EXTRACTION OF WOODS WITH SODIUM BICARBONATE - CARBON DIOXIDE OR WITH LIQUID AMMONIA UNDER PRESSURE

- MAXWELL MENUHIN YAN -

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A Thesis

by

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GENERAL INTRODUCTION

A growing accumulation of experimental evidence indicates that lignin is a polymerization product of polyfunctional phenolic bodies. One of the most characteristic properties of phenols is the Kolbe reaction, whereby they are carboxylated by treatment of the alkali metal salts with carbon dioxide. If carboxyl groups could be introduced into the aromatic nuclei of lignin by the same method, it might be expected that the lignin would be solubilized; hence the Kolbe reaction might function as an effective method for the delignification of wood. A series of spruce wood cooks with aqueous sodium bicarbonate and carbon dioxide under pressure were therefore carried out, but the results were negative. Attention was then turned towards another novel possibility for lignin removal - extraction of wood with anhydrous liquid ammonia.

During the past forty years, the utilization of liquid ammonia as a solvent in a wide variety of chemical reactions has become standard practice, at least on a laboratory scale. Frequently, reactions impossible in aqueous or non-aqueous solutions of ammonia proceed with facility in the anhydrous liquid. The chemistry of liquid ammonia is in many ways similar to that of water, but often reactions which fail in water are easily carried out in liquid ammonia or vice-versa.

Anhydrous liquid ammonia has not only high solvent powers and interesting reaction possibilities but is also an effective intracrystalline swelling agent for cellulose. These considerations suggest that liquid ammonia might swell and permeate wood, and perhaps dissolve the wood lignin.

A general survey of the chemical behaviour of several types of woody materials in liquid ammonia was therefore undertaken. The major effort in the present study was directed towards the elucidation of the nature of the materials extracted by anhydrous liquid ammonia from sugar maple wood, with special reference to the lignin fractions.

HISTORICAL INTRODUCTION

A. Relevant Portions of Lignin Chemistry

Woody tissues represent, in bulk at least, the major result of plant activity on the earth. The elucidation of the chemical structure of wood was therefore one of the earliest obvious targets for attack by chemical science. The first workers quite understandably looked upon wood as a distinct chemical entity, homogeneous in character in a particular plant species but varying in composition from one species to another. With the development of experimental technique, starting particularly with the pioneer work of Payen (1), it became clear that wood consisted not of one, but of three major components - cellulose, hemicellulose and lignin.

As wood chemistry advanced it was very soon recognized that the properties of these three major constituents, and indeed even their amounts, varied substantially, according to the particular method used in their isolation. Intensive effort was therefore directed towards the goal of obtaining pure cellulose and pure lignin in a form identical to that of lignin and cellulose *in situ*. To this day, this ideal, usually attainable in low-molecular weight, classical organic chemistry, has eluded the wood chemist, who is dealing with an exceedingly complex polymeric system. In the case of cellulose close to the ideal preparation has been obtained, so that the major facts in cellulose chemistry rest on a

firm foundation. Lignin chemistry could probably be set on as firm a basis if the preparations studied satisfied two requirements: (1) the lignin must be identical to, or differ only inconsequentially from, lignin *in situ*; (2) the sample studied must be homogeneous in structure, at least as regards the sub-molecules which, combined, make up the complex. Homogeneity in molecular weight may be an unattainable desideratum. The first requirement has been approached for at least a portion of the lignin. The second requires fractionation procedures as yet almost entirely undeveloped.

The methods generally used in the isolation of lignin can be conveniently classified into two groups, in the first of which the cellulose and hemicellulose are extracted as soluble materials leaving insoluble lignin behind (mineral acids, Freudenberg's cuproxam lignin, nitration and acetylation methods). The second group of methods depends upon the solution of the lignin to leave an undissolved residue of wood pulp (sulfite, soda and kraft pulping; organic solvents, usually hydroxylic and acidic in nature).

The many variations of these two general methods have been fully described by Wise in "Wood Chemistry" (2) but the majority of them are of minor interest when one is concerned with the structure of lignin as it occurs in wood, since the resultant lignin preparations have often suffered deep-seated changes in the process of isolation. This remark is

especially true of methods of the first class, since hydrolysis of the carbohydrates usually requires vigorous reaction conditions which convert the very labile lignin complex to a dark amorphous insoluble form. The commercial pulping processes, sulfite, soda and kraft, effectively and almost completely dissolve out lignin from wood but the high temperatures and strongly acid or alkaline media employed, yield lignins far removed in properties from lignin in situ or so-called "protolignin" (3).

More hopeful are the methods of the second class which employ comparatively neutral solvents such as methanol, ethanol, butanol and other hydroxylic solvents (2) to extract the lignin, though even here some hydrogen chloride, generally 1%-3%, is used as catalyst, as well as a temperature of about 100°. The resultant lignins are generally light-colored, amorphous, soluble products which have incorporated in their structure substantial amounts of the hydroxylic solvent used in their extraction.

Despite the uncertainties of the methods for isolating lignin, a growing accumulation of evidence from a variety of approaches allows scant opportunity for doubting the most generally accepted modern view of lignin structure (4) (5) (6) (7); wood lignin is essentially aromatic in nature consisting chiefly of a polymerization product of oxygenated phenylpropane units. An outstanding exception to this view is Hilpert's (8) claim that isolated lignin fractions are

only artifacts, resulting from the action of the isolating media on very sensitive methylated carbohydrate fractions present in wood. Hilpert in reality denies the individual existence of lignin in wood.

An examination of the elementary composition of lignin, however, makes the views of Hilpert quite untenable. Wald, Ritchie and Purves (9) have calculated the composition of lignin *in situ* from the difference in composition of wood, and holocellulose isolated from the wood concerned. This method is free of the uncertainties of most reported lignin analyses caused by changes in lignin composition during isolation. The results for spruce lignin and northern pine, C, 67.5% are far beyond the carbon content expected if lignin were a carbohydrate, even fully methylated, as claimed by Hilpert. The 67.5% carbon content, however, is consistent not only with an aromatic but also a hydroaromatic structure for lignin. However, Wald, Ritchie and Purves reported a hydrogen content for spruce and northern pine wood lignin of 6%, whereas a hydroaromatic structure would require a value of about 9% for hydrogen. The elementary composition of lignin, therefore, points clearly to an aromatic structure.

The refractive index of spruce lignin is 1.61, a high value, which Freudenberg, Zocher and Durr (10) consider to be indicative of the aromatic structure of lignin. The ultraviolet absorption spectra of various lignins show, according to Herzog and Hillmer (11), that the basic unit of lignin is a

di- or tri-hydroxyphenol, partially or wholly etherified, with a three-carbon atom side chain. Lange (12) has recently determined the absorption spectrum of spruce wood and concludes that there is about one aromatic ring for every ten carbon atoms of lignin *in situ*.

The analytical and optical evidence favoring an aromatic character for lignin is strengthened by the variety of aromatic products produced from lignin when it is subjected to dry distillation, alkaline oxidation, alcoholysis and similar degradative processes. Phillips (13) lists some twenty phenolic aromatic compounds which have been obtained in this way, all of which can be considered as having arisen from the guaiacyl type, 2-methoxy-4-propylphenol, in the case of softwoods and from the above named compound, as well as from the syringyl type, 2,6-dimethoxy-4-propylphenol, in the case of hardwoods. Thus 25% of vanillin has been obtained from softwoods (14) and 40%-45% of vanillin and syringaldehyde from hardwoods (50). High-pressure catalytic hydrogenation of lignin has produced the hydrogenated analogs of the phenolic phenylpropane units (15) (16).

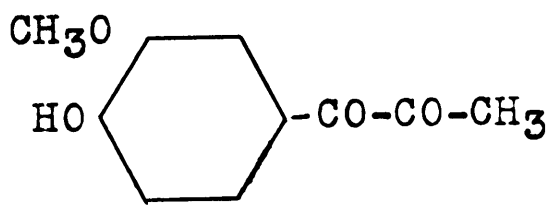
The overwhelming mass of evidence indicates, therefore, that the major part of lignin consists of phenolic phenylpropane units, linked together in some manner as yet not definitely established.

Numerous investigators (17) have put forth the suggestion that lignin was chemically bound to some carbohydrate constituent of wood, the linkage being of the ester, ether, acetal

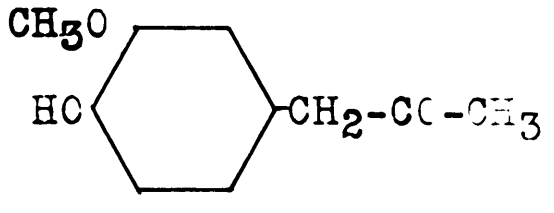
or glycosidic type. Purves (18), on the basis of the susceptibility of such a bond to hydrolytic media, favored the view of a glycosidic bond involving a phenolic or enolic group of the lignin complex. However no definite evidence in favor of any of these suggestions has been presented.

By treating solvent-extracted maple wood with 2% hydrogen chloride in anhydrous ethanol at reflux temperature for forty-eight hours, Hibbert and co-workers (3) (19) obtained, for every 100 grams of Klason lignin originally present, about 38 to 50 grams of water-soluble products and 55 to 60 grams of amorphous ethanol lignin, soluble in ethanol but insoluble in water. About 22 grams of Klason lignin was left unextracted in the wood meal, the total recovery being more than 100% of the original Klason lignin because ethoxyl units from the ethanol had been added to some of the constituents.

From the distillable water-soluble fraction, accounting for about 30% of the total Klason lignin, Hibbert and his school (20) isolated and clearly indentified a variety of pure phenylpropane types containing guaiacyl (I) (II) (III) or syringyl nuclei (IV) (V) (VI) attached to propanone and propandione units. Similar guaiacyl derivatives but no syringyl types were isolated from spruce (21).



(I)



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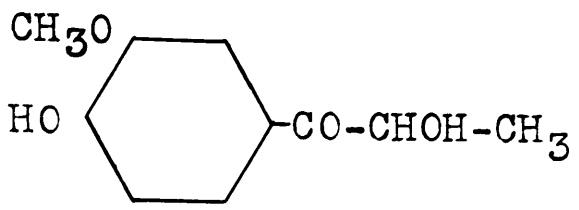

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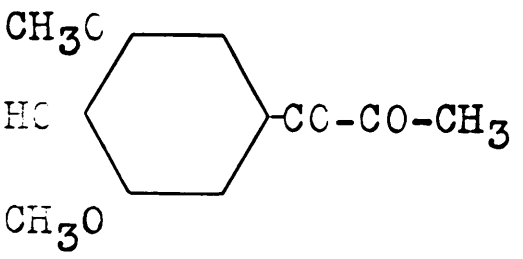

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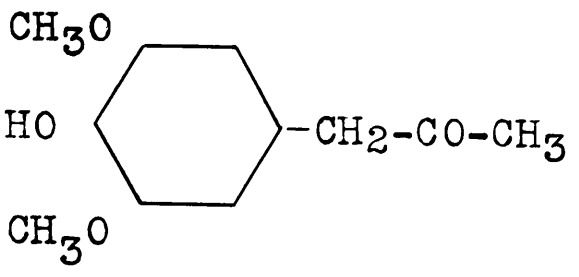

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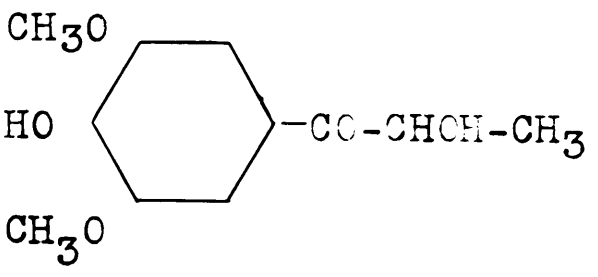
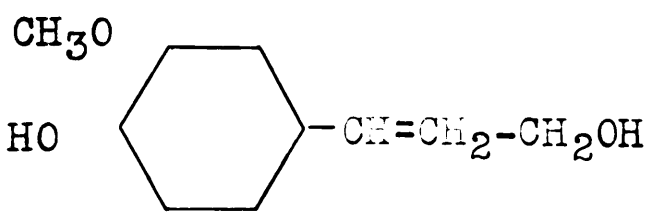


(VI)

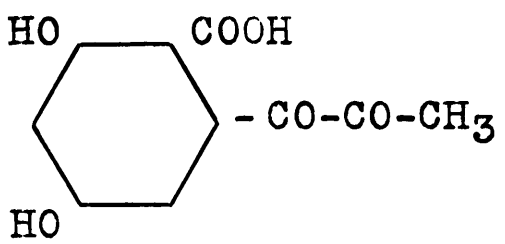
Klason (22) had early suggested that lignin was a polymer of coniferyl alcohol (VII) types. For a number of years previous to Hibbert's work, Freudenberg (4) had held that lignin was a linear polymer of monomer propylphenol units almost identical to those isolated by Hibbert, but he had never succeeded in isolating more than a two-carbon atom side chain. Now for the first time aromatic derivatives bearing fully intact propyl chains had been isolated in appreciable yield, providing experimental confirmation for the early speculations that lignin was a polymer of coniferyl alcohol (VII) type molecules.



(VII)

Hibbert and Kulka (23) pointed out the similarity of these phenylpropane derivatives (I-VI), especially in the struc-

tures of the side chains, to products (VIII) (IX) isolated by Oxford and Raistrick (24) from certain strains of Penicillium brevi-compactum growing on glucose. The mold derivatives have their phenolic hydroxyl groups free, not methylated, but there is one other difference of importance between the two series of derivatives: the mold derivatives (VIII) (IX) possess a carboxyl group ortho to the aliphatic side chain whereas the series from wood (I to VI) lack this carboxyl unit.



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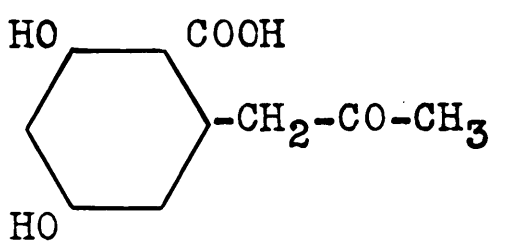


(IX)

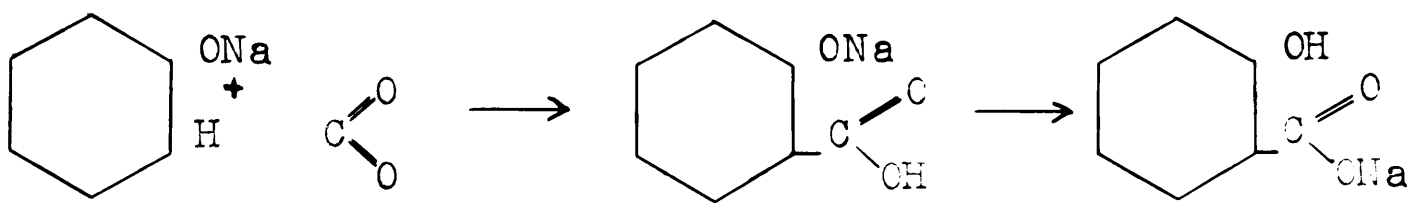
This difference was attributed by Hibbert to the carboxylation of the non-acidic progenitors by a biochemical process analogous to the Kolbe, or Kolbe-Schmitt reaction, so familiar to synthetic organic chemists. In a typical Kolbe reaction the phenol, either dissolved in aqueous bicarbonate or alkali, or in the form of its dry sodium or potassium salt, is treated with carbon dioxide, usually under pressure and at an elevated temperature. Under such conditions a carboxyl group enters ortho, and, more rarely, para to the phenolic hydroxyl group, the yields being generally good. The over-all reaction in the production of salicylic acid,

historically the first application of the Kolbe reaction (25), is

$$C_6H_5 \cdot ONa + CO_2 \longrightarrow o\text{-}C_6H_4 \cdot OH \cdot COONa,$$

and acidification of an aqueous solution of the cooled melt liberates the free salicylic acid in an almost theoretical yield.

The Kolbe reaction is considered to proceed by a preliminary step involving the addition of the aromatic component to a carbonyl group of the carbon dioxide (26):



The Kolbe synthesis goes remarkably easily with polyhydroxyphenols, especially with those which have hydroxyl groups meta to each other. Thus carboxylation of phloroglucinol takes place to a considerable degree even at room temperature if carbon dioxide is passed into a solution of phloroglucinol in glycerol and potassium bicarbonate (27). Resorcinol needs only to be warmed a short while with aqueous bicarbonate at about 60° for conversion to the carboxyl derivative (28). These observations make it easy to see how the carboxyl group could have entered the nuclei of the mold products. The latter have two hydroxyl groups meta to each other and there is generally carbon dioxide available in a living sys-

tem. Both of these factors are extremely favorable for carboxylation by the Kolbe reaction.

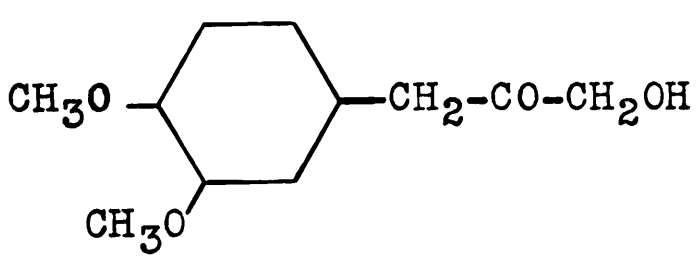
The significance of the work of the Hibbert school was limited by the fact that a maximum of only 30% of the total Klason lignin was isolated as water-soluble distillable oils and indeed only 9.8% of the Klason lignin was accounted for by identifiable products (20). Thus the results could be applied only to the lesser portion of the lignin in maple wood.

Hewson, McCarthy, and Hibbert (29) carried out an extensive investigation of the effects produced by varying the conditions (time, acid concentration and physical state of wood) employed in the ethanolysis of maple wood, in an effort to increase the yield of water-soluble distillable oils, but the results were thoroughly negative. They did succeed, however, in removing 93% of the lignin from maple in alcohol-soluble form by the step-wise removal and replacement of the ethanol-hydrogen chloride medium used as extractant, as contrasted to the 75% removal in the standard single forty-eight hour treatment (19).

However, Hewson and Hibbert (30) showed that when isolated ethanol lignin was subjected to renewed ethanolysis a further amount of distillable oils was formed and that these oils yielded the same lignin building units as were isolated by the regular wood ethanolysis procedure. It must be noted however that even with the additional distillable oils

isolated by re-ethanolysis of ethanol lignins, about one-half of the lignin was still not accounted for by propylphenol units. No clear-cut experimental evidence exists to prove that this half is constituted in a similar fashion.

The isolation of these phenylpropane derivatives made it possible to use them as models of protolignin. It is of special interest in the present study to note that some, when boiled with dilute acids, yielded brown amorphous lignin-like substances and were converted by alkalies into reddish-brown condensation products of unknown composition (3).



(X)

Fisher, Kulka and Hibbert (31) reported that when 3-hydroxy-1-(3,4-dimethoxyphenyl)-2-propanone (X) was treated with 72% sulfuric acid under the conditions of the Klason lignin procedure, over 60% of a dark amorphous lignin-like product was obtained. Even 5% sulfuric produced 20% of the lignin-like material. Sodium hydroxide, 3%, at room temperature for seventy-two hours, produced 80% of amorphous product. West, Hawkins and Hibbert (32) also found that several of the phenylpropane monomers isolated from the ethanolysis products of maple, yielded up to about 30% of polymers when subjected to re-ethanolysis.

These investigations clearly show the dangers attendant in drawing conclusions from results with lignin samples which have been isolated by the use of acid or alkali, since there is a strong possibility that a goodly portion of "extracted lignins" are only secondary condensation products of simple units, which themselves have probably been split off from a more complex lignin occurring in the wood.

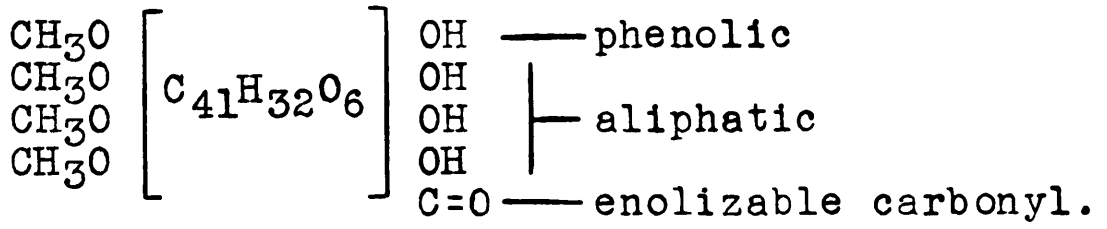
The discussion thus far has been restricted to lignin isolated, and undoubtedly altered, by the use of acid, alkali or high temperature. Brauns (33) has succeeded in extracting 8%-10% of the lignin in black spruce with 96% ethanol at room temperature without added acid or alkali. Brauns called his product "native lignin" which he defined as "a lignin isolated in such a way that the solvent does not react with the lignin or alter it in any way." The light-colored native lignin had a methoxyl content of 14.8% which figure was close to that calculated for the entire spruce lignin. Native lignin gave the typical lignin color reactions (34), a deep purple with phloroglucinol and hydrochloric acid and a bluish-green with phenol and hydrochloric acid, and analyzed 92%-95% lignin by the Klason procedure. It condensed with phenol in the presence of hydrochloric acid to give a phenol lignin with a methoxyl content of 11.5%, a value identical with that of the same derivative (35) prepared from Willstatter lignin or Freudenberg's cuproxam lignin. All these reactions showed that Brauns was dealing with a genuine lignin and, moreover,

that native lignin was closely similar to at least a portion of the lignin in wood. The observation that native lignin dissolved easily and completely in bisulfite solution at 125° was particularly significant, since almost all isolated lignins differ from lignin *in situ* by being insoluble in hot bisulfite (36).

When subjected to methanolysis with 2% hydrogen chloride for about five hours, 35% of the native lignin separated as a resin, insoluble in dioxane, whereas untreated native lignin itself was completely soluble in dioxane. If a concentration of 0.5% instead of 2.0% of hydrogen chloride was used in the methanolysis no insoluble polymer was formed, a soluble methanol lignin being obtained with a methoxyl content of 20.9%.

Brauns assigned the formula $C_{42}H_{36}O_{11}(OCH_3)_4$ to native lignin by relying on the agreement between the observed methoxyl content of 14.8% and the calculated value, and by ignoring a discrepancy of 1.8% in the carbon content. Following earlier work by Brauns and Hibbert (37) on methylated lignin, native lignin was methylated in ether suspension by diazomethane to a methoxyl content of 18.3%. When dioxane was the solvent, diazomethane raised the methoxyl content to 21.4%, and this product was the first to be insoluble in cold alkali and to give a negative response to the lignin color reaction with phloroglucinol and hydrochloric acid. Brauns concluded that diazomethane had selectively methylated two kinds of

phenolic or enolic hydroxyl groups. The original native lignin was then submitted to the methanolysis reaction and the product, although resembling the earlier one in its methoxyl content of 20.9%, was soluble in alkali and was capable of further methylation to a methoxyl value of 25.4% by diazomethane. The inference was that methanolysis had converted a carbonyl group to a dimethyl acetal unit but had left one phenolic hydroxyl group unchanged, as shown by the ready precipitation of the substance from an alkaline solution by carbon dioxide. As a result of much similar work, Brauns expanded his empirical formula into the partial structure:



(XI)

Although such a structure is very useful as a summary of a great mass of analytical data, it tacitly makes the unproven assumption that native lignin is a single chemical individual isolated in a state of purity. It has been repeatedly shown that diazomethane is capable of methylating aliphatic hydroxyl groups, such as those in cellulose (38) and starch (39) and may not be rigorously selective for phenolic, enolic or carboxylic hydroxyl units as Brauns (33) and Brauns and Hibbert (37) have assumed. Moreover, some phenolic groups, especially those which have substituents in the ortho position of the

benzene ring are not methylated by diazomethane (40). For these reasons, it seems prudent to regard the quantitative details of Brauns' structural formula with great reserve. It is interesting to note, however, that both native lignin (33) and wood (41), when methylated with diazomethane to about 21% methoxyl content, lost the capacity to be pulped by bisulfite solution, although the lignins became sulfonated. Hagglund and Holmberg (42) made a similar observation. It appeared that some hydroxyl group in wood, supposed by Brauns to be enolic in nature, was essential for solubility of the lignin in bisulfite and was methylated selectively by diazomethane. Freudenberg, (10) on the other hand, claimed that no free phenolic unit was present in cuproxam lignin.

Though native lignin is one of the least changed forms of lignin isolated thus far, long extraction periods are necessary to obtain even the small yields reported. Wald (43) repeated Brauns' work on northern pine and found that even after four months of continuous extraction with 95% ethanol, native lignin was still steadily, though very slowly, going into solution. Harris (44) suggested that native lignin was not present as such in wood but was the result of attack on the lignin complex by hydrolytic enzymes. If a sample of aspen wood meal was extracted immediately after cutting with 95% ethanol, which would inactivate the enzymes present, only 0.006% of lignin, based on the wood weight, was recovered, whereas 2.2% was obtained if the meal was allowed to air-dry

(giving the hydrolytic enzymes time to act) previous to the ethanol extraction.

Hewson, McCarthy and Hibbert (45) treated carefully extracted and dried maple wood with anhydrous ethanol for nine hours at 150° and found that the wood had liberated acids so that the pH was 4.5 at the conclusion of the cook. Only about 10% of the protolignin was removed in this treatment but as the temperature was increased a larger amount was extracted. It has been shown (46) (47) that acids such as formic and acetic are formed by the action of water on wood at elevated temperatures and in the experiments of Hewson et al. these acids probably catalyzed delignification. A slow release of such acids with time provides an alternative explanation for the gradual solution of native lignin in Brauns' procedure. Indeed there is probably no example of lignin having been isolated without the application of some hydrolytic agent, either added, or already present in the wood.

Since the hydrolytic effect of acids is due essentially to the presence of the hydrogen ion, it might be expected that the effect of these traces of acids liberated would be greater in ethanol-water solution than in anydrous ethanol. This inference was checked by Hewson, McCarthy and Hibbert (45) who found that maple wood extracted with a 1:1 mixture of ethanol-water at 165° for ten hours retained only 14% of the original lignin content while 75% was left if anhydrous ethanol was used under the same conditions. It is

probable therefore that the hydrolytic action in any method for isolating lignin will be least when the solvent used is anhydrous.

A novel approach to the problem of obtaining lignin in a relatively unchanged form was recently described by Wald, Ritchie and Purves (9). What they termed "periodate lignin" was an insoluble residue from black spruce or northern pine wood first oxidized with sodium paraperiodate, $Na_3H_2IO_6$, at pH 4 and 20° and then extracted with boiling water at a pH of about 7. About six oxidation extraction cycles were necessary. Lignin was only slowly attacked under such conditions whereas the holocellulose was converted by the cold paraperiodate solution into products readily solubilized by boiling in neutral water.

