of sulphonation is that proposed by Hägglund (74), and later supported by Freudenberg (76), and which postulated the opening of an oxygen bridge by the sulphurous acid.



The only experimental proof offered by Freudenberg in support of this theory is his work on Erdtman's acid, proof which is open to some criticism because of the fact that the structure of Erdtman's acid has not been definitely established (101), nor is Freudenberg's analogy between this acid and lignin particularly convincing. It is hard to reconcile a theory of this type with the demonstrated action of diazomethane and of the Grignard reagent, on bisulphite soluble lignins. Furthermore, if formic and acetic acid lignins are polymerized through the loss of water between hydroxyl groups, and the evidence seems fairly definite, then it is just this type of a linkage which the Hägglund-Freudenberg theory postulates would be opened by sulphurous acid. However these lignins do not react with sulphurous acid.

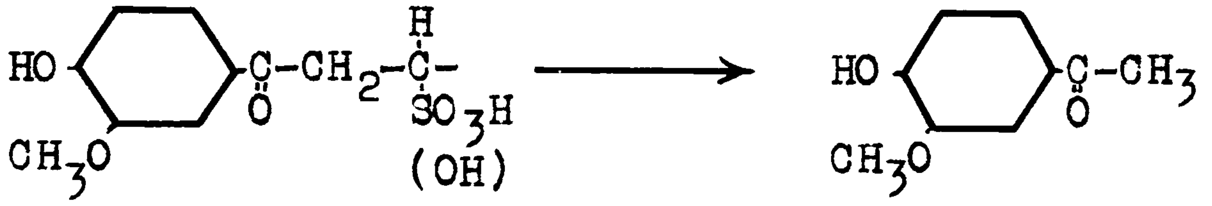
The fact that the lignin must be sulphonated before the alkaline treatment gives rise to such products as vanillin would seem to indicate that the sulphonation reaction is more than merely the addition of the elements of sulphurous acid, but that in all probability some type of intramolecular change is involved as well. The evidence from this investigation points to the fact that this might be the formation of a double bond by the elimination of water between an hydroxyl group and an adjacent hydrogen. Since ozone (which destroys double bonds) creates a bisulphite soluble product it seems hardly likely that the resulting solubility could in any way involve a double bond. However it is known that a part of the sulphur in a lignin sulphonic acid is of the stable type and the only way in which this could arise in the aliphatic portion of the molecule is by addition to an ethylenic double bond since addition to a carbonyl results in the formation of an unstable sulphonic acid.

If a vinyl ketone of the following type were formed by loss of water in the first stage of the sulphonation reaction,

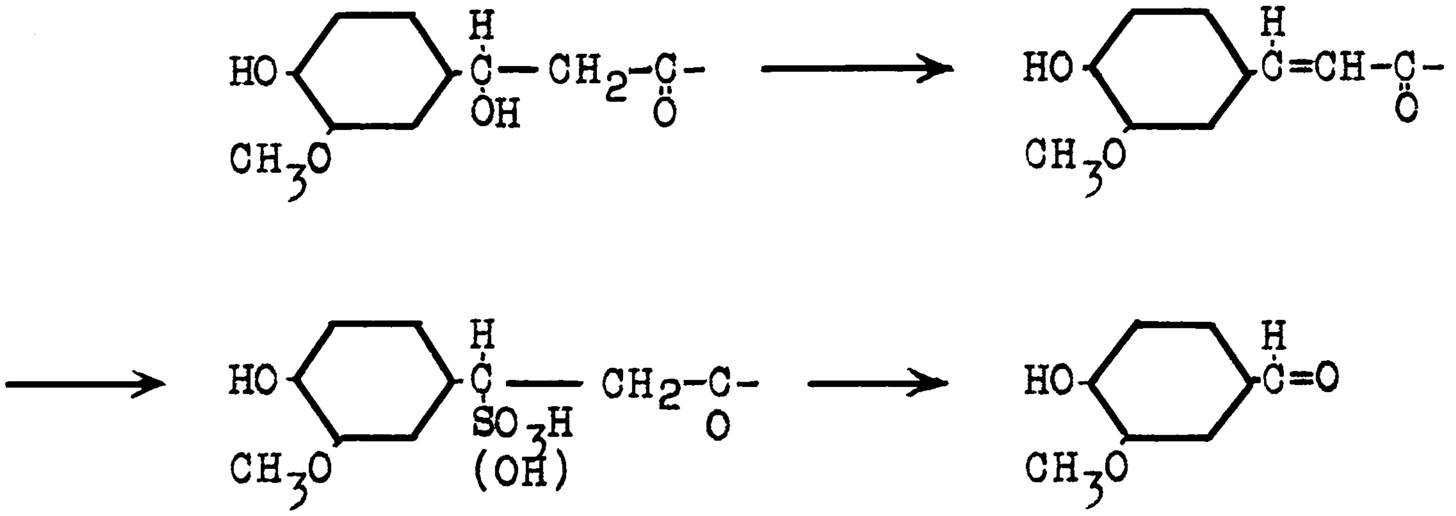


III

then 1,4 addition of sulphurous acid would result in a stable sulphonic acid. Alkali could then give rise to acetovanillone by a reverse aldol as has been shown,



If however the aromatic nucleus were at the other end of the aliphatic chain shown in III, the following reactions would account for the vanillin production in a manner similar to the Tomlinson-Hibbert mechanism,



The predominance of compounds of the C_6-C_3 type from the ethanolysis experiments on wood carried out in these laboratories (90) lends support to the possibility that a unit of this type might be involved in the sulphonation of lignin.

EXPERIMENTAL RESULTS

I. THE PREPARATION OF BIRCH LIGNINS

(a) Previous History of the Wood.

The birch log was cut three feet above the ground from a tree located in a rather open pure hardwood stand in Quebec. The tree was sixty years old and growth had been fairly even and reasonably fast.

The wood was chipped, the shavings air dried and then ground to a fine meal of 60-300 mesh. This wood meal was successively extracted with alcohol-benzene, alcohol and finally water to remove tars and resins. The extracted wood meal was then air dried.

(b) The Preparation of Formic Acid Lignin.

In three separate experiments the birch wood meal was refluxed with formic acid (Schering Kahlbaum, 95%) in a flask equipped with a mercury sealed stirrer and reflux condenser. After 20-22 hours the cooled reaction mixture was filtered through cloth on a Büchner funnel and washed with formic acid until the washings were almost clear. The filtrate was evaporated under reduced pressure to a volume of about 100 cc. To this was added a litre of water and the flask well shaken.

The resulting lignin was filtered through a Büchner funnel and well washed with distilled water until the washings were both neutral to litmus and colorless. This required some 20 litres. The lignin was partially dried under a rubber dam.

The undissolved wood meal from the first filtration was washed with water until the washings were no longer acid to litmus and dried under a rubber dam. The crude lignin and the undissolved wood meal were then dried in the vacuum oven at 55°C. for two days. The weights obtained in the three experiments are recorded in Table I.

(c) The Fractionation of Birch Formic Acid Lignin.

Experiment 1.

The crude lignin was placed in a Sohxlet extractor and extracted for seven days with ether. The ether was removed from the extractor under reduced pressure and the lignin then extracted with chloroform at a pressure of 500 mm. for three days. Upon removal of the chloroform from the apparatus, extraction was next carried out with acetone at a pressure of 350 mm. for three days. The lignin remaining in the Sohxlet was dissolved in purified dioxane. The chloroform solution (250 cc.), the acetone solution (130 cc.) and the dioxane solution (200 cc.) were each filtered and precipitated in a fine stream into a tenfold volume of ether. The resulting precipitates were centrifuged off from the filtrates, washed with ether followed by 30-50° petroleum ether and dried. After thorough drying each fraction was weighed. These results are summarized in Table II.

Methoxyl Analysis:

(1) Chloroform Soluble Fraction -

% OCH_3 - 19.7 (19.7, 19.7)

(0.02600 g. sample required 20.34 cc. of 0.04893 N $Na_2S_2O_3$)

(0.02430 g. sample required 19.01 cc. of 0.04893 N $Na_2S_2O_3$)

(2) Acetone Soluble Fraction -

% OCH_3 - 17.3 (17.3, 17.3)

(0.02490 g. sample required 17.12 cc. of 0.04893 N $Na_2S_2O_3$)

(0.02840 g. sample required 19.46 cc. of 0.04893 N $Na_2S_2O_3$)

(3) Dioxane Soluble Fraction -

% OCH_3 - 16.5 (16.5, 16.5)

(0.02590 g. sample required 18.08 cc. of 0.04585 N $Na_2S_2O_3$)

(0.02370 g. sample required 16.60 cc. of 0.04585 N $Na_2S_2O_3$)

Experiment 2.

This fractionation was carried out in the same manner as described for Experiment 1, with the exception that 90% acetone-water was used as the solvent in the final fractionation rather than dioxane. This acetone-water solution was precipitated into a tenfold volume of dry ether. The results are summarized in Table II.

Methoxyl Analysis:

(1) Chloroform Soluble Fraction -

% OCH_3 - 19.25 (19.3, 19.2)

(0.02530 g. sample required 19.46 cc. of 0.04893 N $Na_2S_2O_3$)

(0.02620 g. sample required 20.00 cc. of 0.04893 N $Na_2S_2O_3$)

(2) Acetone Soluble Fraction -

% OCH_3 - 17.4 (17.4, 17.4)

(0.02510 g. sample required 18.50 cc. of 0.04585 N $Na_2S_2O_3$)

(0.02590 g. sample required 19.09 cc. of 0.04585 N $Na_2S_2O_3$)

(3) 90% acetone-water Soluble Fraction -

% OCH_3 - 15.7 (15.7, 15.7)

(0.02470 g. sample required 16.44 cc. of 0.04585 N $Na_2S_2O_3$)

(0.02740 g. sample required 18.20 cc. of 0.04585 N $Na_2S_2O_3$)

Experiment 3.

The crude lignin was placed in a Sohxlet and extracted for 47 hours with benzene. The benzene was removed under reduced pressure and the resulting residue dissolved in chloroform (60 cc.), filtered and precipitated into a tenfold volume of 30-50° petroleum ether. The lignin was centrifuged, washed with petroleum ether and dried.

After removing the benzene from the Sohxlet under reduced pressure, extraction was carried out with acetone for 24 hours at a pressure of 500 mm. The acetone solution (500 cc.) was filtered and precipitated into a twenty-fold volume of water containing 50 cc. of concentrated hydrochloric acid. The lignin was filtered through a Büchner funnel, washed with water and dried thoroughly in the vacuum oven.

The lignin remaining in the Sohxlet was dissolved in one litre of 90% acetone-water solution and precipitated into a twenty-fold volume of water containing 50 cc. of concentrated hydrochloric acid. As before, the lignin was filtered through a Büchner funnel, washed with water and dried in the vacuum oven.

For analytical purposes a 2 gram sample of the acetone soluble fraction was dissolved in 20 cc. of anhydrous dioxane, the solution filtered and precipitated into a tenfold volume of 30-50° petroleum ether. The precipitated lignin was washed with petroleum ether and dried. In a similar manner, 2 grams of the acetone-water soluble fraction was purified by dissolving it in 90% acetone-water and precipitating the filtered solution into ether. The product was washed with ether followed by petroleum ether and dried. A summary of this fractionation is presented in Table II.

