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FRACTIONATION OF OAT HULL LIGNIN

by

Wilbur Scheirich Claus

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for the Degree of

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Approved:

Signature was redacted for privacy.

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INTRODUCTION AND HISTORICAL

General Definition of Lignin

Plant or woody tissues consist principally of cellulose, hemicelluloses, lignin, and small amounts of such constituents as ash, resins, gums, tannins, and proteins. The cellulose and hemicelluloses (pentosans, hexosans, etc.) are polysaccharides, while the lignin represents the non-carbohydrate portion of the plant. Approximately 25% of wood is lignin (35), an organic material which is present in the cell walls of the plant (3, 14, 21).

To the chemist, lignin represents the high molecular weight, non-carbohydrate portion of the cellular tissue after it has been freed of tannins, resins, and gums. It is very resistant to the usual carbohydrate hydrolysis but is soluble in hot alkali sulfites and insoluble in concentrated acids. It can be further defined as that portion which contains methoxyl groups and approximately 20% more carbon than the carbohydrates. The presence of methoxyl groups has usually been considered as one of the most important characteristics of the lignin molecule.

To the botanist, lignin represents that woody or lignified cellular tissue abundant in the plant kingdom. By lignified cellular tissue, the botanist means those tissues which show no customary cellulose reactions and which are insoluble in ammoniacal cupric hydroxide. Lignin is the predominate substance in the lignified cellular tissue.

The chemistry of lignin is at the present time in a state of considerable doubt and uncertainty. Among the most obscure plant constituents, lignin occupies a most prominent place. This substance or mixture of substances is characterized largely by the method of preparation, as well as by the presence of certain characteristic groups, viz., methoxyl and hydroxyl groups.

The methods of lignin preparation are based upon (a) its dispersion in dilute alkali solutions, (b) its insolubility in concentrated acids, such as 64-80% sulfuric and 42% HCl, and (c) its combination with certain organic solvents, such as phenol, methoxy glycol, and other hydroxylic compounds. All investigators agree that it is most unlikely for the lignin to be separated from the wood in its original condition. Many support the view that lignin is chemically combined with the cellulose, either in the form of an ester-type union between an acidic group in the lignin and a hydroxyl group of the carbohydrate, or an ether-type linkage between the lignin and the cellulose or other carbohydrates. Just what changes occur during isolation are unknown at the present time.

The Question of the Existence of Lignin

The question as to whether lignin exists pre-formed in the woody tissue or alternately arises from simpler types of molecules during the extraction process is still an open debate. Investigators, in general, have considered lignin to be a substance pre-formed in the woody tissue, which is altered to a considerable extent by the extraction process.

Recently, there have appeared certain contributions by Hilpert and associates (15-23) which have tended to question the validity of the time-honored ideas on the distribution and properties of lignin. He has pointed out that various sugars (fructose, pentose) give, on prolonged boiling with acids, dark colored compounds which have properties similar to lignin, especially their insolubility in concentrated acids. This fact was interpreted by Hilpert as indicating that the origin of lignin should be looked for in the interaction of certain, unknown as yet, methylated sugars with the acid used in the preparation of the lignin.

It has long been recognized by almost all investigators that in the preparation of "acid lignin" a short period of treatment just sufficient to hydrolyze the carbohydrates is essential; continued contact with the concentrated acid will result in the caramelization of some of the dissolved carbohydrates. In some cases this process was believed to lead to the formation of "synthetic lignin" (13). The similarity of the "synthetic lignin" to the natural lignins was shown by (a) its resistance to hydrolysis with 72% sulfuric acid, (b) its solubility in sulphite reagent after chlorination, (c) its ultra violet absorption spectrum, and (d) its reducing value.

Hilpert (17) further suggested that his proof of the non-existence of lignins in plant materials is substantiated by the fact that on treatment of straw with acids at a very low temperature (-12°), practically all the organic matter was brought into solution. On diluting the extract with water, a flakly precipitate was obtained, amounting to 8-20% of the total material in the case of straw, and to 49-50% in the case of wood; on treating the latter with concentrated acid at room temperature, a product was obtained which contained 61% carbon and 17.7% methoxyl, namely a substance which showed characteristics of lignin. This fact was interpreted as indicating that lignin is produced by the action of acid on certain carbohydrates originally present in the plant material, brought into solution at the low temperatures, and transformed into lignin at room temperature.

Storch (36), however, has shown that a part of the lignin in beech wood is soluble in acid (64% sulfuric) and is precipitated out on dilution with water. The methoxyl content of the insoluble and soluble portions of the lignin is practically the same, pointing merely to a difference in the solubility of some of the lignin but not in its chemical nature. The acid soluble portion of the lignin was also soluble in cold sodium hydroxide, pyridine, and aqueous acetone. A similar amount of lignin could be dissolved out directly from the wood by treatment with 5% sodium hydroxide.

Wedekind (41) treated wood flour with Schweitzer's reagent, (which dissolves the cellulose) and obtained a lignin preparation which was not different in appearance and chemical properties from the lignin prepared by treatment with acids. This led Wedekind to conclude that the existence of beech wood lignin was beyond any question.

Waksman and Cordon (37) have made a study of the yield and chemical nature of lignin prepared from wheat straw by treatment with concentrated sulfuric acid at different temperatures, with and without preliminary treatment with dilute acid. They concluded that the hydrolysis of the carbohydrates was incomplete at the lower temperature, and that this incomplete hydrolysis tended to mask the characteristic properties of the lignin. When lignin obtained from straw at 2° C. was again treated with the same acid at room temperature, the same yield of both lignin and methoxyl was obtained as by the single treatment of a straw with acid at room temperature. Preliminary treatment of the straw with dilute acid, however, did reduce the lignin yield. This was probably due to the destruction of some hemicelluloses, which would otherwise remain with the acid lignin residue. The necessity of hydrolyzing the carbohydrates and hemicelluloses with dilute acid before the determination of lignin has long been recognized and has been utilized in the standard methods of analyses. These facts led Waksman to conclude that Hilpert's theory of the formation of lignin from carbohydrates during the isolation process had no foundation.

Fractionation of Lignin

Many investigators have considered the problem of the existence or non-existence of lignin pre-formed in the woody tissue as of secondary importance compared with the study of the constitution of lignin as it comes to hand. Early investigators in this field seemingly assumed their products to be homogeneous, and upon this assumption based their studies and their conclusions.

It was found, however, that the lignins isolated by various hydroxylic compounds in the presence of catalysts were easily fractionated by various organic solvents. The fact that these fractions, so obtained, were found to differ considerably in structure and properties, indicated that the previously reported lignins were mixtures and not homogeneous products.

Due to the fact that the alkali and acid lignins were insoluble in certain organic solvents, nothing has been reported on the action of other common organic solvents. It would appear that the fractionation of alkali or an acid lignin with some suitable organic solvent would lead to fruitful results in regard to the constitution of these lignins.

Brauns and Hibbert (5) noticed in preparing methanol lignin from spruce wood meal that the crude methanol lignin could be purified by dissolving the prepared lignin in anhydrous dioxane and reprecipitating from ether. This ether-dioxane insoluble portion was used in the determination of the "native building unit" of lignin, which they showed had a formula of $C_{42}H_{32}O_6(OCH_3)_5(OH)_5$. The ether-dioxane soluble fraction was later reported on by MacKinney and Hibbert (25). The last named authors found that the ratio of the methoxyl to hydroxyl in the ether-dioxane soluble was 5:3 as compared with 5:5 in the ether-dioxane insoluble fraction.

Buckland, Brauns, and Hibbert (4) have prepared a phenol lignin by the condensation of lignin with phenol using HCl as a catalyst. They were thus able to obtain by fractionation two chemically different phenol lignins, namely, an ether-dioxane insoluble and an ether-dioxane soluble fraction, in a ratio of approximately 3 to 1. They concluded that the ether insoluble phenol lignin contained 3 new phenolic hydroxyl groups which were capable of methylation with CH_2N_2, while one phenol group had reacted with one hydroxyl group in the lignin unit with the formation of a phenyl-oxygen ether linkage.

The analytical results of the ether-dioxane soluble phenol lignin indicated that a much larger quantity of phenol had condensed with the "native" lignin building unit than in the case of the ether-dioxane insoluble fraction. The correlation found to exist in the investigation between the phenol condensation products, Freudenberg lignin, and Willstatter lignin was regarded by these authors as providing definite experimental evidence of the presence of a chemical unit common to the various lignins.

Gray, King, Brauns, and Hibbert (12) reported that they were able to separate a prepared glycol lignin into a $CHCl_3$ insoluble and a $CHCl_3$ soluble fraction, each having the same chemical composition and yielding the same methylated products.