The importance of the procedure lay in the fact that high temperatures and strongly acid or alkaline media had been avoided. Brauns' (33) native lignin was also isolated with a neutral solvent at low temperatures but the reported yield did not exceed 10%, so that native lignin could not be considered representative of fully 90% of the lignin content. In contrast, Ritchie (48) obtained an almost quantitative yield of periodate lignin analyzing 86%-96% Klason lignin from spruce and 75%-85% yields from hardwoods such as maple, birch and beech. However, the periodate lignin had suffered changes of an oxidative nature in the process of isolation, as reflected, in the case of spruce, by a fall in methoxyl

and carbon content from 14.0% and 67.5% calculated for lignin *in situ*, to 12.2% and 61.4% respectively, and in the case of the hardwoods, by the low yields and the low (54.6%-58.4%) carbon content, though the methoxyl value remained near the calculated value.

It should also be pointed out that even the acidity at pH 4 at room temperature, as used in the isolation of periodate lignin, might well have produced changes of a hydrolytic nature on prolonged exposure. Such changes were apparently minor in character, since Ritchie showed that spruce periodate lignin very closely resembled lignin *in situ* in its insolubility in neutral solvents and in its response toward oxidation with alkaline nitrobenzene, toward high-pressure catalytic hydrogenation, ethanolysis, and standard sulfite and alkali cooking conditions. Periodate lignin given even such a mild pretreatment as heating with water for six hours at 135°, dissolved to the extent of only 20% in a standard sulfite cook, whereas 97% of untreated periodate lignin dissolved under similar cooking conditions. This observation emphasized what was already generally known, that the vigorous conditions employed in standard methods of lignin isolation caused an inhibition of its response to a sulfite cook. This effect was clearly shown for the first time to be dependent on the lignin alone and not to be involved with the carbohydrate portion of wood.

An interesting conclusion also resulted from Ritchie's

work on the alkaline nitrobenzene oxidation of spruce periodate lignin. Freudenberg and his co-workers (49) first used a mixture of nitrobenzene and aqueous alkali at 160° to obtain vanillin from spruce wood in 25% yield. Hibbert and co-workers (50) (51) confirmed Freudenberg's 25% yield of vanillin from spruce and extended the method to a wide variety of plant materials. They found that softwoods gave a 35%-51% yield of a mixture consisting of vanillin and syringaldehyde in a ratio of 1:3, while gymnosperms gave only vanillin. Despite the fact that spruce periodate lignin had 3%-4% less methoxyl content than lignin in situ, it gave the same yield of 25% of vanillin when oxidized with alkaline nitrobenzene. This concordance meant that the periodate treatment was removing methoxyl from some part of the structure other than that which gave the normal 25% yield of vanillin. These results were considered to show that the precursor of this 25% yield of vanillin was chemically combined in the native state in a manner that offered protection against the oxidative attack of periodate solution, since isolated vanillin (52) was rapidly attacked by aqueous periodate.

In 1942, Hess and Heumann (53) introduced a new group of solvents, the amino bases, for dissolving lignin out of woody materials. In approaching the problem of isolating lignin without change or loss of functional groups, these workers were guided by the following principles. Due to the natural course of development of the cell wall, the lig-

nin in wood was completely enclosed by carbohydrate material. Therefore, in order to render the lignin easily accessible to dissolving media, thorough disintegration of the cell wall bonds by choice of a suitable comminuting process was indicated. The accessibility of the lignin would be still further increased if the lignin solvent was an agent which swelled cellulose and hence the microscopic wood fragments produced by the comminuting process. Lignin in vivo undoubtedly contained active groups which were lost by secondary reactions in the standard methods of lignin isolation. The ideal solvent for lignin extraction would be one that protected such active groups by reacting with them, hence preventing secondary reactions at these points. The use of acids, alkalies, oxidative action or elevated temperatures was to be avoided.

By the use of a vibratory mill for disintegration of the cell wall, and of hydrazine at room temperature as the solvent which would both swell cellulose and simultaneously condense with highly reactive portions of the natural lignin, Hess and Heumann believed they had satisfied the principles they had set forth, and that they had obtained the least changed form of lignin ever isolated.

The procedure finally adopted for the extraction of lignin from winter rye straw (the main object of the preliminary investigations) was as follows. Rye straw, 10 grams, was ground, in the dry condition, in a vibratory mill for

twenty-four hours. Hydrazine hydrate, 24 grams, and water, 75 grams, were added and the grinding continued for a further fifteen hour period. The slurry obtained was then given a nine hour treatment in the mill with 200 cc. of ethanol. The filtrate was evaporated to dryness in vacuo and the residue of hydrazine lignin was dissolved in 80% ethanol. The alcohol solution was poured into 1:1 ethanol-ether, when the hydrazine lignin was thrown out in a yield of 13.5% based on the total rye used. The lignin content of the rye fell from 19.5% to 2.2% and if the extraction were repeated twice more the lignin content dropped to almost zero. In an almost similar procedure aqueous ethylenediamine extracted 18% of rye straw. Such amino bases as monoethanolamine, triethanolamine, tetramethylammonium hydroxide, and guanidonium hydroxide, all used in aqueous solution, extracted, in one treatment, 7%-10% of the rye weight, presumably as lignin.

Hess and Heumann described control experiments which clearly showed the value of the vibratory mill in increasing the amount of lignin extracted by hydrazine. While there can be no doubt as to the efficacy of the vibratory mill in increasing the accessibility of lignin to solvents, there is a distinct possibility that not only physical changes but also chemical changes of a degradative nature were being produced by the vibratory mill, especially in the dry grinding treatment where there would be inefficient dissipation of the

heat evolved. The degree of polymerization of native cellulose exceeds 3000 (54), a figure which means that the average length of a native cellulose molecule completely stretched out is in excess of 15,000Å, the length of a single glucose anhydride unit being about 5Å.(55). Hess and Heumann reported that the particles of rye straw after grinding in the vibratory mill had a diameter of 10,000Å.and less. One can easily see therefore that the vibratory mill had begun to break up cellulose molecules, an action no longer of a physical but of a chemical hydrolytic or oxidative nature. Hess and Steurer (56) found that the viscosity of cellulose did decrease after grinding in a vibratory mill, and there is no reason to suppose that the lignin would escape a similar mechano-chemical attack when similarly ground. Indeed, the probability is perhaps greater, since lignin is more sensitive than cellulose to heat and oxidative influences.

More valuable than the use of the vibratory mill was the recommendation by Hess and Heumann (53) of liquids which swelled cellulose as solvents for the extraction of lignin. Hess and Trogus (57) found in hydrazine and in the homologous alkylene-diamines a group of bases that were permutoid swelling agents, actually penetrating the crystal lattice of cellulose and forming characteristic double compounds. Quaternary ammonium bases, such as tetramethylammonium hydroxide and guanidonium hydroxide had a similar effect (58). Such solvents would be expected to increase the liability of lignin to ex-

traction, by swelling the cellulose lattice. But when Hess and Heumann used hydrazine and the other nitrogen bases mentioned in aqueous solution, they controverted one of the principles they had themselves set up - that is, the avoidance of alkaline reagents. Tetramethylammonium hydroxide in water solution is as strong a base as sodium hydroxide (59). Even hydrazine in aqueous solution has a basicity close to that of aqueous ammonia (60).

Many isolated attempts have been made to determine the size of the lignin molecule, but the results reported vary from several hundred to several thousand (61). Any molecular weight for lignin may be only an average value, since probably lignin in situ, like any other polymer, is made up not of one definite size of molecule, but of a whole range of sizes. The variations in the reported molecular weights are, however, too extreme to be explained away in this fashion, but are a reflection of the drastic conditions used to obtain the lignins for molecular weight determinations. Just as petroleum is converted by a high-temperature cracking process both to very light gases and to very heavy tars, so lignin in vivo responds to drastic methods of isolation by both depolymerization and polymerization reactions, so that the molecular weight of the lignin finally isolated bears only casual relation to the true molecular weight of lignin.

Probably the most careful investigation in this field

was that of Loughborough and Stamm (61) in 1936. They studied maple lignin samples isolated either by sulfuric acid as in a standard Klason lignin determination, by methanolysis using hydrogen chloride as catalyst or by an alkaline cook with ethanol-sodium hydroxide. Using the osmotic-pressure and boiling-point methods in a variety of solvents (methanol, ethanol, chloroform and acetone) they obtained for lignin prepared in any of the three ways an average molecular weight of 3900 ± 300. Diffusion measurements gave a value of 10,000 ± 1000 for all three lignin preparations in a variety of solvents. Two possible explanations were offered for the wide difference between the two results. First, the lignin solution might have been a mixture of different molecular species or "polydisperse," so that the osmotic-pressure and boiling-point methods yielded number-average values while the diffusion method fundamentally gave a weight-average value (62). However, Loughborough and Stamm presented a number of reasons to support their belief that the lignin solutions being studied were not polydisperse but almost monodisperse, consisting of only a small range of molecular sizes, so that differences between weight-average and number-average values would be too slight to explain the discrepancy. They considered that the discrepancy was probably due to the influence of shape on the accuracy of the diffusion method, as contrasted to the osmotic-pressure and boiling-point methods, which gave values independent of the shape of the solute particles. The formulae used to derive molecular

weight from diffusion data were based on the assumption that the solute particles were spherical, which was not the case with lignin. When an approximation was made to correct the diffusion data for this shape factor the answer approximated the value of 3900 for the molecular weight of lignin.

Conner (63), by measuring the high frequency energy losses in solutions of lignin prepared by methanolysis from aspen, maple and spruce, confirmed this value of 3900 and established the value of the shape factor at 8.

Gralen (64) has recently reported molecular weights obtained by the use of the ultracentrifuge (65). He worked mainly with thioglycolic acid lignin prepared by Holmberg's (66) method and clearly showed how the results could vary with the conditions used in isolation.

Spruce thioglycolic acid lignin prepared by extracting the wood with thioglycolic acid and 2N hydrochloric acid at water-bath temperature, had a molecular weight of about 7000, after correction for the thioglycolic acid which had condensed with the lignin. Spruce hypobromite lignin (67) and also spruce alkali lignin (prepared by heating wood in 1N caustic at 170°) also gave values of about 7000. But if thioglycolic acid lignin was prepared in the normal way from spruce which had been pretreated with boiling hydrochloric acid or with aqueous alkali, the molecular weight jumped to about 29,000 and 11,000 respectively. This increase was ascribed to self-condensation and etherification and confirmed

a conclusion that had been previously drawn from analytical data. If spruce lignin was treated with hydrochloric acid after it had been extracted in the normal way by the thioglycolic acid procedure, the molecular weight dropped to about 3500, or by one-half, and an analysis of the ultracentrifuge data showed that the "spread" in molecular weights was less, indicating that the 3500 - 4000 figure might be a more stable value. It will be noticed that this value is in the range of that quoted by Loughborough and Stamm (61). Spruce acetic acid lignin, prepared by the action of hot glacial acetic acid containing a trace of hydrogen chloride catalyst (68), gave the divergent value of 14,000.

Patterson et al. (69), found that the apparent viscometric chain length of maple ethanol lignin was changed considerably even by heating under reflux in neutral organic solvents. All these results demonstrate that the observed properties of any lignin sample depend to a very considerable extent on its history and emphasize the need for developing methods of isolation which produce the minimum of chemical change.

B. Pertinent Portions of Liquid Ammonia Chemistry

Even concentrated aqueous ammonia, 23%-28%, has no effect on cellulose (70) under ordinary conditions, but Bernardy (71) did succeed in reducing purified cellulose to a brown powder containing 20% nitrogen by heating at 200° and 40 at-

mospheres pressure for forty-eight hours with 22% aqueous ammonia. Like most polar gases, ammonia is absorbed in large amounts by cellulose. Thus cotton will absorb 4.0% and white spruce 7.4% by weight of ammonia at 22° and atmospheric pressure (72). Heuser (70) states that cotton will occlude 115 times its own volume of ammonia. After evaporation of the ammonia the cellulose appears to be unchanged.

Liquid ammonia produces quite different results. Bernardy (71) (73) first observed that cellulose became swollen in liquid ammonia, the fibres being mercerized in a way similar to the action produced by strong aqueous alkali. Barry, Peterson and King (74) confirmed this swelling in the anhydrous liquid, and showed that a distinct crystalline entity which they called ammonia-cellulose was formed. They worked with bundles of ramie fibres which were immersed in liquid ammonia for five to ten hours at -33.3°. Free evaporation was permitted at atmospheric pressure, and the ramie fibres were then immediately covered with paraffin oil to avoid loss of the more tenaciously retained portion of the ammonia and subjected at once to x-ray examination. Clark and Parker (75) showed later that the use of the paraffin was ineffectual.

The fibres were found to be swollen at this stage and to contain one mole of ammonia to one mole of anhydro-glucose unit in the cellulose. X-ray analysis showed a distinct structure similar to that produced by Hess and Trogus (57) in

treating cellulose with certain diamines such as hydrazine and ethylenediamine. The volume of the unit cell was increased from 671 cu.Å. for normal cellulose to 801 cu.Å. for the ammonia-swollen cellulose. Barry, Peterson and King considered this swelling to be caused by the entry of ammonia molecules into the unit cell after the regular manner of a permutoid swelling agent. The a-axis of the unit cell increased from 8.3 Å. to 9.83 Å., and the c-axis from 7.9 Å. to 10.95 Å., while angle β decreased from 84° to 53.5°. The b-axis was unchanged. Ammonia-cellulose could not be prepared by soaking cellulose in saturated aqueous ammonia for several hours or in ammonia gas at atmospheric pressure for fifty hours.

If, after treatment with liquid ammonia, the ammonia-cellulose was heated at 105° for fifteen hours instead of being coated with paraffin, all ammonia was lost and a new cellulose, cellulose II was formed, definitely not the original cellulose but resembling hydrate cellulose in its structure and enhanced chemical activity. X-ray analysis showed a lattice volume of 702 cu.Å., smaller than that of ammonia-cellulose but still larger than that of normal cellulose. The a:b:c ratio was found to be 7.87:10.31:10.13 as compared to 8.3:10.30:7.9 for normal cellulose, while angle β was 58°. A similar cellulose II was formed by allowing the paraffin covered fibres to stand forty-eight hours at room temperature. They found further that ammonia-cellulose reverted to normal

cellulose when immersed in dilute acetic acid, or in either dilute or concentrated aqueous ammonia. Cellulose II was unaffected by these agents, though with liquid ammonia it reverted to ammonia-cellulose which could then be changed to the normal cellulose. Barry, Peterson and King summarized their conception of the relation between these forms of cellulose by the equation below.

$$\text{n-Cellulose} \underset{H_2O}{\overset{\text{liq.}NH_3}{\rightleftarrows}} NH_3 - \text{Cellulose} \underset{\text{liq.}NH_3}{\overset{\text{Heating}}{\rightleftarrows}} \text{Cellulose II}$$

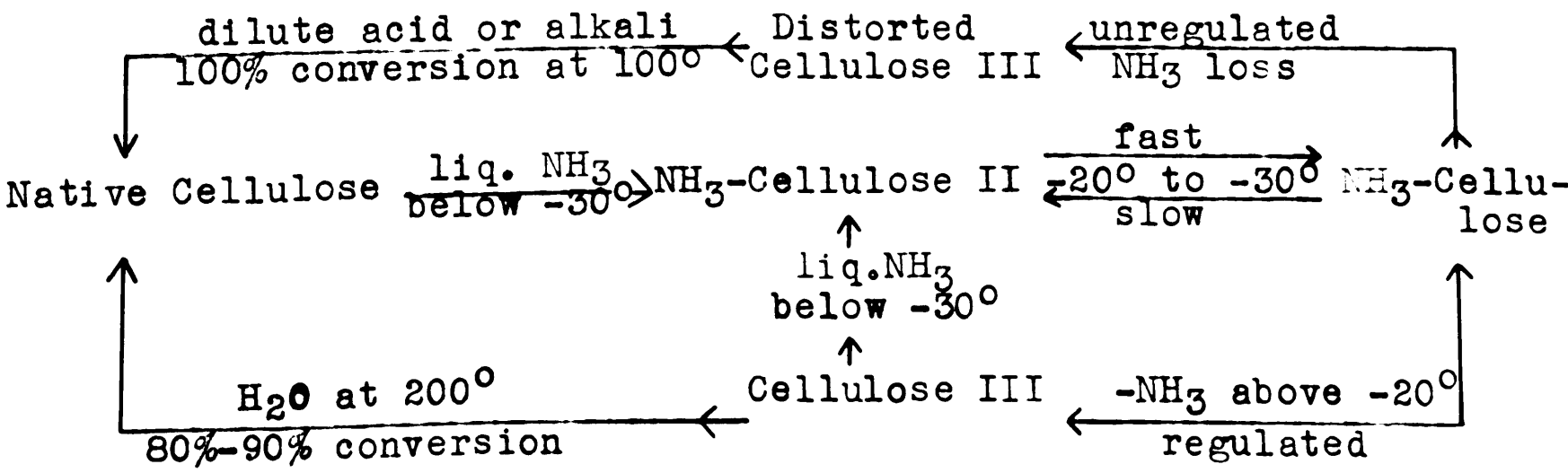
Meanwhile Hess and Trogus (76) had been carrying on similar studies, and on hearing of the work of Barry *et al.* before it had actually been published in full, issued a preliminary communication which, although itself later corrected and extended, indicated that the interpretation given by the American investigators was probably by no means complete. Hess and Trogus treated cellulose with dry liquid ammonia for an unstated period at -77° to -80°. The failure of the fibres to swell may have been connected with the very low temperature used (ammonia freezes at -77.7° according to the International Critical Tables). However, when the ammonia was distilled away, the cellulose did give a new x-ray pattern which they considered to be either ammonia-poor, ammonia-cellulose or a third modification, provisionally called cellulose III. Contrary to the ammonia-cellulose behaviour observed by Barry *et al.* neither methanol, water or concentrated

aqueous ammonia restored the normal cellulose pattern. Indeed, entirely different patterns were produced whose nature was not clear. Hess and Trogus also made the important observation that the x-ray diffraction pattern given by their cellulose III varied with the speed of the evaporation of ammonia from the fibre, indicating that, under liquid ammonia, ammonia-cellulose existed in a form not identical to cellulose III. In view of the contradictory findings reported, Clark and Parker (75) carried out further studies using liquid ammonia at -75°. They found that the cellulose fibres swelled to three times their normal size. However, even when protected by paraffin oil, the fibres, when removed from the ammonia, rapidly lost ammonia, regaining their normal size. Swollen ammonia-cellulose, taken directly from liquid ammonia and immersed in concentrated aqueous ammonia, reverted to the original native cellulose.

A little reflection leads to the conclusion that it is not enough to study ammonia-cellulose after it has been removed from liquid ammonia but that it is essential to conduct the x-ray examination while the fibres are still immersed. Hess and Gundermann (77) adopted this view and their work served to clarify to some degree the confused relations of the various celluloses to one another. They found that ramie fibre gave two ammonia-celluloses, mutually interchangeable between -20° and -30°. When the form stable at lower temperatures, ammonia-cellulose II, was warmed, it changed rapidly to ammonia-cellulose I, stable above -20°, but the reverse change

was slow. Cellulose II was taken to be $C_6H_{10}O_5(NH_3)_6$ but they were not able to determine the amount of ammonia in cellulose I, so it is not known whether I and II are polymorphs or different chemical compounds. As before, with loss of ammonia from the ammonia-cellulose a form of cellulose resembling hydrate cellulose was produced, called cellulose III by Hess and Gundermann. They found that the exact nature of cellulose III was very greatly influenced by the method used in its production, a slow regulated ammonia evaporation giving a more ordered x-ray picture and a product which reverted in 80% or 90% yield to natural cellulose when heated with water at 200°. Less ordered cellulose III, formed when the fibres were removed rapidly from the liquid ammonia and the excess ammonia was pumped off, or by permitting the ammonia to evaporate at will under atmospheric pressure and then allowing the product obtained to stand in the air for several weeks, was converted more easily and more completely to natural cellulose by boiling with dilute acid or alkali at atmospheric pressure.

The Hess and Gundermann scheme for the ammonia-cellulose system is given below.



This chart best explains, though still not completely, the interconnections among the various celluloses discussed. Hess and Gundermann considered the ammonia-cellulose and "cellulose II" of Barry, Peterson and King to correspond respectively to a partially decomposed ammonia-cellulose I, and to cellulose III mixed with natural cellulose. Later work by Barry *et al.* (78) reported the formation of an ammonia-cellulose more closely equivalent to ammonia-cellulose I when cellulose was sealed in a glass tube with liquid ammonia at room temperature. The a-axis was given as 11.97 Å., and the c-axis was given as 11.15 Å., a very considerable expansion. The incorrectly named "cellulose III" of Hess and Trogus appeared to be a partially degraded ammonia-cellulose I, the result of insufficient control in its production. The other celluloses obtained by use of various aqueous solutions, methanol and so on, were not considered to be pure types but rather distorted forms of one or more of the basic lattices - natural cellulose, ammonia-cellulose I and hydrate-like cellulose III.

A limited amount of work has been carried out at the Iowa State College by Peterson and Hixon (79) and others (80) on the pulping of cereal straws by aqueous ammonia at pressures not exceeding 100 pounds per square inch. Since these conditions are essentially those of an aqueous alkaline pulping process which would produce chemical change, this study will not be further discussed here.

Only two papers dealing with the chemical behaviour of lignin or wood in anhydrous liquid ammonia have been discovered. These papers are by Freudenberg and co-workers (81) (82) who studied the action of alkali metals and alkali metal amides dissolved in liquid ammonia on a limited variety of lignins and woods. Freudenberg was not directly concerned with wood pulping but used liquid ammonia in an attempt to extract lignin with its carbon skeleton relatively intact, the ammonia extracts being studied by methylation and oxidative techniques in order to throw light on the chemistry of lignin. Spruce lignin, isolated by the Urban hydrochloric-phosphoric acid method (83), when treated for twenty-four hours at 20° with a solution of potassium or potassium amide in excess liquid ammonia, yielded a residue completely soluble in the strong alkaline solution produced by decomposition of the residue with water (81). Spruce wood meal, extracted with metallic sodium dissolved in liquid ammonia, became 50% soluble in aqueous alkali, the soluble portion containing two-thirds of the lignin in the wood. Sodium amide reacted similarly though less vigorously (81).

In Freudenberg's later paper (82) the use of alkali amide was avoided since it caused side reactions and complicated the course of the reaction. Spruce wood meal was treated with a solution of potassium in ammonia for several hours at 20°. Only 1.9% of the meal dissolved in the liquid ammonia but lignin amounting to 16%-18% of the meal was now

soluble in absolute methanol. The remaining 8% of the lignin, although methanol-insoluble, could be extracted by boiling 1% sodium hydroxide. A residue of 41.8% of undegraded Cross and Bevan cellulose was recovered. Beech wood, similarly treated, reacted in a more complicated fashion, an appreciable amount of cellulose being degraded to a methanol-soluble form, in contrast to the result with spruce wood. Even isolated spruce cellulose was quite stable to liquid ammonia solutions of potassium. Cuproxam lignin was rendered completely soluble, some 6% dissolving in the ammonia, and the rest in absolute methanol. Phenol ethers generally underwent partial or complete demethylation to yield phenols. The solubilization of wood lignin was probably connected with a similar action.

Water occupies an outstanding position among liquids. In its capacity as a solvent for salts, as an ionizing agent and in many of its other physical properties it stands almost in a class by itself. Of all other liquids, anhydrous liquid ammonia most closely approaches water in those properties which give to water its distinctive role as a solvent. Liquid ammonia approaches water as an ionizing solvent. Although it does not dissolve all water-soluble salts, some salts, for example the iodides of mercury, lead and silver, which are quite insoluble in water, are easily soluble in liquid ammonia. Also, ammonia will dissolve many organic compounds unaffected by water, ammonia generally resembling

alcohol in its powers as a solvent in the organic field. Like water, ammonia frequently unites with salts as "ammonia of crystallization" comparable to water of crystallization.

These resemblances extend to compounds which can be considered as being analogously derived from the parent substances ammonia and water. Such comparisons of the water and ammonia series, as well as the techniques of handling liquid ammonia, were developed and popularized chiefly by E.C. Franklin, along with C.A. Kraus, H.P. Cady and a few others. The concept of an ammonia system of compounds analogous to a water system of compounds has proven to be a very fertile one and has given rise to a large volume of experimental work, most of which has been amply reviewed in a book by Franklin (84) and in other reviews appearing in the Journal of Chemical Education (85) and in Chemical Reviews (86). The analogy is especially complete as regards acids, bases and salts, which are of particular interest in this investigation. Acids and bases can be considered as arising from the parent substances, ammonia and water, by replacement of part of the hydrogen by either a strongly electropositive atom to form a base, or by an electronegative atom or radical to give an acid. The relationships involved are sometimes complicated by the greater possibilities for hydrogen substitution afforded by ammonia as well as by deammonation reactions comparable to dehydration, but the fundamental analogy is generally traceable.

Thus both an alkali amide such as $LiNH_2$, and an alkali

imide such as Li_2NH, when considered in liquid ammonia solution, are bases in the full sense of the word as it is applied say, to KOH, in water. Acids, bases and salts of the nitrogen system, in liquid ammonia solutions, bring about the same reciprocal color changes of indicators so well known in analogous water solutions and they react stoichiometrically with each other in normal fashion. For example, soluble ammonium salts, which in aqueous solution are neutral or at best faintly acid by incipient hydrolysis, are fairly strong acids in liquid ammonia, attacking many metals, reacting with bases and catalyzing ester hydrolyses after the regular manner of an acid in water, although, as is generally the case with reactions in ammonia, such action is more sluggish. This acid action is easily understood when it is recalled that the acidity of hydrogen chloride in water is caused not by H^+ but by the hydrated hydrogen ion, H_3O^+. The ammonium ion, in liquid ammonia, represents an analogous ammonation of the H^+ ion, giving rise to a similar acid character, NH_4^+, which however has a lesser acidity than H_3O^+.

It is of interest in this study to indicate the versatility of liquid ammonia as a solvent (84). Most of the solubilities given below are those at room temperature and lower. The alkali metals dissolve very readily and, most surprisingly, are recoverable unchanged when the ammonia is evaporated. Such alkali metal solutions are the strongest homogeneous reducing agents known. Iodine, sulfur, and phosphorus dissolve

in liquid ammonia, but react at the same time. Amongst the salts, ammonium and sodium chloride have a substantial solubility in liquid ammonia while the other chlorides are generally insoluble. The chlorides of the alkaline earth metals react with ammonia to form addition products so that calcium chloride cannot be used as a dehydrating agent for ammonia. The sulfates, including ammonium sulfate, are generally insoluble, as are the sulfites. Ammonium sulfide is readily soluble. Most of the nitrates and nitrites dissolve abundantly. The alkali cyanides are soluble in liquid ammonia. Most of the metallic oxides and hydroxides are however insoluble.

Aliphatic hydrocarbons, (84) are practically immiscible with liquid ammonia at the boiling point of ammonia, -33.5°, but become partially miscible at higher temperatures. The lower aliphatic alcohols, also benzyl and cinnamic alcohols are completely miscible with liquid ammonia. Phenols, cresols, pyrogallol - all are very soluble. Most ethers aldehydes and ketones, aliphatic or aromatic, are soluble, though aldehydes and ketones frequently react with the ammonia by ammonolysis to give imino type derivatives (87). Acetals, such as dimethyl and diethyl acetal, dissolve readily, but in contrast to their ready hydrolysis by aqueous acid, acetals are almost unaffected by liquid ammonia even over several years and in the presence of ammonium salts (87). Acetic acid, benzoic acid and the toluic acids dissolve readily, but the higher aliphatic acids and the dicaboxylic

acids are almost insoluble. The amides of most acids are quite soluble. Esters are in general soluble, but most of them undergo an ammonolysis reaction, analogous to hydrolysis in the aqueous system.