<u>Methoxyl Analysis:</u>

(1) Acetone Soluble Fraction -

% OCH_3 - 18.55 (18.5, 18.6)

(0.02460 g. sample required 18.95 cc. of 0.07459 N $Na_2S_2O_3$)

(0.01640 g. sample required 12.75 cc. of 0.07459 N $Na_2S_2O_3$)

(2) 90% acetone-water Soluble Fraction -

% OCH_3 - 15.5 (15.6, 15.4)
(0.02761 g. sample required 17.80 cc. of 0.04684 N $Na_2S_2O_3$)
(0.02507 g. sample required 15.95 cc. of 0.04684 N $Na_2S_2O_3$)

TABLE I

THE PREPARATION OF BIRCH FORMIC ACID LIGNIN

	Run 1.	Run 2.	Run 3.
Wt. of wood meal.	200 g.	200 g.	400 g.
Moisture Content.	17.4%	4.6%	10.9%
Wt. of dry meal.	165 g.	191 g.	356 g.
Volume of formic acid.	1500 cc.	1500 cc.	3000 cc.
Time of refluxing.	20 hrs.	22 hrs.	22 hrs.
Wt. of undissolved wood meal.	52 g.	63 g.	97 g.
Wt. of crude lignin.	43 g.	50 g.	96 g.
Yield of crude lignin based on original dry wood meal.	26%	26%	27%

TABLE II

THE FRACTIONATION OF BIRCH FORMIC ACID LIGNIN

RUN 1			RUN 2		
Fraction	Weight	% OCH_3	Fraction	Weight	% OCH_3
Chloroform	5.1 g.	19.7	Chloroform	4.3 g.	19.25
Acetone	7.4 g.	17.3	Acetone	11.1 g.	17.4
Dioxane	23.0 g.	16.5	90% acetone-water	28.0 g.	15.7

RUN 3		
Fraction	Weight	% OCH_3
Benzene	4.0 g.	-
Acetone	28.0 g.	18.55
90% acetone-water	57.0 g.	15.5

(d) The Preparation of Acetic Acid Lignin

Four hundred grams of the extracted birch wood meal were refluxed with 4 litres of glacial acetic acid in a flask equipped with a mercury sealed stirrer and reflux condenser for 105 hours. Upon cooling the reaction mixture was filtered through cloth on a Büchner funnel and washed with glacial acetic acid until the washings were colorless. The filtrate and washings were evaporated under reduced pressure to a volume of 500 cc. and this was poured slowly into 6 litres of vigorously stirred water. After standing overnight the supernatant liquid was siphoned off and the lignin filtered on a Büchner funnel and washed with water until free from acid. It was then dried in the vacuum oven.

The undissolved wood meal from the first filtration was thoroughly washed with water until free from acid, partially dried under a rubber dam and finally in the vacuum oven. The results are summarized in Table III.

(e) The Fractionation of Acetic Acid Lignin

The crude lignin was placed in a Soxhlet and extracted with benzene for 30 hours. The benzene was then removed under reduced pressure and the residue dissolved in 20 cc. of chloroform. This chloroform solution was filtered and precipitated into a tenfold volume of 30-50° petroleum ether. The lignin was washed with petroleum ether and dried.

The benzene was removed from the Soxhlet under reduced pressure and the next extraction carried out with acetone for 26 hours. Inspection of the Soxhlet at this point revealed that all of the lignin had been dissolved. The acetone solution (500 cc.) was precipitated into a twenty fold volume of water containing 50 cc. of concentrated hydrochloric acid. The lignin was filtered on a Büchner funnel, washed with water and dried in the vacuum oven. The results are summarized in Table III.

<u>Methoxyl Analysis</u>:

(1) Benzene Soluble Fraction -

%OCH_3 - 20.7

(0.001453 g. sample required 6.31 cc. of 0.00923 N $Na_2S_2O_3$)

(2) Acetone Soluble Fraction -

% OCH_3 - 18.8 (18.7, 18.9)

(0.02584 g. sample required 20.30 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02059 g. sample required 16.30 cc. of 0.04613 N $Na_2S_2O_3$)

TABLE III

THE PREPARATION AND FRACTIONATION OF BIRCH ACETIC ACID LIGNIN

PREPARATION	
Wt. of wood meal	400 g.
Moisture content	6.1%
Wt. of dry wood meal	376 g.
Wt. of undissolved wood meal	225 g.
Wt. of crude lignin	72 g.
Yield of crude lignin based on dry meal	19%

FRACTIONATION

Fraction	Weight	% OCH_3
Benzene	4.9 g.	20.7
A cetone	61.0 g.	18.8

II. THE OZONE MACHINE AND ITS USE.

The ozone machine was built in these laboratories and has been described by Bell (42). Two modifications were introduced by the writer. In the first place a sensitive needle valve was set up so that the flow of oxygen passing from the tank through the machine could be regulated to give an absolutely invariable rate of flow at any desired speed. Furthermore a two way stopcock was introduced at the outlet of the machine so tha t the ozone-oxygen mixture could be passed at will through the reaction mixture or could be passed into another flask for a definite period of time in order to determine the amount of ozone being produced by the machine.

Smith (87) has shown that the following reaction takes place very rapidly and very completely when ozone is passed in to neutral potassium iodide solution:

$$O_3 + KI + H_2O \longrightarrow I_2 + 2KOH + O_2$$

Thus in order to determine the amount of ozone being produced at any given rate of flow, the ozone is passed through the potassium iodide solution for a definite length of time, then the solution is acidified and titrated with standardized thiosulphate solution. This same method applied to the gases coming from the reaction mixture also gives a measure of the amount of ozone that is being absorbed by the solution.

III. THE OZONIZATION OF ORGANIC COMPOUNDS.

(a) Maleic Acid.

(i) Formic Acid Solvent.

Two grams of maleic acid were dissolved in 35 cc. of formic acid. Ozone was then passed through the solution at 0°C for a period of five hours. During this time 1.64 grams of ozone were passed into the solution, of which 0.12 grams were unabsorbed. Upon completion of the ozonization the formic acid was removed under reduced pressure. The remaining white solid was refluxed with 30 cc. of water and thereby passed into solution. The cooled solution was filtered into a 100 cc. volumetric flask and the volume brought to exactly 100 cc. with water.

The absence of oxalic acid in this solution was determined in the following manner. To 3 cc. of the solution was added 10 cc. of water and 2 cc. of a saturated solution of calcium hydroxide. No precipitate or cloudiness was observed on standing. A series of tests with this reagent on solutions of oxalic acid of known concentration showed that this test is sensitive to the extent of giving a definite precipitate in the presence of 0.0005 grams of oxalic acid.

The yield of glyoxylic acid was determined as follows: 1.2 grams of 2,4-dinitrophenylhydrazine and 80 cc. of water were heated to boiling and sufficient concentrated HCl added to the solution to cause the hydrazine to go into solution as its hydrochloride. The solution was then removed from the flame and while still warm 20 cc. of the solution from the volumetric flask added. A yellow precipitate separated out almost immediately. After standing at room temperature for a few hours the precipitate was filtered through a tared sintered glass crucible and dried at 105°C. Weight of derivative - 1.697 grams. Melting point - 195°C. Upon recrystallization from methanol the melting point was 196°C. The mixed melting point with an authentic sample of the 2,4-dinitrophenylhydrazone of glyoxylic acid (M.P. 198°C.) was 196°C.

Because of the fact that this derivative was soluble in water it was necessary to determine this solubility in order to evaluate the yield. 0.121 grams of the derivative were added to a mixture of 100 cc. of water and 15 cc. of concentrated HCl (the same conditions used previously) and the solution heated to boiling and allowed to stand at room temperature for a few hours. Filtration and drying gave rise to 0.073 grams of undissolved material, and thus 0.048 grams were soluble under these conditions.

The theoretical yield of the 2,4-dinitrophenylhydrazone of glyoxylic acid from 2 grams of maleic acid would be 8.88 grams. Actually there was obtained 1.745 grams (1.697 plus 0.048 g.) from an aliquot of one-fifth of the total solution. This corresponds to a yield of 98%.

(ii) Ethyl Acetate Solvent.

Ethyl acetate was prepared for use as an ozonisation solvent by distilling stock ethyl acetate from sodium carbonate. This distillate was then dried over calcium chloride and again distilled. A stream of ozone was then passed through the solvent for one and one-half hours and it was distilled from fresh calcium chloride into clean dry bottles.

In 20 cc. of ethyl acetate were dissolved 0.5 grams of maleic acid and while this solution was maintained at 0°C. ozone (0.57 grams) was passed through the solution for a period of one and one-half hours. The solvent was removed under reduced pressure and the resulting residue dissolved in 20 cc. of water. This solution was filtered and the volume made up to 25 cc. exactly.

To a 5 cc. aliquot of this solution was added calcium hydroxide solution until there was no further precipitate. This precipitate was filtered, dried at 195°C. and weighed as calcium oxalate. The theoretical yield of calcium oxalate possible would be 1.10 grams. The weight obtained was 0.176 grams, which corresponds to a yield of 80%.

To another 5 cc. aliquot of the reaction mixture was added an excess of a water solution of 2,4-dinitrophenylhydrazine hydrachloride. The precipitate was filtered, dried and weighed. The melting point was 197-198°C. The mixed melting point with an authentic sample of the 2,4-dinitrophenylhydrazone of glyoxylic acid (M.P. 198°C.) was 197°C. The weight obtained was 0.064 grams, which corresponds to a yield of 14.5% of glyoxylic acid based on the original maleic acid.

(b) Vanillin.

(i) Formic Acid Solvent.

One gram of vanillin was dissolved in 100 cc. of formic acid and 1.14 grams of ozone passed through the solution over a period of two hours, the flask being maintained at a temperature of 0°C. During the ozone treatment the solution turned pale yellow in color. The formic acid was removed under reduced pressure leaving a dark brown syrup to which was added 150 cc. of water. Upon slight warming the syrup went into solution. The solution was filtered and the volume made up to exactly 250 cc.

A portion of this solution gave no test for oxalic acid with calcium hydroxide. A 25 cc. aliquot was diluted to 50 cc. with water and 3.5 grams of sodium acetate and 2 cc. of 10% sulphuric acid added. The solution was warmed to 60°C. and to this was added 10 cc. of water at 60°C. containing 0.1 gram of meta-nitrobenzoyl hydrazine. Upon standing overnight the precipitate was filtered through a tared sintered glass crucible and dried at 105°C. The melting point of the derivative was 205°C. The mixed melting point with an authentic sample of the metanitrobenzoylhydrazone of vanillin (M.P. 210-211°C.) was 207°C. The weight of the derivative 0.08642 grams which corresponds to a recovery of 42% of the original vanillin.

Another 25 cc. aliquot of the reaction solution was extracted continuously with ether for 12 hours. The ethereal extract was dried over sodium sulphate and the ether removed under reduced pressure. The residue was sublimed at 12 mm. pressure and 100-115°C. This sublimate was then resublimed under the same conditions and the second sublimate carefully washed into a tared weighing bottle with anhydrous ether. The ether was removed under reduced pressure and gave 0.04826 grams of material. The melting point was 76°C. The mixed melting point with an authentic sample of vanillin (M.P. 82°C.) was 81°C. Thus this corresponds to a recovery of 48% of the original vanillin. The average value for these two methods of determining the amount of vanillin is 45%.

(ii) Ethyl Acetate Solvent.

One gram of vanillin was dissolved in 100 cc. of ethyl acetate and at 0°C. was subjected to the action of 1.08 grams of ozone over a period of two hours. The solvent was removed under reduced pressure giving rise to a brownish oil. Upon the addition of 125 cc. of water, and warming, the oil went into solution. The solution was then filtered and the volume made up to exactly 250 cc.

No precipitate of calcium oxalate was obtained on a portion of this solution upon the addition of calcium hydroxide solution. A 100 cc. portion of the reaction solution was continuously extracted with ether for 18 hours, the ethereal extract dried, and the ether removed under reduced pressure. Sublimation at 10 mm. and 100°C. gave only a very minute amount of oily material on the cold finger. At a temperature of 140°C. a yellowish-white sublimate collected, the melting point of which was 197-199°C. Two recrystallizations from water gave a white, crystalline product which melted at 206-207°C. The mixed melting point of this material with an authentic sample of vanillic acid (M.P. 207°C.) was 205-206°C. Thus no vanillin was left in the solution this having been converted to vanillic acid. The yield of the latter was determined by extracting a further 60 cc. portion of the original solution with ether and subliming the acid at 8 mm. and 110-120°C. after removal of the ether. By this means 0.0563 grams were obtained which corresponds to a yield of 21% based on the original vanillin.