Marshall, Brauns, and Hibbert (26) prepared an alkali lignin by the method of Mehta (27). They separated the alkali lignin into two fractions: Alkali Lignin A, insoluble in dioxane-ether, and Alkali Lignin B, soluble in dioxane-ether. The alkali Lignin A had a methoxyl content of 15.0% which differed markedly from that obtained by other investigators

(3, 27). The Alkali Lignin B had a methoxyl content of 14.0% and was present in considerable quantity (20%). A molecular weight of 1244, or $C_{67}H_{71}O_{23}$, was deduced for the Alkali Lignin A from methylation experiments using diazomethane and dimethyl sulfate. The Alkali Lignin B seemed to have a molecular weight of 1996, or an expanded formula of $C_{108}H_{107}O_{37}$. These empirical formulas for Alkali Lignin A and Alkali Lignin B were considerably larger than the formula for the "native building unit" postulated by Brauns and Hibbert (5). The authors stated that their results indicated clearly the more complex character of alkali lignin in comparison with lignins extracted by organic solvents, eg., methanol lignin (5).

Hilpert and Wisselinck (22) have reported that fructose and xylose, when treated under conditions similar to those used by Hibbert and co-workers for the isolation of lignin products by use of phenol and HCl, change into substances which in composition and properties are very similar to those obtained by treatment of plant materials with stronger mineral acids. Hilpert maintains that the action of phenol and dioxane on lignin has been underestimated, and that the sensitivity of the sugars to these last two named reagents has been overlooked by Hibbert and his co-workers.

Phillips (29) isolated two lignin fractions from oat hulls by extracting them successively and exhaustively, first with 2% alcoholic sodium hydroxide solution at room temperature, and then by refluxing with 4% aqueous sodium hydroxide solution. All the results agree with the formula $C_{36}H_{31}O_9(OCH_3)_4(OH)_3$ for the first lignin fraction and $C_{38}H_{25}O_8(OCH_3)_4(OH)_3$ for the second lignin fraction. The lignin fraction isolated by the alcoholic sodium hydroxide method was found to have the same composition as that isolated from corn cobs by the same method, namely, $C_{40}H_{46}O_{16}$. In the lignin fraction isolated from corn cobs, however, four hydroxyl and three methoxyl groups were shown to be present, whereas the results on the lignin fraction isolated from oat hulls indicated the presence of three hydroxyl and four methoxyl groups.

Phillips and Goss (31) isolated two fractions from barley straw in the same manner as described above. The lignin fraction isolated by use of alcoholic NaOH had five hydroxyl and four methoxyl groups, while the fraction isolated by the aqueous NaOH had four methoxyl and four hydroxyl groups. There was also a slight difference in the number of carbon and hydrogen atoms of the two fractions.

Phillips and Goss (30) have fractionated oat straw into three fractions by successive treatment with 2% alcoholic NaOH at room temperature, refluxing with 4% aqueous NaOH, and finally treating the residue with fuming HCl. The first and second fraction both contained four methoxyl and four hydroxyl groups. The third lignin fraction contained higher percentage of carbon than the others but the same percentage of methoxyl.

Sarkar (34) treated jute lignin with acetone in the presence of P_2O_5 at a temperature below 10° C. The material that he obtained was no longer soluble in dilute NaOH. He postulated the view that this acetone condensation product was probably a cyclic compound and indicated the presence of two hydroxyl groups in the o-position in the benzene ring, which was formed by the cleavage of the methylene dioxide group present in the lignin molecule.

Statement of Problem

The preceding discussion has brought out the following points:

1. The uncertainty as to whether lignin exists as a definite entity in the woody tissue or is a product formed during the extraction process has been indicated.
2. The improbability of isolating unchanged lignin from the woody tissue is still apparent.
3. The homogeneity of an isolated lignin depends upon the source of material, method of isolation, and the technique used by the investigator.
4. The hitherto reported lignins indicate that they are mixtures and can thus be fractionated by organic solvents.

5. These fractionated lignins vary considerably in structure and properties.

The first three points seem to be fairly well established by the data already quoted. To test the validity of the last two parts, on a lignin isolated from oat hulls, was the purpose of the present work. This work continues the investigation begun by Walde (38).

The experimental part divides itself into three main sections:

A. The preparation of various lignins.

B. The fractionation and alkaline oxidation of the isolated lignins.

C. The preparation of methylated and saponified derivatives of various isolated lignins.

EXPERIMENTAL

The Preparation of Various Lignins

The preparation of ammonia lignin from oat hulls.

The ammonia lignin was prepared from oat hulls according to the procedure of Walde and Hixon (40). The analytical results of the isolated product compared favorably with the results reported by these authors. The analyses, calculated on the ash-free basis, are: C, 62.4; H, 6.6; OCH_3, 11.5; ash, 7.7. Analyses of Walde's ammonia lignin, calculated on the ash-free basis, are: C, 63.8; H, 6.02; OCH_3, 13.1; ash, 3.7.

The preparation of oxidized lignins by alkaline hypoiodite.

Oxidized lignins may be prepared by two different methods. To differentiate these two lignins and their derivatives, appropriate abbreviations have been used throughout the thesis. A list of these abbreviations is given in Table I.

Oxidized lignin from acid-hydrolyzed oat hulls. The first method used to prepare an oxidized lignin was as follows: 1750 grams of air-dry acid-hydrolyzed oat hulls

Table I

List of Abbreviations Used in the Thesis.

1. Iodo-carboxy lignin # I	lignin prepared from acid-hydrolyzed oat hulls by use of NaOI. Also labeled I.C.L. # I.
2. Iodo-carboxy lignin # II	lignin prepared from ammonia lignin by use of NaOI. Also labeled I.C.L. # II.
3. Bromo-carboxy lignin # II.	lignin prepared from ammonia lignin by use of NaOBr. Also labeled Br.C.L. # II.
4. Re-ox.^{1} # I	iodo-carboxy lignin, prepared from acid-hydrolyzed oat hulls, re-oxidized with NaOI.
5. Re-ox.^{2} # I	the preceding product re-oxidized with NaOI.
6. A. Insol. # I	acetone insoluble fraction of the iodo-carboxy lignin prepared from acid-hydrolyzed oat hulls.
7. A. Sol. # I	acetone soluble fraction of the iodo-carboxy lignin prepared from the acid-hydrolyzed oat hulls.
8. Re-ox. A. Insol. # I	acetone insoluble fraction of the iodo-carboxy lignin, prepared from acid-hydrolyzed oat hulls, which was re-oxidized with NaOI.
9. U^{x}	the exponent x represents the number of times methylation was performed by Urban's (U) method (33).
10. di^{x}	the number x represents the number of times methylation was performed by the diazomethane (di).

were treated with 18 liters of cold 4% NaOH. After the solution was allowed to stand overnight, it was filtered by suction using a Buchner funnel containing a cloth filter. To determine quantitatively the theoretical amount of iodine needed to oxidize the alkaline lignin solution, 10.00 cc. portions of the alkaline lignin solution were treated with 20.00 cc. of 0.2N I_2-KI solution, according to the procedure of Goodwin (11), and the excess I_2 titrated with standard $Na_2S_2O_3$. The excess iodine titrated in three samples, expressed in cc. of 0.1014N $Na_2S_2O_3$, was 20.55, 20.20, and 20.40. The titration of the blank was equivalent to 42.70 cc. of the 0.1014N $Na_2S_2O_3$. The lignin in 10.00 cc. of the alkaline solution therefore required 22.40 cc. of 0.1014N $Na_2S_2O_3$.

From the above result a calculated amount of 317 grams of iodine and 418 grams of KI, dissolved in two liter of water, were added with vigorous stirring to 11 liters of the alkaline lignin solution during a two hour period. The solution was allowed to stand overnight. When the oxidation was complete a known amount of the mixture was tested for the presence of free iodine by acidifying the solution. The alkaline lignin solution was heated to 55° C. and concentrated HCl was added, with vigorous stirring, until the solution was slightly acid to litmus. The precipitated lignin was filtered by suction and washed free of halides. A 15% yield of the air-dry oxidized lignin was obtained; the calculations were based on the air-dry, acid-hydrolyzed oat hulls. The freshly prepared oxidized lignin liberated CO_2 from a $NaHCO_3$ solution and thus indicated the presence of a carboxyl group. Walde (38) showed that the iodine was firmly bound to the lignin molecule. For convenience of reference the lignin prepared from the acid-hydrolyzed oat hulls has been tentatively designated as iodo-carboxy lignin # I. Analysis: C, 44.25; H, 4.3; OCH_3, 9.63; ash, 0.18; I_2, 11.4. The lignin was insoluble in petroleum ether, ethyl ether, chloroform, lactic acid, and o-nitrotoluene; slightly soluble in dioxane, ethyl alcohol, and isopropyl alcohol; it was partially soluble in methyl alcohol and acetone.