Ammonolysis $XCOOR + HNH_2 \longrightarrow XCOONH_2 + HOR$

Hydrolysis $XCOOR + HOH \longrightarrow XCOOH + HOR$

All the ordinary sugars, arabinose, glucose, fructose, galactose, sucrose, maltose, lactose, as well as their methylated, acetylated and acetone derivatives are quite soluble (88) (89) in liquid ammonia at its boiling point (-33.5°). Many polysaccharides (but not cellulose) are also soluble. With the exception of the sugars with free potential carbonyl groups, liquid ammonia at -33.5° has no effect on these carbohydrates (89).

The reducing sugars, when dissolved in liquid ammonia at -33.5°, first form an aldehyde addition product and then the corresponding amine. Glucose for example forms 1-aminoglucose (88). Muskat (89) found that liquid ammonia at -33.5° dissolved the acetylated and benzoylated derivatives of any sugar without removing the acyl groups, provided that the reducing group was suitably blocked by forming the methyl glucoside or 1,2-acetone compound. If, however, the acetyl group was attached to the aldehydic or ketonic carbon atom, it was readily removed to form an amino sugar. At room temperature liquid ammonia removed all acetyl groups from the sugars (90). Similarly Zechmeister and Toth (91) found that

when cellobiose octoacetate was heated with liquid ammonia at 55° for forty-eight hours all the acetyl groups were removed and 1-aminocellobiose was formed.

The use of liquid ammonia promises to become standard procedure in the organic laboratory, because, to quote Fernelius (87): "(1) Liquid ammonia can bring about reactions that are not possible with aqueous or non-aqueous solutions of ammonia: (2) because there are inherent differences in the extent and type of reactions taking place in solutions of ammonia and in the anhydrous solvent."

Bearing in mind the frequent parallel behaviour of liquid ammonia and water solutions, it should be possible to choose reagents which, in liquid ammonia, would have an action theoretically equivalent to that of the customary wood pulping agents used in water. Thus Freudenberg's use of alkali amide in liquid ammonia could be regarded as equivalent to aqueous caustic in a normal alkali cook. Involving the use of free alkali metal as it did, Freudenberg's method of rendering wood lignin soluble could hardly be considered from a practical aspect, but it did point to the possibility of finding an agent which in liquid ammonia would attack lignin and yet leave the cellulose comparatively untouched. It was not unreasonable to hope further that any lignin solubilizing action possessed by liquid ammonia or by substances dissolved in it would be enhanced by the intimate permutoid penetration by ammonia of the cellulose lattice work as demonstrated by the

x-ray studies previously discussed. It seemed possible therefore that a method for pulping wood with liquid ammonia might be developed. The unusual solvent powers of liquid ammonia also gave reasonable hope that some of the lignin might dissolve in liquid ammonia alone without the use of heat, or added acid, alkali, or oxidizing agents.

EXPERIMENTAL

A. Analytical Methods

I. Ash

Ash content was determined in duplicate by ignition of 0.5 to 2.0 gram samples to constant weight in a tared crucible, first over a Bunsen flame, then in an electric muffle at a dull-red heat. Ash contents are reported as a percentage of the oven-dry weight of the wood used.

II. Water

The loss of weight in 0.1 to 1.0 gram samples when kept in an oven at 105° for sixteen hours was determined. The weighings were carried out in glass-stoppered containers. All determinations were done in duplicate or triplicate.

III. Klason Lignin

Klason lignin was determined in either duplicate or triplicate by the standard T.A.P.P.I. method (92) which uses a digestion period of two hours at 18°-20° with 72% sulfuric acid, followed by dilution to 3% acid with water and a further hydrolysis at reflux temperature for four hours. The sample weight for an individual Klason lignin determination was reduced from the 2 grams required by the official T.A.P.P.I. method to about 0.1-0.6 grams. The Klason lignin values of the rye straw and of the maple used in the large-scale cooks were corrected for the ash content of the Klason lignin and for the ash content of the rye or maple.

IV. Pentosan

Pentosan determinations were carried out in duplicate by distilling 0.1 to 0.4 gram samples with 12% hydrochloric acid and collecting 360 cc. of distillate as recommended by the U.S. Forest Laboratory (93), and then estimating the furfural in the distillate by Powell and Whittaker's volumetric potassium bromate-bromide method at room temperature (94) (95). Fifty cc. of potassium bromate-bromide solution, containing about 3 grams of potassium bromate and 50 grams of potassium bromide per litre of water, was added to the entire distillate and the whole was allowed to stand in the dark for one hour at room temperature, during which time each mole of furfural present consumed 2 moles of bromine. After adding 10 cc. of 10% potassium iodide solution, the liberated iodine was titrated with 0.100N sodium thiosulfate. The entire procedure, distillation and titration, was repeated without a sample in order to determine the blank under test conditions. The pentosan content was calculated by substitution in the following expression:

$$\% \text{ Pentosan} = \frac{0.0412 \times N \times (v_2 - v_1) \times 100}{w}$$

where N = normality of thiosulfate

v_2 = blank titration in cc. of thiosulfate

v_1 = cc. of thiosulfate required in actual determination

w = weight of sample in grams.

The factor 0.0412 equals 0.024/0.582 where 0.024 is the weight

of furfural in grams equivalent to 1 cc. of N sodium thiosulfate, and the figure 0.582 is the factor for converting furfural to pentosan content, 80% conversion being assumed. This expression gives results comparable to those in Krober's tables (93).

V. Acetyl

Acetyl in wood was determined by hydrolyzing the wood sample with 72% sulfuric acid and titrating the acetic acid, liberated from the reaction mixture by steam distillation, with standard alkali. Lemieux's (96) semi-micro adaptation of the method described by Genung and Mallatt (97) was used, and gave good duplicates.

VI. Methoxyl

Methoxyl content was determined by E.P. Clark's (98) modification of the Viebock and Schwappach (99) method. Instead of water in the scrubber, as used by Clark, a 1:1 mixture of 5% aqueous soldium thiosulfate and 5% aqueous cadmium sulfate was used as recommended by Friedrich (100). Where the methoxyl content of a wood sample was desired, the very similar method of Hibbert and Peniston (101) was used.

VII. Nitrogen

Nitrogen was determined on a semi-micro scale by an adaptation of the Gunning method modified to include nitrate nitrogen (102), though the nitrogen content was almost certainly proteinaceous or aminoid in nature.

The sample, 0.1 to 0.3 grams, was weighed into the

Kjehldahl flask together with about 0.1 grams of pure salicyclic acid. Concentrated sulfuric acid, 2 cc., was added, and the contents were shaken for a few minutes before the addition of 0.3 grams of sodium thiosulfate. The mixture was heated gently for five minutes. After cooling, 0.6 grams of potassium sulfate and a pinch of copper sulfate were added and the sample was then digested in the usual way until only the faint color of the cupric ion was left. The ammonia was distilled into 5 cc. of 2% boric acid and titrated with standardized hydrochloric acid of about 0.01 normality as described by Ma and Zuazaga (103), using methyl red-bromcresol green mixed indicator. The reagent blank was about 0.25 cc. of 0.01 N hydrochloric.

VIII. Carbon and Hydrogen

Carbon and hydrogen were determined by semi-micro combustion in the usual manner.

IX. Cryoscopic Molecular Weight in Dioxane

The molecular weight of methanol-soluble liquid ammonia lignin was determined by the cryoscopic method using dioxane as solvent.

The method of Eigenberger (104) as described by Fieser (105) was used to prepare pure dioxane, reaction times being doubled since a larger amount of solvent was being purified. A mixture of 6 litres of commercial dioxane, 81 cc. of concentrated hydrochloric acid and 600 cc. of water was heated under reflux for twenty-four hours, during which time a slow

stream of nitrogen was bubbled through the solution to entrain the acetaldehyde which arose from the acid hydrolysis of any glycol acetal, $CH_3-CH\begin{matrix}OCH_2\\ |\\ OCH_2\end{matrix}$, present. The solution was cooled and potassium hydroxide pellets were added slowly, with vigorous mechanical stirring, until no more potassium hydroxide dissolved. A layer of concentrated aqueous potassium hydroxide settled. The dioxane was decanted and the potassium hydroxide treatment repeated to remove an additional amount of water. The dioxane was again decanted into a clean flask and was heated under reflux with sodium for twenty-four hours, by which time the surface of newly added sodium metal remained quite bright. Pure dioxane was then recovered by distillation, the boiling point being correct at 101.5°, and was stored in the dark under an atmosphere of nitrogen, which was renewed whenever the container was opened.

The actual determinations of molecular weight were carried out by the standard Beckmann technique as described in detail by Findlay (106). In order to obtain a steady value for the freezing point, and to avoid the constant drop in its value caused by a slow but steady absorption of moisture from the air, it was necessary to sweep the freezing-point tube continually with a slow stream of nitrogen, previously dried by passage over anhydrous magnesium perchlorate. The nitrogen was led in by the side-arm of the Beckmann freezing-point tube. During the course of a determination, the nitrogen generally entrained with it from 0.5 to 1.0 gram of

the 13-17 grams of dioxane initially weighed out. Any error resulting from this source was minimized by weighing the freezing-point tube and its contents, with thermometer and stirrer assembly removed, at the start and at the end of a determination, so that the actual weight of dioxane present when the freezing-point of the dioxane solution was taken could easily be calculated. Care was taken to expose the dioxane to the air as little as possible during these weighings, the freezing-point tube being kept tightly stoppered. Samples were quickly and conveniently introduced by means of long-handled weighing tubes. About 0.4 gram of lignin was used in each determination, the resulting depression of the freezing-point being 0.10°-0.15°. The results are summarized in Table I.

TABLE I

MOLECULAR WEIGHT OF METHANOL-SOLUBLE LIQUID AMMONIA LIGNIN

Fraction	Weight of Lignin, g.	Weight of Dioxane, g.	F.-P. Depression $^{\circ}$C.	Molecular Weight[b]
IVA	0.625	16.00	0.216	840
IV	0.317	12.23	0.107	1130
IV	0.424	14.34	0.131	1050
IV-2	0.460	13.28	0.179	900
IV-2[a]	0.402	11.28	0.123	1350
IV-3	0.457	13.20	0.142	1140
IV-4	0.407	17.20	0.109	1010
			Average	1060

(a) Redissolved in dioxane and reprecipitated in ether.

(b) From the expression:

$$\text{Molecular Weight} = \frac{4.65 \times 1000 \times \text{sample wt.}}{\text{f.-p. depression} \times \text{dioxane wt.}}$$

Roberts and Bury (107) found that they could overcome difficulties with the hygroscopic nature of nitrobenzene by adding one or two grams of anhydrous sodium sulfate and a drop of water. The salt-hydrate pair of anhydrous and hydrated sodium sulfate maintained a constant vapour pressure of water in the nitrobenzene and hence a constant freezing-point. They claimed that using such a scheme no special precautions against moisture were necessary. This idea was tried with dioxane but did not result in a constant freezing-point. Probably the best method of avoiding moisture would have been the use of an all-enclosed system with a magnetically operated stirrer. Such an apparatus was unfortunately not available but the gas-sweeping method gave freezing-points (determined in triplicate at least) whose values varied not more than 0.002° from the average. The excellent checks obtained from three separate determinations of the freezing-point constant of dioxane by the use of a pure sample of naphthalene as a solute of known molecular weight, bore witness to the reliability of the method chosen. The naphthalene was a pure, recrystallized, thoroughly dried specimen, melting at 80.0° (corr.) as compared to the literature value (108) of 80.1° (corr.).

A freezing-point constant of 4.65 was obtained, in good agreement with the standard value (109). The freezing-point data are detailed in Table II.

TABLE II

FREEZING-POINT CONSTANT OF DIOXANE

Weight of Naphthalene, g. (a)	Weight of Dioxane, g.	F.-P. Depression °C.	Freezing-Point Constant (b)
0.1072	14.92	0.262	4.66°
0.1949	14.88	0.476	4.65
0.2529	14.79	0.620	4.64
		Average	4.65°

(a) Molecular weight of naphthalene, $C_{10}H_8$ was taken as 128.

(b) From expression:

$$\text{Freezing-Point Constant} = \frac{128 \times \text{f.-p. depression} \times \text{dioxane wt.}}{1000 \times \text{sample wt.}}$$

X. Calculation of Data in Wood Meal Cooks

All data were calculated on a moisture-free wood basis. Wood residues from all cooks were air-dried and analyzed in duplicate or triplicate for water. Samples were stored in air-tight containers so that the moisture content would not vary appreciably, but if storage was for more than a few weeks, the moisture content was redetermined if any fresh analyses were required. Data for rye were corrected for ash content as were the figures for the large scale maple cooks.

The extent of the reaction in the liquid ammonia cooks was followed by determining the percentage "f" of Klason lignin remaining in the residue, whose percentage yield on an oven-dry basis was denoted by "r." If the original wood contained "i"% of lignin, the percentage "l," removed in a cook was given by:

$$l = i - \frac{rf}{100}$$

The quantity (100-r-l) equalled "h," the percentage of non-Klason lignin substances (carbohydrate) simultaneously removed in the cook.

The calculation of lignin and holocellulose losses was complicated by an apparent formation of "lignin" in many of the Kolbe cooks. Methods of calculation used in the Kolbe cooks are fully dealt with in the "Discussion of Results."

B. Cooking Procedures

I. Preparation of Extractive-Free Wood Meals

All wood meals used in the preliminary small scale cooks were extracted first with benzene-ethyl alcohol (2:1) for forty-eight hours then with ethyl alcohol for twenty-four hours and finally with hot water for twenty-four hours. After being air-dried, the extracted meals were carefully screened to separate that portion of the spruce samples passing a 40 mesh and being retained on a 100 mesh sieve, and the 60 to 80 mesh fractions in the case of beech, birch and maple. For the rye straw cooks, the 20 to 100 mesh fraction, similarly extracted, was used. These fractions were kept in tightly sealed glass bottles so that their water content would remain approximately constant over long periods.

For the large scale extractions with liquid ammonia, wood meal from a freshly cut log[a] of sugar maple (Acer saccharum,

(a) Kindly supplied by Mr. A.J.Philip of the Canada Paper Co.

Marsh) was used. The log came from a tree which was about 90 years old and had been cut about 4 feet from the ground. It was solid specimen, free of rot or other imperfections, and had a length of 4 feet and a thickness of 9 inches, 4 inches of which was heartwood. The log was chipped, shredded, air-dried and then cut down in a Wiley mill until all the meal passed a 40 mesh screen. The portion passing 80 mesh was discarded and the 40 to 80 mesh portion, amounting to about 65% of the total, was saved for use.

A little over 3 kilograms of this 40 to 80 mesh maple meal was then exhaustively extracted in a large metal Soxhlet which could handle about a kilogram of meal at one time. The meal was extracted first with hot benzene-ethanol (2:1) for seventy-two hours. The greater part of the benzene left in the meal was then removed by soaking the bag containing the meal in ethanol for a few hours, then squeezing out the ethanol with the entrained benzene. This treatment was followed by hot ethanol extraction for forty-eight hours. The bag of meal was then removed from the Soxhlet and hot (55°) tap water was run through the meal for another forty-eight hours. After a four hour soaking in distilled water, the meal was air-dried for three days and vacuum-dried at 50° for another three days. The meal was then ready for the large scale liquid ammonia cook.

II. Small Scale Liquid Ammonia Cooking Procedures

The vapour pressure of liquid ammonia is about 120 pounds per square inch at 20° and 910 pounds per square inch at 100°.

All cooking with liquid ammonia was therefore done in stout steel pressure bombs.

One type of bomb, which could be used for only relatively low pressures, consisted of a 280 cc. capacity heavy stainless steel cup, and a lid with two openings, one leading to an ammonia pressure gauge reading up to 300 pounds per square inch, the other fitted with a 1/8 inch steel needle valve for filling the bomb with liquid ammonia. The lid could be tightly sealed to the rest of the bomb through a lead gasket by the customary arrangement of a bolt threaded through a stout steel yoke encompassing the entire bomb and serving as anchor for the bolt. This low-pressure bomb could not be used above 45°, and indeed was used only when the high-pressure bomb, described below, was being used in another cook.

The high-pressure bomb was likewise of relatively simple construction. A thick-walled (12 mm.) steel cup, of 130 cc. capacity, was fitted with a stout lid and a lead gasket, closure again being effected by a bolt pressing the lid and lead gasket against the main body of the bomb. The bolt was anchored however, not in a yoke, but in a heavy screw-cap, which screwed on to the steel cup, properly threaded on its outer surface so as to engage a similar thread in the inner surface of the screw-cap. The entire bomb assembly was made of stainless steel.

In all small scale cooks using the high-pressure bomb,

50 grams of ammonia and a weight of air-dried wood meal corresponding to 3.33 grams of oven-dry wood were used, to give an ammonia:dry wood ratio of 15:1. If salts were used in the cook, 2.50 grams were added to 50 grams of ammonia to give an ammonia:dry wood:salt ratio of 15.0:1.0:0.75. However, in cook No. 19, using sodium cyanide and spruce, 15 grams of the salt were used to 50 grams of liquid ammonia. Similar ratios were used in the few cooks employing the low-pressure bomb, but here about twice as much wood was used per cook, since the bomb capacity was greater.

The proper amount of air-dry wood meal, and other material, if used, were mixed together in a wide-mouthed weighing bottle and the whole cooled to about -60° in an acetone-dry ice mixture. While the meal was cooling, the high-pressure bomb was charged with commercial liquid ammonia, anhydrous grade, with a quoted analysis of ammonia 99.95%, water 0.03%. During the filling operation, the lid of the bomb and the screw-cap were replaced by a rubber stopper fitting tightly into the cup portion of the bomb, and equipped with inlet and exit glass tubes. On the bomb side the inlet tube barely projected beyond the surface of the stopper, but the corresponding end of the exit tube was so placed that, with the rubber stopper tightly in place, it was just above a point in the bomb corresponding to a volume of 70 cc., equivalent to 50 grams of ammonia measured at -60°. The entire assembly was weighed beforehand and the filling operation was carried

out in the fume cupboard. The inlet was connected by rubber tubing to the ammonia cylinder, set in position to yield the liquid when the cylinder valve was opened. A length of rubber tubing fastened to the exit tube served to carry any ammonia volatilized to the back of the fume cupboard.

The bomb was then chilled to about -60° with an acetone-dry ice mixture. The cylinder valve was opened about a quarter of a turn and liquid ammonia run into the bomb. Once about 50 grams of liquid ammonia had collected, any additional liquid ammonia passed in was forced up the exit tube where its presence was clearly visible. At this point, generally after two to three minutes, the cylinder valve was closed, the rubber tubes disconnected, the bomb removed from the dry ice bath, quickly wiped externally and weighed to the nearest gram. If the weighing showed insufficient ammonia, the bomb was quickly reconnected and more ammonia added. With the ammonia cylinder valve opened a definite amount, and with a little practice, it was quite easy to charge the bomb with 50 ± 1 gram of liquid ammonia.

The weighing bottle containing the wood meal was then taken out of the cooling bath, the rubber stopper was removed from the bomb and the contents of the weighing bottle rapidly but carefully added. The meal had to be well chilled or it caused the ammonia to boil and the resultant rush of ammonia gas would blow away some of the meal. The bomb was then sealed immediately by its lid and screw-cap. Hand tightening

was sufficient for the screw-cap but the bolt was firmly tightened with a wrench, the bomb being held in a vise. Speed was essential in these last steps to avoid the freezing of atmospheric moisture on the very cold threaded surface of the bomb. No more than twenty minutes was needed for the entire filling operation.

After stirring up the contents of the bomb by shaking for a few minutes, the bomb was tested for leaks by immersion in water at room temperature and then heated to the temperature chosen for the cook. Temperatures of 100° were maintained by placing the bomb inside an insulated can heated by steam at atmospheric pressure. For other temperatures, a thermostatically controlled oil-bath was used, care being taken that none of the oil got into the threaded portions of the bomb. Where steam had been used as the heating medium, all water within the threads of the bomb had to be removed by heating for one-half hour in a hot-air oven at 100°, before the bomb could be opened. If this precaution was not taken, the subsequent chilling to -60° froze the various parts of the bomb together and made its opening very difficult. After all the water had been driven off, the bomb was cooled, first in a brine-bath (-15°), and then in acetone-dry ice to -60°, care being taken that the upper threaded portions of the bomb did not come into contact with the cooling mixtures, so that they remained warm and thus free of ice formed from atmospheric moisture. The bomb was then removed from the cooling mixture to the vise and the bomb lid and screw-cap

removed. The lower portion of the bomb was quickly connected to the apparatus shown in Fig. 1.

This assembly was designed for a convenient separation of the meal, and its washing with liquid ammonia, in such a way that no atmospheric moisture could freeze on to the bomb contents and possibly cause complications by the alkaline action of aqueous ammonia. After being unsealed, the bomb (A) with its contents chilled to -60°, was set on the lower flange (B) and the rubber stopper (C) tightly forced into the mouth of the bomb by screwing three wing-nuts (D) on to three long bolts connecting the two flanges (B). During this time the 2-way Pyrex stopcock (E) was closed and the 2-way Pyrex stopcock (F) was open. The bomb was then immersed in acetone-dry ice mixture, thereafter kept at -60° or lower.

With the suction on, (E) was opened, (F) closed and liquid ammonia, together with some wood meal, was pushed up the glass tube (H) on to the coarse sintered glass filter (J) by nitrogen entering by the tube (K). The tube (L) provided a path for the relief of the momentary, excessive ammonia gas pressure developed when the liquid ammonia came into contact with the unchilled glass filter during the first part of the filtration. After some of the liquid ammonia had passed over, this tube was connected with a caustic soda drying tube to prevent the ingress of atmospheric moisture. Stopcock (E) was a very large size, a No. 8 Pyrex being needed to provide a bore as wide as that of the glass tube (H) so as not to be

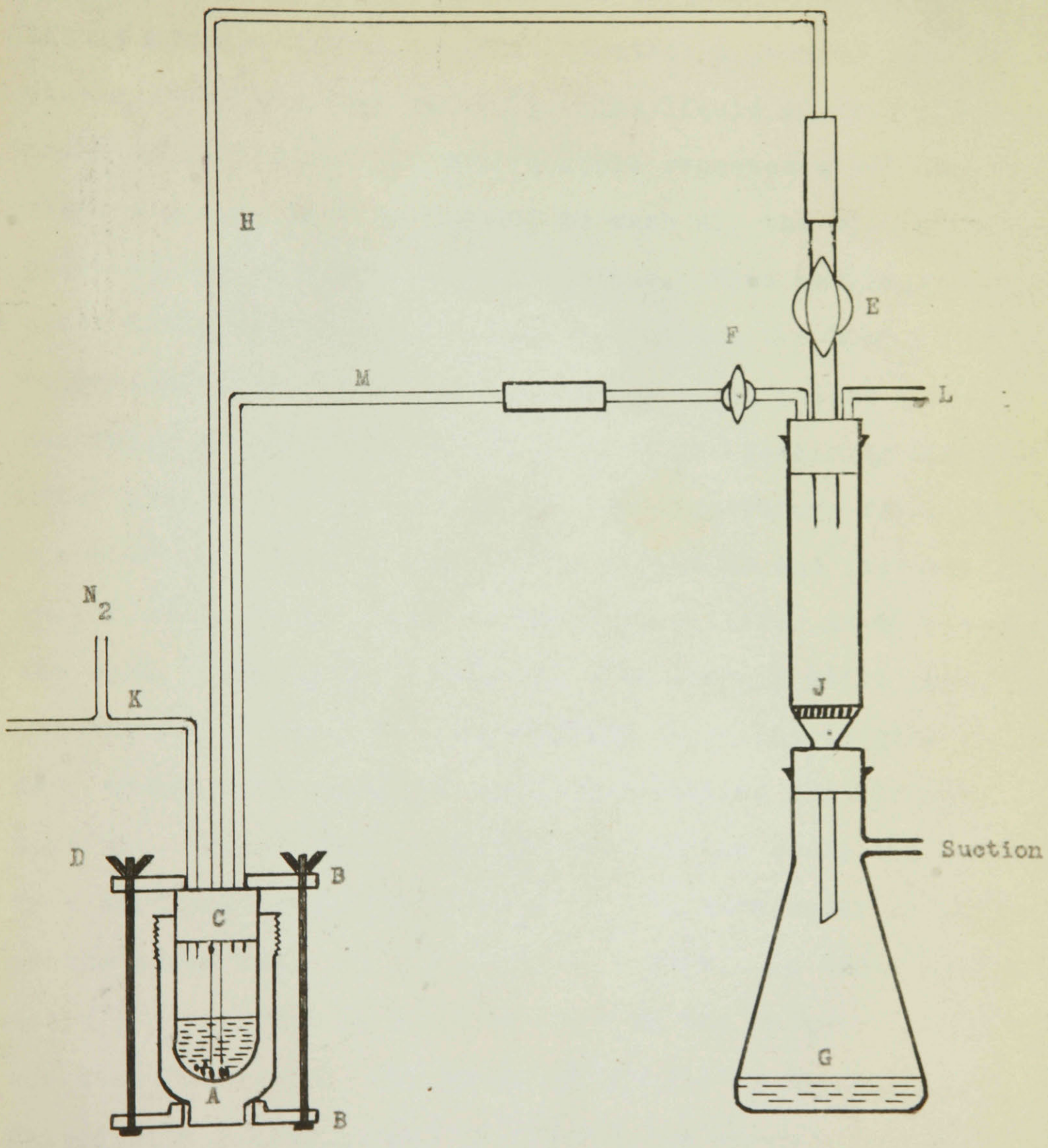


Fig. 1 - Filtration apparatus used in liquid ammonia wood meal cooks.

blocked by wood meal.

When no more liquid ammonia issued from the bomb, the nitrogen flow was cut off, (F) was opened, (E) closed and liquid ammonia run in until it started to pass up tube (M) showing that the bomb was full. The liquid ammonia was removed as before and the entire cycle repeated about six times, which was more than sufficient to wash all the liquid ammonia-solubles out of the wood meal residue. When washing was complete the freezing mixture was removed and the bomb allowed to warm to room temperature, nitrogen being passed through meanwhile to assist in the removal of the remaining ammonia. After standing about an hour at room temperature the bomb was disconnected from the rest of the apparatus and any meal left inside added to the meal in the glass filter. At this stage the wood, although no longer wet with ammonia still gave off ammonia gas. Air was sucked through overnight to give an air-dry, ammonia-free product, which was bottled for further testing. Where a salt had been used in the cook, the ammonia-free wood meal was washed thoroughly in warm water until free of the added salt and air-dried as before. In many, though not all cooks, the material soluble in the ammonia filtrate was also recovered. The mouth of the filter flask (G) was fitted with a drying tube to prevent the entry of atmospheric moisture and suction was continued until the ammonia filtrate had evaporated to a volume of 25 cc. This concentrate was poured into a small beaker, previously weighed, and when all

the ammonia had evaporated the brown semi-liquid residue was thoroughly dried to constant weight over phosphorus pentoxide. The extract was a light brown, transparent, brittle resin, which was often associated with a small amount of a white feathery crystalline material, consisting mostly of acetamide.