(c) Veratric Aldehyde.

(i) Formic Acid Solvent.

One gram of freshly distilled veratric aldehyde (M.P. 45-46°C.) was dissolved in 90 cc. of formic acid and while kept at 0°C. was subjected to the action of 0.64 grams of ozone over a period of one hour. The removal of the formic acid under reduced pressure left a brown syrup. This was dissolved in warm water and a small amount of methanol added to effect complete solution. It was then filtered and the volume made up to exactly 250 cc.

No test for oxalic acid was obtained with calcium hydroxide solution. A 50 cc. aliquot was partially evaporated under reduced pressure to remove the methanol and then diluted with 100 cc. of 2% acetic acid. This solution was warmed to 60°C. and to it was added 25 cc. of water at 60°C. containing 0.3 grams of meta-nitrobenzoylhydrazine. Upon standing overnight the precipitate was filtered through a tared sintered glass crucible and dried at 105°C. The melting point was 213-214°C. One recrystallization from methanol gave a melting point of 222-223°C. The mixed melting point with an authentic sample of the meta-nitrobenzoylhydazone of veratric aldehyde (M.P. 224-225°C.) was found to be 223°C. The weight of the derivative was 0.2470 grams which corresponds to a recovery of 62% of the veratric aldehyde.

(ii) Ethyl Acetate Solvent.

One gram of pure veratric aldehyde was dissolved in 90 cc. of purified ethyl acetate and while kept at 0°C. was subjected to the action of 1.30 grams of ozone over a period of two hours. Removal of the solvent under reduced pressure left a brown syrup which, as before, was taken up in an alcohol-water mixture, filtered and the volume made up to 250 cc.

A 50 cc. aliquot was distilled under reduced pressure to remove the alcohol and then added to 100 cc. of 2% acetic acid. 0.3 grams of metanitro benzoyl hydrazine were then added in the manner already described above. The weight of the derivative formed in this case was 0.0060 grams. In other words a negligible amount of veratric aldehyde remained in the solution after the ozone treatment.

A 100 cc. aliquot of the reaction solution was evaporated to dryness under reduced pressure and the residue sublimed at a pressure of 0.008 mm. and 135°C. This gave rise to 0.1974 grams of a crystalline sublimate. Recrystallization from water gave a white crystalline product which had a melting point of 177°C. The mixed melting point with an authentic sample of veratric acid (M.P. 181°C.) was found to be 180-181°C. The weight of this acid obtained corresponds to a yield of 45% on the basis of the original veratric aldehyde.

IV. THE OZONIZATION OF FORMIC ACID BIRCH LIGNIN.

(a) Run 1.

Ten grams of the dioxane soluble fraction of formic acid lignin (Run 1., Table II, p. 92) were dissolved in 250 cc. of formic acid, and while the solution was maintained at 0°C. ozone was passed through for a period of seven and one-half hours. During this time 6.65 grams of ozone were passed into the lignin solution and the color changed from an opaque brown to a transparent red.

The solvent was then removed under reduced pressure until no odor of formic acid remained. Seven hundred cc. of water were added and boiled until 75 cc. of distillate had been collected (hereafter, Distillate A.). The cooled solution was filtered on a Büchner funnel and the water insoluble fraction washed with water until the washings were colorless and neutral to litmus paper, (hereafter, Water Insoluble Fraction B.). This fraction was dried under reduced pressure at 60°C. Weight, 1.1 grams.

Methoxyl Analysis:

% OCH_3 - 5.3 (5.2, 5.4)

(0.0271 g. sample required 5.74 cc. of 0.04893 N $Na_2S_2O_3$)

(0.0288 g. sample required 6.15 cc. of 0.04893 N $Na_2S_2O_3$)

The filtrate from the above filtration (1000 cc.) was extracted continuously with ether for 37 hours giving rise to Ether Extract C. The water solution was then evaporated to dryness under reduced pressure and the resulting solid material dissolved in 50 cc. of acetone. This acetone solution was filtered and precipitated in a fine stream into 500 cc. of well stirred ether. The flocculent precipitate which settled out was centrifuged off and washed with ether followed by 30-50° petroleum ether and dried under reduced pressure at 60°C. (hereafter Water Soluble Fraction D.). Weight, 5.4 grams.

Methoxyl Analysis:

% OCH_3 - 4.45 (4.3, 4.6)
(0.0311 g. sample required 5.45 cc. of 0.04893 N $Na_2S_2O_3$)
(0.0304 g. sample required 5.55 cc. of 0.04893 N $Na_2S_2O_3$)

(b) Run 2.

An additional 10 grams of the dioxane fraction of birch formic acid lignin was treated with 6.65 grams of ozone in the same manner as has been described for Run 1. Upon the removal of the solvent and distillation of the residue with water 85 cc. of Distillate A. were collected. Filtration, washing and drying gave rise to 1.7 grams of the Water Insoluble Fraction B.

Methoxyl Analysis:

% OCH_3 - 5.6

(0.0287 g. sample required 6.40 cc. of 0.04893 N $Na_2S_2O_3$)

Ether Extract C was obtained by continuous extraction of the filtrate for 66 hours. As in Run 1. the water solution was evaporated to dryness under reduced pressure and the residue precipitated from 50 cc. of acetone into 500 cc. of ether. The Water Soluble Fraction D was centrifuged off, washed with ether followed by 30-50-C. petroleum ether and dried. Weight - 4.8 grams.

Methoxyl Analysis:

% OCH_3 - 4.7 (4.7, 4.7)

(0.002187 g. sample required 2.14 cc. of 0.00923 N $Na_2S_2O_3$)

(0.003188 g. sample required 3.13 cc. of 0.00923 N $Na_2S_2O_3$)

The results obtained are summarized in Table IV.

(c) The Identification of Acetone.

Distillates A. from Run 1 and Run 2 were combined and to this was added 25 cc. of a solution of 2,4-dinitrophenylhydrazine hydrachloride in water prepared according to the following method. 0.4 grams of 2,4-dinitrophenylhydrazine were triturated with 21 cc of concentrated hydrochloric acid and then diluted to 100 cc. with water. After standing for 24 hours the solution was filtered and bottled (88).

The solution of distillates and hydrazine was refluxed for ten minutes and after standing overnight the orange precipitate was filtered, washed and dried. This precipitate was extracted with purified (acetone free) chloroform, the extract filtered and the chloroform removed under reduced pressure. Recrystallization of the residue from methanol gave 0.020 grams of yellow crystals which had a melting point of 123-124°C. The mixed melting point with an authentic sample of the 2,4-dinitrophenylhydrazone of acetone (M.P. 124-125°C.) was found to be 124-125°C. Yield of acetone from 20 grams of lignin = 0.005g. or 0.025%.

(d) The Identification of Oxalic Acid.

The Ether Extracts C. from Run 1 and Run 2 were combined and after drying over sodium sulphate the ether was removed under reduced pressure. Sublimation of the residue at 10 mm. pressure and 120°C. gave 1.2 grams of crystalline material. A portion of this material was resublimed under the same conditions and the white crystals formed had a melting point of 180°C. with decomposition. Pure oxalic acid was sublimed and gave threadlike crystals which melted at 190°C. with decomposition. The mixed melting point was found to be 185°C. It gave the usual tests for oxalic acid, Yield from 20 grams of lignin = 1.29 or 6.0%.

TABLE IV

SUMMARY OF THE OZONIZATION OF BIRCH FORMIC ACID LIGNIN

	RUN 1	RUN 2
Weight of lignin used	10 g.	10 g.
% methoxyl	16.5%	16.5%
Water insoluble fraction		
Weight	1.1 g.	1.7 g.
% methoxyl	5.3%	5.6%
% yield	11.0%	17.0%
Water soluble fraction		
Weight	5.4 g.	4.8 g.
% methoxyl	4.45%	4.7%
% yield	54.0 %	48.0%
Total yield of two fractions	65%	65%

V. METHYLATION OF THE PRODUCTS FROM THE OZONIZATION OF BIRCH FORMIC ACID LIGNIN.

(a) Methylation with Diazomethane of the Water Soluble Fraction.

Four and one half grams of the water soluble fraction from the ozonization of birch formic acid lignin (Run 2. - page 108) were used in this experiment. This fraction had a methoxyl content of 4.7%. The water soluble lignin was dissolved in 100 cc. of methanol and an ethereal solution of diazomethane prepared from 10 grams of nitrosomethylurea was slowly added to the cooled solution. There was considerable gaseous evolution and a portion of the lignin was precipitated from the solution. After standing four hours 100 cc. of methanol were added to effect solution and this was again treated with diazomethane from 10 grams of nitrosomethylurea.

After standing overnight the methanol and ether were removed by evaporation under reduced pressure and the residue dissolved in acetone (60cc.) and centrifuged to remove the insoluble diazomethane polymer. The acetone solution was filtered into a small flask and the solvent removed under reduced pressure. The lignin was then dissolved in chloroform (40 cc.), the solution filtered and precipitated into 30-50° petroleum ether (400 cc.). The lignin was centrifuged, washed with petroleum ether and dried at 60°C. under reduced pressure. Yield, 4.4 g.

Methoxyl Analysis:

% OCH_3 - 20.4

(0.001190 gram sample required 5.09 cc. of 0.00923 N $Na_2S_2O_3$)

The lignin after this methylation was now found to be quite insoluble in methanol, but was readily soluble in chloroform. Consequently for further methylation it was dissolved in 100 cc. of purified chloroform and further treated with diazomethane from 10 grams of the nitroso methyl urea. The reaction mixture was worked up in the same manner as has already been described and the precipitated lignin dried and weighed. Yield, 4.0 g.

Methoxyl Analysis:

% OCH_3 - 21.2

(0.001285 g. sample required 5.72 cc. of 0.00923 N $Na_2S_2O_3$)

The product was very light brown in color and was found to be still soluble in 0.5% sodium hydroxide.

(b) Hydrolysis of the Diazomethane Methylated Water Soluble Fraction

A portion of this lignin (0.564 grams) which had been obtained as described in the preceding section and which had a methoxyl content of 21.2% was dissolved in 25 cc. of 5% sodium hydroxide and kept under an atmosphere of nitrogen at room temperature for 48 hours. At the end of this time the solution was acidified with hydrochloric acid and the precipitated lignin centrifuged off, washed with water and dried.

The product was purified by precipitation from 7 cc. of methanol into 60 cc. of anhydrous ether. The lignin was centrifuged, washed with petroleum ether and dried. Yield - 0.15 grams.

Methoxyl Analysis:

$\%$ OCH_3 - 11.0 (11.0, 11.0)

(0.001910 g. sample required 4.39 cc. of 0.00923 N $Na_2S_2O_3$)

(0.002225 g. sample required 5.11 cc. of 0.00923 N $Na_2S_2O_3$)

(c) Methylation with Dimethylsulphate of the Water Soluble Fraction.

The water soluble fraction from the ozonization of birch formic acid lignin was used in this experiment, (Run 1, p.107). In 100 cc. of 1% sodium hydroxide was dissolved 4.6 grams of the lignin and while this solution was kept well stirred there was added at equal rates over a period of one and one-half hours 32 cc. of 30% sodium hydroxide and 30 cc. of dimethylsulphate, the reaction mixture being kept at room temperature and under nitrogen during the procedure. After stirring overnight the solution was acidified and the lignin filtered off, washed with a small quantity of water and dried. It was observed that the filtrate was rather dark in color. This loss of lignin was undoubtedly due to the high solubility of this fraction of the lignin in water.