Oxidized lignin from ammonia lignin. The second method used for the preparation of an oxidized lignin was the oxidation of the isolated ammonia lignin by use of NaOI, according to the procedure of Walde and Hixon (40). The NaOI oxidation values on three samples of the ammonia lignin, obtained by the method described by Walde (38), were 187.5, 188.3, and 187.5 respectively. These latter values, expresses in cc. N/10 iodine per gram of lignin, are in agreement with the average oxidation value of 188.0 obtained by Walde.

A 75% yield of the oxidized lignin was obtained by this procedure. The lignin thus prepared by the oxidation of the ammonia lignin has been tentatively designated as iodo-carboxy lignin # II. Analyses: C, 49.20; H, 4.41; OCH_3, 7.4; I_2, 14.4; ash, 0.0. The freshly prepared product was completely soluble in 10% NaOH, 5% Na_2CO_3, and 5% $NaHCO_3$ solutions; partially soluble in dioxane and acetone; and insoluble in ether, methyl and ethyl alcohol, carbon tetrachloride, and ethyl acetate.

The preparation of re-oxidized iodo-carboxy lignins.

Re-oxidized iodo-carboxy lignin # I. It was found that the iodo-carboxy lignin # I could be further oxidized by NaOI. A weighed sample of the iodo-carboxy lignin # I was placed in a 250 cc. iodine flask, and 10 cc. of water were added to facilitate dispersion. To this dispersed sample were added 25.00 cc. of 1 N NaOH solution. After solution had been complete, 20.00 cc. of 0.2 N I_2-KI solution were added, using the precautions recommended by Goodwin (11). The flask was stoppered immediately and allowed to stand the allotted time for oxidation. To the alkaline lignin solution were added 25.00 cc. of 2 N H_2SO_4, and the liberated I_2 was titrated with standard sodium thiosulfate. Starch was used as an indicator. The results are shown in Table II. The time of oxidation did not seem to bring about any appreciable change in the oxidation values.

It was found, however, that if there were a preliminary

Table II

The effect of time on the NaOI oxidation of iodo-carboxy lignin # I

Wt. of oxidized lignin (I.C.L.I) g.	Time of oxidation hours	Excess $Na_2S_2O_3$ cc.	cc. of 0.1014*N* $Na_2S_2O_3$	cc. *N*/10 iodine per gram of lignin
0.2018	24	29.50	13.15	67.18
0.2114	24	28.80	13.85	67.55
0.1906	48	30.75	11.90	64.37
0.1910	48	30.60	12.05	65.05
0.1912	48	30.60	12.05	64.98

heating of the alkaline, lignin-containing solution at various elevated temperatures for a one-half hour interval before the addition of the I_2-KI solution, the oxidation value increased from an average of 65.0 to 154.0 cc. of *N*/10 iodine per gram of lignin. The results of these experiments are indicated in Table III. Consistent oxidation values were obtained at both the 100° and 120° C. temperatures.

The theoretical amount of I_2 and KI necessary to reoxidize 10.0 grams of the iodo-carboxy lignin # I was calculated from the average oxidation value obtained from the latter series of experiments. Ten grams of the iodo-carboxy lignin # I were dissolved in 65 cc. of 5 *N* NaOH and heated to 100° C. for one-half hour. The iodine-potassium iodide

Table III

The effect of temperature on the NaOI oxidation of iodo-carboxy lignin # I.

Wt. of oxidized lignin (I.C.L.I) g.	Temp. of heating °C.	Time of heating hours	Time of oxidation hours	Excess $Na_2S_2O_3$ cc.	cc. 0.1023 N $Na_2S_2O_3$	cc. N/10 iodine per gram of lignin
0.2232	120	0.5	0.5	20.85	33.35	153.8
0.2206	120	0.5	0.5	20.90	33.30	155.2
0.2186	100	0.5	0.5	21.65	32.55	153.0
0.2164	100	0.5	0.5	22.15	32.05	152.4
0.2149	100	0.5	0.5	21.80	32.40	154.8

solution was added drop-wise with shaking and the stoppered solution allowed to stand for three hours. The isolation of the re-oxidized product was then carried out in the usual manner. This product has been tentatively designated as re-oxidized^1 iodo-carboxy lignin # I (re-ox.^{1} I.C.L. # I). Yield, 8.0 g. Analysis: C, 48.7; H, 4.74; OCH_3, 8.5; I_2, 14.8; ash, 19.5. During the NaOI oxidation of the iodo-carboxy lignin # I iodoform crystals were observed. The crystals were later characterized and identified as previously reported by Walde and Hixon (40).

Oxidation of the re-oxidized iodo-carboxy lignin # I. The isolated re-oxidized iodo-carboxy lignin # I (OCH_3, 8.5) was found to have an oxidation value of 44.7 cc. N/10 iodine per gram of lignin. The effect of time on the NaOI oxidation of the re-oxidized iodo-carboxy lignin # I is shown in Table IV. It is evident from the table that an increase in the time of oxidation did not bring about an increase in the oxidation value, but tended toward a lower value.

The re-oxidized iodo-carboxy lignin # I (OCH_3, 8.5) was again oxidized with the calculated amount of I_2-KI and isolated in the usual manner. A 20% yield was obtained. The twice oxidized iodo-carboxy lignin, tentatively designated as re-oxidized^2 iodo-carboxy lignin # I (re-ox.^{2} I.C.L. # I), had a very low oxidation value and was not further re-oxidized. Analysis: C, 47.5; H, 3.9; OCH_3, 7.5; I_2, 15.7; ash, 19.1.

Table IV

The effect of time on the NaOI oxidation of the re-oxidized iodo-carboxy lignin # I.

Wt. of re-oxidized lignin g.	Time of oxidation hours	Excess $Na_2S_2O_3$ cc.	cc. of 0.1014N $Na_2S_2O_3$	cc. N/10 iodine per gram of lignin
0.1761	2	34.05	8.08	46.72
0.2060	2	33.80	9.33	46.70
0.2053	4	33.75	8.38	42.34
0.2020	48	33.45	8.68	44.3
0.2010	48	33.58	8.55	43.9

The preparation of an oxidized lignin by alkaline hypobromite.

Oxidized lignin from ammonia lignin. The sodium hypobromite oxidation values on the ammonia lignin were obtained in a manner similar to the values obtained by the NaOI oxidation procedure. There were, however, a few variations. The procedure was as follows: 10 cc. of water were added to a weighed lignin sample which had been placed in a 250 cc. iodine flask. Twenty five cc. of 1 N NaOH were added and the mixture shaken till dispersion was complete. To the alkaline lignin solution were added 50.00 cc. of NaOBr, which had been prepared according to the procedure of Kolthoff (24). The flask was stoppered immediately after the addition of the alkaline hypobromite solution and allowed to stand for ten minutes. About two grams of KI were added to the solution, after which 25.00 cc. of 2 N H_2SO_4 were added. The liberated iodine was immediately titrated with standard $Na_2S_2O_3$ using starch as an indicator. A blank was also obtained using the above standardized procedure. The excess cc. of sodium thiosulfate used in the lignin titration were subtracted from the cc. of thiosulfate used in the blank to give the number of cc. used in oxidizing the weighed lignin sample. The results of the effect of time on the NaOBr oxidation of ammonia lignin at room temperature are shown in Table V. It is evident from the table that an increase in time of oxidation did not bring about an increase in the oxidation value.

Table V
The effect of time on the NaOBr oxidation of ammonia lignin at room temperature.

Wt. of ammonia lignin g.	Time of oxidation hours	Excess $Na_2S_2O_3$ cc.	cc. of 0.1014N $Na_2S_2O_3$	cc. N/10 bromine per gram of lignin
0.1925	1	0.95	44.88	240.4
0.1912	1	1.10	44.73	241.1
0.1898	2	0.90	44.93	244.1
0.1902	2	0.85	44.98	243.8
0.1912	4	0.70	45.13	243.4
0.1898	4	0.70	45.13	245.2
0.1943	18	0.65	45.28	239.8
0.1927	18	0.60	45.23	242.0
0.1951	18	0.65	45.28	238.7

It was found that when there was a preliminary heating of the alkaline lignin-containing solution to 80° and 100° C. for a one-half hour interval prior to the addition of the NaOBr solution, the oxidation values at these two temperatures were practically the same, namely, 242.0. This latter figure was found to be in close agreement with the results obtained by the NaOBr oxidation of the ammonia lignin at room temperature (Table V).

The NaOBr oxidized lignin was prepared according to the following procedure: five liters of N/2 NaOH solution containing 60 cc. of bromine were stirred until all of the bromine was dissolved. Twenty-eight and one-half grams of ammonia lignin, which had been well dispersed with water, were added slowly to the well-stirred alkaline hypobromite solution, and the solution allowed to stand overnight. The lignin was then precipitated with concentrated HCl, filtered, and dried in the usual manner. Yield, 8.2 grams. Analysis: C, 46.1; H, 6.2; OCH_3, 2.3; Br_2, 29.1; ash, 18.5. The lignin prepared from the ammonia lignin by NaOBr has been tentatively designated bromo-carboxy lignin # II (Br.C.L. # II).