When the residue soluble in liquid ammonia was carefully recovered and dried in this manner, its weight was generally slightly higher than the loss in weight of the wood meal recovered from the cook. This discrepancy was caused, at least in part, by the addition of amino groups to acetyl groups of the wood.

The following procedure was used to determine the degree of swelling of spruce chips in liquid ammonia. The length, breadth and width of a dozen air-dried spruce chips were carefully measured with dividers and ruler. The chips, suitably identified by little metal tags, were placed in the low-pressure bomb which was then sealed and evacuated for six hours (to remove any air entrapped in the wood capillaries). Sufficient ammonia was then condensed into the bomb to cover the chips. With the filling valve on the bomb open to the atmosphere to allow the ammonia to boil away slowly, thus maintaining a temperature of -33°, the bomb was set in a vacuum jar, itself held in a brine-bath holding a temperature of -15°. This arrangement made it unnecessary to introduce additional liquid ammonia in order to keep the chips fully immersed. After twenty-two hours the chips were removed and

quickly measured. Except for a slight expansion the chips seemed physically unchanged. They were then allowed to stand twenty-two hours in the air when they were measured again.

A similar method was used to carry out the wood meal cooks at -33° over long periods of time.

III. Attempted Kolbe Cooking Procedure

The attempted Kolbe cooks were carried out in the small thick-walled stainless steel bomb of 130 cc. capacity, previously described. The bomb was loaded with 90 cc. of distilled water containing 4.5 grams of sodium bicarbonate, and an equal weight of air-dried extracted sprucemeal. Carbon dioxide was bubbled through the solution for about ten minutes to remove air, and a piece of dry ice, weighing 4.5 grams was then quickly dropped in, and the bomb sealed. This weight of dry ice was sufficient to give a pressure of about 400 pounds per square inch at 160°. The bomb was heated either in a steam-bath at 100° or in a thermostatically controlled oil-bath when higher temperatures were required. At the end of the heating period the bomb was cooled in water. After the pressure had been carefully released the wood meal was removed, thoroughly washed with water, recovered by filtration and air-dried.

Prehydrolysed wood samples were prepared by treating 7.5 grams of air-dried extracted spruce meal with 150 cc. of the hydrolysing medium for the time and at the temperature

indicated in Table VI. For temperatures of 100° the meal was heated under reflux with the hydrolyzing agent at atmospheric pressure. Prehydrolyses above 100° were carried out in the pressure bomb. The recovered meals were thoroughly washed with water and air-dried before use in the attempted Kolbe cooks.

IV. Large Scale Liquid Ammonia Cooking Procedure

A stainless steel bomb with a volume of 11 litres was employed. The body of the bomb consisted of a pipe, 48 inches long and 6½ inches wide, sealed at one end and threaded on the outside of the open end. This thread engaged a similar thread on the inside of a heavy top. The top carried 8 bolts which, when screwed down through the top, pressed a separate heavy metal lid against the body of the bomb to produce a tight closure. This lid was pierced by two openings connected to ¼ inch steel needle valves serving as inlet and outlet tubes for the liquid ammonia. The ends of the inlet and outlet tubes opening into the bomb were each covered by a disc of 60 mesh steel screen. The wire mesh screens prevented the loss of any wood meal during the filling or emptying of the bomb.

The bomb, charged with about one kilogram of extracted 40 to 80 mesh maple meal, was sealed, chilled to about -50° with acetone-dry ice mixture, then removed from the cooling bath and set on a heavy-capacity platform scale. The inlet was connected to a cylinder of ammonia, set in position to

deliver the liquid, and a tube was run from the exit valve on the bomb to an efficient fume cupboard. After weighing the bomb to the nearest 1/8 of a kilogram, both valves on the bomb were opened and liquid ammonia was run in, slowly at first, since the first portions of ammonia entering the bomb produced a rush of gaseous ammonia on contacting the insufficiently cooled wood meal. But this evaporation served to cool the bomb down further and the rate could soon be increased so that the bomb was charged with the required 5 kilograms of ammonia in about one hour. The course of the addition was carefully followed by repeated weighings. When the filling was completed, the ammonia cylinder was closed first, then the inlet valve and finally the outlet valve on the bomb. The bomb was warmed to room temperature with hot water and then shaken in the cradle of a hydrogenation apparatus for five hours, the temperature of the room being 25°.

At the end of five hours the bomb was removed from the shaker and placed in an inverted position on a stout tripod in the fume cupboard. The outlet valve was connected by a length of thick-walled rubber tubing to a 12-litre Pyrex flask carrying a rubber stopper fitted with glass inlet and outlet tubes, the inlet tube projecting several inches further into the flask than the outlet tube. The exit valve on the bomb was then carefully opened, whereupon the pressure of the ammonia forced the liquid ammonia extract into the flask. The rate of flow was carefully controlled by the exit valve on

the bomb, so that the flow was never fast enough to blow any liquid ammonia extract out of the receiver. Within ten minutes the steady flow of ammonia had dropped to a slow drip. The exit valve was then closed and the bomb allowed to stand for fifteen minutes to allow the ammonia to drip down and collect in the bottom, after which this additional ammonia was run off as before. The bomb was then disconnected from the flask and removed to the scale. Usually about 1.25 kilograms of ammonia remained so that about one-quarter of the material extracted by the ammonia was still in the bomb. To remove this fraction, another 2.5 kilograms of ammonia was added, the bomb was shaken for one-half hour and the liquid ammonia run out again, the procedure already described being followed. The washing with fresh ammonia was repeated with another 2.5 kilograms of ammonia so that finally less than an estimated 5% of the total material extracted by the ammonia remained behind in the bomb with the wood meal. The wood meal was then removed, allowed to stand in the air until all the ammonia evaporated and was stored, without further treatment, in covered metal tins for possible future work. The entire liquid ammonia extract from successive cooks was run into the same 12-litre receiver, which was protected against atmospheric moisture and carbon dioxide by connecting the inlet and outlet tubes to a tube of sodium hydroxide pellets. The ammonia was then allowed to evaporate off freely at room temperature, the greater part of the liquid ammonia collected

from a single cook evaporating in about a day, leaving ample space for the addition of ammonia from succeeding cooks.

Analysis of Maple Meal:

	Before Ammonia	After Ammonia
Water %	1.66, 1.63, 1.62	3.84, 3.93, 3.85
Ash	0.36, 0.35	0.37, 0.36
Klason lignin (ash-free basis)	23.42, 23.73, 23.37	22.78, 23.07, 23.38
Acetyl	4.8	1.4, 1.4
Nitrogen	0.084, 0.083	0.461, 0.458

On an oven-dry basis, 943.1 grams of meal was recovered for every 1000 grams of initial wood meal, a loss of 5.69%. A total of 3085 grams of wood was extracted, 5.69% of which amounted to 175.5 grams of material.

C. Preliminary Fractionation of Liquid Ammonia Extract

When the major portion of the ammonia had boiled away from the ammonia-extract described above, the thick reddish-brown crystal-studded fluid residue was transferred to a smaller flask and subjected to a vacuum distillation at about 50° to remove the remainder. From 3085 grams (oven-dry weight basis) of wood meal, 210 grams of extract containing only traces of ammonia and water (the latter from the wood meal) was obtained.

I. Fraction I - Polysaccharide

Synthetic methanol, 6 litres, was added to the 210 grams of extract and the whole stirred vigorously to assist dispersion. A fine light brown precipitate was immediately thrown out by the methanol. The entire methanol suspension

was then filtered through a sintered glass crucible to separate the precipitate, which was polysaccharide in nature and was labelled Fraction I. To the clear filtrate an additional 4 litres of synthetic methanol was added and the whole set in a cold box at -5° overnight. A small additional amount of Fraction I separated together with a larger amount of a darker brown resinous material that stuck to the bottom of the flask. The entire methanol extract was decanted and filtered to recover the addition to Fraction I, which could easily be scraped off the walls of the container into methanol suspension, the darker resinous material staying behind during the decantation. The whole of Fraction I was then washed carefully with 1 litre of methanol, the methanol washings being added to the main body of the clear methanol filtrate, and was dried by solvent exchange through a light petroleum ether (30°-50°). The yield of this fraction, finally dried *in vacuo* over phosphorus pentoxide, was 26.9 grams, corresponding to 0.87% of the original wood weight.

Analysis: Klason lignin, 24.6, 23.6; Methoxyl, 6.06, 6.19; N, 0.60%.

II. Fraction II - Lignin

The dark brown resinous material thrown out by the methanol was washed with several portions of absolute methanol which were added to the main body of the methanol filtrate. When the methanol had evaporated, Fraction II lost its original tacky nature and became a brittle brown mass which could

easily be removed from the flask. This fraction contained appreciable amounts of admixed polysaccharide (Fraction I). The yield of the thoroughly dried product was 3.5 grams, which was about 0.11% of the original wood.

Analysis: Klason lignin, 74.0; Methoxyl, 20.3; N, 0.64%.

III. Fraction III - Acetamide.

The entire methanol filtrate left after the isolation of Fraction I and Fraction II was subjected to a vacuum distillation at 50° until all the methanol had been removed. Diethyl ether, 5 litres, was then added to the oily residue and the mixture was heated under reflux for one-half hour so as to saturate the ether with acetamide, which was the only ether-soluble component present in the residue. The clear, slightly yellow ether solution was decanted and cooled to -15° for fifteen minutes, whereupon acetamide crystallized and was separated by filtration. The ether filtrate was poured back into the flask containing the residue being extracted and the whole cycle of heating on reflux, cooling and filtration was repeated. After a dozen such treatments the greater part of the acetamide, though not quite all, had been removed. The acetamide was surprisingly pure and melted at 80.4°-80.6° (corr.) compared to 81.1° (corr.) for a genuine sample, the determination being made in the same melting point apparatus. Admixture with an authentic sample did not depress the melting point. The yield of 123.5 grams corresponded to 4.0% of the original wood, but included the added amide groupings which originated not from the wood but from the ammonia. Calculated

on the basis of acetyl the yield corresponded to 2.92% of the wood weight.

Analysis: Calculated for CH_3CONH_2: N, 23.7. Found: N, 23.5%.

IV. Fractions IV and IVA - Lignin

The thick oily residue from the ether extraction, which still contained some acetamide, was redissolved with some difficulty in 1 litre of absolute methanol. After filtration to remove small bits of foreign material, the solution was poured, with stirring, into 3 litres of distilled water. A powdery yellow-colored material, Fraction IV, was precipitated by the water. Besides this precipitate, a thick blob of a brown oil came out of the methanol and floated on the surface of the solution. This oil soon hardened to a crust, Fraction IVA, most of which could easily be picked out mechanically and thus separated from the powdery precipitate which settled on the bottom of the receiver after a few hours standing. Fraction IVA was crushed and well washed with water to remove adherent methanol and acetamide, the water being removed by centrifuging. The yield, after drying over phosphorus pentoxide, was 4.0 grams, or 0.13% of the wood.

Analysis of Fraction IVA: Klason lignin, 84.5; Methoxyl, 20.9; N, 0.58%.

The methanol-water suspension of Fraction IV (containing minor amounts of Fraction IVA) was subjected to a distillation at 50° under vacuum until the greater part of the methanol had been driven off, when an additional amount of Fraction IV came

out of solution. This precipitate was separated and well washed with water to remove all traces of entrained acetamide, the centrifuge being used to recover the precipitate. The ammonia-lignin, Fraction IV, was finally dried over phosphorus pentoxide, the yield being 14.8 grams, or 0.48% of the original wood.

Analysis of Fraction IV: Klason lignin, 91.2, 89.2; Methoxyl, 21.8, 21.0; N, 0.52%.

The aqueous solution from Fractions IV and IVA was evaporated down to dryness at 50° using reduced pressure. The dry dark brown semi-crystalline residue weighing 16 grams had a considerable acetamide content but was not further fractionated.

The flow sheet for the preliminary fractionation together with yields of the liquid ammonia extract is given in Fig. 2, while analytical data for the various fractions are summarized in Table XII.

V. Re-Extraction of Wood Residue

One kilogram of the meal, recovered from the first five-hour extraction, with liquid ammonia, was re-extracted with five parts of ammonia for another twenty-five hours. The residue was recovered in the manner described, and on evaporation of the extract a thick, dark-brown oil was left. This oil was washed with a little methanol to remove a trace of acetamide and, after drying, was recovered as a dark-brown brittle resin. A yield of 0.54% on a wood basis showed that

the original extraction of the wood had been nearly complete.

Analysis: Klason lignin, 71.5, 72.9; Methoxyl, 19.13, 19.15; N, 0.73%.

D. Purification of Fractions from Liquid Ammonia Extract

I. Purification of Liquid Ammonia Lignin - Fraction IV

The crude ammonia lignin contained appreciable amounts of impurities and was therefore purified by extraction with methanol and reprecipitation of the dissolved lignin with water, followed by solution in dioxane, and reprecipitation with diethyl ether. The resultant lignin was finally split into four fractions by fractional precipitation from dioxane-ether solution.

1. Purification from Absolute Methanol

In a preliminary experiment designed to determine quantitatively the action of methanol on Fraction IV, 1.25 grams of the crude ammonia lignin was shaken vigorously with 30 cc. of absolute methanol for one-half hour. The methanol solution was then decanted and the amount of lignin left undissolved determined, adherent methanol being evaporated by a three hour exposure to a water pump vacuum at room temperature. The procedure was repeated with three additional 30 cc. portions of absolute methanol, the shaking time being progressively lengthened. It was found that 52.0% dissolved in the first half hour, 13.6% in the next one and one-half hours, 8.8% in the next fourteen hours and 7.2% in the final extraction

period of fourteen hours. Thus, after thirty hours, 18.4% of the original crude lignin remained undissolved. The high methoxyl content, 19.9%, of the residue, indicated that the insoluble fraction was almost entirely lignin. There was a small but definite difference between the methoxyl content of the first fraction, obtained in one-half hour, and the last fraction, obtained in the final fourteen hour extraction, the values being 21.7% and 21.2% respectively. The difference was not considered sufficient to justify separation and individual consideration in this preliminary study of ammonia lignin.

The procedure finally adopted for the extraction of 6 grams of crude ammonia lignin with absolute methanol was as follows: 75 cc. of methanol for ten minutes, 50 cc. for twenty minutes, 50 cc. for one and one-half hours, 50 cc. for twenty-two hours and 50 cc. for forty-eight hours. The results closely parallelled those of the preliminary experiment, 1.36 grams (22.6%) of the lignin remaining undissolved after a total extraction period of seventy-two hours.

Initially all five methanol extracts showed a faint turbidity and in a few hours a thin brown layer of resinous material settled out on the wall of the container. These small portions of resinous material were later added to the material insoluble in methanol after seventy-two hours. Each of the clear amber-colored methanol extracts was separately poured with stirring into two volumes of water. Some of the lignin separated as a very light, faintly yellow, precipitate

which remained in suspension, while another portion of the lignin, especially in the first and more concentrated methanol solutions, appeared as a slightly darker and denser material, resinous at first, but soon hardening to a crumbly solid. This latter material tended to stick to the walls of the container or float about as discrete, easily separable, pieces. The larger pieces were picked out mechanically and the smaller fragments were removed by alternate settling and decantation, since they tended to stick to the container. This denser form of the ammonia lignin from all five methanol extracts was combined, washed well with water to remove methanol, and dried overnight in a vacuum oven at 55°. The yield was 1.13 grams.

The less dense form was easily separated from its suspension in methanol-water by centrifuging and a further amount was obtained by distilling the methanol from the turbid mother liquors at 50° under reduced pressure. The combined precipitates were well washed with several portions of water and dried overnight in a vacuum oven at 55°. An additional 0.08 grams of methanol-soluble lignin was obtained by a further seventy-two hour extraction of the 22.6% of methanol-insoluble lignin. The total yield was 3.54 grams or 59%.

2. Purification from Dioxane

Both the lighter and denser lignin fractions mentioned above dissolved quickly and completely in anhydrous dioxane to give clear red-brown solutions. Since there seemed to be

no difference in the color of the two solutions made up in equivalent concentrations, the two dioxane solutions were combined and treated as one thereafter. The solution was filtered to remove a trace of foreign matter and enough dioxane was added to make the concentration 1%. This dilute solution was poured drop by drop into three times its volume of absolute diethyl ether, the ether being rapidly stirred throughout the precipitation. A very finely divided flocculent pale buff-colored precipitate separated and was recovered by use of the centrifuge. When the supernatant ether-dioxane solution was being poured off after a period of centrifuging, it was important to add fresh ether-dioxane or ether immediately, since otherwise the precipitate quickly lost its ether content, by evaporation, thereby retaining a sufficient concentration of dioxane to cause softening into an undesirable oily form. The precipitate was washed with three 200 cc. portions of diethyl ether to remove dioxane and finally with three 200 cc. portions of purified petroleum ether (30°-50°) to remove the polar solvent, diethyl ether. After drying for twenty-four hours in a vacuum oven at 55°, the yield was 3.00 grams or 50% of the original crude Fraction IV.

Analysis: Klason lignin, 88.8; Methoxyl, 21.6, 21.6, 21.6; C, 58.83, 58.96, 58.87, 58.83, 59.03; H, 6.52, 6.55, 6.46, 6.45, 6.46; N, $<0.01\%$

3. Fractionation with Dioxane-Ether

Liquid ammonia lignin (Fraction IV), 2.054 grams, which had been put through the methanol and the dioxane purification, was dissolved in 45 cc. of pure anhydrous dioxane contained in a 200 cc. centrifuge cup. Purified chilled diethyl ether, 7 cc., was then added drop by drop, the solution being vigorously stirred throughout the addition. The precipitate, given the designation Fraction IV-I, was centrifuged out and the supernatant liquor, containing about 10% of ether by weight, was decanted into a fresh centrifuge cup and an additional portion of ether, 9 cc., sufficient to bring the concentration of ether in the ether-dioxane solution to about 20%, was added. The precipitate, Fraction IV-2 was separated as before. Fraction IV-3 was obtained in a similar manner from a solution finally containing about 40% by weight of ether and Fraction IV-4 on flooding the mother liquor with diethyl ether, sufficient in amount to bring the ether content to about 85% by weight.

Each of the fractions was redissolved in 60 cc. of dioxane, filtered, and reprecipitated by slow addition with stirring to two volumes of ether. The resultant precipitates were washed well with diethyl ether, then with a light petroleum ether (30°-50°) and thoroughly dried over phosphorus pentoxide at 50°. All the fractions were very light-colored highly electrostatic powders, although Fraction IV-1 was perceptibly darker than the rest. The yields of these fractions, together with analytical data are given in Table III.

TABLE III

FRACTIONATION OF METHANOL-SOLUBLE FRACTION IV WITH DIOXANE-ETHER

Fraction No.	% (a) Yield	% By Weight of Ether in Mother Liquor	Cryoscopic Molecular Weight	% Methoxyl		% C			% H		
				1	2	1	2	3	1	2	3
IV - 1	6.3	10	...	19.8	19.6	57.90	...		6.74	...	...
IV - 2	29.0	20	900 1350(b)	21.6	...	57.65	58.09		6.38	6.41	...
IV - 3	46.2	40	1140	21.6	...	58.22	...		6.37	...	...
IV - 4	15.3	85	1010	21.8	21.8	58.32	58.57	58.59	6.38	6.32	6.53

(a) Based on methanol-soluble Fraction IV.

(b) Redissolved in dioxane and reprecipitated with ether.

4. Fractionation of Methanol-Insoluble Liquid Ammonia Lignin - Fraction IV

As described above, 22.6% of the crude ammonia lignin was insoluble in absolute methanol even after seventy-two hours. The major portion dissolved quickly in dioxane, but there was a distinct amount of insoluble material and the solution was darker in color than the equivalent dioxane solutions of the lignin which dissolved in the methanol. The dioxane solution was filtered through a sintered glass crucible and the retained precipitate washed with dioxane and ether and then dried. The weight of the methanol-insoluble, dioxane-insoluble Fraction IV, was 0.20 grams. An additional amount of a similar material was also obtained later, as described below.

The lignin in the clear dioxane filtrate was precipitated in diethyl ether, solvent exchanged and dried as previously described for the methanol-soluble lignin. Yield, 1.06 grams or 17.7% of the crude weight of the ammonia lignin, and methoxyl, 20.84%. This product was further fractionated by a repetition of the entire procedure used in its isolation - a seventy-two hour extraction with methanol at room temperature, followed by extraction of the methanol-insoluble residue with dioxane.

The results were as follows: 0.08 grams dissolved in the methanol, and was added to the main methanol-soluble portion; of the undissolved residue, 0.21 grams failed also to dissolve in dioxane; the total yield of methanol-insoluble, dioxane-

insoluble lignin was therefore 0.41 grams or 6.8% of the 6.0 grams of crude Fraction IV originally put into the purification. The remainder of the methanol-insoluble Fraction IV was soluble in dioxane and was recovered by precipitation with ether in the usual way. Yield, 0.70 grams or 11.7%. Of the two methanol-insoluble fractions only the dioxane-soluble one was analysed.

Analysis: Klason lignin, 86.4; Methoxyl, 21.0, 21.2; C, 58.48, 58.33; H, 6.89, 6.75%.

The complete fractionation procedure, together with yields, of Fraction IV is outlined in Fig. 3.

II. Purification of Liquid Ammonia Lignin - Fraction IVA

The sample, 3.65 grams, was dissolved in 250 cc. of dioxane and the red-brown solution was filtered to get rid of a trace of insoluble material which was discarded. The clear filtrate was poured, drop by drop, into 750 cc. of diethyl ether which was vigorously stirred throughout the precipitation. A very light-colored precipitate was separated by use of the centrifuge and washed with two 250 cc. portions of ether and then with two 250 cc. portions of petroleum ether (30°-50°). The lignin was finally dried over phosphorus pentoxide under reduced pressure at 50°. The yield was 3.18 grams, with methoxyl, 21.3, 20.9%.

Absolute methanol, 50 cc., was now added to 3.07 grams of this sample, and as in the case of Fraction IV, the lig-

nin was changed immediately to a thick brown resin. After shaking this resin with methanol for twenty-four hours, the reddish-brown methanol solution was decanted, 50 cc. of fresh methanol was added and the procedure was repeated. At the end of forty-eight hours the methanol was replaced by a fresh 50 cc. portion. The total extraction period was seventy-two hours.

The residue from the combined methanol extracts was dissolved in dioxane and the amber-colored solution filtered to remove a very small amount of extraneous substance. The lignin was precipitated from dioxane solution by the technique, already described, of pouring into diethyl ether, and the solid was washed first with diethyl ether and then with a light petroleum ether (30°-50°). After drying at 50° over phosphorus pentoxide at reduced pressure, the yield was 1.73 grams, corresponding to 49.1% of the original crude Fraction IVA.

Analysis: Klason lignin, 87.4, 87.9; Methoxyl, 20.7, 20.7, N, 0.32%.

That portion of Fraction IVA, which did not dissolve in methanol after seventy-two hours, was freed of methanol under reduced pressure, and to the resulting 1.20 grams of reddish-brown resin, 100 cc. of dioxane was added. A substantial amount of the methanol-insoluble lignin did not dissolve in the dioxane even after long shaking and this methanol-insoluble, dioxane-insoluble dark-brown gummy precipitate was recovered by

filtration, washed with dioxane and then with ethyl ether. It was rather difficult to wash the precipitate free of dioxane, since it formed a rubbery mass on the sintered glass filter and was almost impervious to the ethyl ether. The yield of vacuum and phosphorus pentoxide-dried product was 0.20 grams, or 5.7% of the original crude lignin Fraction IVA.

The dioxane-soluble portion of methanol-insoluble Fraction IVA was precipitated in diethyl ether, washed with ethyl ether and petroleum ether and dried at 50° in a manner already described. The yield was 0.93 grams or 26.4%.

Analysis: Methoxyl, 20.5, 21.1%.

A summary of the fractionation of Fraction IVA is given in Fig. 4.

III. Purification of Liquid Ammonia Lignin - Fraction II

Before the use of dioxane and methanol had been decided upon as the method for purifying liquid ammonia lignin, all of Fraction II had been treated with 1% sodium hydroxide, a procedure which had no advantages over the dioxane technique and indeed raised the possibility of alteration of the chemical properties of the lignin. Fraction II dissolved completely in 1% sodium hydroxide on shaking overnight. The lignin was recovered by acidification with hydrochloric acid followed by centrifuging and thorough washing with water. To 3.02 grams of the dry lignin, 200 cc. of dioxane was added and the whole was shaken overnight. A considerable amount did not dissolve and was separated by filtration, washed with

dioxane and diethyl ether and dried at 50° under reduced pressure. The yield of this dark brown amorphous substance was 1.74 grams. An additional amount of this material was also isolated later in the fractionation.

Analysis: Klason lignin, 79.8, 79.9; Methoxyl, 21.7, 21.3; C, 58.51, 58.79; H, 6.90, 7.00%.

The dioxane-soluble portion was precipitated with diethyl ether, washed with diethyl ether and petroleum ether and dried in the usual way, the 1.21 grams recovered being then shaken with 50 cc. of absolute methanol for seventy-two hours. As in the case of the other fractions of lignin isolated, methanol immediately resinified Fraction II. The methanol extract was evaporated to dryness under reduced pressure at room temperature, the residue was redissolved in dioxane and the lignin was recovered by precipitation with diethyl ether, washed with diethyl ether and a light petroleum ether and dried under reduced pressure at 50° over phosphorus pentoxide. The yield was 0.15 grams or 5.0% of the crude weight of Fraction II; the methoxyl content was 20.5, 20.3%.

That portion of Fraction II which did not dissolve in methanol was dried under reduced pressure and was then shaken with 100 cc. of dioxane but only a portion dissolved. Further treatment was exactly like that given the corresponding products obtained from Fraction IVA. The yield of methanol-insoluble dioxane-soluble Fraction II was 0.79 grams or 26.2%;

the methoxyl content was 21.3, 21.2%.

The yield of methanol-insoluble and dioxane-insoluble Fraction II, obtained at this point, was 0.23 grams. This amount was added to the main bulk of the dioxane-insoluble material to make a total yield of 1.97 grams or 65.2%. A summary of the fractionation of Fraction II can be found in Fig. 5.

IV. Purification of Fraction I - Polysaccharide

The polysaccharide fraction was insoluble in the following solvents whether cold or boiling; dioxane, ethyl acetate, toluene, water, ethanol and carbon tetrachloride. It was, however, partially soluble in anhydrous pyridine and this solvent was chosen for its further purification.

Fraction I, 0.66 grams, was shaken for one-half hour with each of 6 successive 30 cc. portions of anhydrous pyridine. The pyridine immediately turned yellow. Even after these six treatments a small amount of Fraction I was going into solution, but at a very slow rate. The various pyridine extracts were combined and filtered, both the filtrate and undissolved polysaccharide being saved. The undissolved portion was washed free of adherent pyridine with 300 cc. of diethyl ether, which, on addition to the pyridine filtrate, precipitated the pyridine-soluble material as yellow curds. This precipitate was separated by filtration and washed with ether. Both the pyridine-soluble and pyridine-insoluble fractions were dried to constant weight in vacuo.