The dried lignin was redissolved in 100 cc. of 1% sodium hydroxide and again treated with dimethylsulphate in the same manner as already described above. The lignin was recovered by acidification of the reaction mixture and filtration. After washing with water the lignin was dried. Yield - 2.0 g.

Methoxyl Analysis:

% OCH_3 - 12.5%

(0.02665 g. sample required 13.73 cc. of 0.04704 N $Na_2S_2O_3$)

This partially methylated lignin was then dissolved in 50 cc. of 1% sodium hydroxide and again methylated according to the procedure described. Upon acidification it was observed that the precipitate did not coagulate and it was believed that possibly the methanol formed in the reaction kept the lignin in partial solution. Consequently the solution was distilled under reduced pressure to about half of its original volume. The lignin which separated out on this treatment was filtered, washed with water and dried.

A fourth methylation was carried out in 100 cc. of dioxane and after recovering the product in the usual manner it was purified by precipitation from 90% acetone-water solution (20 cc.) into ether (200 cc.). The precipitated lignin was washed with ether, followed by petroleum ether, and was dried at 60°C. under reduced pressure. Yield.- 1.5 g. The product was still soluble in 5% sodium hydroxide.

Methoxyl Analysis:

% OCH_3 - 16.2

(0.02611 g. sample required 17.41 cc. of 0.04704 N $Na_2S_2O_3$)

(d) Diazomethane Treatment of the Dimethylsulphate Methylated Water Soluble Fraction.

The water soluble fraction which had been methylated with dimethyl sulphate (1.15 g.) was dissolved in 50 cc. of methanol and at 0°C. treated with an ethereal solution of diazomethane from 5 g. of nitrosomethylurea. Nine hours later it was treated with diazomethane from 3 g. of urea and the reaction mixture let stand overnight.

The methanol-ether solvent was removed under reduced pressure and the residue dissolved in acetone. The diazomethane polymer was centrifuged off and the solution filtered into a flask and evaporated to dryness under reduced pressure. The residue was dissolved in 20 cc. of purified dioxane, filtered and precipitated into 200 cc. of 30-50°C. petroleum ether. The precipitate was washed and dried. The product was then redissolved in 60 cc. of dioxane and treated with diazomethane from 3 g. of urea. After standing for four hours the treatment was repeated and the reaction mixture allowed to stand overnight. It was worked up in the same manner as already described and the precipitated lignin after washing was dried at 60°C. and weighed. Yield,-0.9 g.

The product was insoluble in 5% sodium hydroxide.

Methoxyl Analysis:

% OCH_3 - 25.2 (25.2, 25.2)
(0.02460 g. sample required 25.52 cc. of 0.04704 N $Na_2S_2O_3$)
(0.02906 g. sample required 30.10 cc. of 0.04704 N $Na_2S_2O_3$)

(e) Methylation with Diazomethane of the Water Insoluble Fraction.

The water insoluble fraction from the ozonization of birch formic acid lignin (Run 1, page 106) was used in this experiment. This fraction had a methoxyl content of 5.3%. Because of the great insolubility of this fraction in the usual solvents 0.9 g. of the material was finely ground and partially suspended and partially dissolved in 100 cc. of 90% dioxane-water. At 0°C. this suspension was treated with an ethereal solution of diazomethane from 5 g. of nitrosomethylurea. Five hours later the treatment was repeated.

Upon removal of the solvents under reduced pressure after the reaction mixture had stood overnight it was found that the great insolubility of the lignin in the common organic solvents made it impossible to remove the diazomethane polymer in the usual manner. Consequently it was subjected to a further methylation in the hope that this treatment might increase the solubility in organic solvents. This treatment was again carried out in suspension in 90% dioxane-water and consisted of diazomethane from 3 g. of nitrosomethylurea, followed by that from 2 g. eight hours later. The product obtained on removal of the solvents was found to be still insoluble in organic solvents and for this reason could not be purified for methoxyl analysis. That methylation had taken place, however, was indicated by fact that, although the original material had been easily soluble in 5% sodium hydroxide, the methylated product was completely insoluble in this medium.

(f) Methylation with Dimethylsulphate of the Water Insoluble Fraction.

The water insoluble fraction from the ozonization of birch formic acid lignin (Run 2, page 107) was used in this experiment. This fraction had a methoxyl content of 5.6%. The product (1.5 g.) was dissolved in 100 cc. of 1% sodium hydroxide and over a period of one and one-half hours with constant stirring under an atmosphere of nitrogen, and at room temperature was added 33 cc. of 30% sodium hydroxide and 30 cc. of dimethylsulphate at equal rates. After standing overnight the reaction mixture was acidified and the lignin filtered off, washed with water and dried. The product was then redissolved in 1% alkali and the methylation repeated as already described. The resulting product was found to be insoluble in organic solvents and consequently could not be purified by reprecipitation. It was however still soluble in 5% sodium hydroxide. Yield - 0.9 g.

Methoxyl Analysis:

% OCH_3 - 12.4 (12.4, 12.4)
(0.02474 g. sample required 12.58 cc. of 0.04704 N $Na_2S_2O_3$)
(0.02532 g. sample required 12.95 cc. of 0.04704 N $Na_2S_2O_3$)

(g) Methylation with Diazomethane of the Dimethylsulphate Methylated Water Insoluble Fraction.

The water insoluble fraction which had been methylated with dimethylsulphate (0.6 g.) was suspended in 50 cc. of methanol and at 0°C. treated with diazomethane from 2 g. of nitrosomethylurea. After standing overnight the product was worked up in the usual manner and purified by precipitation from dioxane (15 cc.) into a tenfold volume of 30-50°C. petroleum ether. The precipitated lignin was washed with petroleum ether and dried at 60°C. The product was insoluble in 5% alkali.

Methoxyl Analysis:

% OCH_3 - 23.7
(0.02895 g.sample required 27.92 cc. of 0.04759 N $Na_2S_2O_3$)

VI. THE DISAPPEARANCE OF METHOXYL ON OZONIZATION.

(a) The Ozonization of Formic Acid Lignin.

The acetone soluble fraction of birch formic acid lignin was used in this experiment, (Table II, page 93). This fraction had a methoxyl content of 17.3%. Two and one-half grams were dissolved in 100 cc. of formic acid and at 0°C. this solution was subjected to the action of 1.067 g. of ozone over a period of two and one-half hours. It was found that 0.266 g. of ozone were unabsorbed and passed out of the solution in the effluent stream of oxygen.

The formic acid was removed under reduced pressure and the resulting residue refluxed with 500 cc. of water for one hour. The water insoluble fraction was obtained by filtration and after washing with water was dried under a rubber dam and finally at 60°C. Weight - 0.77 g. (31% yield)

Methoxyl Analysis:

% OCH_3 - 7.05 (7.0, 7.1)
(0.02625 g. sample required 7.60 cc. of 0.04704 N $Na_2S_2O_3$)
(0.02629 g. sample required 7.66 cc. of 0.04704 N $Na_2S_2O_3$)

The filtrate was evaporated to dryness under reduced pressure and the residue dissolved in acetone (20 cc.) and precipitated into a tenfold volume of ether. This precipitate of the water soluble fraction was centrifuged, washed well with ether and finally petroleum ether and dried. Weight - 1.0 g. (40% yield)

Methoxyl Analysis:

% OCH_3 - 6.25 (6.1, 6.4)

(0.02697 g. sample required 6.78 cc. of 0.04704 N $Na_2S_2O_3$)

(0.02636 g. sample required 6.80 cc. of 0.04704 N $Na_2S_2O_3$)

The combined ether solutions from the precipitation of the water soluble lignin fraction were evaporated to dryness in a tared flask under reduced pressure. Weight - 0.75 g. (30% yield) This ether soluble fraction was then subjected to a sublimation at 10 mm. and 140°C. and resulted in a white crystalline sublimate. Weight - 0.12 g. (4.8% yield)
This product was resublimed and identified as oxalic acid by a mixed melting point with a sample of anhydrous oxalic acid.

(b) The Diazomethane Methylation of Birch Formic Acid Lignin.

The lignin fraction used in this experiment was a portion of the same fraction that was used in the ozonization described in the previous section, namely the acetone soluble fraction (Table II, page 93), and having a methoxyl content of 17.3%. This lignin (3.68 g.) was dissolved in 100 cc. of dioxane and treated with diazomethane at 0°C. from 5 g. of nitrosomethyl urea. Five hours later this treatment was repeated and the reaction mixture allowed to stand overnight.

The solvent was removed under reduced pressure and the diazomethane polymer removed in the usual manner. The product was precipitated from dioxane (40 cc.) into a tenfold volume of 30-50°C. petroleum ether, washed with petroleum ether and dried at 60°C.

Methoxyl Analysis:

% OCH_3 - 22.1

(0.02345 g. sample required 21.28 cc. of 0.04704 N $Na_2S_2O_3$)

The product was then again methylated in dioxane solution (60 cc.) using 2 g. of nitrosomethylurea and the product worked up in the manner previously described.

Methoxyl Analysis:

% OCH_3 - 23.8

(0.02667 g. sample required 26.08 cc. of 0.04704 N $Na_2S_2O_3$)

A final methylation was carried out in the same manner described above using 3 g. of the nitrosomethylurea. The product was washed with 30-50°C. petroleum ether and dried at 60°C. and found to be insoluble in 5% sodium hydroxide. Yield (overall) 3.21 g. or 87%.

Methoxyl Analysis:

% OCH_3 - 24.05 (24.0, 24.1)

(0.02683 g. sample required 26.50 cc. of 0.04704 N $Na_2S_2O_3$)

(0.02712 g. sample required 26.90 cc. of 0.04704 N $Na_2S_2O_3$)

(c) Ozonization of Diazomethane Methylated Birch Formic Acid Lignin.

Two and one-half grams of the diazomethane methylated lignin obtained as described in the previous section (b), were dissolved in 100 cc. of formic acid and at 0°C. this solution was subjected to the action of 1.15 g. of ozone over a period of one and one-half hours. During this time 0.39 g. of ozone were not absorbed by the solution.

Upon removing the formic acid under reduced pressure, and refluxing the residue with 500 cc. of water for one hour, the water insoluble fraction was filtered off, well washed with water and dried, first under a rubber dam and finally at 60°C. Weight - 1.41 g. (56% yield)

Methoxyl Analysis:

% OCH_3 - 14.2 (14.3, 14.1)
(0.02628 g. sample required 15.45 cc. of 0.04704 N $Na_2S_2O_3$)
(0.02824 g. sample required 16.38 cc. of 0.04704 N $Na_2S_2O_3$)

The aqueous filtrate was evaporated to dryness under reduced pressure and the water soluble fraction precipitated from acetone (15 cc.) into a tenfold volume of ether. After washing the lignin with ether it was washed with 30-50°C. petroleum ether and dried at 60°C. Weight - 0.35 g. (14% yield)

Methoxyl Analysis:

% OCH_3 - 10.8 (10.8, 10.8)

(0.02366 g. sample required 10.48 cc. of 0.04704 N $Na_2S_2O_3$)

(0.02496 g. sample required 11.12 cc. of 0.04704 N $Na_2S_2O_3$)

The combined ether solutions from the precipitation of the water soluble fraction were evaporated to dryness under reduced pressure in a tared flask. Weight - 0.71 g. (28% yield)
This ether soluble fraction was then subjected to a sublimation at 0.070 mm. pressure and 100°C. and resulted in a white crystalline sublimate. Weight - 0.11 g. (4.4% yield)
The majority of this product was resublimed and identified as oxalic acid by a mixed melting point with anhydrous oxalic acid.

A methoxyl determination was carried out on the material remaining in the flask after the sublimation. This material had a lignin-like appearance.

Methoxyl Analysis:

% OCH_3 - 10.1

(0.03350 g. sample required 13.75 cc. of 0.04759 N $Na_2S_2O_3$)

(d) The Dimethylsulphate Methylation of Birch Formic Acid Lignin.