Oxidation values on the isolated bromo-carboxy lignin # II were obtained in the same manner as described above. The oxidation value decreased from an average of 242.0, as obtained on the ammonia lignin, to 68.0, the value obtained on the isolated bromo-carboxy lignin # II. The time of oxidation made no appreciable difference on the NaOBr oxidation value of the isolated bromo-carboxy lignin # II.

A schematic summary of these various lignin preparations are shown in Diagram I.

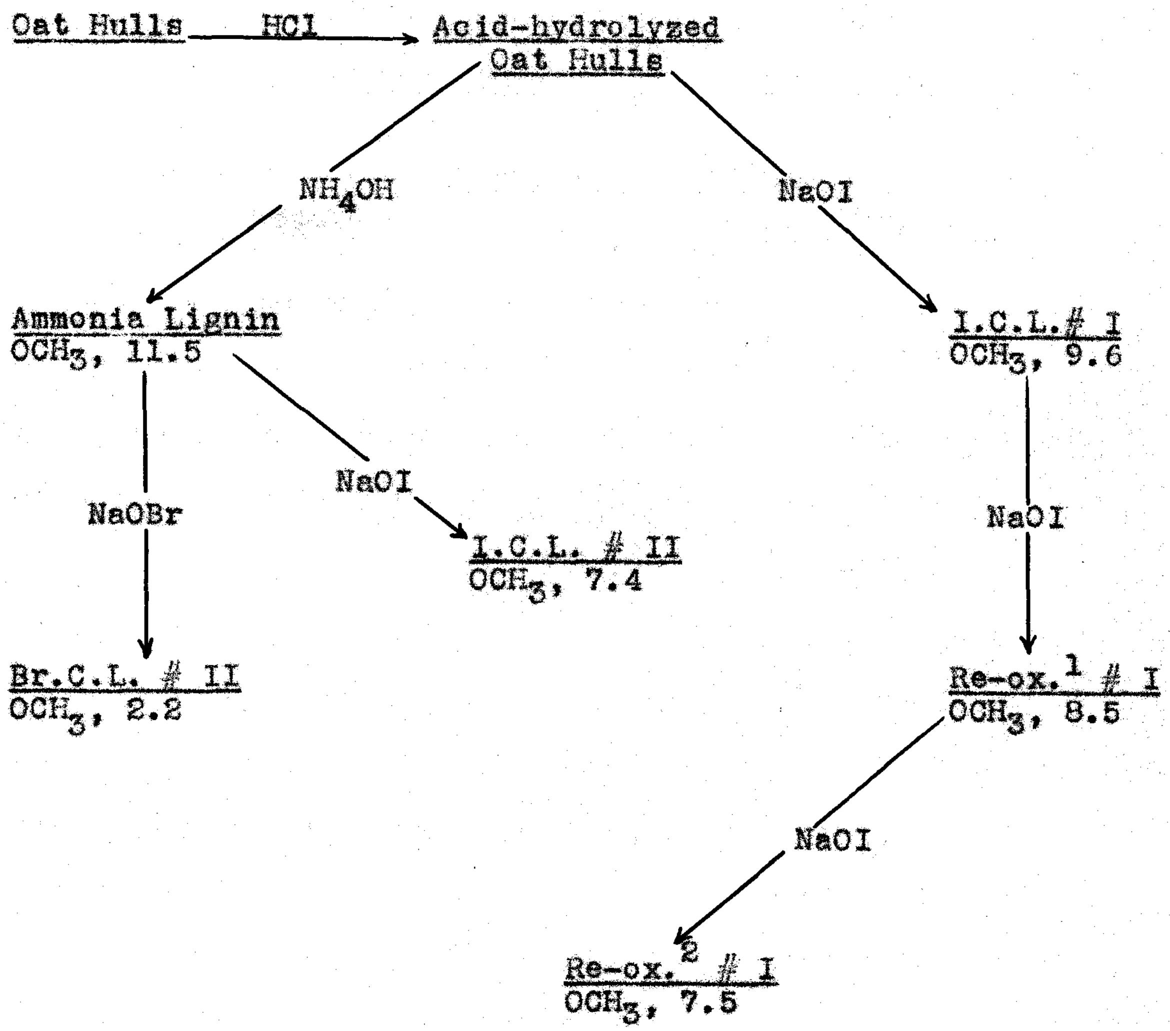
The Fractionation and Alkaline Oxidation of Isolated Lignin Preparations.

The fractionation and alkaline oxidation of ammonia lignin.

Air-dry ammonia lignin (22.5 g.) was exhaustively extracted with acetone for 9 hours in a Soxhlet extractor. The dark colored solution was replaced by fresh acetone until the extractant liquor was colorless. The acetone soluble fraction was then concentrated by vacuum distillation and allowed to evaporate to dryness. The gummy-like residue was dissolved in NH_4OH and slowly heated to 40° C. Concentrated HCl was added to the hot ammoniacal solution until slightly acid to litmus. A light brown flocculent lignin was precipitated. The lignin was filtered, redissolved, and reprecipitated in the above manner. Care was exercised in precipitating this lignin fraction so that the temperature did not rise above 50° C. On the addition of the concentrated HCl to the ammoniacal solution heated to higher temperatures, the lignin coalesced and became a heavy, black, gummy mass. The acetone soluble fraction (air-dry) weighed 6.5 grams, whereas the acetone insoluble fraction (air-dry) weighed 12.1 grams. A

Diagram I

Diagrammatic summary of
various lignin preparations.



repeated run on 21.0 grams of ammonia lignin yielded 10.7 grams of the acetone insoluble fraction and 9.5 grams of the acetone soluble fraction.

The acetone soluble and insoluble fractions were oxidized by NaOI in the same manner as described for the NaOI oxidation of the ammonia lignin. In each case an air-dry sample was used and all the precautions observed as recommended by Goodwin (11) and Walde (38).

Some experiments were also run by giving the alkaline lignin solution a preliminary heating at various temperatures for a period of one-half hour before the addition of the I_2-KI solution. The results of the NaOI oxidation of the acetone soluble fraction of the ammonia lignin under these varying conditions, are shown in Table VI. An increase in the time of oxidation at room temperature did not bring about any change in the oxidation values. Approximately the same oxidation values were obtained when the alkaline lignin solution was treated to a preliminary heating at 120° C. for a half-hour before the NaOI oxidation procedure.

When the same procedure as described above for the acetone soluble fraction was applied to the acetone insoluble fraction, the oxidation values increased with an increase in temperature and reached a constant value at 80° C. The results are shown in Table VII.

A series of similar experiments were carried out on the

Table VI

The effects of time and temperature on the NaOI oxidation of the acetone soluble fraction of ammonia lignin.

Wt. of acetone soluble lignin g.	Time of heating hours	Temp. of heating °C.	Time of oxidation hours	Excess $Na_2S_2O_3$ cc.	cc. of 0.1031N $Na_2S_2O_3$	cc. of N/10 iodine per g. of lignin
0.1947	--	room temp.	1	1.85	40.10	212.8
0.1930	--	" "	1	2.35	39.60	212.0
0.1949	--	" "	48	1.80	40.15	213.8
0.1914	--	" "	48	1.75	40.20	215.8
0.1902	0.5	120	0.5	1.80	40.15	217.8
0.2022	0.5	120	0.5	2.25	39.70	202.2

Table VII

The effect of temperature on the NaCI oxidation of the acetone insoluble fraction of ammonia lignin.

Wt. of acetone insoluble lignin g.	Time of heating hours	Temp. of heating °C.	Time of oxidation hours	Excess $Na_2S_2O_3$ cc.	cc. of 0.1031$\underline{N}$ $Na_2S_2O_3$	cc. of $\underline{N}$/10 iodine per g. of lignin
0.1982	--	room temp.	0.5	11.05	31.08	161.7
0.1949	--	" "	0.5	10.90	31.23	165.7
0.2025	0.5	60	0.5	7.90	35.23	174.2
0.2048	0.5	60	0.5	7.60	35.53	173.9
0.2025	0.5	80	0.5	5.70	36.43	187.2
0.2006	0.5	80	0.5	6.50	35.63	184.0
0.1946	0.5	100	0.5	6.90	35.23	186.7
0.1989	0.5	100	0.5	6.45	35.68	185.2

non-fractionated ammonia lignin. The samples, dissolved in alkali, were heated at various temperatures for one-half hour, and then treated with I_2-KI in the usual manner. The results of these experiments are shown in Table VIII. The oxidation values at -5° and 120° C. were 152.0 and 200.0, respectively. A corresponding gradation in the oxidation values was obtained between these two temperatures. At a temperature higher than 120° C. there was a slight decrease in the oxidation value.