Some 16% of the crude polysaccharide had dissolved in the pyridine and was recovered almost quantitatively (0.105 grams) by the precipitation with ether.

Analysis of Pyridine-Insoluble Fraction I: Klason lignin, 18.0; Methoxyl, 4.32, 4.48; Pentosan, 7.8, 5.9; C, 43.70, 43.77; H, 6.95, 6.79%.

Analysis of Pyridine-Soluble Fraction I: Methoxyl, 16.2, 16.2; C, 54.9; H, 6.41%.

E. Some Reactions of Liquid Ammonia Lignin

I. Methylation

Liquid ammonia lignin, Fraction IV, 0.60 grams, was dissolved in 35 cc. of pure dioxane held in a 3-necked flask. Dimethyl sulfate, 8 cc., and 35% aqueous sodium hydroxide, 12 cc. were then added drop by drop over a period of about twenty minutes, the solution being vigorously stirred throughout the addition. Two layers were formed but vigorous agitation dispersed these into a fine emulsion. The mixture was stirred for an additional two hours and then boiled for about one-half hour. The reaction mixture was acidified with dilute sulfuric acid, poured into 500 cc. of water and the very light-colored precipitate of methylated lignin recovered by means of the centrifuge and washed with water.

The lignin was then remethylated by the same procedure with the exception that the use of dioxane was abandoned in favor of 35 cc. of 1% aqueous sodium hydroxide, which failed

to dissolve the sample. After six methylations in all, the product was recovered and dried in vacuo. Small samples were removed at the end of the second and of the fifth methylation. Making allowance for these samples the yield was 0.41 grams.

Analysis: Second methylation; Methoxyl, 32.6, 32.6
Fifth methylation; Methoxyl, 34.4, 34.6
Sixth methylation; Methoxyl, 34.6, 34.8; C, 60.99, 61.13; H, 6.96, 6.94%.

II. Methanolysis

Liquid ammonia lignin, Fraction IV, 0.318 gram, was heated under reflux with 25 cc. of anhydrous methanol containing 2% of hydrogen chloride for forty-eight hours. The reflux condenser was protected by a drying tube to prevent the access of moisture and before the start of the methanolysis the apparatus was swept free of air with dry nitrogen. A slow stream of dry nitrogen was passed through the apparatus during the entire reflux period.

The liquid ammonia lignin soon dissolved almost completely, forming a deep brownish-red solution. At the conclusion of the reflux period the small amount of insoluble material was removed by filtration, and washed with a little methanol. The dry weight of this solid was only 0.006 gram or 1.9% of the original. Sodium bicarbonate was added to the filtrate and the now neutral methanol solution, 40 cc., was poured, with stirring, into 400 cc. of water. A chocolate-

brown precipitate of methanol lignin was thrown out. The yield, dried *in vacuo*, was 0.168 gram or 53% of the original sample weight.

Analysis: Methoxyl, 28.4, 28.8%.

III. Sulfite Cook

The cooking liquor, containing 1% combined and 4.7% total sulfur dioxide, was prepared by adding 0.87 grams of calcium oxide to 100 cc. of water, then bubbling sulfur dioxide through the solution until the weight of the solution had increased by about 5 grams. The weight of calcium oxide used ensured 1% combined sulfur dioxide. The total sulfur dioxide percentage was checked by titrating 1 cc. of the sulfite liquor with 0.1N iodine and then applying the formula:

$$\text{Total } SO_2\% = \frac{100 \times \text{cc. } I_2 \text{ titre} \times N \text{ of } I_2 \times 32}{1000}$$

The small high-pressure bomb, previously described, but fitted with a glass liner, was used for the sulfite cooking experiments. Liquid ammonia lignin, Fraction IV, about 0.1 gram, was placed in the glass liner and 20 cc. of the sulfite liquor was added. The bomb was then sealed and heated for the required period at 130° in a thermostatically controlled oil-bath, after which the lignin residue was separated by filtration, thoroughly washed with water and dried *in vacuo* to constant weight.

The lignin did not darken in color but the original powdery form became agglomerated to a few large amorphous

pieces during the sulfite cook. Only slightly more than one-third of the lignin dissolved both with a six and a fourteen hour treatment at 130° with the sulfite liquor, the loss in weight being 39% and 37% respectively.

IV. Periodate Oxidation of Liquid Ammonia Lignin

The solution of trisodium paraperiodate, $NaIO_4.2NaOH$, was prepared by shaking the salt (a quantity slightly in excess of the calculated amount for the desired percentage) in water acidified with acetic acid, and was buffered to a pH of about 4.0 by the addition of glacial acetic acid. Even after filtration through the finest sintered glass (paper filters must be avoided) additional solid settled out of the solution when it was allowed to stand overnight, the solution apparently being supersaturated. The solution was again filtered and the clear filtrate used in the oxidations.

In a typical periodate oxidation, 2 cc. of periodate solution was added to 60 to 100 milligrams, carefully weighed, of methanol-soluble liquid ammonia lignin (Fraction IV) contained in a 5 cc. weighing bottle. The temperature was kept at about 25° and the weighing bottle was occasionally shaken during the course of the oxidation. When the required time had elapsed the unconsumed periodate was estimated by the arsenite-iodine titration method of Fleury and Lange (110).

Very little of the lignin disappeared in any of the periodate oxidations carried out. When the titration was

complete, the undissolved lignin was filtered and washed free of periodate and iodate with water. Without being dried, the recovered lignin was heated under reflux with distilled water for three hours in accordance with Ritchie's procedure for the hydrolysis following periodate oxidation of wood. Three short runs, with oxidation periods of two, twenty-four and seventy-two hours, and one long run, involving three cycles of oxidation and hydrolysis were made. The analytical data are given in Table IV.

TABLE IV

PERIODATE OXIDATION OF LIQUID AMMONIA LIGNIN - FRACTION IV

Sample Weight, g.	Time Hours	Periodate Strength, %	Titre of 0.1N I_2	$Na_3H_2IO_6$ Consumed[a] per Gram of Sample		Recovered Lignin after Hydrolysis[d]	
				g.	Moles x 10^3	g.	%
Blank	...	4.0	4.63	...	...	...	...
0.0622	2	4.0	6.10	0.348	1.19	...	...
0.0586	24	4.0	7.10	0.620	2.11	...	...
0.0564	72	4.0	8.18	0.925	3.15	0.0360	63.8
Blank	...	2.4	7.20	...	...	...	...
0.1126[b]	60	2.4	10.00[c]	0.366	1.24	...	...
	60	2.4	8.90	0.222	0.76	0.0686	61.1
	60	2.4	8.85	0.215	0.73	0.0590	52.4

(a) All oxidations at 25° with 2 cc. of periodate solution of pH 4.0 - 4.1.

(b) Three cycles of oxidation and hydrolysis.

(c) Represents total consumption of periodate present.

(d) With boiling water after the oxidation was over.

DISCUSSION OF RESULTS

A. Attempted Kolbe Pulping

Since it is generally accepted that lignin is a polymerization product of polyfunctional phenolic bodies, it might reasonably be expected that lignin would undergo the Kolbe reaction when treated with carbon dioxide and aqueous sodium bicarbonate under pressure. If lignin did undergo the Kolbe reaction, it would gain carboxyl groups and hence probably solubility in mild alkali. This possibility was investigated by a series of cooks using spruce wood. The degree of pulping was followed by determining the total loss in wood weight, and the change in Klason lignin and methoxyl content. The results of these cooks are given in Tables V and VI. It will be noticed that in several cases the lignin content of the wood residue from a cook, calculated as a percentage of the original wood, is greater than the lignin content of the initial wood. In cook No. 5, for example, the percentage of lignin recovered (column 10) in the residue is 30.9%, which is 2.4% higher than the original lignin content of 28.5%. The percentage loss of lignin, calculated from the Klason lignin, is therefore entered as a minus value, -2.4, in column 14. All such anomalous results are underlined.

Of course no true lignin can be formed during the cook, unless we accept Hilpert's view that lignin is merely the result of acid action on a sensitive carbohydrate component of wood. While Hilpert's opinions regarding the nature of lignin(3) need

not be accepted, his observation that such materials as arabinose, xylose, glucose, mannose and fructose give insoluble humin-like residues when treated with 72% sulfuric acid at room temperature for two days (111), may explain the artificially high Klason lignin values obtained in the present study, since undoubtedly a substantial amount of free sugars are formed during the cook by hydrolysis of carbohydrate material.

Corey, Calhoun and Maass (112) reported that when spruce meal was heated in water at a buffered pH of 3, at 140°, the Klason lignin in the residue, expressed as a percentage of the original wood increased from 26.3% to 28.6% in ninety-six hours. Aronovsky (113) found similarly that when jack pine was cooked with water at 170° or higher, the sum of the lignin contents of the residual wood and liquors was greater than the amount of lignin in the original wood. Thus twelve hours heating at 170° produced an apparent increase in the total lignin of 6.5%. The apparent increase in the lignin content of the spruce during the Kolbe cooks was probably of a similar nature.

An approximate estimate of the lignin removed by the Kolbe conditions in cook Nos. 2 - 4 on non-prehydrolysed spruce meal (Table V) was made by following the change in methoxyl content. Most of the methoxyl groups in wood are associated with lignin but determinations of the methoxyl value of various holocellulose preparations have shown that about 10% - 16% of the total methoxyl content is associated with the carbohydrate

portion of wood (114)(115). However, there was no basis available for determining how much of the methoxyl loss in any particular Kolbe cook should be assigned to holocellulose loss and how much to lignin loss. It was therefore assumed, for purposes of approximate calculation, that the entire methoxyl was associated with lignin. The method of calculating the lignin loss on this basis is best illustrated by a specific case, e.g. cook No. 2 in Table V. The spruce meal used had a methoxyl content of 4.71% and a Klason lignin content of 28.5%. One part of methoxyl was therefore equivalent to (28.5/4.71) or 6.05 parts of lignin. In No. 2, 16.6% of the wood was lost during the cook (column 9). The residue had a Klason lignin content of 29.1% (column 10) and a methoxyl content.of 4.17% (column 11) both expressed as a percentage of the original wood. This methoxyl content corresponded to (4.17 x 6.05) or 25.2% of lignin and this value was entered in column 12. The loss of lignin during a cook was therefore (28.5 - 25.2) or 3.3% (column 13). In contrast, the loss of lignin calculated from the Klason lignin determination shown in column 10 was (28.5 - 29.1) or -0.6%, entered in column 14; that is 0.6% of "lignin" had been created during the cook. The holocellulose loss was then calculated by subtracting the lignin loss, obtained by the methoxyl method, from the total loss in wood weight shown in column 9. In this particular case the holocellulose loss calculated by the methoxyl method was (16.6 - 3.3) or 13.3%, entered in column 15. Values of holocellulose loss based on Klason lignin could of course not

be calculated where there was an apparent increase in the Klason lignin. Calculations of the lignin removed in a Kolbe cook based on methoxyl values represented maximum values but they gave a better picture than the values based on the Klason lignin determination.

Table V shows the results obtained from spruce meal, previously extracted with ethanol : benzene, ethanol, and water, when cooked with aqueous sodium bicarbonate-carbon dioxide in a sealed bomb under pressure. The water : wood : carbon dioxide : bicarbonate ratio was kept at about 20:1:1:1, the pressure of carbon dioxide in the bomb ranging from 215 to 400 pounds per square inch. Except for cook No. 1 a heating period of twenty-four hours at about 160° was used.

Contrary to expectation, not more than about 3%-4% of lignin was removed, though the loss of carbohydrate material was high, 16.6%-23.3%. Spruce meal treated with water alone at 160° for twenty-four hours showed a similar behaviour, with a low loss of lignin and an even higher loss in carbohydrate substance, 25% in Nos. 4 and 5. These results made it clear that the spruce wood lignin was not undergoing a Kolbe reaction. The action of the aqueous carbon dioxide-bicarbonate solution was only one of aqueous acid prehydrolysis. It has been shown that when wood is digested with water, acids such as formic and acetic are released (46), and that these acids catalyze hydrolytic attack, pentosans being especially sensitive (113). The fact that water alone removed more of the wood substance than the carbon dioxide-bicarbonate solution showed that the bicarbonate

TABLE V

ATTEMPTED KOLBE COOKS WITH SPRUCE MEAL[a]

Cook No.	Bomb Charge: Ratio by Weight H_2O	Wood	CO_2	$NaHCO_3$	Cooking Conditions: Temp. °C.	Time hrs.	CO_2 press. lbs.sq.in.	Analyses - Found[b]: % Loss in wood weight	% in Residue K.L.	MeO	Analytical Data - Calculated (b): % Lignin in Residue calc. from MeO	Lignin % Loss from (e) MeO	Lignin % Loss from (f) K.L.	Holocell.% Loss from (g) MeO	Holocell.% Loss from (h) K.L.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
1	20	1.0	1.0	1.0	100	20	265	slight	26.5[d]	...	...	...	...	...	...
2	20	1.0	0.7	1.0	158	24	215	16.6	29.1	4.17	25.2	3.3	-0.6	13.3	...
3	20	1.0	1.0	1.0	163	24	400	23.3	26.2	4.00	24.2	4.3	2.3	19.0	21.0
4	20	1.0	0	0	159	24	0	24.9	31.4	3.94	23.8	4.6	-2.9	20.3	...
5	20	1.0	0	0	163	24	0	24.5	30.9	...	...	...	-2.4	...	...

(a) Analysis: Klason lignin, 28.5; Methoxyl, 4.71%.

(b) All figures, but for cook No. 1, are based on the original oven-dry weight of wood used.

(c) K.L. signifies Klason lignin, and MeO, methoxyl.

(d) This value is based on residue weight.

(e) Calculated from: 28.5 minus (column 12).

(f) Calculated from: 28.5 minus (column 10); minus values indicate apparent increase in lignin.

(g) Calculated from: (column 9) minus (column 13).

(h) Calculated from: (column 9) minus (column 14).

served only to moderate the hydrolytic action of water. Jahn (116) concluded generally that alkaline salts repressed the acidic hydrolytic action of water on the polysaccharides of wood. The attempt to apply the Kolbe reaction to spruce wood did nothing but what might have been expected from the action of water, modified slightly by the presence of sodium bicarbonate.

Consideration of the causes of the failure of spruce wood to respond to the conditions of the Kolbe reaction brought out the thought that perhaps sufficient free phenolic hydroxyls, so essential to a Kolbe reaction, were not present in lignin as it existed in situ. If this conception were correct then prehydrolysis of the wood prior to the carbon dioxide-bicarbonate treatment might split off carbohydrate, creating sufficient free phenolic hydroxyl groups in the lignin residue to make the Kolbe reaction possible.

Table VI summarizes experiments with extracted spruce wood meal prehydrolysed by 0.5% hydrochloric acid, 1% sodium hydroxide, or with water alone. The prehydrolysed wood meals lost only minor amounts of lignin when heated with aqueous sodium bicarbonate at 160° for twenty-four hours, the carbon dioxide pressure being 400 pounds per square inch. Spruce meal prehydrolysed by treatment with 1% aqueous sodium hydroxide at 100° for three hours lost 15.1% of its weight but only 2.2% of this was Klason lignin. When this prehydrolysed meal was subjected to the conditions of a Kolbe cook for

TABLE VI

ATTEMPTED KOLBE COOKS WITH PREHYDROLYSED SPRUCE MEAL

Cook No.	Prehydrolysis Conditions: Medium	Time hrs.	Temp. °C.	Kolbe[a] Cook Temp. °C.	Stage of Cook	Analyses - Found[b]: % Loss in wood weight	% Klason lignin: Initial	% Klason lignin: Final	Analytical Data Calculated[b]: % Loss[c] of lignin	% Loss[d] of holocell.
6	H_2O	24	162	100	prehydrolysis	24.7	28.5	31.2	-2.7	...
					Kolbe cook	2.4	31.2	30.0	1.2	...
					total	27.1	28.5	30.0	-1.5	...
7	aqueous 0.5% HCl	4	130	158	prehydrolysis	32.2	28.5	25.2	3.3	...
					Kolbe cook	15.2	25.2	25.6	-0.4	...
					total	47.4	28.5	25.6	2.9	44.5
8	aqueous 1.0% NaOH	3	100	160	prehydrolysis	15.1	28.5	26.3	2.2	12.9
					Kolbe cook	10.0	26.3	24.6	1.7	8.3
					total	25.1	28.5	24.6	3.9	21.2
9	aqueous 0.5% HCl	2	100	160	prehydrolysis	18.4	28.5	26.9	1.6	16.8
					Kolbe cook	21.8	26.9	25.6	1.3	20.5
					total	40.2	28.5	25.6	2.9	37.3
10	aqueous 0.5% HCl	2	100	160	prehydrolysis	20.0	28.5	26.8	1.7	...
					Kolbe cook	16.2	26.8	27.0	-0.2	...
					total	36.2	28.5	27.0	1.5	34.7
11[e]	1 part NaOH - 2 parts meal, P_2O_5 - dried			160	total	31.6	29.4	24.4	5.0	26.6

See next page for notes.

(a) In all Kolbe cooks but No. 11, H_2O : wood : CO_2 • $NaHCO_3$ ratios of 20:1:1:1, and a cooking period of twenty-four hours with a carbon dioxide pressure of about 400 pounds per square inch, were used.

(b) All figures are based on the original oven-dry weight of wood used.

(c) Calculated from (column 8) minus (column 9).

(d) Calculated from (column 7) minus (column 10).

(e) Data based on weight of wood in lye-impregnated wood sample. The cooking time was sixty-five hours, with a carbon dioxide pressure of about 400 pounds per square inch.

twenty-four hours at 160°, with a carbon dioxide pressure of about 400 pounds per square inch (No. 8, Table VI), an additional 10%, based on the original wood, was lost, but again only a minor amount of this was lignin. The total amount of lignin lost in the prehydrolysis and in the Kolbe cook was 3.9%, of an original Klason lignin content of 28.5%.

A similar low removal of lignin, not exceeding 3.0% of the original wood, was produced by the Kolbe cook with spruce meal prehydrolysed with water alone (No. 6), 0.5% hydrochloric acid at 100° for two hours (Nos. 9 and 10), and 0.5% hydrochloric acid at 130° for four hours (No. 7). These results - low lignin and high holocellulose removal - are in general similar to those on non-prehydrolysed spruce meal shown in Table V.

As previously mentioned, the Kolbe reaction is sometimes carried out with the dry phenol. Also, the mechanism proposed by Tijmstra (26) for the Kolbe reaction pictures the sodium phenolate, and not the free phenol, undergoing carboxylation. It was conceivable that in the experimental cooks already discussed (Nos. 1 - 10) the free phenolic hydroxyl groups in spruce, if present at all, were not converted by the bicarbonate used to sodium salts, and that the use of sodium hydroxide was necessary for this purpose. Cooking the spruce wood with aqueous caustic soda at 160° was inadvisable, since the alkali alone would pulp and thereby make it difficult to detect any additional Kolbe pulping action. Extracted spruce meal was accordingly soaked in 17.5% aqueous sodium hydroxide for one hour in a nitrogen atmosphere at room temperature. The meal was recovered by filtration, pressed and then dried for

several weeks *in vacuo* over phosphorus pentoxide. The yellow, nearly anhydrous meal, contained about 33% of caustic soda. A sample of this meal was then heated for sixty-five hours at 160° with a carbon dioxide pressure of 400 pounds per square inch, but only 5%, of the 29.4% total indication of Klason lignin in the original lye-impregnated meal, dissolved when the meal was stirred with water after completion of the cook (No.11).

The possibility existed that the carbohydrate content of the wood was in some way interfering with the response of the lignin to the Kolbe reaction. A few experiments were therefore conducted with lignins isolated by the comparatively mild methods of ethanolysis (117) and periodate oxidation (48). Maple ethanol lignin, when treated with aqueous carbon dioxide-sodium bicarbonate at 100° for twenty-four hours, dissolved to the extent of only 10%. Since a somewhat greater amount of the lignin, 13.4%, was solubilized when water alone was used at 100° for twenty-four hours, it was clear that no Kolbe reaction was taking place. In both cases the light-colored, powdery ethanol lignin was changed to a very dark, hard, pebbly, coal-like form. Probably the elevated temperature converted the labile lignin complex to a condensed inactive form. Spruce periodate lignin cooked with aqueous carbon dioxide-sodium bicarbonate at 160° for twenty-four hours lost only 22% of its weight, so that in this case too the Kolbe reaction failed to produce a pulping action.

The attempts to cook wood by means of the Kolbe reaction were therefore abandoned. No explanation can be offered for this inactivity of wood lignin to the Kolbe reaction, beyond the one that none of the treatments succeeded in releasing sufficient of the free phenolic groups essential for carboxylation by the Kolbe reaction.

B. Preliminary Pulping Experiments With Liquid Ammonia

The first phase of the present study of the action of anhydrous liquid ammonia was to determine whether liquid ammonia, or its solutions of simple salts, would extract sufficient lignin from wood to warrant further consideration as a practical pulping agent, and whether a relatively unchanged form of lignin could be extracted.

To this end, an extensive series of anhydrous liquid ammonia cooks was carried out in small pressure bombs under a variety of conditions (wood type, time and temperature of cook, added salts). The ammonia : dry wood ratio was usually 15:1, and where salts were used in the cook, the ammonia : dry wood : salt ratio was 15.0:1.0:0.75. The meals were air-dried 20 to 100 mesh samples, which had been carefully extracted with hot benzene-ethanol, ethanol, and water, so that none of the substance extracted by the ammonia could be merely extraneous wood substance, but was definitely a part of the wood holocellulose or lignin.

At the conclusion of the cooking period the residual meal was separated by a specially developed filtration technique (Fig. 1) designed to prevent the absorption of atmospheric moisture by the liquid ammonia during filtration. Possible complications caused by the alkaline action of aqueous ammonia, as contrasted to the "neutrality" of anhydrous liquid ammonia, were avoided in this way. Where a salt had been used in a cook the ammonia-free wood residue was washed thoroughly with warm water until free of the added salt.

The total amount of wood substance solubilized in a cook was determined by carefully weighing the wood residue. This total loss was divided into holocellulose and lignin losses by an algebraic consideration of the change in Klason lignin content of the meal when cooked, combined with the total loss in wood weight, as has already been described in the "Experimental" section. The anomaly met with in the Kolbe cooks, involving the apparent creation of Klason lignin, was not experienced in the liquid ammonia experiments, probably because of the lower temperatures used in a less acidic medium.

Spruce wood meal cooked with anhydrous ammonia at 25° and 45° (Table V11 Nos. 1 and 2) showed a negligiole loss in Klason lignin and wood weight (columns 10 and 11). Even sixty-five hours soaking in ammonia at 100° (No. 3) removed only a maximum of 2.6 grams of lignin per 100 grams of dry wood used, together with 0.6 grams of holocellulose. Under the same conditions, 14.3% of a birch meal sample (No. 6) dissolved in liquid

ammonia, the 5.5% which was lignin corresponding to 30% of the total Klason lignin originally present. This value was much higher than the corresponding figure for spruce, 9.1%. Beech and maple meals lost 24.2% and 24.8% respectively, of their total Klason lignin content when treated for twenty-four hours at 100° (Nos. 8 and 10). There was therefore a sharp distinction between the behaviour of softwood and hardwood in liquid ammonia, the hardwoods losing about three times as much of their lignin content as the softwood. Rye straw, chosen as typical of a third class of lignified materials, the grasses, showed the highest lignin loss of all, 50.7% of the total lignin being removed by a sixty-five hour treatment at 100° (No. 11).

The data in Table V11 indicate, that, as might be expected, the action of liquid ammonia on woody materials increased as temperature increased. This effect was particularly noticeable with the less resistant rye straw, but was minor in nature with hardwoods, and almost completely absent in the case of spruce, a softwood. Thus a sixty-five hour treatment at 25° removed 11.4% by weight of rye straw (No. 14) while the same treating period at 100° removed 19.9% (No. 11). Cooked for forty hours at 75°, beech and maple samples lost 9.3% (No. 7) and 10.4% (No. 9) by weight respectively. When beech and maple wood meals were cooked in liquid ammonia at 100° for only twenty-four hours, the losses increased by about 1.2% (Nos. 8 and 10), the corresponding increase for the rye cooks, though not strictly comparable as regards conditions, being 8.5%. Birch

TABLE VII

LIQUID AMMONIA - WOOD MEAL COOKS

Cook No.	Wood	Cooking Conditions		Experimental Data				Calculated Data			
		Temp. °C	Time hrs.	% Klason[a] lignin in wood Initial	Final	% of wood meal recovered	% Residue from NH_3 filtrate	Loss in % on wood basis Holocell.	Lignin	Total	Lignin loss % on Klason lignin basis
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
1	spruce	25	16	28.5	...	97.0	...	...	...	3.0	...
2	spruce	45	44	28.5	28.4	...	...	...	...	...	...
3	spruce	100	65	28.5	26.8	96.8	...	0.6	2.6	3.2	9.1
4	birch	75	40	19.2	17.3	88.0	...	8.0	4.0	12.0	20.8
5	birch	100	3	19.2	17.8	91.7	8.4	5.4	2.9	8.3	15.1
6	birch	100	65	18.3	14.9	85.7	...	8.8	5.5	14.3	30.0
7	beech	75	40	22.7	20.6	90.7	8.5	5.3	4.0	9.3	17.6
8	beech	100	24	22.7	19.2	89.5	10.8	5.0	5.5	10.5	24.2
9	maple	75	40	21.4	18.8	89.6	8.2	5.8	4.6	10.4	21.5
10	maple	100	24	21.4	18.2	88.4	10.6	6.3	5.3	11.6	24.8
11	rye[b]	100	65	19.7	12.2	80.1	...	9.9	10.0	19.9	50.7
12	rye[c]	100	24	19.7	12.4	76.5	...	13.3	10.2	23.5	51.8
13	rye[c]	100	3	19.7	11.7	81.6	...	8.2	10.2	18.4	51.8
14	rye[c]	25	65	19.7	15.0	88.6	...	5.0	6.4	11.4	32.5

(a) Average of two results.

(b) Data corrected for ash content of rye, 6.46%, and for ash content of Klason lignin.

(c) Total moisture content of system less than 0.1%.

wood showed a similar small increase in the amount extracted by liquid ammonia with increasing temperature: a forty hour treatment at 75° removed 12.0% (No. 4); a sixty hour cook, at 100° extracted 14.3% (No. 6). The increase in loss suffered by spruce when the cooking temperature was increased from 25° (No. 1) to 100° (No. 3) was negligible, 3.0% and 3.2% respectively being extracted.

The low response of spruce wood to the action of liquid ammonia as compared to the hardwoods, beech, maple and birch, is another example of the generally greater resistance to solubilization of softwoods as compared to hardwoods (118). Yorston (119) in a recent review remarked that softwoods were resistant to the action of many media which produced a substantial pulping action on hardwoods and which readily pulped grasses. McMillen et al. (120) found a distinct difference in the reaction of coniferous and deciduous woods in butanol pulping: the deciduous woods pulped readily, giving products with a low lignin content, whereas the pulps from coniferous woods contained large amounts of lignin.