The lignin fraction used in this experiment was a portion of the same fraction used in the two previous experiments described in this section, namely the acetone soluble fraction

(Table II, page93), having a methoxyl value of 17.3%. This lignin (3.8 g.) was dissolved in 100 cc. of dioxane and treated in the usual manner with 35 cc. of sodium hydroxide and 30 cc. of dimethylsulphate. After standing overnight the reaction mixture was acidified with dilute hydrochloric acid and the dioxane removed by distillation under reduced pressure. The precipitated lignin was filtered off and washed with water.

The lignin was redissolved in dioxane and the treatment with dimethylsulphate repeated four times. Finally the product was purified by precipitation from dioxane (40 cc.) into a tenfold volume of 30-50°C. petroleum ether and dried at 60°C. Yield - 2.7 g. The product was found to be insoluble in 5% alkali.

Methoxyl Analysis:

% OCH_3 - 31.55 (31.6, 31.5)
(0.02962 g. sample required 28.42 cc. of 0.04704 N $Na_2S_2O_3$)
(0.03002 g. sample required 38.88 cc. of 0.04704 N $Na_2S_2O_3$)

(e) Ozonization of Dimethylsulphate Methylated Birch Formic Acid Lignin.

Two and one-half grams of the dimethylsulphate methylated lignin, obtained as described in the previous section, were dissolved in 100 cc. of formic acid and at 0°C. this solution was subjected to the action of 0.97 g. of ozone over a period of one and one-half hours. During this time 0.32 g. of ozone were not absorbed by the solution.

Upon removing the formic acid under reduced pressure, and refluxing the residue with 500 cc. of water for one hour, the water insoluble fraction was filtered off, well washed with water and dried, first under a rubber dam and finally at 60°C.
Weight - 1.65 g. (66% yield)

Methoxyl Analysis:

% OCH_3 - 19.6 (19.7, 19.5)
(0.02746 g. sample required 22.30 cc. of 0.04704 N $Na_2S_2O_3$)
(0.03062 g. sample required 24.50 cc. of 0.04704 N $Na_2S_2O_3$)

The filtrate was evaporated to dryness under reduced pressure and the water soluble fraction precipitated from acetone (15 cc.) into a tenfold volume of ether. After washing the lignin with ether it was washed with 30-50°C. petroleum ether and dried at 60°C. Weight - 0.14 g. (5%yield)

Methoxyl Analysis:

% OCH_3 - 12.75 (12.7, 12.8)
(0.02684 g. sample required 13.84 cc. of 0.04759 N $Na_2S_2O_3$)
(0.02450 g. sample required 12.74 cc. of 0.04759 N $Na_2S_2O_3$)

The combined ether solutions from the precipitation of the water soluble fraction were evaporated to dryness under reduced pressure in a tared flask. Weight - 0.12 g. (4.8% yield)

This ether soluble fraction was then subjected to a sublimation at 0.070 mm. pressure and 100°C. and resulted in a white crystalline sublimate. Weight - 0.12 g. (4.8% yield)
The majority of this product was resublimed and identified as oxalic acid by a mixed melting point with anhydrous oxalic acid. A methoxyl determination was carried out on the lignin-like material remaining in the flask after the sublimation.

Methoxyl Analysis:

% OCH_3 - 15.1
(0.03317 g. sample required 20.35 cc. of 0.04759 N $Na_2S_2O_3$)

VII. THE SOLUBILITY IN AQUEOUS BISULPHITES OF OZONIZED LIGNINS AND THEIR METHYLATED PRODUCTS.

(a) The Preparation of Standard Sulphite Liquor.

Forty-three and three tenths grams of sodium sulphite were dissolved in two litres of distilled water and sulphur dioxide bubbled into this well stirred solution until frequent titrations of small samples of the liquor showed that a value of 6% had been reached for the total sulphur dioxide content. The analysis of the liquor was carried out by the method of Palmrose (89).

Analysis: 6.0% total SO_2 and 1.15 combined SO_2

The liquor thus prepared was stored in a well stoppered bottle in the cold room and titrations from time to time indicated that practically no loss of sulphur dioxide occurred.

(b) Determination of the Solubility in the Sulphite Liquor.

A length of soft glass tubing (7 mm. bore) was drawn out into a capillary at one end and a small loose wad of cotton placed in the bottom of the tube. Then 50 milligrams of the lignin sample was placed in the tube and carefully brushed down onto the cotton. The other end of the tube was drawn into a capillary at such a position that the bomb would hold 3 cc. The glass tube was then placed through a stopper into a side arm test tube containing the sulphite liquor and by blowing in the side arm the liquor was forced up into the bomb. A small air space was left for the liquor expansion and the end of the capillaries carefully sealed in a small flame. These bombs were then heated for 12 hours at a temperature of 110°C. in a toluene vapor bath. The following determinations were carried out in this manner, and the samples were found to be soluble or insoluble in the bisulphite solution under standard conditions as indicated.

1. Birch Formic Acid Lignin, Acetone Soluble Fraction - Insoluble.
2. Maple Ethanol Lignin° - Insoluble.
3. Maple Propanol Lignin°° - Insoluble.
4. Maple Butanol Lignin°°° - Insoluble.
5. Birch Acetic Acid Lignin - Insoluble.
6. Ozonization Products from Birch Formic Acid Lignin -
    (a) Water Insoluble Fraction - Soluble.
    (b) Water Soluble Fraction - Soluble.
7. Methylated Products from the Ozonization of Birch Formic Acid Lignin.
    (a) Water Soluble Fraction methylated with Diazomethane - Insoluble.
    (b) Hydrolysed product from (a) above - Soluble.

° This sample was obtained from Dr. J. McCarthy and had been pre-prepared by the alcoholysis of maple wood meal (90).

°° This lignin was prepared by the author by the alcoholysis of maple wood meal with n-propanol containing 2% HCl.

°°° This Lignin was prepared by the author by the alcoholysis of maple wood meal with n-butanol containing 2% HCl.

(c) Water Soluble Fraction methylated with Dimethylsulphate - <u>Soluble</u>.

(d) Diazomethane methylated product of (c) above - <u>Insoluble</u>.

(e) Water Insoluble Fraction methylated with Dimethylsulphate - <u>Insoluble</u>.

(f) Diazomethane methylated product of (e) above - <u>Insoluble</u>.

8. Birch Formic Acid Lignin methylated with Diazomethane - <u>Insoluble</u>.

(a) Water Soluble Fraction after Ozonization - <u>Soluble</u>.

(b) Water Insoluble Fraction after Ozonization - <u>Soluble</u>.

9. Birch Formic Acid Lignin methylated with Dimethylsulphate - <u>Insoluble</u>.

(a) Water Soluble Fraction after Ozonization - <u>Soluble</u>.

(b) Water Insoluble Fraction after Ozonization - <u>Soluble</u>.

VIII. A STUDY OF THE LOSS OF METHOXYL AND INCREASE IN SOLUBILITY IN SULPHITE LIQUOR WITH RESPECT TO THE DEGREE OF OZONIZATION.

The standard procedure to be described was followed in every case in the various runs that were made in order to determine the loss of methoxyl and increase in solubility in sulphite liquor in relation to the degree of ozonization of the lignin. A 2.0 g. sample of the lignin was dissolved in 50 cc. of formic acid and at 0°C. ozone was passed into this solution for varying lengths of time. The amount of ozone delivered by the machine was determined at intervals by passing the gas through a neutral potassium iodide solution for a definite period of time and titrating the iodine liberated on acidification with standardized thiosulphate. The gas passing out of the lignin solution was also passed through potassium iodide in order to determine the amount of ozone that was not absorbed by the solution of the lignin. When the ozonization was completed the formic acid was removed by distillation under reduced pressure, 300 cc. of water added to the residue and the solution refluxed for 30 minutes. On cooling the water insoluble lignin was filtered off, washed with water, dried under reduced pressure at 60°C. and weighed.

Methoxyl determinations were carried out on the water insoluble fraction and the solubility of this fraction in sulphite liquor was determined in the following manner. Exactly 100 milligrams of the lignin sample were placed in a glass bomb with 6 cc.

of standard liquor (6.0% total and 1.15% combined SO_2). This bomb was then cooked under the standard conditions of 110°C. for 12 hours. On the completion of the cook the bomb was cooled to 0°C., the tips broken and the lignin solution allowed to run into a small beaker. The tube was then broken in two and the contents washed into the beaker with water. The small piece of cotton was removed and washed and the solution placed in a small tared centrifuge cup and the insoluble lignin centrifuged off. The solid material in the bottom was then stirred with water and, after centrifuging, the water was decanted off. The tube and contents were then dried overnight at 60°C. under reduced pressure over phosphorus pentoxide. The difference in weight gave the amount of insoluble lignin and from this was calculated the percent soluble in the sulphite liquor.

RUN - 1.

Birch Formic Acid Lignin - Acetone Soluble Fraction - 18.55% OCH_3

Weight of ozone added = 0.189 g.)) Weight used = 0.167 g.
Weight of ozone not absorbed = 0.022 g.)

Weight of water insoluble fraction = 1.90 g. (95% yield)

Methoxyl Analysis:

% OCH_3 - 15.9 (15.8, 16.0, 15.9)

(0.02760 g. sample required 18.25 cc. of 0.07459 N $Na_2S_2O_3$)
(0.02055 g. sample required 13.74 cc. of 0.07459 N $Na_2S_2O_3$)
(0.02850 g. sample required 25.30 cc. of 0.04684 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor.

Weight of insoluble lignin = 0.0641 g.

Solubility = 36%

RUN - 2.

Birch Formic Acid Lignin - Acetone Soluble Fraction - 18.55% OCH_3.

Weight of ozone added = 0.341 g.)
) Weight used = 0.290 g.
Weight of ozone not absorbed = 0.051 g.)

Weight of water insoluble fraction = 1.75 g. (87.5% yield)

Methoxyl Analysis:

% OCH_3 - 13.85 (13.9, 13.8)

(0.02365 g. sample required 13.75 cc. of 0.07459 N $Na_2S_2O_3$)

(0.03185 g. sample required 18.20 cc. of 0.04684 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0394 g.

Solubility = 61%.

RUN - 3.

Birch Formic Acid Lignin - Acetone Soluble Fraction - 18.55% OCH_3.

Weight of ozone added = 0.426 g.)
) Weight used = 0.268 g.
Weight of ozone not absorbed = 0.058 g.)

Weight of water insoluble fraction = 1.26 g. (68% yield)

Methoxyl Analysis:

% OCH_3 - 11.65 (11.7, 11.6)

(0.02983 g. sample required 14.35 cc. of 0.04684 N $Na_2S_2O_3$)

(0.02498 g. sample required 12.00 cc. of 0.04684 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0122 g.

Solubility = 88%.

RUN - 4.

Birch Formic Acid Lignin - Acetone Soluble Fraction - 18.55% OCH_3.

Weight of ozone added = 0.485 g.)
) Weight used = 0.404 g.
Weight of ozone not absorbed = 0.081 g.)

Weight of water insoluble fraction = 1.44 g. (72% yield)

Methoxyl Analysis:

% OCH_3 - 12.5 (12.5, 12.5)

(0.02792 g. sample required 14.40 cc. of 0.04684 N $Na_2S_2O_3$)

(0.02802 g. sample required 14.50 cc. of 0,04684 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0224 g.

Solubility = 78%.

RUN -5.

Birch Formic Acid Lignin - 90% acetone-water soluble fraction - 15.5% OCH_3.

Weight of ozone added = 0.488 g.)
) Weight used = 0.394 g.
Weight of ozone not absorbed = 0.094 g.)

Weight of water insoluble fraction = 1.64 g. (82% yield)

Methoxyl Analysis:

% OCH_3 - 10.4 (10.4, 10.4)

(0.02548 g. sample required 10.95 cc. of 0.04684 N $Na_2S_2O_3$)

(0.02470 g. sample required 10.60 cc. of 0.04684 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0237 g.