When the concentration of the iodine used for the NaOI oxidation of the ammonia lignin was varied, a higher oxidation value was obtained. The conditions of oxidation were in each case: a time allottment of 24 hours, and a temperature approximating 20° C. The results are tabulated in Table IX. The effect of 0.1 N, 0.2 N, and 0.3 N iodine solution on the oxidation values of ammonia lignin varied from 99.0, 165.5, and 175.5, respectively. Higher concentrations than 0.3 N were not attempted.

Oxidation of the acetone soluble fraction of the ammonia lignin with NaOBr at room temperature gave a consistent oxidation value of 240.0 cc. N/10 bromine per gram of lignin. This figure is in close agreement with the values on the non-fractionated ammonia lignin. The NaOBr oxidation values on the acetone insoluble fraction of the ammonia lignin were somewhat lower, but gave a consistent oxidation value of 226.0 cc. N/10 bromine per gram of lignin.

Table IX

The effect of concentration of I_2-KI on the oxidation values of ammonia lignin.

Wt. of ammonia lignin g.	Time of oxidation hours	Excess $Na_2S_2O_3$ cc.	cc. of 0.1023N $Na_2S_2O_3$	N of I_2-KI soln.	cc. of N/10 iodine per g. of lignin
0.2201	24	0.25	21.00	0.1	98.5
0.2161	"	0.25	21.00	0.1	99.6
0.2220	"	5.80	35.75	0.2	165.0
0.2153	"	6.60	34.95	0.2	166.1
0.2202	"	23.45	37.45	0.3	174.1
0.2141	"	23.95	36.95	0.3	176.0

With a preliminary heating at various temperatures, followed by the NaOBr oxidation in the above described manner, the oxidation values of both the acetone soluble and insoluble fractions remained the same, namely, 240.0 and 226.0, respectively.

The fractionation and alkaline oxidation of the iodo-carboxy lignin prepared from the acid-hydrolyzed oat hulls.

The iodo-carboxy lignin # I was exhaustively treated with acetone in the same manner as described above for the fractionation of the ammonia lignin. The acetone fraction, after the evaporation of the excess acetone, was dissolved in 1 N NaOH, and the alkaline solution heated to 80° C. To the hot alkaline solution was added concentrated HCl till the solution was slightly acid. The precipitated acetone soluble fraction was filtered, washed, and dried in the usual manner after which it was dried over P_2O_5 in a vacuum dessicator.

The acetone insoluble fraction was likewise reprecipitated from an alkaline solution by concentrated HCl and dried in the same manner. Twenty three and two tenths grams of air-dry iodo-carboxy lignin # I gave 23.5 grams of the vacuum-dried acetone insoluble fraction and 1.8 grams of the vacuum-dried acetone soluble fraction, or a gain in weight of 9.1%. A duplicate run showed an 11% weight increase when carried out in an analgous manner. The same weight increase was noted when the acetone extraction was performed on an air-dry or oven-dry (120°; 18 hrs.) sample of the iodo-carboxy lignin # I. Analyses on the acetone soluble fraction are: C, 52.5; H, 4.8; OCH_3, 5.2; I_2, 17.3. Analyses, on the acetone insoluble fraction are: C, 51.6; H, 4.8; OCH_3, 9.76; I_2, 12.8.

Oxidation of the acetone soluble fraction of the iodo-carboxy lignin # I with NaOI at room temperatures gave a consistent value of 110.0 and 111.4, whether the time of oxidation were one hour or forty eight hours. The NaOI oxidation of the acetone insoluble fraction gave an oxidation value of 104.0 at room temperature, regardless of the time of oxidation. Preliminary heating at 100° C. for one-half hour, of the alkaline solution containing the acetone insoluble fraction, followed by the NaOI oxidation in the usual manner, gave a consistent oxidation value of 144.0. The oxidation value of the non-fractionated iodo-carboxy lignin # I was only 65.0 when oxidized at room temperature, but rose to 154.0 when treated to a preliminary heating at 100° C. as described above. A summary of the NaOI oxidation of the iodo-carboxy lignin # I and its acetone fractions is shown in Table X. Insufficient quantities of the acetone soluble fraction prevented any series of experiments using the preliminary heating method described above.

Table X

The effect of temperature on the NaOI oxidation of the iodo-carboxy lignin # I and its acetone fractions.

	cc. N/10 iodine per gram of lignin	
	Room Temp.	100° C.
Iodo-carboxy lignin # I	65.0	154.0
Acetone soluble fraction	111.0	—
Acetone insoluble fraction	104.0	144.0

The preparation of re-oxidized acetone insoluble iodo-carboxy lignin # I.

The theoretical amount of I_2 necessary to oxidize 5.0 grams of the acetone insoluble iodo-carboxy lignin # I (OCH_3, 8.8; I_2, 12.8) was calculated from the oxidation value of 144.0 cc. of N/10 iodine per gram of lignin. The lignin was dissolved in 100 cc. of water containing 23 grams of NaOH, to which was added, with shaking, a solution containing 9.0 grams of iodine and 11.8 grams of potassium iodide. The alkaline solution was immediately stoppered and allowed to stand for six days. Iodoform was observed in the alkaline solution and identified and characterized as reported above. The alkaline oxidized solution was then acidified with concentrated HCl, filtered, washed, and dried in the usual manner. Yield, 4.0 grams. Analysis: OCH_3, 8.2; I_2, 17.6; ash, 0.0.

The acid filtrate from the above oxidation was concentrated on the steam bath to one-half its original volume. The solution was then extracted with ether. The ether extract containing the excess iodine was washed with sodium thiosulfate until the thiosulfate was not further reduced by the iodine. The ether extract was then dried over anhydrous Na_2SO_4. After filtering off the Na_2SO_4 the ether solution was concentrated, and a gummy-like residue was obtained. Benzene was added to this gummy residue, which gave a benzene soluble and a benzene insoluble fraction. The benzene insoluble fraction was completely soluble in 3 cc. of acetone. To the acetone solution was added a large excess of CH_2N_2. The benzene soluble fraction was methylated in like manner.

After allowing the CH_2N_2 methylated solutions to stand in the cold for 18 hours, the excess CH_2N_2 was removed by heating at 50° C. for 15 minutes. On cooling the acetone fraction which had been methylated with diazomethane, a small quantity (1-5 mgms.) of crystals were obtained, which have not as yet been identified. The benzene portion, methylated with diazomethane, failed to give any characteristic crystals. A slight residue, however, was obtained.

The Preparation of Methylated and Saponified Derivatives of Various Lignin Preparations.

Methylation of all types of lignin has been regarded as one of the fundamental reactions in this field of study. A recent publication of Compton and Hibbert (7) has pointed out that the use of dimethyl sulfate and caustic as a methylating agent requires caution in the interpretation of data when applied to the structure of lignin. These authors pointed out that high temperatures and large excess of alkali caused the formation of free hydroxyl groups during the methylation process. The best methylating conditions for avoiding structural changes, according to these authors, are: (a) use of acetone as a solvent; (b) a slight excess of alkali (5-10%); and (c) a temperature of 20° C. It was fortunate that prior to this publication of Compton and Hibbert the procedure used below in methylating various lignin preparations and fractions was that recommended by these authors, with the omission of the use of acetone as a solvent.

Methylation by diazomethane was also performed on the various isolated lignin preparations. The diazomethane, prepared according to the method of Arndt (2), was allowed to react with the dry lignin product in the cold for an appropriate time. The excess diazomethane and ether were then removed by heating at 50° C. for 15 minutes. Before each analysis the product was dried in a vacuum oven (20-25 mm.) over P_2O_5 at temperatures varying for the different products. A more detailed description of the methylation and treatment with alkali of the various lignin preparations is described below. Appropriate schematic diagrams are also included to show these series of reactions. All of the analyses reported below were obtained by the micro methods of Pregl and Roth (32).

The preparation of methylated and saponified derivatives of the ammonia lignin.

Methylation of ammonia lignin. One gram of air-dry ammonia lignin (OCH_3, 11.5; ash, 0.0) was treated with 40 cc. of CH_2N_2-ether solution. The lignin solution was allowed to stand overnight, after which the supernatant ether layer was decanted. The methylated product was washed twice with low boiling petroleum ether (30-40° C.) and decanted each time. The product was finally dried over P_2O_5 in a vacuum oven at 56° C. for 4 hours. Analysis: OCH_3, 24.68. After a repeated methylation with CH_2N_2, purification, and drying, as reported in the above experiment, the methoxyl analysis was 24.14%. Yield, 1.2 grams. The diazomethane methylated product was insoluble in cold and hot 10% NaOH, but completely soluble in alcoholic NaOH.