The removal of lignin by use of anhydrous ammonia, a "neutral" solvent, is not to be confused with pulping methods which employ aqueous solutions of ammonia. Peterson and Hixon (79), for example, delignified corn stalks and oat straw by cooking with aqueous ammonia under a pressure of 90-110 pounds per square inch, the action depending on the alkaline character of their reagent. While the ammonia used in the

present experiments was practically anhydrous, the wood meal and straw samples were only air-dried and contained on the average about 7% moisture. With an ammonia : wood ratio of 15:1 the moisture content in a cook was therefore about 0.5%. Was this sufficient water to convert the action of liquid ammonia to that of an alkaline pulping agent? To settle this question a sample of rye straw was dried over phosphorus pentoxide for several weeks until the moisture content fell to less than 0.7%, before being used in several cooks (Table V11, Nos. 12, 13, 14). The overall moisture content in these cooks was less than 0.1%, an insignificant amount. Treated at 100° for twenty-four hours (No. 12), 51.8% of the lignin was lost, a loss even greater than that suffered by the air-dried rye. Similar results were later obtained on comparison of air-dried and phosphorus pentoxide-dried maple meals. These experiments showed that the presence of small amounts of water did not modify the solvent action of liquid ammonia.

As has been previously explained, when liquid ammonia is allowed to act on pure cellulose, two ammonia-celluloses can result. Below -30° ammonia-cellulose II is formed, which, when warmed above -20° changes rapidly to ammonia-cellulose I, stable above -20°. In all the cooks tabulated in Table V11, the ammonia-cellulose, if produced in the cellulose of wood as in pure cellulose, would presumably be of the ammonia-cellulose I type, since the temperatures were well above -20°. It was desirable therefore to extend the investigations to the

temperature range below -30°, since at these lower temperatures a different form of ammonia-cellulose, ammonia-cellulose II, would exist, which might respond in a manner different to that of ammonia-cellulose I. To test this idea a few exploratory cooks were done at -33° for 200 hours, using air-dried spruce meal and rye straw. The results were quite negative, with only negligible amounts, even of rye, being removed. An experiment on the swelling of wood by liquid ammonia revealed a possible reason for these unsatisfactory results. Black spruce chips, when soaked in liquid ammonia at -33° for twenty-two hours swelled only 6% in volume. In contrast to this, Clark and Parker (75) found that pure cellulose fibres swelled to three times their normal size in similar circumstances. When allowed to stand in air for twenty-two hours the ammonia-soaked chips lost their ammonia content and contracted to a volume slightly less than that of the original untreated chips. The lignin in the wood apparently prevented the swelling of the cellulose to the extent that pure cellulose was swollen.

No cooks above 100° were carried out since the critical temperature for ammonia, above which the liquid phase is indistinguishable from the gas phase, is 133° or only a little higher than the temperature range already investigated. It has also already been pointed out that the increase in the amounts of wood solubilized with liquid ammonia increases only slightly with increasing temperature.

Though liquid ammonia by itself appeared to be useless as a practical pulping agent for wood, there still remained the possibility of producing a pulping action by the addition of simple salts to the anhydrous ammonia. A series of cooks was carried out with spruce and birch wood meals at 100° for sixty-five hours, using ammonium salts (Table VIII), which, as previously explained, are acidic in nature when dissolved in liquid ammonia.

Of these, ammonium chloride had the most pronounced action, dissolving 12.7% of the spruce meal (No. 16) and 22.7% of the birch meal (No. 20). In both cases only about one-third of the total loss in wood weight was lignin, the rest being holocellulose, probably hemicellulose hydrolyzed by the acid action of the ammonia solution of ammonium chloride. This one-third of lignin represented a removal of 16.1% of the total lignin in spruce, and 39.4% of the total lignin in birch, as compared to 9.1% and 30% (Table VII, Nos. 3 and 6) when liquid ammonia alone was used. Rye straw lost 58.3% of its Klason lignin content (Table VIII, No. 22) under similar conditions.

Ammonium sulfite in liquid ammonia, which was considered to be the nearest equivalent to the sulfite cook of the aqueous system, attacked birch (No. 21) in a way similar to ammonium chloride. The 20.4% loss in wood weight was presumably due merely to the acid character of ammonium sulfite in ammonia, since no sulfonic acid groups could be detected in the recovered wood by a color test with an acetic solution of azodimethylaniline (121). Spruce wood cooked with ammonia and ammonium sulfite

TABLE VIII

INFLUENCE OF ADDED SALTS ON WOOD COOKING ACTION OF LIQUID AMMONIA

Cook No.	Wood	Cooking Conditions: Temp. °C.	Time hrs.	Added salt	Experimental Data: % Klason[(a)] lignin in wood Initial	Final	% of wood meal recovered	Calculated Data: Loss in % on wood basis Holocell.	Lignin	Total	Lignin loss % on Klason lignin basis
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
15	spruce	100	65	NH_4Cl	28.5	27.9	88.0	8.1	3.9	12.0	13.7
16	spruce	100	65	NH_4Cl	28.5	27.4	87.3	8.1	4.6	12.7	16.1
17	spruce	100	65	$(NH_4)_2SO_3$	28.5	26.5	97.0	0.2	2.8	3.0	9.8
18	spruce	100	65	$(NH_4)_2SO_4$	28.5	28.0	96.4	2.1	1.5	3.6	5.3
19	spruce	100	65	NaCN	28.5	31.3	75.3	19.8	4.9	24.7	17.2
20	birch	100	65	NH_4Cl	18.3	14.4	77.3	15.5	7.2	22.7	39.4
21	birch	100	65	$(NH_4)_2SO_3$	18.3	14.5	79.6	13.6	6.8	20.4	37.2
22	rye	100	65	NH_4Cl	19.7	10.3	79.1	9.4	11.5	20.9	58.3

(a) Average of two results.

(No. 17) lost only 3% by weight, of which 2.8% was lignin. Ammonium sulfate solubilized no more spruce wood (No. 18) than did ammonium sulfite. The low reactivity of spruce to ammonium sulfate and ammonium sulfite solutions was probably due in part to the low solubilities of these salts in liquid ammonia, the more reactive ammonium chloride being much more soluble. Franklin (84) marked them as insoluble in liquid ammonia at room temperature, though cook No. 21 shows that the solubility of ammonium sulfite at 100° is sufficient substantially to increase the solubilizing action of liquid ammonia on birch.

A last attempt at cooking with added salts was made with sodium cyanide (No. 19) which is highly soluble in liquid ammonia, in the hope that the active cyanide group might produce the desired result. Spruce meal when treated for sixty-five hours at 100° with a 30% solution of sodium cyanide in ammonia, lost 24.7% of its weight, double the loss with ammonium chloride, but only 4.9% of this 24.7% was Klason lignin, the other 19.8% being holocellulose. This lignin loss amounted to only 17.2% of the total lignin, a quantity which was considered insufficient to warrant further study of the action of sodium cyanide.

Before abandoning the attempts to pulp wood with liquid ammonia, several representative wood residues from the ammonia cooks were treated with 1% sodium hydroxide at 99° for two hours. These experiments were done in order to test the

TABLE IX

ACTION OF 1% NaOH (99°, two hours) ON WOOD RESIDUES FROM AMMONIA COOKS

Cook No.	Wood Residue Wood	% Klason Lignin(b) Initial	After NH_3	After NaOH	Losses in Grams per 100 Grams of Original Untreated Wood: With liquid NH_3 Holocell.	Klason lignin	Total	With 1% NaOH Holocell.	Klason lignin	Total	Overall-NH_3+1%NaOH Holocell.	Klason lignin	Total	Lignin loss % on Klason lignin basis: With NH_3	With 1% NaOH	NH_3+ 1% NaOH
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
3	spruce	28.5	26.8	29.4	0.6	2.6	3.2	11.8	1.4	13.2	12.4	4.0	16.4	9.1	4.9	14.0
16	spruce	28.5	27.4	27.5	8.1	4.6	12.7	3.2	1.0	4.2	11.3	5.6	16.9	16.1	3.5	19.6
17	spruce	28.5	26.5	27.3	0.2	2.8	3.0	7.1	1.6	8.7	7.3	4.4	11.7	9.8	5.6	15.4
	spruce	28.5	(a)	31.0	...	...	...	12.9	2.2	15.1	...	...	...	...	7.7	...
6	birch	18.3	14.9	17.2	8.8	5.5	14.3	13.7	0.4	14.1	22.5	5.9	28.4	30.0	2.2	32.2
20	birch	18.3	14.4	15.4	15.5	7.2	22.7	13.3	1.5	14.8	28.8	8.7	37.5	39.4	8.2	47.6
21	birch	18.3	14.5	14.9	13.6	6.8	20.4	11.4	1.6	13.0	25.0	8.4	33.4	37.2	8.7	45.9

(a) No ammonia pretreatment.

(b) Average of two results.

possibility that liquid ammonia, while not in itself a pulping agent, might pretreat wood in such a way that it would afterwards pulp very easily with mild aqueous alkali.

The results with spruce and birch wood residues from cooks using ammonia alone, ammonia with ammonium chloride and ammonia with ammonium sulfite were of little significance, with only minor amounts of lignin, from 2.2 to 8.7% (Table IX column 16) of the total lignin, being removed by the mild alkali treatment; even this relatively small removal of lignin was accompanied by a severe attack on the holocellulose portion, the loss of the latter being from five to ten times the lignin loss. A test with spruce which had not been previously cooked in liquid ammonia showed an even greater loss of lignin with 1% sodium hydroxide than that suffered by the ammonia pretreated samples. The total loss of lignin in the ammonia cook, added to the loss in the alkali treatment, was about double the 7.7% loss suffered by spruce treated with alkali only (No. 17 columns 16,17).

Further endeavours to produce a pulping action were therefore discontinued and investigations were started on the materials extracted by the ammonia

C. The Large Scale Liquid Ammonia Extraction of Maple Wood

The liquid ammonia extract of sugar maple was selected as the subject for an intensive study. To determine the best conditions for extraction, a series of cooks were carried out

on two different samples of sugar maple, the results of which are given in Table X. As has already been discussed in connection with Table V11, the cooks showed that the amount extracted by liquid ammonia from maple increased slightly with an increase in temperature. The figures for total loss in wood weight at 25° and 45° were 6.4% (No. 23) and 6.9% (No. 27) respectively, the treating time being 110 hours. A cooking period of twenty-four hours at 100° removed 8.9% (No. 29). Given sufficient time, liquid ammonia extracted as much from maple at 25°, as at 45°, that is, about 6.5 to 7.0% of the total wood weight, this quantity containing 14% of the total original Klason lignin. Once this amount had dissolved, even extremely long extractions increased the amount removed to only a minor degree. Treated for 110 hours at 25° the loss in wood weight was 6.4% (No. 23); when the time was increased to 760 hours at the same temperature (No. 25) the loss in wood weight rose only by about 1%. An increase in the cooking time from forty to 500 hours at 45° caused a rise in wood loss from 6.4% (No. 26) to 7.3% (No. 28).

Since the present study aimed at obtaining the constituents extracted from maple wood by liquid ammonia in a comparatively unchanged form, the temperature of 25° was selected for the liquid ammonia extraction. Bearing in mind the labile nature of the lignin complex, it was decided that the minor increase, 2% to 3%, in the yield of extract obtained at 100° as compared to 25°, did not warrant risking the probability of greater

TABLE X

MAPLE WOOD - LIQUID AMMONIA COOKS

Cook No.	Cooking Conditions Temp. °C.	Time hrs.	Experimental Data: % Klason[a] lignin in wood Initial	Final	% of wood meal recovered	% residue from NH_3 filtrate	Calculated Data: Loss in % on wood basis Holocell.	Lignin	Total	Lignin loss % on Klason lignin basis
23	25	110	20.0	18.4	93.6	7.8	3.6	2.8	6.4	14.0
24	25	250	20.0	18.4	93.4	7.2	3.8	2.8	6.6	14.0
25	25	760	20.0	...	92.5	8.4	...	...	7.5	...
26	45	40	20.0	18.4	93.6	6.8	3.6	2.8	6.4	14.0
27	45	110	20.0	18.6	93.1	7.4	4.2	2.7	6.9	13.5
28	45	500	20.0	...	92.7	8.8	...	...	7.3	...
29[b]	100	24	20.0	17.4	91.1	9.4	4.8	4.1	8.9	20.5
30[b]	100	24	19.5	15.9	89.4	10.3	5.3	5.3	10.6	27.2
31[c]	25	1	23.5	...	97.2	...	...	...	2.8	...
32	25	5	23.5	...	94.4	...	...	...	5.6	...
33	25	5	23.5	...	94.4	...	...	...	5.6	...
34	25	24	23.5	...	94.5	...	...	...	5.5	...
35	25	48	23.5	...	94.6	...	...	...	5.6	...
36	25	96	23.5	...	94.0	...	...	...	6.0	...

(a) Average of two results

(b) Finely ground 140 - 180 mesh maple meal was used in this cook.

(c) Maple meal used in Nos. 31-36 was the same as that used in the large scale cooks to obtain large amounts of extract.

sedondary reactions at the higher temperature.

In order to determine the best extraction period at the chosen temperature of 25°, a series of cooks (Nos. 31 - 36) were carried out using the maple meal from which the final extract was to be obtained. A one-hour extraction removed only 2.8% (No. 31) of the wood. When the extraction period was increased to five hours (Nos. 32 and 33), the amount removed was 5.6% and no increase could be detected when the period was raised to twenty-four (No. 34) and to forty-eight (No. 35) hours. Only when the treating time reached ninety-six hours (No. 36) did the amount extracted increase, to 6.0%. This result confirmed what has already been shown in connection with the other sample of maple studied in Table X (Nos. 23-28).

It was clear, therefore, that at 25° an initial short extraction period, greater than one hour but less than five hours, was sufficient to solubilize about 6% of the wood. Once this 6% had dissolved in the liquid ammonia, the rate of additional solution was very slow. There was therefore two distinct processes going on in a liquid ammonia extraction, the first, a rapid one complete in five hours, the other, a very slow one proceeding indefinitely at the rate of about 1% in several hundred hours. The obvious choice for the extraction period was five hours at 25°.

Hess and Heumann (53) showed that very fine grinding of wood, down to colloidal dimensions, greatly increased its chemical reactivity. To test the influence of particle size, a sample of maple wood was repeatedly passed through a Wiley mill until a meal mostly consisting of 140 to 180 mesh material

was obtained. When cooked at 100° for twenty-four hours (No. 30) this meal lost 10.6% by weight, only slightly more than the 8.9% loss (No. 29) suffered under the same conditions by the 60 to 80 mesh sample regularly used. There appeared to be no spedial advantage, therefore, in using the more finely cut meal, especially since handling was more difficult and there was always the risk of chemical change which might be induced in the wood by excessive grinding (56).

About three kilograms of carefully solvent-extracted 40 to 80 mesh sugar maple meal was shaken in kilogram lots with 5 parts by weight of anhydrous liquid ammonia in an autoclave at room temperature under pressure for five hours. Both the residual wood meal and the clear amber-colored liquid ammonia filtrate were freed of ammonia, care being taken to prevent the absorption of moisture by the extract. For every 1000 grams of meal extracted, 943.1 grams of meal was recovered, a loss of 5.69% as compared to the figure of 5.6% obtained in the preliminary small scale cooks with the same wood (Table X, Nos. 32 and 33). The analytical data for the maple meal are summarized in Table XI.

The water content of the initial meal, 1.64%, was sufficient to bring the per cent of moisture in a cook to 0.3, but it has already been shown that such small amounts of water do not alter the essentially anhydrous character of the ammonia extraction. No ash was removed by liquid ammonia, a fact to be expected in view of the prior extraction of the meal with hot

water and the insolubility of the common constituents of wood ash, calcium, magnesium and potassium in the form of carbonate, sulfate, phosphate and silicate (122), in liquid ammonia (84).

TABLE XI

ANALYTICAL DATA FOR MAPLE MEAL

Constituent - %	Before Ammonia	After Ammonia
Water	1.64	3.87
Ash	0.36	0.37
Klason lignin (ash-free basis)	23.51	23.08
Acetyl	4.8	1.4
Nitrogen	0.084	0.460

There was a small but definite increase in nitrogen content from 0.084% to 0.46% as compared to an increase for spruce from 0.05% to 0.76% in cook No. 3 (Table VII) and for rye, from 0.24% to 0.54% in cook No. 11 (Table VII). The Klason lignin content dropped by about 0.4%. The most pronounced change in the wood was that of the acetyl content which fell from 4.8% to 1.4%. These figures will be referred to again.

The total weight of the ammonia extract, after removal of all but traces of ammonia and water by a vacuum distillation at about 50°, was 210 grams. The entire thick amber-colored fluid residue was fractionated as shown in Fig. 2. In broad outline, the extract consisted of three constituents, poly-

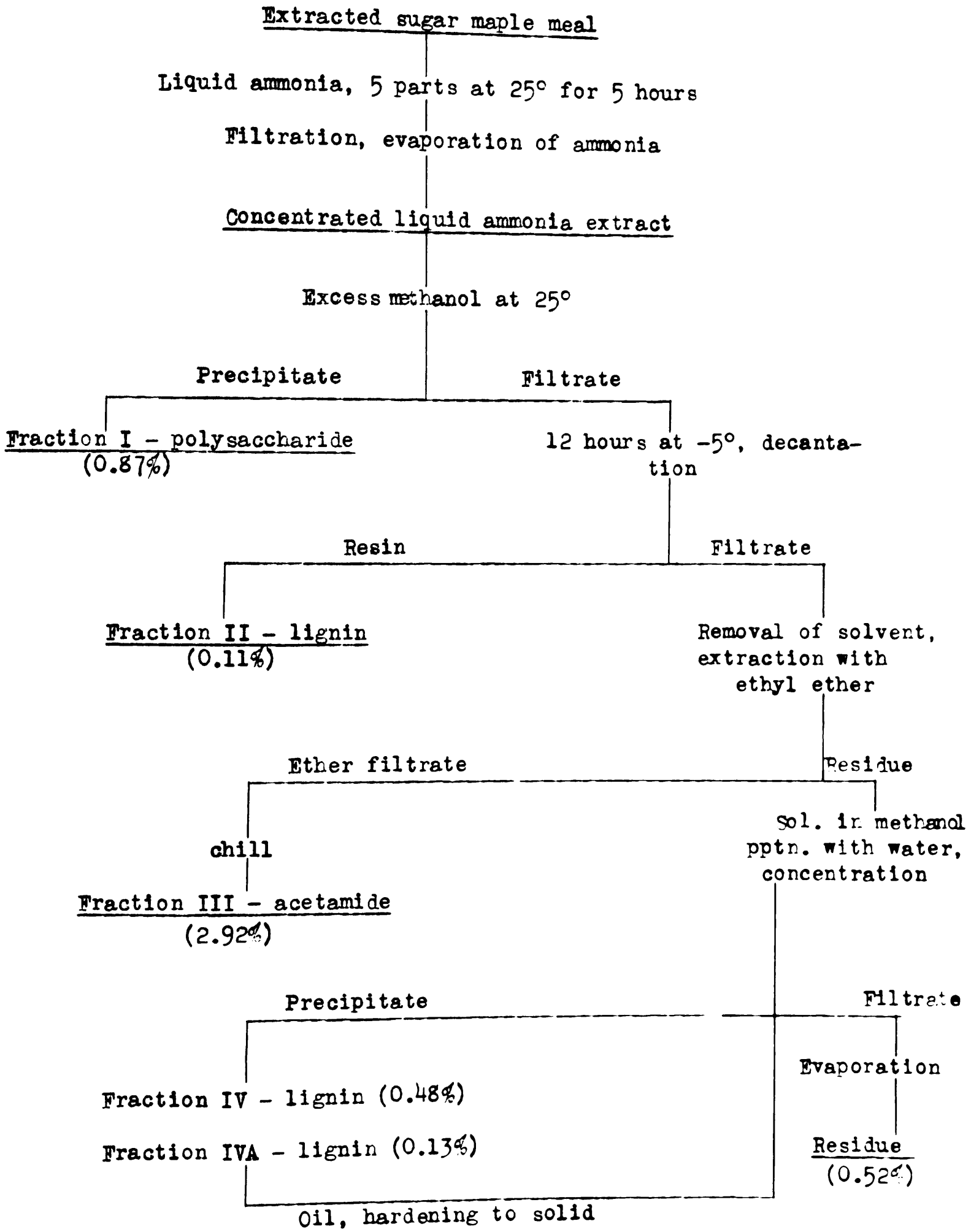


Fig. 2. Crude fractionation of liquid ammonia extract of sugar maple

saccharide, lignin and acetamide. The polysaccharide, Fraction I, was thrown out as a fine, light tan-colored precipitate when the ammonia extract was stirred into excess methanol at room temperature. When the methanol filtrate was chilled, a dark-brown resinous material separated. This was a lignin fraction of low methanol solubility, Fraction II. The major part of the extract was acetamide, Fraction III, which was isolated in a relatively pure, white crystalline form by ether extraction of the residue from the methanol filtrates. The thick oil remaining after the ether extraction, still containing some acetamide, was redissolved in methanol with some difficulty and the lignin Fractions IV and IVA isolated from methanol solution by precipitation with water. The distinction between Fractions IV and IVA was based on the fact that Fraction IV was initially thrown out of methanol by water as a solid, while Fraction IVA appeared first as an oil, which soon hardened to a solid. Evaporation of the filtrate from the isolation of Fractions IV and IVA left a dark brown semicrystalline gummy mass, which had a high acetamide content, and which was not investigated further. The yields and analytical data obtained for the crude fractions are given in Table XII. A total of 5.03% of material in crude form was recovered, or 88.4% of the total loss in wood weight.

The identity of the white crystalline material recovered by ether extraction was established as acetamide by a mixed melting point determination with a genuine sample and by an analysis for nitrogen, the value found being 23.5% as compared to a calculated value of 23.7%. Even without recrystallization,

the acetamide was substantially pure, melting at 80.4° - 80.6° (corr.) as compared to 81.1° (corr.) for an authentic specimen.

The obvious source of the high yield of acetamide, was the decrease in acetyl content of the maple wood from 4.8% to 1.4%, a drop of 3.4%. Calculated as acetyl, the 4.0% acetamide yield corresponded to 2.92% of the wood weight. Thus 86% of the acetyl lost by the wood was recovered as relatively pure acetamide. Considering manipulative losses and the acetamide left unrecovered in the 16 grams of unfractionated residue, it could safely be assumed that almost all the loss of acetyl by the wood was the result of ammonolytic conversion to acetamide. Fernelius, in his review of ammonolysis reactions (87) gave numerous examples of the production of acid amides by the ammonolysis of a wide variety of esters, though the yields varied from nothing to 100% depending on the particular ester concerned. While early wood chemists believed that lignin carried acetyl groups (123) (124), the generally accepted modern view (114) (115) is that almost the entire acetyl content of wood is associated with the polysaccharide portion. Muskat (90) found that at room temperature liquid ammonia removed all acyl groups from a variety of sugars. The production of acetamide from maple wood was another example of the ammonolysis of an acetylated polysaccharide, thus -

$$CH_3CO\cdot OR + HNH_2 \longrightarrow CH_3CO\cdot NH_2 + ROH$$

- where "R" is a polysaccharide residue.

Over one-half (2.92/5.69) of the total loss in weight by the maple meal was acetamide. It might be expected therefore that a wood with a low acetyl content would lose less weight when extracted with liquid ammonia. Data presented by the U.S. Forest Products Laboratory (125) show clearly that the acetic acid content of hardwoods is much higher than that of softwoods: spruce, 1.59%; sugar maple, 4.46%; yellow birch 4.30%. The greater sensitivity of the hardwoods, maple, beech and birch, to the action of liquid ammonia as compared to the softwood spruce has already been discussed (Tables VII and VIII) and associated with the general greater chemical resistance of the softwoods which is apparently related to their lower content of sensitive hemicellulose. In the case of ammonia, part of the difference in response can probably be more directly associated with the lower acetyl content of the softwoods.

Fraction I, insoluble in methanol, was evidently, from its low methoxyl value, 6.13%, and low Klason lignin content, 24.1%, not a lignin fraction. The fact that it was insoluble in alcohol suggested that it consisted chiefly of polysaccharide, and this was confirmed later by determining the carbon content of a partially purified sample. The polysaccharide fraction, amounting to about 0.87% of the wood, was not of major interest in the present study, which was more directly concerned with lignin, and was therefore subjected to only minor investigation in order to determine its general nature. It was insoluble in water, toluene, ethyl acetate, methanol, ethanol, carbon tetrachloride and dioxane. The polysaccharide did, however,

appear to be partially soluble in anhydrous pyridine, and it was therefore treated with this solvent for three hours at room temperature. Some 16% of the polysaccharide fraction dissolved in the pyridine and this portion was recovered as a crude amorphous brown solid by precipitation with ether. The pyridine-soluble fraction, about one-sixth of the whole, was closely related to lignin as shown by methoxyl, carbon and hydrogen values of 16.2%, 54.9% and 6.41%. The corresponding values of 4.40%, 43.7% and 6.88% for the pyridine-insoluble polysaccharide, reflected its polysaccharide nature. Maple hemicellulose preparations have a high pentosan content (126), but in the present instance, the partially purified polysaccharide contained only 6.9%. It has also been shown that some hemicellulose preparations have an appreciable methoxyl content, a portion of which, however, may sometimes be due to the presence of lignin as impurity. Kurth and Ritter (114) found that 15.6% of the total methoxyl content of maple wood was associated with the lignin-free holocellulose portion. More study is needed before it can be definitely decided whether the 4.4% of methoxyl found in the above polysaccharide is the result of lignin impurity or is an integral part of the polysaccharide structure. The evidence available suggests that the former alternative is probably correct. If the 6.1% methoxyl content of the crude polysaccharide was associated entirely with lignin, then the reduction of methoxyl produced by the pyridine treatment should be accompanied by a corresponding reduction in the Klason lignin. The value expected would

TABLE XII

CRUDE FRACTIONS FROM THE LIQUID AMMONIA EXTRACT OF SUGAR MAPLE

Fraction	Description	%[a] Yield	% Klason Lignin	% Methoxyl	% N
I	polysaccharide	0.87	24.1	6.13	0.60
II	lignin	0.11	74.0	20.3	0.64
III	acetamide	2.92[b]	... m.p.	80.4°-80.6° (corr.)	23.5[c]
IV	lignin	0.48	90.2	21.4	0.52
IVA	lignin	0.13	84.5	20.9	0.58
unfract. residue	mostly acetamide	0.52	...	...	...
	Total	5.03			

(a) Based on oven-dry wood.

(b) Calculated as acetyl.

(c) Required for CH_3CONH_2 : N, 23.7; m.p. of authentic acetamide, 81.1° (corr.).

TABLE XIII

SUMMARY OF DATA FOR MAPLE EXTRACT FRACTIONATION

Main Fraction	Sub-Fraction	%(a) Yield	% Klason Lignin	% Methoxyl	% N	% C	% H
I	pyridine-insol.	0.73	18.0	4.4	...	43.7	6.88
I	pyridine-sol.	0.14	...	16.2	...	54.9	6.41
II	dioxane-and methanol-insol.	0.07	79.9	21.5	...	58.7	6.95
II	dioxane-sol., methanol-insol.	0.03	....	21.3	...	...	...
II	dioxane-and methanol-sol.	0.006	...	20.4	...	...	...
III	acetamide	2.92(b)	...	...	23.5	...	...
IV(c)	dioxane-and methanol-insol.	0.033	...	...	...	...	...
IV	dioxane-sol,, methanol-insol.	0.056	86.4	21.1	...	58.4	6.82
IV	dioxane-and methanol-sol.	0.24	88.8	21.6	<0.01	58.9	6.49
IVA	dioxane-and methanol-insol.	0.007	...	...	...	...	...
IVA	dioxane-sol. methanol-insol.	0.03	...	20.8	...	...	...
IVA	dioxane-and methanol-sol.	0.06	87.7	20.7	0.32	...	...