Solubility = 76%.

RUN - 6.

Birch Formic Acid Lignin - Acetone Soluble Fraction - 18.55% OCH_3.

Weight of ozone added = 0.502 g.)
) Weight used = 0.396 g.
Weight of ozone not absorbed = 0.106 g.)

Weight of water insoluble fraction = 1.45 g. (73% yield)

Methoxyl Analysis:

% OCH_3 - 12.6 (12.6, 12.6)

(0.02760 g. sample required 14.40 cc. of 0.04684 N $Na_2S_2O_3$)

(0.02643 g. sample required 13.80 cc. of 0.04684 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0266 g.

Solubility = 73%.

RUN - 7.

Birch Formic Acid Lignin - Acetone Soluble Fraction - 18.55% OCH_3.

Weight of ozone added = 0.505 g.)
) Weight used = 0.405 g.
Weight of ozone not absorbed = 0.100 g.)

Weight of water insoluble fraction = 1.30 g. (65% yield)

Methoxyl Analysis:

% OCH_3 - 11.45 (11.4, 11.5)

(0.03290 g. sample required 11.30 cc. of 0.04684 N $Na_2S_2O_3$)

(0.02745 g. sample required 12.55 cc. of 0.04684 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0181 g.

Solubility = 82%.

RUN - 8.

Birch Formic Acid Lignin - Acetone Soluble Fraction - 18.55% OCH_3.

Weight of ozone added = 0.590 g.)
)
Weight of ozone not absorbed = 0.127 g.) Weight used = 0.463 g.

Weight of water insoluble fraction = 1.20 g. (60% yield)

Methoxyl Analysis:

% OCH_3 - 11.8 (11.9, 11.8, 11.8)

(0.02396 g. sample required 11.92 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02846 g. sample required 14.08 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02259 g. sample required 11.18 cc. of 0.04613 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0177 g.

Solubility = 82%.

RUN - 9.

Birch Formic Acid Lignin - Acetone Soluble Fraction - 18.55% OCH_3.

Weight of ozone added = 0.705 g.)
) Weight used = 0.544 g.
Weight of ozone not absorbed = 0.161 g.)

Weight of water insoluble fraction = 1.06 g. (53% yield)

Methoxyl Analysis:

% OCH_3 - 10.85 (10.9, 10.8)

(0.02506 g. sample required 11.45 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02;89 g. sample required 9.90 cc. of 0.04613 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0073 g.

Solubility = 93%.

RUN - 10.

Birch Formic Acid Lignin - 90% acetone-water soluble Fraction - 15.5% OCH_3.

Weight of ozone added = 0.606 g.)
) Weight used 0.462 g.
Weight of ozone not absorbed = 0.144 g.)

Weight of water insoluble fraction = 1.48 g. (74% yield)

Methoxyl Analysis:

% OCH_3 - 9.5 (9.5, 9.5)

(0.02588 g. sample required 10.26 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02867 g. sample required 11.42 cc. of 0.04613 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0117 g.

Solubility = 88%.

RUN - 11.

Birch Acetic Acid Lignin - Acetone Soluble Fraction - 18.7% OCH_3.

Weight of ozone added = 0.280 g.)
) Weight used = 0.223 g.
Weight of ozone not absorbed = 0.057 g.)

Weight of water insoluble fraction = 1.31 g. (66% yield)

Methoxyl Analysis:

% OCH_3 - 14.5 (14.4, 14.6)

(0.02279 g. sample required 13.74 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02300 g. sample required 14.08 cc. of 0.04613 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor.

Weight of insoluble lignin = 0.0382 g.

Solubility = 62%.

RUN - 12;

Birch Acetic Acid Lignin - Acetone Soluble Fraction - 18.7% OCH_3.

Weight of ozone added = 0.498 g.)
) Weight used = 0.371 g.
Weight of ozone not absorbed = 0.127 g.)

Weight of water insoluble fraction = 1.06 g. (52% yield)

Methoxyl Analysis:

% OCH_3 - 12.85 (12.9, 12.8)

(0.02652 g. sample required 14.38 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02285 g. sample required 12.29 cc. of 0.04613 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0136 g.

Solubility = 86%.

RUN - 13.

Birch Acetic Acid Lignin - Acetone Soluble Fraction - 18.7% OCH_3

Weight of ozone added = 0.350 g.)
) Weight used = 0.278 g.
Weight of ozone not absorbed = 0.072 g.)

Weight of water in soluble fraction = 1.19 g. (60% yield)

Methoxyl Analysis:

% OCH_3 - 14.2 (14.2, 14.2)

(0.02316 g. sample required 13.80 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02313 g. sample required 13.75 cc. of 0.04613 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0553. g.

Solubility = 45%.

RUN - 14.

Birch Formic Acid Lignin - 90% acetone-water soluble fraction - 15.5% OCH_3.

Weight of ozone added = 0.538 g.)
) Weight used = 0.432 g.
Weight of ozone unabsorbed = 0.106 g.)

Weight of water insoluble fraction = 1.51 g. (75% yield)

Methoxyl Analysis:

% OCH_3 - 9.6 (9.6, 9.6)

(0.03171 g. sample required 12.70 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02445 g. sample required 9.80 cc. of 0.04613 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0133 g.

Solubility = 87%.

RUN - 15;

Maple Ethanol Lignin.

Methoxyl Analysis:

% OCH_3 - 26.0 (26.0, 25.8, 26.2)

(0.02978 g. sample required 32.40 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02308 g. sample required 24.95 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02247 g. sample required 24.65 cc. of 0.04613 N $Na_2S_2O_3$)

Weight of ozone added = 0.580 g.)
) Wt. of ozone used
Weight of ozone not absorbed = 0.146 g.) 0.434 g.

Weight of water insoluble fraction = 1.23 g. (62% yield)

Methoxyl Analysis:

% OCH_3 - 17.5, (17.5, 17.5)

(0.02304 g. sample required 16.85 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02484 g. sample required 18.20 cc. of 0.04613 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of Insoluble Lignin = 0.0276 g.

Solubility = 72%.

The Effect of Additional Refluxing with water on the Water Insoluble Fraction.

A number of the water insoluble fractions obtained by the ozonizations just described were treated further with water at 100°C. for 45 minutes. The lignin was then filtered off, washed and dried in the usual manner and quantitative sulphite cooks and methoxyl determinations carried out on these products.

(1) Product from Run 3.

Methoxyl Analysis:

% OCH_3 - 11.5 (11.5, 11.5)

(0.02402 g sample required 11.54 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02635 g. sample required 12.75 cc. of 0.04613 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0006 g.

Solubility = 100% (Previous solubility = 88%)

(2) Product from Run 4.

Methoxyl Analysis:

% OCH_3 - 11.75 (11.7, 11.8)

(0.02390 g. sample required 11.70 cc. of 0.04613 N $Na_2S_2O_3$)

(0.02174 g. sample required 10.72 cc. of 0.04613 N $Na_2S_2O_2$)

Solubility in Sulphite Liquor:

Weight of Insoluble Lignin = 0.0151 g.

Solubility = 85% (Previous solubility = 78%)

(3) Product from Run 7.

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0022 g.

Solubility = 98% (Previous solubility = 82%)

(4) Product from Run 11.

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0345 g.

Solubility = 65% (Previous solubility = 62%)

IX. GRIGNARD ANALYSES OF BIRCH "FORMIC ACID" LIGNIN BEFORE AND AFTER OZONIZATION

It was found that check analyses could be obtained in the Grignard Machine only after careful purification of the lignin samples. Until this was done a rather wide variation in the results was observed.

Two grams of birch formic acid lignin (acetone soluble fraction, Run 3, page 93) were purified by reprecipitation from anhydrous dioxane (20 cc.) into a tenfold volume of 30-50° petroleum ether. The precipitated lignin was washed thoroughly with petroleum ether and dried overnight at 60°C. over phosphorous pentoxide.

Grignard Analysis:

(1) 0.159 g. sample. Used 3.00 cc. of CH_3MgI

Volume of gas at 285°A. and 762 mm., 27.7 cc.

Corrected volume, 21.9 cc.

$\therefore$ Active hydrogen per kilo is 6.2

Added 2 cc. of water.

Volume of gas at 285°A. and 762 mm., 45 cc.

Corrected volume, 38.4 cc.

3.00 cc. of CH_3MgI is equal to 45.0 cc. of gas

$\therefore$ Addition of CH_3MgI per kilo is 1.9

(2) 0.159 g. sample. Used 3.15 cc. of CH_3MgI

Volume of gas at 285°A. and 761 mm., 28.5 cc.

Corrected volume, 22.4 cc.

$\therefore$ Active hydrogen per kilo is 6.3

Added 2 cc. of water.

Volume of gas at 285°A. and 761 mm., 47.2 cc.

Corrected volume, 40.5 cc.

3.15 cc. of CH_3MgI is equal to 47.2 cc. of gas.

$\therefore$ Addition of CH_3MgI per kilo is 1.9

The water insoluble fraction from the ozonization of birch formic acid lignin (the same fraction used in the above analyses) from Run 6, page 134 was purified by dissolving it in dry pyridine (1.4 g. in 15 cc.) and precipitation into a tenfold volume of 30-50° petroleum ether. The precipitated lignin was thoroughly washed with petroleum ether and dried overnight at 60°C. over phosphorous pentoxide.

Grignard Analysis:

(1) 0.163 g. sample. Used 3.10 cc. of CH_3MgI

Volume of gas at 285°A. and 761 mm., 24.4 cc.

Corrected volume, 18.7 cc.

$\therefore$ Active hydrogen per kilo is 5.1

Added 2 cc. of water.

Volume of gas at 285°A. and 761 mm., 44.3 cc.

Corrected volume, 37.5 cc.

3.10 cc. of CH_3MgI is equal to 46.5 cc. of gas.

$\therefore$ Addition of CH_3MgI per kilo is 2.5

(2) 0.156 g. sample. Used 3.16 cc. of CH_3MgI

Volume of gas at 283°A. and 762 MM., 26.1 cc.

Corrected volume, 20.3 cc.

∴ <u>Active hydrogen per kilo is 5.8</u>

Added 2 cc. of water.

Volume of gas at 283 °A. and 762 mm., 45.1 cc.

Corrected volume, 38.6 cc.

3.16 cc. of CH_3MgI is equal to 47.4 cc. of gas.

∴ <u>Addition of CH_3MgI per kilo is 2.2</u>

(3) 0.156 g. sample. Used 3.18 cc. of CH_3MgI

Volume of gas at 283°A. and 760 mm., 25.0 cc.

Corrected volume, 19.2 cc.

∴ <u>Active hydrogen per kilo is 5.5</u>

Added 2 cc. of water.

Volume of gas at 283°A. and 760 mm., 44.0 cc.

Corrected volume, 37.4 cc.

3.18 cc. of CH_3MgI is equal to 47.7 cc. of gas.

∴ <u>Addition of CH_3MgI per kilo is 2.9</u>

X. THE OZONIZATION OF METHYLATED OAK LIGNIN

A sample of fully methylated oak lignin° was found to be completely insoluble in sulphite liquor under the standard conditions of 110°C. for 12 hours.

One gram of this lignin was dissolved in 50 cc. of formic acid and at 0°C. this solution was subjected to the action of 0.348 g. of ozone, of which 0.246 g. was absorbed. Upon removal of the solvent under reduced pressure the residue was refluxed for half an hour with 300 cc. of water. The water insoluble fraction was filtered off, washed with water and dried.

A sample of this water insoluble fraction was found to be completely insoluble in sulphite liquor under the standard conditions.

° This sample was obtained from Dr. H. Hibbert and had been prepared by W.H. Steeves (82). Methoxyl content - 37.6%

XI. EXPERIMENTS ON THE GRIGNARDIZATION OF LIGNIN

(a) Native Oak Lignin

A sample of native oak lignin* was found to be completely soluble in sulphite liquor under the standard conditions of 110°C. for 12 hours.