Two grams of air-dry ammonia lignin and 70 cc. of 45%

KOH (655 grams KOH per liter of water) were treated with 65 cc. of Me_2SO_4 according to the procedure described by Urban (33). The solution was slightly alkaline after the addition of the sulfate. The product was filtered, washed free of sulfates, and dried in the air. The product was again methylated in the same manner. The twice methylated product, after drying in a vacuum oven (20-25 mm.) over P_2O_5 at 56° C. for 4 hours, had a methoxyl content of 27.5%. This product was again methylated with Me_2SO_4 and caustic which gave a product, when treated as described above, having a methoxyl value of 27.8%. Yield of thrice methylated ammonia lignin, 2.1 grams. The fully methylated product was completely soluble in $CHCl_3$ and cold alcoholic NaOH, but insoluble in hot 10% NaOH.

<u>Methylation of the acetone soluble fraction of ammonia lignin.</u> The acetone soluble fraction of ammonia lignin (OCH_3, 10.6) was in like manner methylated with CH_2N_2 until a constant methoxyl content was obtained. The fully diazomethane methylated product had a constant methoxyl value of 23.3%. Complete methylation of the acetone soluble fraction of the ammonia lignin by Urban's method, as described above, yielded a product having a constant methoxyl content of 28.9%.

<u>Methylation of the acetone insoluble fraction of the ammonia lignin.</u> The acetone insoluble fraction of ammonia

lignin (OCH_3, 11.1) was methylated with CH_2N_2 until a constant methoxyl content was obtained. The final methoxyl content of the diazomethane methylated product was 22.8%. Complete methylation of the acetone insoluble fraction by Urban's method gave a methylated derivative with a methoxyl content of 23.0%. After two methylations with Me_2SO_4, according to the method described above, the methoxyl content was 23.1%, and after the third methylation, the methoxyl content was 23.0%. Analysis, calculated on the ash-free basis: C, 58.5; H, 6.33; OCH_3, 23.0; ash, 3.62. The fully methylated acetone insoluble fraction was completely soluble in $CHCl_3$, from which it could be precipitated by use of anhydrous ether. Such purification, by use of $CHCl_3$ and ether, did not affect the methoxyl content if proper precautions were observed in washing and drying.

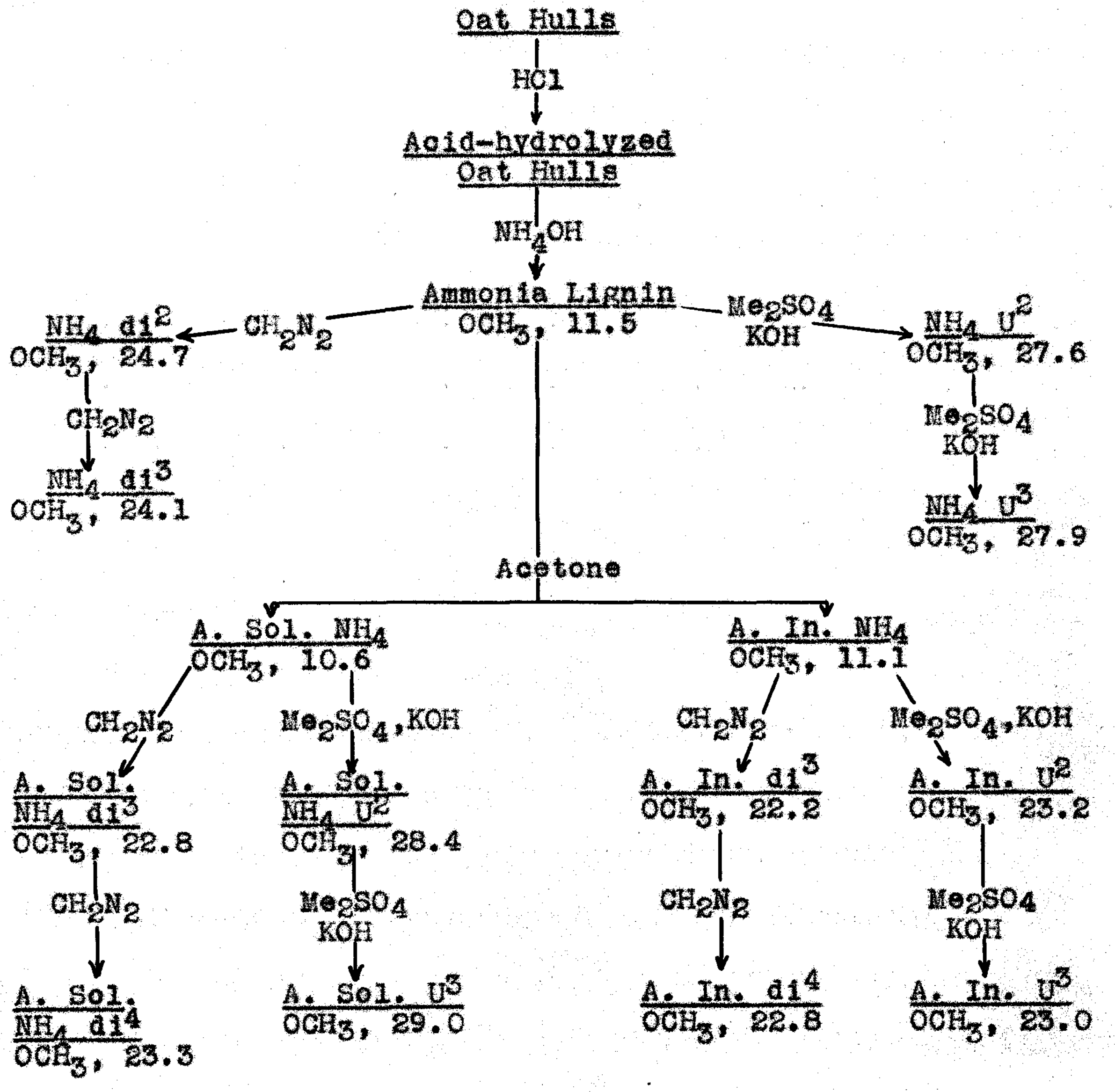
The results of the methylation of ammonia lignin and its acetone fractions are tabulated in Diagram II. It is evident from these results that the methylation of the acetone insoluble fraction by Urban's method and by diazomethane give a product having the same methoxyl content.

The preparation of methylated and saponified derivatives of iodo-carboxy lignin # II.

Methylation of iodo-carboxy lignin # II. Iodo-carboxy lignin # II (OCH_3, 7.4; I_2, 14.4) was methylated three times

Diagram II

The methylation of ammonia lignin and its acetone fractions.



with CH_2N_2 in the usual manner. After each methylation the product was allowed to stand in the cold (15° C.) for 18 hours. The excess ether was then decanted, and the product washed twice with low boiling petroleum ether (30-40° C.). Each time before the analysis the product was dried in a vacuum oven (20-25 mm.) over P_2O_5 at 78° C. for 2-6 hours. The methoxyl content after the second and third methylations was 16.68 and 16.30, respectively. The iodine analysis on the thrice methylated derivative was 12.2%. The product was insoluble in cold and hot 10% NaOH and only slightly soluble in alcoholic NaOH.

Four grams of the iodo-carboxy lignin # II were methylated three times with dimethyl sulfate and caustic according to the procedure described above. The products were dried before each analysis in a vacuum oven (20-25 mm.) over P_2O_5 at 78° C. for 2-6 hours. After the second and third methylations the methoxyl content was 19.86 and 19.32%, respectively. Found, I_2, 7.7%. The product was soluble in dioxane, $CHCl_3$, CH_3OH, and C_6H_6, and only slightly soluble in dioxane-ether and hot 10% NaOH.

Treatment of the Me_2SO_4 methylated iodo-carboxy lignin # II with 10% NaOH. Two grams of the Me_2SO_4 methylated iodo-carboxy lignin # II (OCH_3, 19.5), dispersed with water, were finely pulverized in an agate mortar. Fifty cubic centimeters of 10% NaOH were then added to the lignin suspension. The alkaline lignin suspension was placed in a 125 cc. round bottomed flask and refluxed for two hours. The solution was filtered through filter paper and then re-filtered through diatomaceous earth. The filtrate containing the soluble lignin portion was heated to 55° C. and the lignin precipitated with concentrated HCl. The alkaline soluble portion (A) (see Diagram III) was isolated and dried in the usual manner. Yield, 0.5 grams. Analysis; OCH_3, 17.2; I_2, 11.0; ash, 0.0.