(a) Based on oven-dry wood.

(b) Calculated as acetyl.

(c) See Fig. 2 and Table III for more detail on Fraction IV.

The writer wishes to thank Mr. L.G. Neubauer for his cooperation in obtaining much of these analytical data.

be (4.40 x 24.1 / 6.13) or 17.4%. The actual Klason lignin value of the polysaccharide after pyridine treatment was 18.0%. This result, coupled with the observation that the polysaccharide still gave a response, though weak, to the Maule (127) color reaction, which is specific for angiosperm lignin (128) (129), suggested that lignin was still present in the polysaccharide fraction. The lignin was of the dioxane-insoluble type, or was chemically bound with the polysaccharide, since the latter was quantitatively insoluble in dioxane both before and after the pyridine treatment. Fraction II, which was thrown out by cooling the methanol solution from which the crude polysaccharide was precipitated, had a 65.2% content of dioxane-insoluble lignin. It is easy to see therefore how the polysaccharide could have absorbed some of this material.

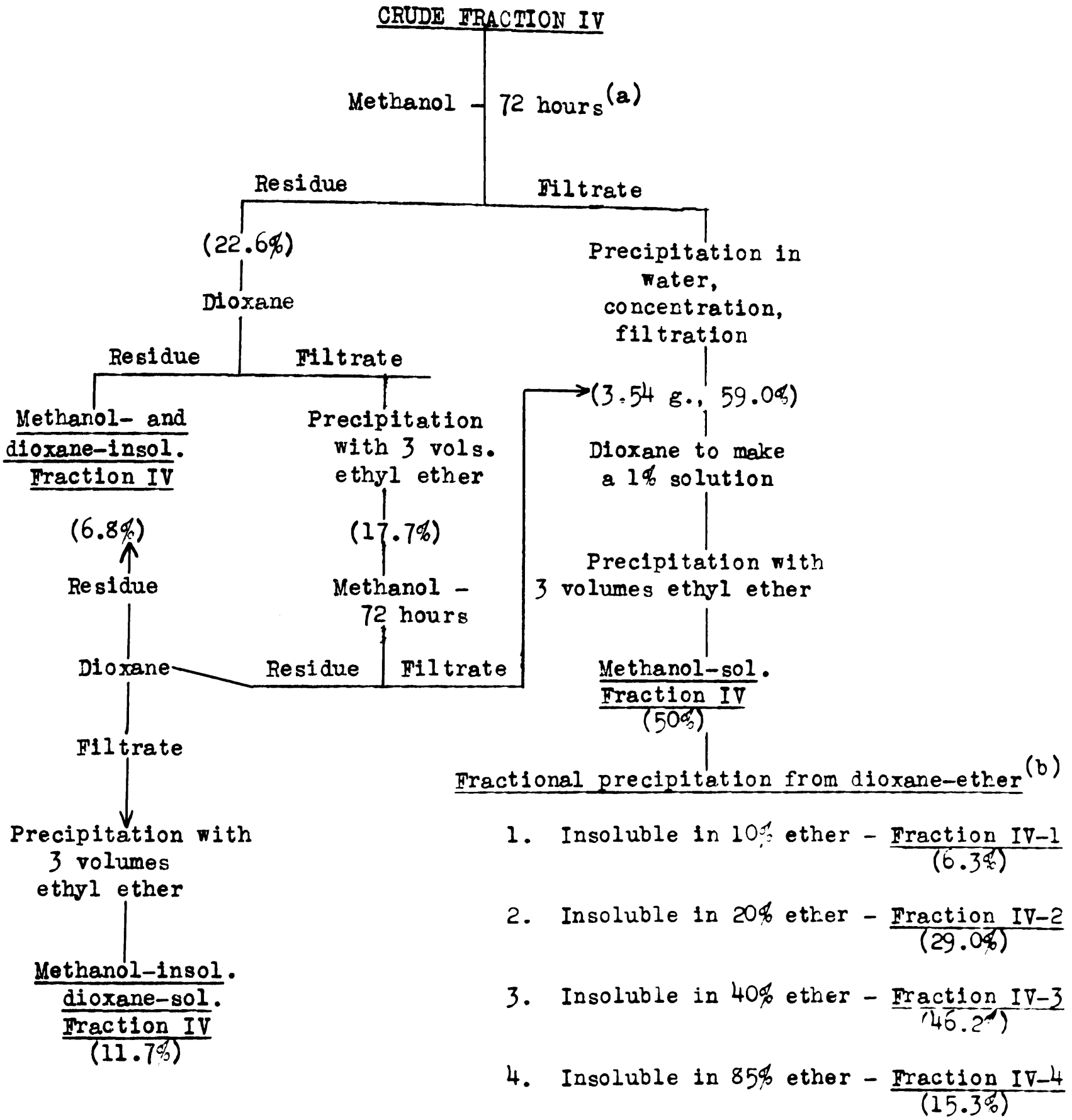
The carbon content of the pyridine-insoluble portion of Fraction I, 43.7% was slightly low for a hexosan $(C_6H_{10}O_5)_x$, which requires a value of 44.4%. This deficit may have been due to the presence of uronic acid groups, which are commonly found (126) in hemicellulose preparations. In line with this suggestion was the observation that the polysaccharide was soluble in dilute alkali, from which it was precipitated on acidification. Further studies on the nature of this polysaccharide fraction are being carried out by Neubauer in these laboratories.

Of the three lignin samples isolated from the liquid ammonia extract of maple, Fraction IV was obtained in the greatest yield, 0.48% of the original wood. A tedious series

of fractional extractions and precipitations involving methanol, ether, dioxane and water, summarized in Fig. 3, eventually yielded half of Fraction IV as a dry, very light-colored powder completely soluble in methanol and in dioxane. Care had been taken during the entire process of isolation, from the initial extraction of the wood with liquid ammonia, to the point where the separation of the final samples of all fractions was complete, to keep the temperature below 60° during all extractions, distillations, drying periods etc. This precaution made sure that changes occurring in any of the constituents during isolation and purification were at a minimum.

The half of Fraction IV which was soluble in methanol was split into four fractions by precipitation from ether-dioxane solution 10, 20, 40 and 85% ether (Fig. 3). The yields and concordant analytical data given in Table XIV, p. 129.

The dioxane-soluble lignin fractions were also soluble in pyridine and glacial acetic acid. They were insoluble in water, ethyl acetate, petroleum ether (30° - 50°), chloroform, acetone and benzene. The dioxane-insoluble fraction of Fig. 3 dissolved slowly in acetic acid and in pyridine. All lignin fractions were soluble in 1% alkali, though the rate of solution depended on the physical state of the lignin: the denser, more resinous, dioxane-insoluble fractions dissolved much more slowly than the very finely divided and accessible methanol-soluble lignins prepared by precipitation with ether from dilute dioxane solution. All lignin fractions could be recovered from alkaline solution



(a) All extractions at room temperature

(b) Yields on basis of methanol-soluble Fraction IV.

Fig. 3. Fractionation of liquid ammonia lignin Fraction IV.

upon acidification, and indeed even by bubbling carbon dioxide through the solution. The solubility in alkali and reprecipitation with carbon dioxide suggested the presence of weakly acidic hydroxyl groups of the type often found in soluble lignin preparations.

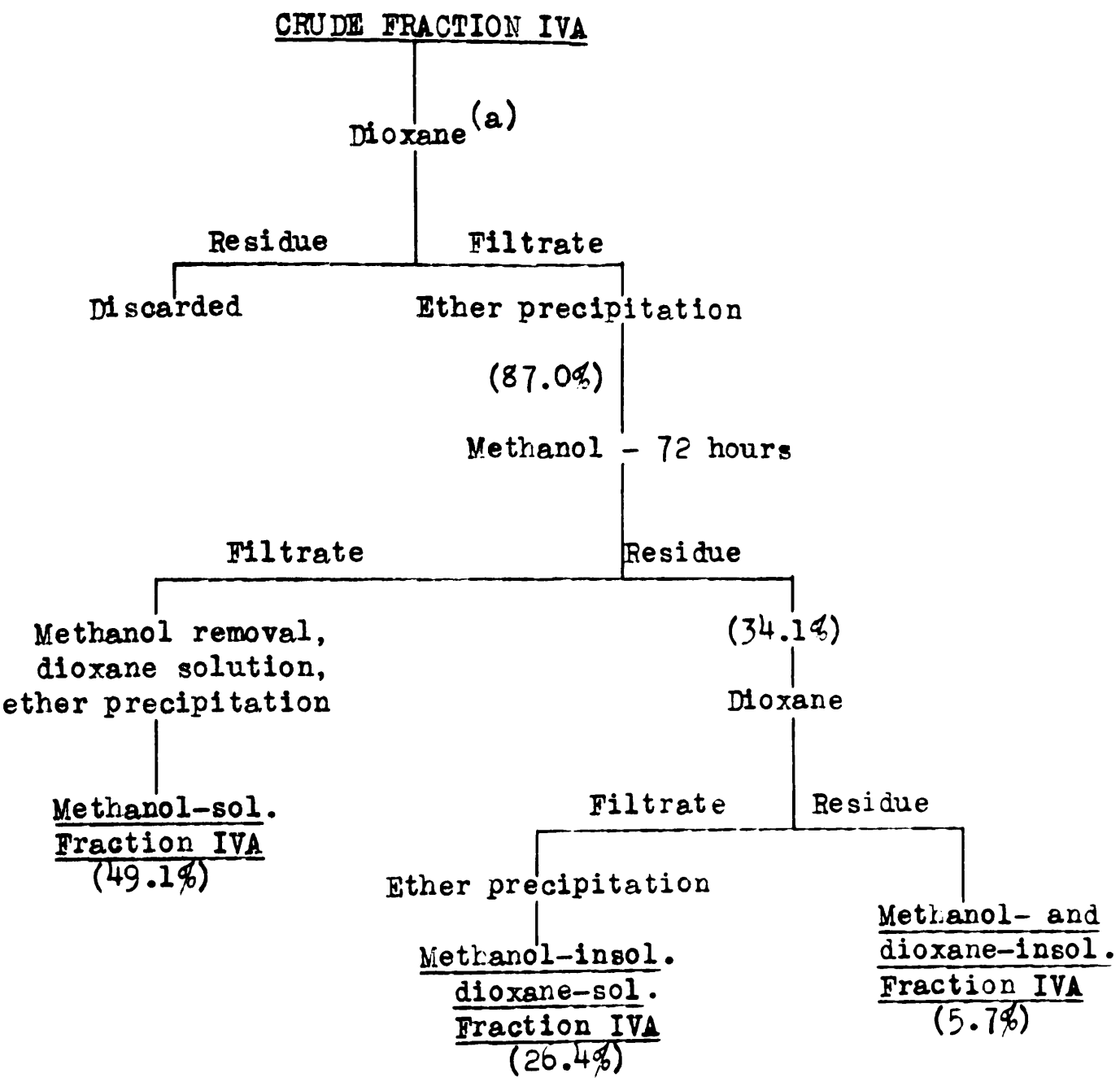
The dioxane-soluble lignin sub-fractions of Fig. 3 reacted positively, though not intensely so, in the typical phloroglucinol-hydrochloric acid lignin color test (34), giving a reddish-violet color, but no evident test was given by the dioxane-insoluble fractions. All the lignin fractions gave a red color in the Maule reaction (127) which is specific for angiosperm lignin (129) though the response of the methanol-soluble fractions was intense, while that of the dioxane-insoluble portion was weaker. This difference was probably connected with the resinous, impervious character of the latter lignin fraction, which would prevent intimate contact with the phloroglucinol or Maule test reagents. In contrast, the methanol-soluble fractions, when precipitated from dilute dioxane with ether, were extremely light and porous, weighing only about 0.2 grams per cc.

Advantage was taken of the clear dioxane solutions of lignins which could be prepared, to determine whether lignin had any optical activity. The observed rotation of a 2.4% solution of methanol-soluble Fraction IV in a 1-decimeter polarimeter tube was only -0.03°, a reading which was barely outside the limits of experimental error. The optical activity of liquid ammonia

lignin was therefore substantially zero, a result which agreed with previous findings, which, however, were based on dark solutions with which accurate measurements could not be made (130).

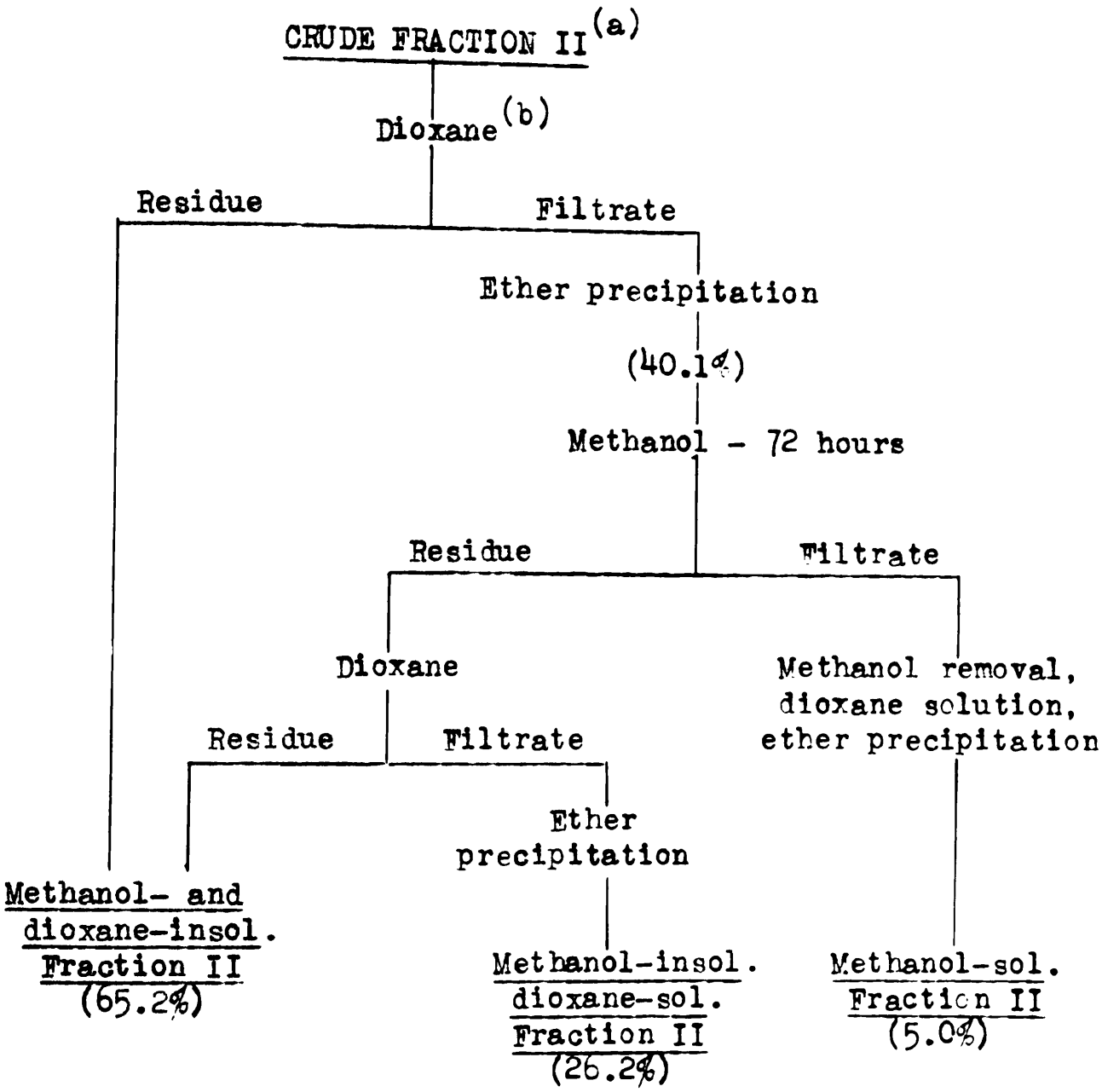
The fractionation of Fractions IVA and II closely followed the methods adopted for Fraction IV and are outlined in Fig. 4 and Fig. 5 respectively. Fraction IVA proved to be substantially similar to Fraction IV: from both lignin fractions about 50% of the methanol-soluble sub-fraction and about 6% of the dioxane-insoluble sub-fraction were isolated (though some of the latter was discarded in the case of Fraction IVA). The chief difference between Fraction IV and Fraction IVA was the greater content of the methanol-insoluble, dioxane-soluble sub-fraction of IVA, 26.4%, as compared to 11.7% for Fraction IV. The methanol-soluble portion of both Fractions IV and IVA was similar in nature. Fraction IV had a Klason lignin content of 88.8% and a methoxyl content of 21.6% where the corresponding values for Fraction IVA were 87.7% and 20.7%.

Fraction II, however, was of a distinctly different nature. The yield of methanol-insoluble, dioxane-soluble material was 26.2%, about the same as for Fraction IVA, but only 5%, instead of 50% of the methanol-soluble sub-fraction was separated. The methanol-soluble material was replaced by a large proportion, 65.2%, of the methanol- and dioxane-insoluble sub-fraction, which dried to a dark-brown, intractable rubbery mass, similar in appearance to the corresponding portions of



(a) All extractions at room temperature

Fig. 4. Fractionation of liquid ammonia lignin, Fraction IVA



(a) Sample previously dissolved in 1% sodium hydroxide and recovered by acidification

(b) All extractions at room temperature

Fig. 5. Fractionation of liquid ammonia lignin, Fraction II.

Fractions IV and IVA. The different character of the dioxane-insoluble part of the isolated lignins was made evident by a Klason lignin content of 79.9% or 10% lower than that of the methanol-soluble fractions. A determination of the hydrogen content gave a value of 6.95% as compared to 6.49% for methanol-soluble Fraction IV. This same tendency for high hydrogen was found in the methanol-insoluble, dioxane-soluble portion of Fraction IVA which had a hydrogen content of 6.82%, though the Klason lignin content of 86.4% was close to the 88.8% value for methanol-soluble Fraction IV.

One kilogram sample of meal, after having been extracted once with ammonia for five hours, was treated for a further twenty-five hours with five parts by weight of ammonia in order to determine whether the step-wise use of ammonia would increase the yield of extracted material. When the ammonia had evaporated away from this ammonia extract, a dark-brown brittle resin, resembling Fraction II in appearance, was left, in a yield of 0.54%. Like Fraction II it had a Klason lignin content of 72.2% and its methoxyl content was 19.2%, slightly lower than that of Fraction II, 20.3%. The isolation of this fraction, closely resembling Fraction II, by a more protracted liquid ammonia extraction, suggested that the dioxane-soluble portions of the lignin in wood were quickly and completely removed in the initial stages of the ammonia extraction, while the extraction of the dioxane-insoluble lignin was a slow, continuous process. Such a conception would explain the fact that the second extraction

yielded none of the dioxane-soluble fraction.

The fact that methanol-soluble liquid ammonia lignin dissolved quickly and completely in dioxane at room temperature to give a clear, amber-colored solution, suggested the use of this solvent for the cryoscopic determination of molecular weight. Numerous estimations of the molecular weight of lignin by the cryoscopic method have been reported, but all have been made on lignins isolated by the use of acid, alkali or elevated temperature. The most common cryoscopic solvents employed were camphor (131), acetic acid (132) (133) and phenol (133). The known sensitivity of lignin to heat severely restricts the theoretical value of lignin molecular weights obtained by the Rast camphor method, where the temperatures exceed 150°. Acetic acid gave anomalous results. Fuchs (132) reported that the freezing-point of a solution of spruce methylglycol lignin in acetic acid was variable and could be changed with energetic stirring. Rassow (133) obtained the obviously low value of 80 for the molecular weight of spruce glycol lignin in acetic acid, but in phenol the value was generally seven times as great. Hagglund and Urban (134) reported values from 427 to 846 for the molecular weight of amyl lignins in acetic acid. It is clear that some unknown factor, perhaps solvent-association or colloidal phenomena, prevented a clear-cut response in the cryoscopic determination of lignin molecular weights in acetic acid.

Anschutz and Broeker (135) initially recommended the use of 1,4-dioxane as an excellent solvent for the cryoscopic determination of molecular weights. Hertz and Lorentz (136),

who carried out the first subsequent study of importance, unfortunately overlooked the highly hygroscopic nature, and slow decomposition with time, of dioxane, a fact later pointed out by Roth and Meyer (137). Payne et al. (138) used dioxane to determine the molecular weight of lignin extracted from bagasse by dilute nitric acid. Virasoro (139) reported molecular weights of lignins extracted by ethylacetoacetate, obtained by dioxane cryoscopy. These are, however, isolated examples, for, generally speaking, the possible use of dioxane in the determination of molecular weights of lignins has been overlooked.

In the present study dioxane purified by the method of Eigenberger (104) was used. The freezing point constant, K, was found by determining the depression produced by known amounts of pure naphthalene, the average of three excellently concordant results being 4.65°. Anschutz and Broeker (135) reported a value of 4.98°; Hertz and Lorentz (136), 5.01°; Roth and Meyer (137), 4.71°. Kraus and Vingee (109), using a very highly refined cryoscopic technique, quoted a result of 4.63°. The value for the dioxane used in the present instance was therefore in excellent agreement with the Kraus and Vingee figure.

The results for the molecular weights of the methanol-soluble Fractions IV and IVA, and of Fractions IV-2, IV-3, IV-4, are summarized in Table XIV, which also includes analytical data for the dioxane-ether fractionation.

TABLE XIV

MOLECULAR WEIGHT OF METHANOL-SOLUBLE LIQUID AMMONIA LIGNIN

Fraction	% Yield[a]	Mol. Wt.	% Methoxyl	% C.	% H.
IVA	...	840	20.7	...	...
IV	...	1130, 1050	21.6	58.9	6.50
IV-1	6.3	...	19.4	57.9	6.74
IV-2	29.0	900, 1350[b]	21.6	57.9	6.40
IV-3	46.2	1140	21.6	58.2	6.37
IV-4	15.3	1010	21.8	58.5	6.41

(a) From ether-dioxane fractionation, on basis of methanol-soluble Fraction IV.

(b) The 1350 value was obtained for a sample redissolved in dioxane and reprecipitated with ether.

The average molecular weight of methanol-soluble liquid ammonia lignin was 1060, with an average error of about 115. Payne et al. (138) reported a similar average error, 125, in their determination of the molecular weight of bagasse lignin in dioxane, although their values ranged from a low of 1490 to a high of 1860.

Successive determinations of the freezing-point of any given solution of liquid ammonia lignin in dioxane never differed from each other by more than 0.002°, out of a total freezing point depression of 0.10° to 0.15° and as mentioned, the results for naphthalene were eminently satisfactory. For these reasons the spread in the molecular weights obtained was

not caused by a lack of precision in the determination of the freezing-point, such as was often experienced when glacial acetic acid was used. Some other unknown factor was concerned. Hagglund and Urban (134) found that the molecular weight of amyl lignin varied according to the method of precipitation of the lignin. Sufficient liquid ammonia lignin was not available to test the applicability of this possibility in the present instance. However, the molecular weight of Fraction IV-2 jumped from 900 to 1350 when it was redissolved in dioxane and reprecipitated with ether. This variation was probably not caused by a change in composition, since the recovery from such a process was almost 100%, and the methoxyl value remained constant. The variations may have been caused by associative effects in dioxane or by tiny amounts of ether tenaciously retained by the very fine, highly electrostatic lignin, despite the solvent-exchange with a light petroleum ether and the long drying period, as much as a week, over phosphorus pentoxide at about 55°.

As Table XIV shows, the sub-fractions IV-2, IV-3 and IV-4 agreed so closely with each other not only in molecular weight, but also in composition, that the probability of their chemical identity was high. Sub-fraction IV-1 diverged somewhat in having slightly low methoxyl and carbon contents and a rather high hydrogen content. These discrepancies, together with a slightly darker color, suggested that sub-fraction IV-1 might have been contaminated with traces of the methanol-insoluble ammonia lignin. The carbon contents of the

original Fraction IV (58.9%) was too high to be completely accounted for by the carbon contents (58.2%) of the fractionated material, all of which are based on closely agreeing duplicate or triplicate analyses. Since five combustions of Fraction IV gave analyses within the limits C, 58.9 to 59.0%; H, 6.45 to 6.55%, the discrepancy could not be attributed to analytical error and was tentatively ascribed to the loss of a small amount of a high carbon constituent during the fractionation from ether-dioxane.

If the conclusion that Fraction IV was a homogeneous chemical individual with the analysis C, 58.8; H, 6.49; OCH_3, 21.6% and molecular weight 1060, be entertained, the corresponding molecular formula is -

$$C_{42}H_{43}O_{15}(OCH_3)_7 \qquad \text{(XII)}$$

This formula has the composition C, 58.5; H, 6.42; OCH_3, 21.6 and molecular weight, 1005.

Six methylations of the entire methanol-soluble Fraction IV with dimethyl sulfate and caustic soda at room temperature yielded an alkali-insoluble product with the analysis C, 61.1; H, 6.95 and OCH_3, 34.7%. These data agreed best with the formula $C_{42}H_{38}O_{10}(OCH_3)_{12}$, although this structure requires a carbon content lower by 0.8%. Comparison with this result shows that (XII) can be rewritten as -

$$C_{42}H_{38}O_{10}(OH)_5(OCH_3)_7 \qquad \text{(XIII)}$$

Brauns' structure for the enol form of spruce native lignin (33) was -

$$C_{42}H_{31}O_6(OH)_5(OCH_3)_4 \qquad \text{(XIV)}$$

but it must be remembered that he depended upon an uncertain chemical method to determine equivalent weight (840) and a physical molecular weight was not obtained. In each case the basic carbon skeleton was a C_{42} unit, but a discrepancy of seven hydrogen and four oxygen atoms is apparent. This discrepancy might well have been four molecules of water.

This difference could be due to the presence of four -C-O-C- bonds in spruce native lignin which are open in maple liquid ammonia lignin as -C-OH OH-C-. The resistance of these hydroxyl groups to alkaline dimethylsulfate methylation can be understood if it be assumed that they are tertiary hydroxyl groups. According to Freudenberg (140) some of the hydroxyl groups in spruce lignin are tertiary groups incapable of methylation in the normal fashion. The resistance of such hypothetical extra hydroxyl groups in maple to alkaline dimethysulfate methylation, can thus be accounted for if it be assumed that these hydroxyl groups are tertiary.

Methylation in each case revealed the presence of five free hydroxyl groups, although this result should be regarded with caution since Compton and Hibbert (141) and Wright and Hibbert (142) claimed that alkaline methylation could create new hydroxyl groups. Indeed, Wright and Hibbert expressed the view that "alkaline methylation is unreliable both as a measure of the hydroxyl groups present in the lignin and of complete methoxylation". The fact that native lignin from spruce has three methoxyl groups less than liquid ammonia

lignin from maple is easy to understand since the former is based upon vanillin, and the latter presumably upon the more highly methoxylated syringyl units.