One gram of this lignin was dissolved in 35 cc. of dry pyridine in a three-necked flask equipped with mercury sealed stirrer and a condenser. Under an atmosphere of dry nitrogen 30 cc. of an isoamylether solution of methyl magnesium iodide was added in three 10 cc. portions. The reaction mixture was then heated on the water bath at 100°C. for one and one-half hours. Upon cooling, 500 cc. of water was added to the reaction mixture and the precipitated lignin filtered off, well washed with water and dried. Yield - 1.36 g.

A sample of this grignardized oak lignin was found to be completely <u>insoluble</u> in sulphite liquor under the standard conditions.

One gram of the grignardized oak lignin was dissolved in 50 cc. of formic acid and at 0°C. this solution was subjected to the action of 0.331 g. of ozone, of which 0.151 g. was not absorbed by the solution.

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* This sample of native oak lignin had been prepared by the acetylation technique (82) and was kindly donated by Mr. Q.Peniston

Upon removal of the solvent under reduced pressure and refluxing the residue for half an hour with 300 cc. of water, the water insoluble fraction was filtered off, washed and dried.

A sample of this water insoluble fraction was found to be completely insoluble in sulphite liquor under the standard conditions.

(b) Birch Formic Acid Lignin

One gram of birch formic acid lignin (acetone soluble fraction Table II, Run 3) was dissolved in 35 cc. of dry pyridine and treated with 30 cc. of methyl magnesium iodide solution in the same manner described in the previous section, (a).
Yield - 1.46 g.

A sample of this grignardized birch lignin was found to be completely insoluble in sulphite liquor under standard conditions.

One gram of this lignin was dissolved in 50 cc. of formic acid and at 0°C. was subjected to the action of 0.341 g. of ozone, of which 0.120 g. was not absorbed. Upon removal of the solvent under reduced pressure and refluxing the residue for half an hour with 300 cc. of water, the water insoluble fraction was filtered off, washed and dried.

A sample of this water insoluble fraction was found to be completely insoluble in sulphite liquor under standard conditions.

XII. THE ISOLATION OF VANILLIN AND ACETOVANILLONE FROM OZONIZED BIRCH 'FORMIC ACID' LIGNIN

(a) The Ozonization of Birch 'Formic Acid' Lignin

Ten grams of birch 'formic acid' lignin (the 90% acetone-water soluble fraction from Run 3, Table II.) were dissolved in 400 cc. of formic acid and at 0°C. this solution was treated with 2.56 g. of ozone over a period of eight hours, during which time 2.34 g. of the ozone was absorbed by the solution. The solvent was then removed under reduced pressure and the residue refluxed for one hour with one litre of water. Upon cooling, the water insoluble fraction was filtered off, washed with water and dried. This product was ground to a fine powder and again refluxed with one litre of water for forty-five minutes. The water insoluble fraction was then filtered, washed and dried. Yield - 6.9 g.

Methoxyl Analysis:

% OCH_3 - 8.3 (8.3, 8.3)
(0.02068 g. sample required 6.65 cc. of 0.04978 N $Na_2S_2O_3$)
(0.02777 g. sample required 8.95 cc. of 0.04978 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0051 g. from 0.1000 g. sample.
Solubility = 95%

(b) The Preparation and Cleavage of the Lignin Sulphonic Acid

Two hundred cc. of sulphite liquor (6.0% total and 1.15% combined SO_2) and 6.8 g. of the water insoluble fraction, obtained as described in the previous section (a), were placed in a stainless steel bomb which was heated to a temperature of 110°C. over a period of one and one-half hours. The bath was held at 110°C. for four hours and then raised to 130°C. for an additional eight hours. Upon cooling the bomb was opened and the contents filtered and the volume of the filtrate made up to exactly 500 cc. by the addition of water. The insoluble material was dried and weighed. Weight = 0.8 g.

A portion of the filtrate (100 cc.) was freed from inorganic material by dialysis against running distilled water for seven days. By evaporating the solution to dryness a relatively pure sample of the lignin sulphonic acid was obtained. This was analyzed for methoxyl and sulphur, the latter by the method of Wolesensky (91).

Methoxyl Analysis:

% OCH_3 - 3.25 (3.3, 3.2)

(0.02591 g. sample required 3.40 cc. of 0.04978 N $Na_2S_2O_3$)

(0.03152 g. sample required 3.90 cc. of 0.04978 N $Na_2S_2O_3$)

Sulphur Analysis:

% S - 6.45 (6.5, 6.4)

(0.10322 g. sample gave 0.04872 g. of $BaSO_4$)

(0.10283 g. sample gave 0.04807 g. of $BaSO_4$)

The alkaline cleavage of the lignin sulphonic acid prepared in the manner described was carried out by adding 70 g. of sodium hydroxide to 290 cc. of the crude liquor and heating this at a temperature of 135°C. for 12 hours under an atmosphere of oxygen-free nitrogen. Upon cooling the solution was acidified with 50% sulphuric acid.

(c) The Isolation of Vanillin

The acidified reaction mixture from the alkaline cleavage was extracted continuously with benzene for a period of 48 hours. The benzene extract was then shaken ten times with 15 cc. portions of 5% sodium bisulphite solution. This latter solution was acidified with 3 cc. of concentrated sulphuric acid and the sulphur dioxide removed under reduced pressure. The solution was then filtered and the volume adjusted to exactly 200 cc.

The determination of the total aldehydes was carried out as follows. To a 20 cc. aliquot of the acidified bisulphite solution was added 10 cc. of a water solution of 2,4 dinitrophenylhydrazine hydrochloride. After standing for 6 hours the precipitate was filtered through a tared sintered glass Pregl filter and dried at 105°C.

Weight of derivative = 0.1005 g.

Weight calculated as vanillin for total solution = 0.0445 g.

Yield based on lignin in liquor = 1.3%

The remaining 180 cc. of the solution was extracted continuously with ether for 48 hours, the ethereal extract dried over anhydrous sodium sulphate and the solvent removed under reduced pressure. The residue was transferred to a small tared flask and again evaporated to dryness. Weight of residue = 0.075 g. This residue was then sublimed at 2 mm. pressure and 61°C. and yielded 0.040 g. of a crystalline product which melted at 65-70°C. A further sublimation of the 0.030 g. residue gave only a slight oiliness at increased temperatures.

The sublimate was recrystallized from 80-90°C. petroleum ether and gave 0.030 g. of white feathery crystals. M.P. 81-82°C. The mixed melting point with an authentic sample of vanillin (M.P. 82-83°C.) was found to be 81-82°C.

Yield of crude vanillin = 1.1% based on the lignin.

Yield of pure vanillin = 0.9% based on the lignin.

(d) The Isolation of Acetovanillone

The benzene extract of the acidified alkaline cleavage product left after the extraction with bisulphite was evaporated to dryness under reduced pressure. The residue was transferred to a small tared flask and again evaporated to dryness. Weight of residue = 0.080 g.

This residue was sublimed at 2 mm. pressure and 150°C., yielding 0.065 g. of sublimate. This product was transferred to a clean flask and again sublimed at the same pressure and a temperature of 90°C. This gave 0.055 g. of crystalline material. M.P. 105-110°C.

The product was recrystallized from 80-90°C petroleum ether and gave 0.030 g. of white crystals. M.P. 111-112°C. The mixed melting point with an authentic sample of acetovanillone (M.P. 114-115°C.) was found to be 111-112°C.

Yield of crude acetovanillone = 1.6% based on the lignin.

Yield of pure acetovanillone = 0.9% based on the lignin.

XIII. THE ISOLATION OF VANILLIN AND ACETOVANILLONE FROM OZONIZED BIRCH 'ACETIC ACID' LIGNIN

(a)The Ozonization of Birch 'Acetic Acid' Lignin

Twelve grams of birch 'acetic acid' lignin (acetone soluble fraction, Table III.) were dissolved in 300 cc. of formic acid and at 0°C. this solution was subjected to the action of 2.16 g. of ozone over a period of five and one-half hours, during which time 1.84 g. of ozone was absorbed by the solution. The solvent was then removed under reduced pressure and the residue refluxed with one litre of water for forty-five minutes. Upon cooling, the water insoluble fraction was filtered off, washed with water and dried. This product was ground to a fine powder and again refluxed with one litre of water for forty-five minutes.
The water insoluble fraction was then filtered, washed and dried.
Yield - 5.4 g.

Methoxyl Analysis:

% OCH_3 - 13.25 (13.3, 13.2)
(0.02197 g. sample required 12.2 cc. of 0.04613 N $Na_2S_2O_3$)
(0.02216 g. sample required 12.3 cc. of 0.04613 N $Na_2S_2O_3$)

Solubility in Sulphite Liquor:

Weight of insoluble lignin = 0.0050 g. from 0.1000 g. sample.
Solubility = 95%

(b) The Preparation and Cleavage of the Lignin Sulphonic Acid

Five grams of the water insoluble fraction, obtained as described above, and 200 cc. of sulphite liquor (6.0% total and 1.15% combined SO_2) were placed in a stainless steel bomb. The bomb was placed in a bath and the temperature raised to 110°C. over a period of one and one-half hours and maintained at that temperature for 12 hours. After cooling the bomb, it was opened and the contents filtered and the volume of the filtrate made up exactly to 250 cc. with water. The insoluble material was dried and weighed. Weight = 0.02 g.

A portion of the filtrate (50 cc.) was freed from inorganic material by dialysis against running distilled water for seven days. The lignin sulphonic acid was obtained by evaporating the solution to dryness and was analyzed for methoxyl and sulphur (91).

Methoxyl Analysis:

% OCH_3 - 6.4 (6.5, 6.3)

(0.02727 g. sample required 6.85 cc. of 0.04978 N $Na_2S_2O_3$)

(0.02611 g. sample required 6.42 cc. of 0.04978 N $Na_2S_2O_3$)

Sulphur Analysis:

% S - 6.15 (6.1, 6.2)

(0.10200 g. sample gave 0.04563 g. of $BaSO_4$)

(0.10303 g. sample gave 0.04502 g. of $BaSO_4$)

The alkaline cleavage of this lignin sulphonic acid was carried out by adding 33.6 g. of sodium hydroxide to 140 cc. of the crude liquor. This solution was maintained at a temperature of 135°C. for a period of 12 hours under an atmosphere of oxygen-free nitrogen. The reaction mixture was then cooled and acidified with 50% sulphuric acid.

(c) The Isolation of Vanillin

The acidified reaction mixture from the alkaline cleavage was extracted continuously with benzene for 48 hours. This benzen extract was then shaken ten times with 15 cc. portions of 5% sodium bisulphite solution. This latter solution was acidified with 3 cc. of concentrated sulphuric acid and the sulphur dioxide removed under reduced pressure. The solution was filtered and the volume adjusted to exactly 250 cc.

The determination of the total aldehydes was carried out in the following manner. To a 20 cc. aliquot of the acidified bisulphite solution was added 10 cc. of a water solution of 2,4-dinitrophenylhydrazine hydrochloride. After standing for 6 hours the precipitate was filtered through a tared sintered glass Pregl filter and dried at 105°C.

Weight of derivative = 0.02084 g.

Weight calculated as vanillin for total solution = 0.1158 g.

Yield based on lignin content of liquor = 4%

The remaining 230 cc. of the solution was extracted continuously with ether for 48 hours, the ethereal extract dried over anhydrous sodium sulphate and the solvent removed under reduced pressure. The residue was transferred to a small tared flask and again evaporated to dryness. Weight of residue = 0.160 g. This residue was then sublimed at 2 mm. pressure and 61°C. and yielded 0.070 g. of a crystalline product which melted at 70-75°C. This was recrystallized from 80-90° petroleum ether giving <u>0.065 g</u>. of white feathery crystals. M.P. 82-83°C.
The mixed melting point with an authentic sample of vanillin (M.P. 82-83°C.) was found to be 82-83°C.
Sublimation of the residue at a temperature of 140°C. yielded 0.030 g. of additional material which was resublimed at 61°C. and then crystallized from 80-90° petroleum ether. Yield = <u>0.010 g</u>. M.P. 81-82°C. Mixed melting point with vanillin - 81-82°C.
Thus the total yield of pure vanillin was <u>0.075 g</u>.