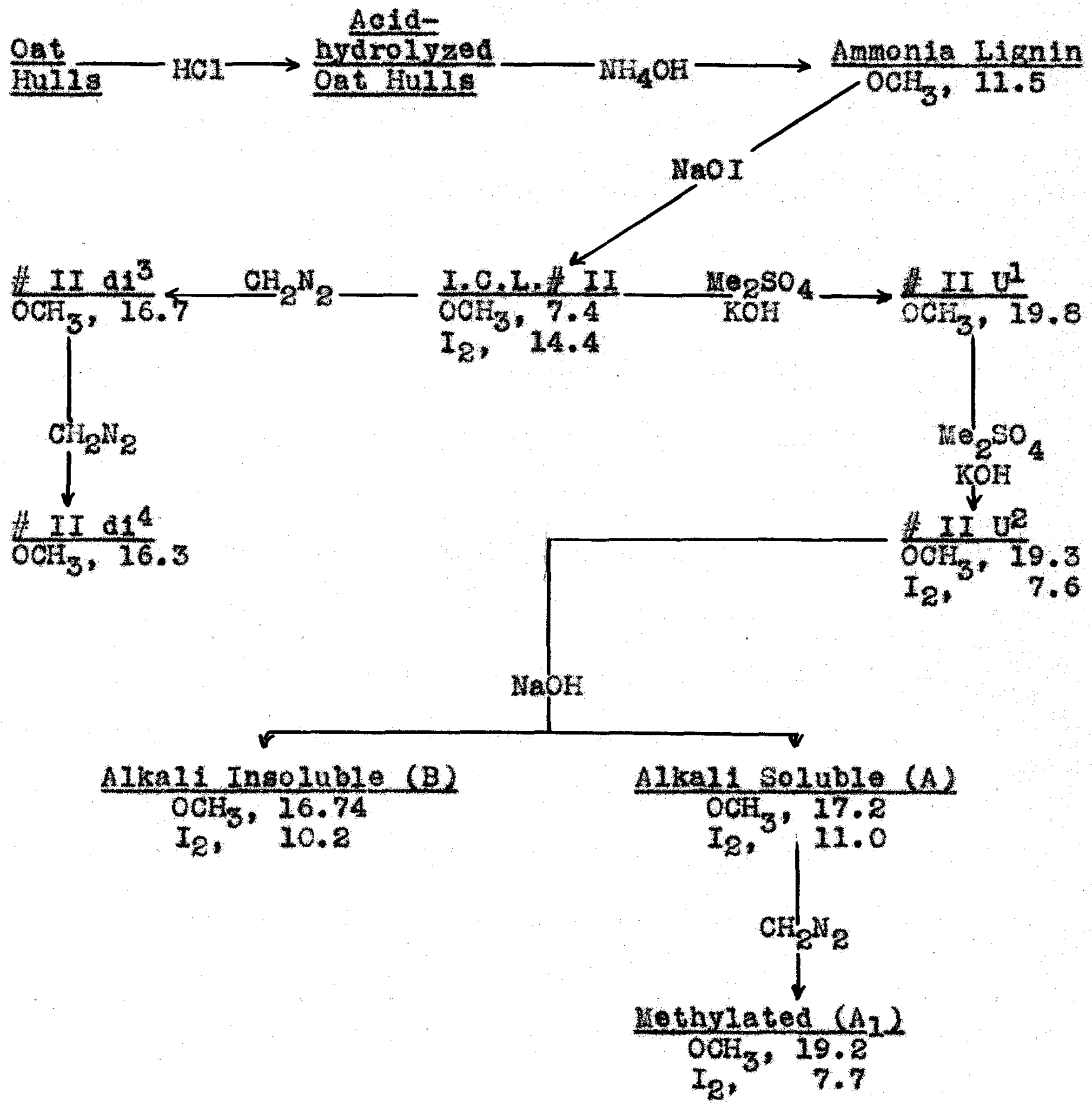
The alkaline insoluble fraction (B) was washed with water and dilute HCl until the lignin was free of alkali. Yield of the insoluble fraction (B), 1.0 gram. Analysis: OCH_3, 16.7; I_2, 10.2; ash, 0.0.

The alkaline soluble fraction (A) (OCH_3, 17.2) was twice methylated with CH_2N_2. After the first methylation the methoxyl content was 19.0%, and 19.2% after the second methylation. This latter value agrees, within experimental error, with the value of 19.5% methoxyl, which was the value on the fully Me_2SO_4 methylated product. The iodine analysis on the twice re-methylated alkaline soluble product (A_1) was 7.7%.

The results of the methylation and treatment with alkali of the iodo-carboxy lignin # <u>II</u> are shown in Diagram III. It is noteworthy that in treating the Me_2SO_4 methylated product with 10% NaOH there is a distinct loss in the

Diagram III

The methylation and saponification of iodo-carboxy lignin # II.



methoxyl content in both the alkali soluble (A) and insoluble (B) fractions.

The preparation of methylated and saponified derivatives of iodo-carboxy lignin # I and its various fractions.

Methylation of iodo-carboxy lignin # I. Diazomethane methylation of the iodo-carboxy lignin # I (OCH_3, 8.1; I_2, 11.4) yielded a product which after the third methylation had a methoxyl content of 19.4% and after the fourth methylation a methoxyl value of 19.1%. The iodine analysis on the fully methylated product was 9.3%. The methylated derivative was insoluble in cold and hot 10% NaOH but fairly soluble in alcoholic NaOH and $CHCl_3$.

Repeated methylations of the iodo-carboxy lignin # I with Me_2SO_4 according to Urban's method (33) yielded a product having a constant methoxyl content of 23.0%. Other analyses: C, 55.4; H, 5.8; I_2, 9.3; ash, 2.0. The fully methylated product was completely soluble in dioxane, but insoluble in $CHCl_3$, ether, and cold 10% NaOH. Purification of the product fully methylated with dimethyl sulfate by dissolving it in dioxane and reprecipitating it with ether did not change its methoxyl content.

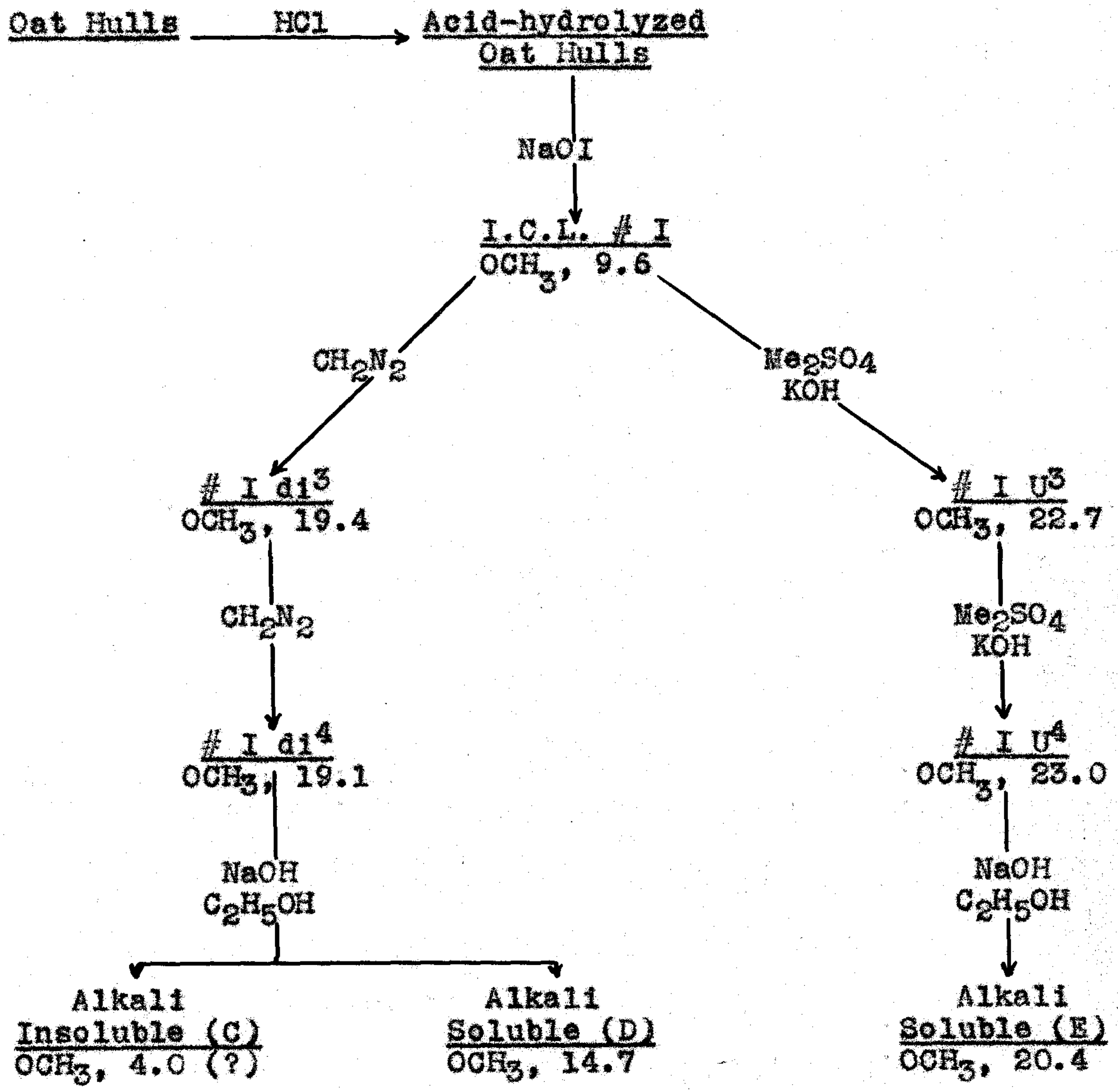
Fractionation of the iodo-carboxy lignin # I methylated by diazomethane. The iodo-carboxy lignin # I fully methylated with diazomethane (OCH_3, 19.1) was fractionated by treatment with $CHCl_3$. This separation gave a 65% yield of a $CHCl_3$ soluble fraction. The $CHCl_3$ soluble portion was precipitated with anhydrous ether in a manner similar to the method described by Brauns and Hibbert (6). There were no marked differences in the analyses of these two fractions. The analyses for the $CHCl_3$ insoluble fraction are: OCH_3, 19.0; I_2, 9.3. Analyses for the $CHCl_3$-ether insoluble fraction are: OCH_3, 18.9; I_2, 9.5.