Including Fractions II, IV and IVA, the total amount of liquid ammonia lignin isolated was only 0.72% of the total wood weight or about 3.1% of the original Klason lignin content. Liquid ammonia lignin can therefore obviously not be taken as representative of the major part of lignin <u>in situ</u>, particularly when the carbon content is quite lower than the values quoted for the bulk of the lignin in wood. It must also be borne in mind that formula (XII), assigned to liquid ammonia lignin, assumes the homogeneity of the sample, and it is possible that other fractionation methods may reveal a greater degree of heterogeneity than has been detected thus far. These limitations, however, do not detract from the basic accomplishment; for the first time a molecular formula has been assigned to a lignin extracted without the use of acid, alkali, oxidant or elevated temperatures and on the basis of purely physical evidence involving no assumptions and no chemical reactions.

Brauns showed that spruce native lignin dissolved completely in a sulfite cook at 125° for twelve to fourteen hours. Maple liquid ammonia lignin, however, when heated at 130° with calcium bisulfite solution containing 1% combined and 4.7% total sulfur dioxide dissolved only to the extent of 39% in six hours and 37% in fourteen hours. The undissolved residue

responded positively in a color test for sulfonic acid groups. Yet, in fourteen hours at room temperature, liquid ammonia lignin dissolved to the extent of some 17% in 20% aqueous sodium bisulfite solution, with no added sulfur dioxide. While the nature of the reactions occurring in sulfite cooking has not been completely established, the work of Brauns and Brown (41) who showed that wood methylated with diazomethane no longer readily underwent sulfonation, and of Brauns (33), who found that when spruce native lignin was methylated it lost its ability to dissolve in bisulfite, indicate clearly that some oxygen-containing group, capable of methylation, is involved in sulfite cooking. It is difficult to see how the liquid ammonia treatment at room temperature would affect such an oxygen grouping. Since there is no nitrogen in liquid ammonia lignin, any ammonolytic reactions involving oxygen atoms are limited to those in which only hydrogen enters the lignin molecule, as for example, in the ammonolysis of a uronic acid lignin ester. Such an ammonolysis would only create, and would hardly block, hydroxyl groups. The explanation of the failure of liquid ammonia lignin to dissolve quickly and completely in the sulfite cook is therefore not likely to be found in this direction. Very probably the acid conditions of the sulfite cook convert the labile and highly oxygenated liquid ammonia lignin to an inactive form before the solubilizing sulfite process is complete.

Liquid ammonia lignin differs also from Brauns' native lignin in its response to the methanolysis reaction. Following

Hibbert's (117) alcoholysis procedure, Fraction IV was heated under reflux for forty-eight hours with anhydrous methanol containing 2% of hydrogen chloride, in an atmosphere of nitrogen. Only 1.9% of the liquid ammonia lignin remained insoluble in the acid methanol. The methanol filtrate, containing 98% of the lignin, was poured into water to precipitate methanol liquid ammonia lignin which had a methoxyl content of 28.6%. The weight of this methanol-lignin was 53% of the original lignin weight, leaving some 45% of the lignin as water-soluble material. These figures ignore the minor correction required by the addition of methoxyl groups during methanolysis. When maple wood is subjected to a similar ethanolysis procedure, only 60% of the lignin is dissolved, one-half of which is obtained as water-insoluble ethanol lignin, the other half being water-soluble (3). Brauns, on the other hand, reported that methanolysis of spruce native lignin gave an almost quantitative yield of the water-insoluble methanol lignin. Thus liquid ammonia lignin is a relatively soluble lignin, a fact which may be due to its rather low carbon (59%) and high oxygen content. Most lignin preparations have 63% - 64% carbon and the lowest figures range about 61% (143), but a recent report by Wald, Ritchie and Purves (9) indicates that the carbon content of spruce lignin <u>in situ</u> is 67.5%.

The response of liquid ammonia lignin towards oxidation with sodium paraperiodate at 25° and pH 4 was studied.

After determining the unconsumed periodate in the entire sample by titration, the recovered lignin was carefully washed with water, and then boiled with water under reflux for three hours. The undissolved lignin was subjected to another cycle of oxidation and water-digestion. By the end of the second cycle, some 39% of the lignin had been solubilized by the periodate treatment. A third oxidation increased this amount to 48%. In another experiment, using an oxidation period of seventy-two hours , 36% of the lignin was removed. Ritchie (48) found that after five oxidations, 25% of the lignin in maple wood had been converted to soluble products. Spruce Klason lignin, under the more drastic conditions of a longer oxidation period, seventy-two hours, and a greater excess of periodate, also lost about 25% by weight. Liquid ammonia lignin by comparison was, in general, more sensitive to the action of periodate, since 40% - 50% of the lignin was made soluble. The greater periodate sensitivity was probably due to the fact that liquid ammonia lignin represented the more soluble part of the lignin in wood and hence the transition to a water-soluble form was easier.

Since a reliable molecular weight, 1060, had been obtained for methanol-soluble liquid ammonia lignin, it was possible to determine whether there was any distinct whole-numoer ratio between the moles of periodate consumed and the moles of lignin present. An analysis of the oxidation data however (Table XV) showed clearly that, by 180 hours at any rate, no such whole-number ratio was reached, the molar ratio of

periodate consumed to initial lignin present increasing steadily with time and also varying with the concentration of the periodate solution used. Ritchie (48) noticed a similar variation of periodate consumption with the strength of the periodate solution.

TABLE XV

MOLAR RATIO OF PERIODATE CONSUMED TO LIGNIN PRESENT

Periodate % Strength	Time Hours	Moles of Periodate Consumed per Mole of Lignin
4.0	2	1.3
4.0	24	2.2
4.0	72	3.5
2.4	60	1.4
2.4	120	2.2
2.4	180	3.0

In the mildness of the extraction conditions, the isolation of liquid ammonia lignin is best compared to Brauns' procedure for obtaining native lignin from spruce by the use of 96% ethanol at room temperature. The greater part of what must have corresponded to Brauns' native lignin in maple wood was probably removed prior to the liquid ammonia treatment by the very thorough extraction with hot ethanol-benzene for three days and with hot ethanol for two days. A re-extraction of the maple meal with hot ethanol for two days,

removed only 0.28% of the wood, as compared to about 6% removed by liquid ammonia at room temperature in five hours. Wald (43) found that even after four months pine native lignin was still very slowly extracted by ethanol. In contrast, liquid ammonia extracted a sharply defined quantity of material from maple in five hours while thereafter the rate of additional solution was extremely slow.

If liquid ammonia lignin exists as such in wood, the initial rapid action can be ascribed to the great penetrating and solvent powers of liquid ammonia. There is no experimental evidence, however, for adopting the view that the action of liquid ammonia is strictly limited to the physical process of solution. Since no less than 60% of the acetyl groups in the maple wood can be ammonolyzed off as acetamide in five hours, it is quite conceivable that in the same period of time ester linkages involving lignin and carbohydrate can also be split. The initial rapid action of the liquid ammonia would then be due to the occurrence of somewhat less than 1% of lignin in a linkage easily ammonolyzed. The resultant lignin fragment, freed of the polysaccharide, then dissolves in liquid ammonia.

It has been variously suggested (18) that the bond of lignin to carbohydrate has the nature of an acetal, ester, glycoside or ether linkage. Of these alternatives, the ester linkage is most likely the ammonia-sensitive bond concerned in the extraction, since ester linkages are far more sensitive to ammonolytic attack than are ethers, glycosides or acetals.

The nitrogen content of purified methanol-soluble Fraction IV was less than 0.01%, and the carboxylic half of any ammonia-sensitive linkage was therefore not in the lignin fraction. This result was to be expected since it has been generally accepted that lignin has no carboxyl groups (144). Any amide was therefore probably part of the holocellulose structure, now under investigation by Neubauer.

If the lignin from polysaccharide Fraction I is included, the total yield of crude lignin from the ammonia extraction is 3.6% on a Klason lignin basis and about 0.86% on the wood basis. Adopting the experimentally determined molecular weight of 1060 for the entire quantity of extracted lignin, this 0.86% corresponds to less than 0.001 moles of lignin per 100 grams of wood. Therefore an increase in the nitrogen content of the maple meal of 0.001 moles would be sufficient to account for the origin of liquid ammonia lignin by ammonolysis. The actual increase in the nitrogen content of the maple wood meal was 0.39% or 0.28 moles of amide which is far more than is required. The additional nitrogen content is probably present as an amide or ammonium salt of polyuronide units present in the maple.

SUMMARY

Little or none of the lignin in spruce wood was extracted by aqueous sodium bicarbonate and carbon dioxide under pressure and at elevated temperatures. This Kolbe reaction, which is characteristic of simple polyhydric phenols, also failed with wood samples which had been prehydrolysed with either acid or alkali. The results were those to be expected of a wood hydrolysis by water at an elevated temperature, and were only slightly moderated by the presence of bicarbonate. The failure of lignin in wood to undergo a Kolbe reaction suggests that few free phenolic hydroxyl groups are present and that very few more are created by mild prehydrolysis of the wood.

Anhydrous liquid ammonia, used either alone, or with added ammonium chloride, sulfite, sulfate or potassium cyanide, at temperatures from -33° to 100°, did not remove enough lignin from spruce, birch, beech or maple wood to warrant consideration as pulping agents. Hardwoods were solubilized to a greater extent than softwoods but both were less sensitive than rye straw, which lost as much as one-half of its lignin when extracted with liquid ammonia.

Liquid ammonia extracted 5.7% by weight from three kilograms of sugar maple wood at 25° in five hours, and thereafter the rate of extraction became very slow. The extract was separated into the following constituents, yields being on a wood basis: (1) a polysaccharide of unknown nature which was partially purified to a light-colored powder with C, 43.7;

H, 6.88; OCH_3, 4.4; Pentosan, 6.9; yield, 0.73%; (2) pure acetamide in a yield of 4.0%, representing the 2.9% of acetyl units removed from the wood; (3)"liquid ammonia lignin" in a yield of 0.72% or 3% on a Klason lignin basis.

The lignin fraction was carefully fractionated into three main portions, the first being soluble in methanol and dioxane, the second insoluble in methanol but soluble in dioxane, and the third, insoluble in both of these liquids. The methanol-soluble fraction which comprised about half of the total, was separated into four sub-fractions with closely similar molecular weights and elementary analyses. It was therefore considered that the methanol-soluble lignin possessed a high degree of homogeneity. This fraction of the lignin was assigned the formula $C_{42}H_{43}O_{15}(OCH_3)_7$ on the basis of its molecular weight of 1060, obtained by dioxane cryoscopy, (average error ±115) and its elementary composition, C, 58.9; H, 6.49; OCH_3, 21.6%. Alkaline methylation introduced five additional methoxyl groups. This formula seems to be the first that has been assigned on the basis of analysis plus a physical determination of molecular weight at a low temperature, to a lignin isolated without the use of elevated temperatures or added acid, alkali or oxidizing agent. However methanol-soluble liquid ammonia lignin was not considered representative of all the lignin in maple wood since the yield was only 1.5% on a Klason lignin basis and its elementary composition was distinctly different from the average for the whole lignin.

Moreover, only 40% of the ammonia lignin dissolved in a standard sulfite cook, capable of removing practically all of the lignin from maple wood. Liquid ammonia lignin was somewhat more sensitive to oxidation by aqueous sodium periodate than maple lignin in situ. Its generally more soluble character was shown also by its complete solution during acid-catalyzed methanolysis, which would have removed not more than half of the lignin from wood. This greater solubility was perhaps connected with a lower molecular weight and a higher oxygen content. Since the nitrogen content of liquid ammonia lignin is negligible, its extraction from wood by liquid ammonia was either a purely physical process of solution, or a chemical process involving the ammonolysis of ammonia-sensitive links. If present at all, the latter were probably of an ester nature, involving hydroxyl groups in lignin and carboxyl groups in the holocellulose portion.

CLAIMS TO ORIGINAL RESEARCH

1. It has been demonstrated that aqueous sodium bicarbonate and carbon dioxide at temperatures up to 160°, is not a system that is capable of pulping wood.

2. The behaviour of solvent-extracted woody materials in anhydrous liquid ammonia under pressure at temperatures ranging from -33° to 100° has been studied for the first time. These extractions removed up to 3.2, 8.9, and 23.5% from spruce, maple and rye straw respectively.

3. The extract originating from three kilograms of maple wood in liquid ammonia at room temperature was separated into three main fractions. The major component was identified as pure acetamide, obtained in a yield of 4% on a wood basis. A polysaccharide with a pentosan content of 6.9%, but otherwise of unknown nature, constituted the second fraction, and the third consisted of about 3% of the total original lignin. This "liquid ammonia lignin" was shown, by careful fractionation from chemically inert solvents, to contain a methanol-soluble portion with a high degree of homogeneity. A molecular formula, $C_{42}H_{43}O_{15}(OCH_3)_7$, was assigned to this portion on the basis of elementary analysis and cryoscopic molecular weight determinations in dioxane. This work is the first in which a true molecular formula has been assigned to a lignin obtained without the use of heat, or added acid, alkali, or oxidizing agent.

4. The response of liquid ammonia lignin to periodate oxidation, methanolysis, acid bisulfite cooks and methylation with dimethyl sulfate and alkali was also investigated.

REFERENCES

1. Purves, C.B., in Ott, E., "Cellulose and Cellulose Derivatives," p. 29, Interscience Publishers, New York, 1943.

2. Phillips, M., in Wise, L.E., "Wood Chemistry," p. 282, Reinhold, New York, 1944.

3. Hibbert, H., Paper Trade J., 113, No. 4, 35 (1941).

4. Freudenberg, K., Ann. Rev. Biochem., 8, 81 (1939).

5. Wacek, A., Experientia, 2, 171 (1946).

6. Percival, E.G.V., Ann. Repts. Progress Chem. (Chem. Soc. London) 39, 142 (1942).

7. Erdtman, H., Svensk Papperstidn., 44, 243 (1941); Pulp Paper Mag. Can., 43, 253 (1942).

8. Hilpert, R.S., and Hellwage, H., Ber., 68, 380 (1935).

9. Wald, W.J., Ritchie, P.F., and Purves, C.B., J. Am. Chem. Soc., 69, 1371 (1947).

10. Freudenberg, K., Zocher, H., and Durr, W., Ber., 62, 1814 (1929).

11. Herzog, R.O., and Hillmer, H., Ber., 60, 365, 1600 (1927); Ber., 64, 1283 (1931).

12. Lange, P.W., Svensk Papperstidn., 47, 262 (1944).

13. Reference 2, p. 346.

14. Freudenberg, K., Lautsch, W., and Engler, K., Ber., 73, 167 (1940).

15. Harris, E.E., D'Ianni, J., and Adkins, H., J. Am. Chem. Soc., 60, 1467 (1938).

16. Godard, H.P., McCarthy, J.L., and Hibbert, H., J. Am. Chem. Soc., 63, 3061 (1941).

17. Reference 2, p. 273.

18. Reference 1, p. 104.

19. West, E., MacGregor, W.S., Evans, T.H., Levi, I., and Hibbert, H., J. Am. Chem. Soc., 65, 1176 (1943).

20. Kulka, M., Fisher, H.E., Baker, S.B., and Hibbert, H., J. Am. Chem. Soc., 66, 39 (1944).

21. West, E., MacInnes, A.S., and Hibbert, H., J. Am. Chem. Soc., 65, 1187 (1943).

22. Klason, P., Paper Trade J., 74, No. 18, 45 (1922).

23. Kulka, M., and Hibbert, H., J. Am. Chem. Soc., 65, 1180 (1943).

24. Oxford, A.E., and Raistrick, H., Biochem. J., 27, 634 (1933).

25. Kolbe, H., Ann., 113, 125 (1860).

26. Tijmstra, S., Ber., 38, 1375 (1905).

27. Brunner, K., Ann., 351, 313 (1907).

28. Bistrzycki, A., and Kostanecki, St., Ber., 18, 1983 (1885).

29. Hewson, W.B., McCarthy, J.L., and Hibbert, H., J. Am. Chem. Soc., 63, 3041 (1941).

30. Hewson, W.B., and Hibbert, H., J. Am. Chem. Soc., 65, 1173 (1943).

31. Fisher, H.E., Kulka, M., and Hibbert, H., J. Am. Chem. Soc., 66, 598 (1944).

32. West, K.A., Hawkins, W.L., and Hibbert, H., J. Am. Chem. Soc., 63, 3038 (1941).

33. Brauns, F.E., J. Am. Chem. Soc., 61, 2120 (1939).

34. Reference 2, p. 277.

35. Buckland, I.K., Brauns, F., and Hibbert, H., Can. J. Research., B13, 61 (1935).

36. Reference 2, p. 339.

37. Brauns, F.E., and Hibbert, H., Can. J. Research, B13, 28 (1935).

38. Reeves, R.E., and Thompson, H.J., Contrib. Boyce Thompson Inst., 11, 55 (1939); C.A., 34, 3923 (1940).

39. Schmid, L., Ber., 58, 1963 (1925).

40. Smith, L.T., Chem. Rev., 23, 193 (1938).

41. Brauns, F.E., and Brown, D.S., Ind. Eng. Chem., 30, 779 (1938).

42. Hagglund, E., "Holzchemie," p. 149, Akademische Verlagsgesellschaft, Leipzig, 1939.

43. Wald, W.J., Doctoral Dissertation, Massachusetts Institute of Technology, 1940.

44. Harris, E.E., Ind. Eng. Chem. 32, 1049 (1940).

45. Hewson, W.B., McCarthy, J.L., and Hibbert, H., J. Am. Chem. Soc., 63, 3045 (1941).

46. Corey, A.J., and Maass, O., Can. J. Research, B13, 289 (1935).

47. Aronovsky, S.I., and Gortner, R.A., Ind. Eng. Chem., 27 451 (1935).

48. Ritchie, P.F., Doctoral Dissertation, McGill, 1947.

49. Lautsch, W., Plankenhorn, E., Klink, F., Angew. Chem. 53, 450 (1940); see also reference 14.

50. Creighton, R.H.J., McCarthy, J.L., and Hibbert, H., J. Am. Chem. Soc., 63, 3049, (1941).

51. Creighton, R.H.J., Gibbs, R.D., and Hibbert, H., J. Am. Chem. Soc., 65, 32 (1944).

52. Pennington, D.E., and Ritter, D.M., J. Am. Chem. Soc., 69, 187 (1947).

53. Hess, K. and Heumann, K.E., Ber. 75, 1802 (1942).

54. Stamm, A.J., in Wise, L.E., "Wood Chemistry," p. 180, Reinhold, New York, 1940.

55. Sisson, W.A., in Ott, E., "Cellulose and Cellulose Derivatives," p. 209, Interscience Publishers, New York, 1943.

56. Hess, K., and Steurer, E., Z. physik. Chem. 193, 234 (1944).

57. Hess, K., and Trogus, C., physik. Chem., B14, 387 (1931).

58. Dehnert, F., and Konig, W., Cellulosechemie, 6, No. 1, 1 (1925).

59. Fieser, L.F., and Fieser, M., "Organic Chemistry," p. 229, D.C. Heath, Boston, 1944.

60. Mellor, J.W., "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," 8, p. 312, Longmans, Green and Co., London, 1928.

61. Loughborough, D.L., and Stamm, A.J., J. Phys. Chem., 40, 1113 (1936).

62. Lansing, W.D., and Kraemer, E.O., J. Am. Chem. Soc., 57, 1369 (1935).

63. Conner, W.P., J. Chem. Phys., 9, 591 (1941).

64. Gralen, N., J. Colloid Sci., 1, 453 (1946).

65. Svedberg, T., and Pedersen, K.O., "The Ultracentrifuge," Clarendon Press, Oxford, 1940.

66. Holmberg, B., Ber., 69, 115 (1936).

67. Holmberg, B., Ber., 75, 1760 (1942).

68. Friedrich, A., Z. physiol. Chem., 176, 127 (1928).

69. Patterson, R.F., West, K.A., Lovell, E.L., Hawkins, W.L., and Hibbert, H., J. Am. Chem. Soc., 63, 2065 (1941).

70. Heuser, E.F.F., "Cellulose Chemistry," p. 138, Wiley and Sons, New York, 1944.

71. Bernardy, G., Z. angew. Chem., 38, 1195 (1925).

72. Grace, N.H., and Maass, O., J. Phys. Chem. 36, 3046 (1932).

73. Bernardy, G., Z. angew. Chem., 38, 838 (1925).

74. Barry, A.J., Peterson, F.C., and King, A.J., J. Am. Chem. Soc., 58, 333 (1936).

75. Clark, G.L., and Parker, E.A., J. Phys. Chem., 41, 777 (1937).

76. Hess, K., and Trogus, C., Ber., 68, 1986 (1935).

77. Hess, K., and Gundermann, J., Ber., 70, 1788 (1937).

78. Davis, W.E., Barry, A.J., Peterson, F.C., and King, A.J., J. Am. Chem. Soc., 65, 1294 (1943).

79. Peterson, C.J., and Hixon, R.M., Iowa State Coll. J. Sci., 7, No. 1, 25 (1932); C.A. 27, 1505 (1933).

80. Claus, W.S., and Hixon, R.M., Iowa State Coll. J. Sci., 12, 205 (1938); C.A. 32, 4956 (1938).

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

82. Freudenberg, K., Lautsch, W., and Piazolo, G., Ber., 74, 1879 (1941).

83. Urban, H., Cellulosechemie, 7, No. 5, 73 (1926).

84. Franklin, E.C., "The Nitrogen System of Compounds," Reinhold, New York, 1935.

85. Watt, G.W., and Leslie, W.B., J. Chem. Education, 18, 210 (1941).

86. Watt, G.W., Leslie, W.B., and Moore, T.E., Chem. Rev., 32, 219 (1943).

87. Fernelius, W.C., and Bowman, G.B., Chem. Rev., 26, 3 (1940).

88. Miller, C.O., and Siehrs, A.E., Proc. Soc. Exptl. Biol. and Med., 29, 535 (1931-2).

89. Muskat, I.E., J. Am. Chem. Soc., 56, 693 (1934).

90. Muskat, I.E., J. Am. Chem. Soc., 56, 2449 (1934).

91. Zechmeister, L., and Toth, G., Naturwissenschaften, 23, 35 (1935).

92. T.A.P.P.I. Official Standard, T 13 m-45 (April, 1945).

93. Bray, M.W., "Methods used at the Forest Products Laboratory for the Chemical analysis of pulps and pulpwoods," p. 32, United States Department of Agriculture, Forest Service, Revised 1939.

94. Powell, W.J., and Whittaker, H.J., J. Soc. Chem. Ind., 43, 35T (1924).

95. Kline, G.M., and Acree, S.F., Bur. Standards J. Research, 8, 25 (1932).

96. Lemieux, R.W., Ph.D. Dissertation, McGill University, 1946.

97. Genung, L.B., and Mallatt, R.C., Ind. Eng. Chem., Anal. Ed., 13, 369 (1941).

98. Clark, E.P., J. Assoc. Offic. Agr. Chemists, 15, 136 (1932).

99. Viebock, R. and Schwappach, A., Ber., 63, 2818 (1930).

100. Friedrich, A ., Z. physiol. Chem., 163, 141 (1927).

101. Peniston, Q.P., and Hibbert, H., Paper Trade J., 109, No. 17, 46 (1939).

102. Association of Official Agricultural Chemists, "Official and Tentative Methods of Analysis," p.28, Washington, D.C., 1945.

103. Ma, T.S., and Zuazaga, G., Ind. Eng. Chem., Anal. Ed., 14, 280 (1942).

104. Eigenberger, E., J. prakt. Chem., 130, 75 (1931).

105. Fieser, L.F., "Experiments in Organic Chemistry," p. 369, D.C. Heath, New York, 1941.

106. Findlay, A., "Practical Physical Chemistry," p. 118, Longmans, Green and Co., London, 1935.

107. Roberts, H.M., and Bury, C.R., J. Chem. Soc., 123, 2037 (1923).

108. Marti, F.B., Bull. soc. chim. Belg., 39, 590 (1930).

109. Kraus, C.A., and Vingee, R.A., J. Am. Chem. Soc., 56, 511 (1934).

110. Fleury, P., and Lange, J., J. pharm. chim., 17, 107, 196 (1933).

111. Hilpert, R.S., and Litmann, E., Ber., 67, 1551 (1934).

112. Corey, A.J., Calhoun, J.M., and Maass, O., B15, 168 (1937).

113. Jahn, E.C., in Wise, L.E., "Wood Chemistry," p. 766, Reinhold, New York, 1944.

114. Ritter, G.J., and Kurth, E.F., Ind. Eng. Chem., 25, 1250 (1933).

115. Freeman, R.D., and Peterson, F.C., Ind. Eng. Chem., Anal. Ed., 13, 803 (1941).

116. Reference 113, p. 770.

117. Brickman, L., Pyle, J.J., McCarthy, J.L., and Hibbert, H., J. Am. Chem. Soc., 61, 868 (1939).

118. Reference 113, p. 806.

119. Yorston, F.H., Pulp Paper Mag. Can., 48, 296 (1947).

120. McMillen, J.M., Gortner, R.A., Schmitz, H., and Bailey, A.J., Ind. Eng. Chem., 30, 1407 (1938).

121. Yorston, F.H., private communication..

122. Kurth, E.F., in Wise, L.E., "Wood Chemistry," p. 435, Reinhold, New York, 1944.

123. Klason, P., Ber., 53, 1864 (1920).

124. Cross, C.F., and Bevan, E.J., Ber., 43, 1526 (1910).

125. Freeman, R.D., in Wise, L.E., "Wood Chemistry," p. 658, Reinhold, New York, 1944.

126. Mitchell, R.L., and Ritter, G.J., J. Am. Chem. Soc., 62, 1958 (1940).

127. Maule, C., Beitrage, wiss. Bot., 4, 166 (1900).

128. de Lamarliere, L.G., Rev. gen. botan., 15, 149 (1903).

129. Sharma, P.D., J. Forestry, 20, 476 (1922).

130. Freudenberg, K., and Sohns, F., Ber., 66, 262 (1933).

131. Friedrich, A., and Diwald, J., Monatsh. 46, 31 (1925).

132. Fuchs, W., and Daur, R., Cellulosechemie, 12, 103 (1931).

133. Rassow, B., and Wagner, K., Wochbl. Papierfabr. 63, 103 (1932).

134. Hagglund, E., and Urban, H., Cellulosechemie, 9, 79 (1928).

135. Anschutz, L., and Broeker, W., Ber., 59B, 2844 (1926).

136. Hertz, W., and Lorentz, E., Z. physik. Chem., A140, 406 (1929).

137. Roth, W.A. and Meyer, I., Z. Elektrochem., 39, 35 (1933).

138. Payne, J.H., Fukanaga, E., and Kojima, R., J. Am. Chem. Soc., 59, 1210 (1937).

139. Virasoro, E., C.A., 38, 6553 (1934).

140. Freudenberg, K., Sohns, F., and Janson, A., Ann., 518, 62 (1935).

141. Compton, J., and Hibbert, H., Can. J. Research, B15, 38 (1937).

142. Wright, G.W., and Hibbert, H., J. Am. Chem. Soc., 59, 125 (1937).

143. Reference 2, p. 296.

144. Reference 42, p. 200, p. 457.