Yield of pure vanillin = 2.7% based on the lignin.

(d) The Isolation of Acetovanillone

The benzene extract of the acidified alkaline cleavage reaction product, after the extraction with the bisulphite, was evaporated to dryness under reduced pressure. The residue was transferred to a small tared flask and again evaporated to dryness. Weight of residue = 0.170 g.

This residue was sublimed at 2 mm. pressure and 120°C. yielding 0.080 g. of crystalline material. Further sublimation of the residue at temperatures up to 170°C. gave only a small oiliness. The sublimate was resublimed at a pressure of 2 mm. and 95°C. giving 0.075 g. of product. M.P. 109-112°C. Recrystallization from 80-90° petroleum ether gave 0.050 g. of material. M.P. 110-111°C.
An additional recrystallization gave 0.030 g. M.P. 112-113°C. The mixed melting point with an authentic sample of acetovanillone (M.P. 114-115°C.) was found to be 112-113°C.

Yield of crude acetovanillone = 2.7% based on the lignin.

Yield of pure acetovanillone = 1.1% based on the lignin.

XIV. MISCELLANEOUS EXPERIMENTS

(a) The Action of Formic Acid on Ozonized Lignin

Eight-tenths of a gram of the water insoluble fraction from the ozonization of birch formic acid lignin (Run 8, page135) was refluxed for 48 hours with 100 cc. of formic acid. The solution was then evaporated under reduced pressure to a volume of 25 cc. and poured in a fine stream into 2 litres of water. The precipitated lignin was centrifuged off, washed and dried. Yield - 0.56 g.

This product was found to be completely insoluble in sulphite liquor under the standard conditions of 110°C. for 12 hours.

(b) The Action of Formic Acid on α-hydroxypropioveratrone.

α-hydroxypropioveratrone (2.9 g.) was refluxed for 48 hours with 180 cc. of formic acid. The reaction mixture was then evaporated to a volume of 80 cc. and poured in a fine stream into 2 litres of water. The precipitate was filtered off, washed and dried. Weight - 1.4 g. Yield - 50%

Methoxyl Analysis:

% OCH_3 - 31.6
(0.02948 g. sample required 39.10 cc. of 0.04613 N $Na_2S_2O_3$)

This product was found to be completely insoluble in sulphite liquor under the standard conditions.

(c) The Action of Ozone on "α-hydroxypropioveratrone Formic Acid Lignin"

One gram of the product which was obtained as described in the previous section was dissolved in 50 cc. of formic acid and at 0°C. was subjected to the action of 0.445 g. of ozone over a period of 45 minutes, of which 0.369 g. was absorbed by the solution. The solvent was then removed under reduced pressure and the residue refluxed with 300 cc. of water for 45 minutes. The water insoluble fraction was filtered off, washed and dried. Yield - 0.80 g.

This product was found to be completely insoluble in sulphite liquor under the standard conditions.

(d) The Action of Ozone on "α-hydroxypropiovanillone Sulphuric Acid Lignin"

This "lignin" was obtained by refluxing α-hydroxypropiovanillone with 1% sulphuric acid for 36 hours*, and was completely insoluble in hot bisulphite solution. Three-tenths of one gram of this material was dissolved in 15 cc. of formic acid and at 0°C. was subjected to the action of 0.061 g. of ozone over a period of ten minutes. The solvent was then removed under reduced pressure and the residue refluxed with 15 cc. of water for five minutes. The water insoluble fraction was filtered off, washed and dried. Yield - 0.22 g.

This product was completely insoluble in sulphite liquor under the standard conditions.

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* The writer is indebted to Mr. A.Cramer for this sample.

(e) The Action of Hydrogen Peroxide on Birch 'Formic Acid' Lignin

(i) Two grams of birch formic acid lignin (acetone soluble fraction) were dissolved in 50 cc. of formic acid and while the solution was kept well stirred 2 cc. of 30% hydrogen peroxide was added dropwise. Stirring was continued for one hour and then the solvent was removed by evaporation under reduced pressure. The residue was refluxed for one hour with 300 cc. of water and the water insoluble fraction filtered off, washed with water and dried. Yield - 1.40 g.

Solubility in Sulphite Liquor:

Weight of insoluble lignin - 0.0354 g.
Solubility - 65%

(ii) One gram of birch formic acid lignin (acetone soluble fraction) was dissolved in 25 cc. of formic acid and was treated with 2 cc. of 30% hydrogen peroxide in the same manner as described in the previous section.

Weight of water insoluble fraction - 0.51 g.

Solubility in Sulphite Liquor:

Weight of insoluble lignin - 0.0 g.
Solubility - 100%

XV. THE ACTION OF SULPHITE LIQUOR ON VARIOUS ORGANIC COMPOUNDS

(1) Benzoin

Three cc. of sulphite liquor and 0.080 g. of benzoin were cooked in a glass bomb for 12 hours at 110°C. Upon cooling, the bomb was opened and the insoluble crystalline material was filtered off, washed with water, dried and weighed. Yield - 0.080

M.P. 132-133°C. The mixed melting point with the original benzoin (M.P. 133-134°C.) was found to be 132-133°C. Thus benzoin does not form a sulphonic acid under these conditions.

(2) α-ethoxypropioveratrone

Three cc. of sulphite liquor and 0.080 g. of α-ethoxypropioveratrone were cooked for 12 hours at 110°C in a sealed tube. Upon cooling a green insoluble globule was observed in the tube. The contents were washed out into a beaker and the oily globule crystallized on scratching. This material was filtered, washed with water and dried. Yield - 0.053 g.

M.P. 79-80°C. The mixed melting point with α-ethoxypropioveratrone (M.P. 81-82°C.) was found to be 80-81°C. Thus the compound does not form a sulphonic acid under the conditions employed and is stable to hot bisulphite solutions.

(3) α-hydroxypropiovanillone

Six cc. of sulphite liquor and 0.200 g. of α-hydroxypropiovanillone were cooked for 12 hours at 110°C. Upon cooling the bomb no insoluble material separated out. The contents were washed into a small beaker and allowed to evaporate to dryness. The residue was then extracted three times with 3 cc. portions of dry chloroform. The chloroform extract was allowed to evaporate in a tared dish and yielded 0.200 g. of an oil which slowly crystallized on standing. This product was recrystallized from 80-90°C. petroleum ether and gave 0.050 g. of crystalline material. M.P. 96-101°C. The mixed melting point with a sample of α-hydroxypropiovanillone (M.P. 100-101°C.) was found to be 98-99°C. Thus this compound did not form a sulphonic acid under the conditions employed and was stable under the conditions of a 'sulphite cook'.

(4) α-hydroxypropiosyringone

Three cc. of sulphite liquor and 0.200 g. of α-hydroxypropiosyringone were cooked for 12 hours at 110°C. No insoluble material was obtained upon cooling the bomb. The contents were washed into a small beaker and allowed to evaporate to dryness. The residue was then extracted three times with 3 cc. portions of dry chloroform. Evaporation of the chloroform extract in a tared dish yielded 0.180 g. of crystalline material which was recrystallized from benzene. M.P. 122-123°C. The mixed melting point with a sample of α-hydroxypropiosyringone (M.P. 123-124°C.) was found to be 123-124°C. Thus this compound did not form a sulphonic acid under the conditions employed and was stable to hot bisulphite solutions.

XVI. EXPERIMENTS WITH SPRUCE WOOD MEAL

A Grignard analysis was carried out in the usual manner on a carefully dried sample of extracted spruce wood meal, with the exception that the reagent was allowed to stand in contact with the wood meal for 18 hours at room temperature before the addition of the water. Another analysis in which this procedure was not followed gave no addition of the reagent.

Grignard Analysis: (pyridine solvent)

0.147 g. sample. Used 3.25 cc. of CH_3MgI

Volume of gas at 275°A. and 763 mm., 12.8 cc.

Corrected volume, 9.5 cc.

$\therefore$ Active hydrogen per kilo is 0.21

Added 2 cc. of water.

Volume of gas at 275°A. and 760 mm., 47.4 cc.

Corrected volume, 41.7 cc.

3.25 cc. of CH_3MgI is equal to 43.5 cc. of gas.

$\therefore$ Addition of CH_3MgI per kilo is 0.06

The reaction mixture from this analysis was poured into 150 cc. of water and the wood meal reaction product filtered off, well washed with water and dried. Yield - 0.150 g.

This product was then subjected to a standard sulphite cook and the pulp filtered off, washed with water and dried.

Weight - 0.122 g. (80% yield)

Methoxyl Analysis of Residual Pulp:

% OCH_3 - 4.1

(0.03470 g. sample required 5.55 cc. of 0.04978 N $Na_2S_2O_3$)

Sulphite Cook on Spruce Wood Meal

A sample of extracted spruce wood meal (4.8% OCH_3) weighing 0.400 g. was subjected to a standard sulphite cook at 110°C for 12 hours with 6 cc. of liquor. The pulp was then filtered off, washed with water and dried. Weight of residual wood meal - 0.308 g. (77% yield)

Methoxyl Analysis:

% OCH_3 - 2.4

(0.05058 g. sample required 5.90 cc. of 0.04978 N $Na_2S_2O_3$)

Sulphite Cook on Diazomethane methylated Spruce Wood Meal

A sample of extracted spruce wood meal which had been methylated with diazomethane (7.2% OCH_3)* and which weighed 0.400 g. was cooked for 12 hours at 110°C. with 6 cc. of sulphite liquor. The pulp was then filtered off, washed with water and dried. Weight of residual wood meal - 0.336 g. (88% yield)

Methoxyl Analysis:

% OCH_3 - 5.8

(0.05163 g. sample required 11.70 cc. of 0.04978 N $Na_2S_2O_3$)

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* The writer is indebted to Mr. A.Cramer for this sample.

SUMMARY

1. The use of formic acid as a solvent for ozonization has been developed and its characteristics in this capacity demonstrated by experiments on organic compounds of known structure.

2. This solvent shows a remarkable tendency to protect the aldehyde group from oxidation to the carboxyl group. The stability of the aromatic nucleus is somewhat greater in this solvent than in ethyl acetate.

3. Isolated lignins, obtained by extraction with formic and acetic acids, and by alcoholysis with ethanol-hydrogen chloride, which are normally insoluble in aqueous bisulphite solution, have been shown to yield lignin sulphonic acids after a prior treatment of the lignin with ozone.

4. Lignin sulphonic acids obtained in this manner from formic and acetic acid lignins have been shown to give rise to vanillin and acetovanillone on alkaline cleavage. Presumably this is the first time that acetovanillone has been obtained from a hard wood.

5. The increase in solubility in aqueous bisulphite solution and the decrease in methoxyl content of formic acid lignin with increasing ozonization has been studied.

6. A methylation study of the water soluble and water insoluble lignin fractions obtained upon ozonization has shown the importance of an hydroxyl group, methylatable with diazomethane, upon the solubility of these fractions in aqueous bisulphite solution.

7. Direct chemical evidence has been obtained in a number of cases for the necessity of a carbonyl group for the solubilization of lignin by means of aqueous bisulphite solution.

8. A study of various lignins has produced evidence to show that the carbonyl group is associated with an hydroxyl group and that both these groups are necessary for the formation of a lignin sulphonic acid.

9. The action of aqueous bisulphite solution on a number of organic compounds of known structure has been studied.

10. A new interpretation of the process of delignification by means of aqueous bisulphite solution has been postulated in view of these new experimental findings.

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