<u>Treatment of the methylated derivatives of iodo-carboxy lignin # I with alcoholic NaOH.</u> The iodo-carboxy lignin # I fully methylated with diazomethane (OCH_3, 19.2) was carefully refluxed with alcoholic NaOH for two hours. The alcohol was then removed by distillation. The alkaline insoluble portion (C) (see Diagram IV), after filtering off the soluble liquors, was again treated with fresh portions of alcoholic NaOH until no more of the product went into solution. The alkaline soluble portion was treated in the usual manner for the isolation of the lignin fraction. Analyses of the alkaline soluble (D) fraction are: OCH_3, 14.7; I_2, 9.5; neutralization equivalent (1 COOH), 384. An insufficient amount of the alkali insoluble (C) fraction prevented any extensive analyses. One methoxyl determination on the alkali insoluble fraction (C), however, gave a value of 4.0%.

The iodo-carboxy lignin # I methylated with dimethyl

Diagram IV

The methylation and saponification of iodo-carboxy lignin prepared from acid-hydrolyzed oat hulls.



sulfate (OCH_3, 23.0), when treated with alcoholic NaOH as described above, did not leave any insoluble fraction. The alkaline soluble fraction (E), when isolated and dried in the usual manner, was soluble in $NaHCO_3$ and liberated CO_2. Analyses: OCH_3, 18.6; I_2, 9.5; neutralization equivalent (2 COOH), 898.

Diagram IV gives the results of these above described reactions on the iodo-carboxy lignin # I. Caution must be exercised in evaluating the methoxyl content of the alkali insoluble fraction (C), as has been already mentioned above. However, it is noteworthy that in the treatment of the diazomethane methylated product with alcoholic NaOH, the alkali insoluble (C) and alkali soluble (D) both show a decided loss in the methoxyl content.

The methylation of the acetone soluble fraction of the iodo-carboxy lignin # I. Diazomethane methylation of the acetone soluble fraction of the iodo-carboxy lignin # I (OCH_3, 5.2) in the usual manner yielded a product which, after the third and fourth methylations, had methoxyl values of 16.6 and 16.4% respectively. The iodine analysis was 12.6%.

Another portion of the acetone soluble fraction was twice methylated with Me_2SO_4 and caustic according to Urban's method (33). The methoxyl content after the first and second methylations was 13.1 and 21.5% respectively. An insufficient amount of the product prevented a re-methylation. It is not at all unlikely that this fraction was not completely methylated.

Methylation of the acetone insoluble fraction of the iodo-carboxy lignin # I. Complete methylation of the acetone insoluble fraction of the iodo-carboxy lignin (OCH_3, 8.8) with diazomethane gave a product with 18.2% methoxyl, and with Me_2SO_4, as described above, a product with 23.9% methoxyl. The product fully methylated with dimethyl sulfate (OCH_3, 23.9), when methylated with diazomethane, had a methoxyl content of 23.8%, and when acylated with acetic anhydride and pyridine, had a methoxyl content of 22.4%.

Treatment of the methylated derivatives of the acetone insoluble iodo-carboxy lignin # I with alcoholic NaOH. The acetone insoluble derivative fully methylated by diazomethane (OCH_3, 18.2) was completely soluble in hot 10% NaOH. The soluble product (F) (see Diagram V), when isolated and dried in the usual manner, had a methoxyl content of 13.8%, and a neutralization equivalent of 510 (1 COOH).

The acetone insoluble derivative fully methylated with dimethyl sulfate (OCH_3, 23.9) was also completely soluble in hot 10% NaOH. The alkali soluble product (G), when isolated and dried in the usual manner, had a methoxyl content of 18.0% and a neutralization equivalent of 499 (1 COOH).

Diagram V

The methylation and saponification of the acetone soluble and insoluble fractions of the iodo-carboxy lignin # I.

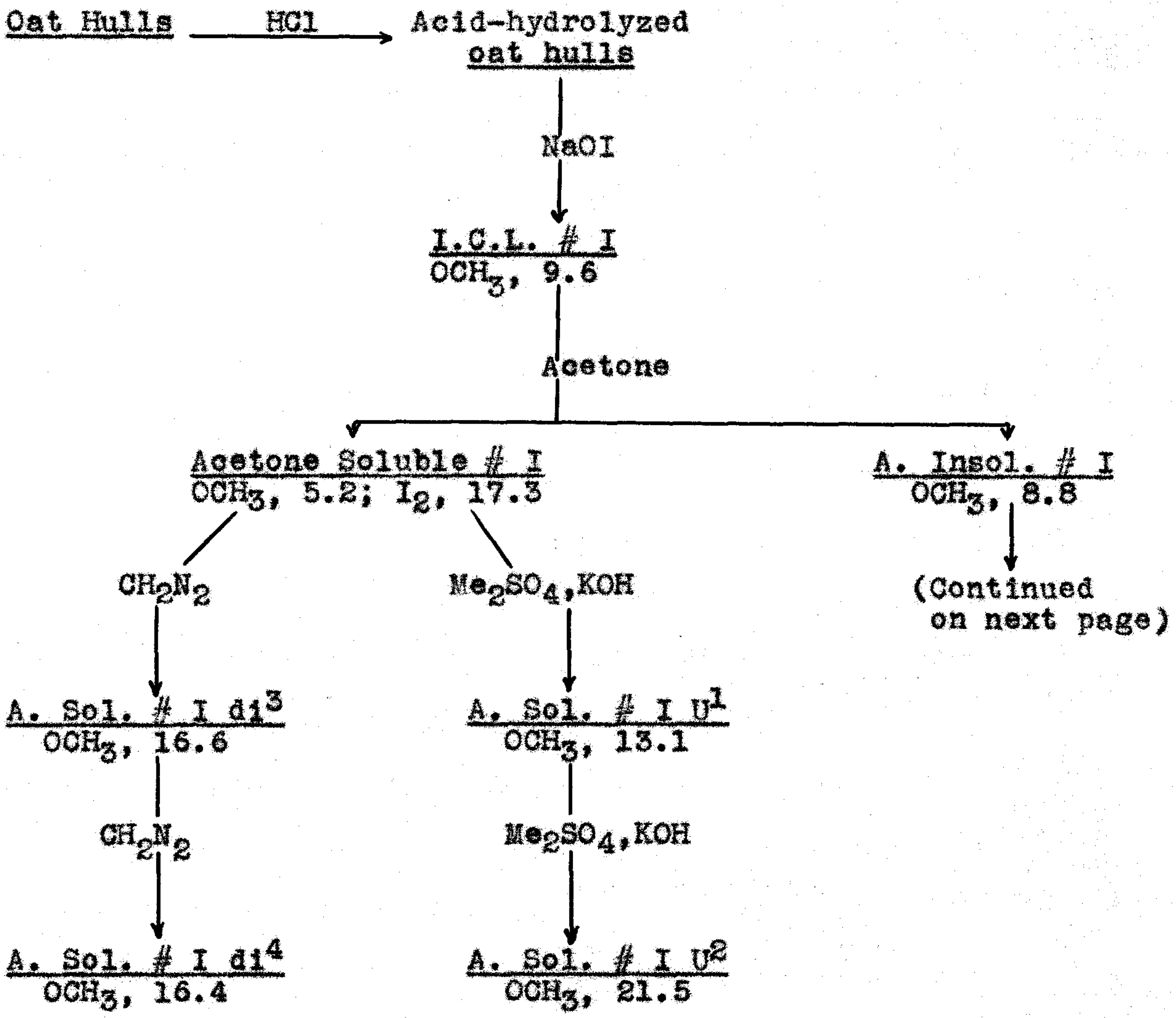
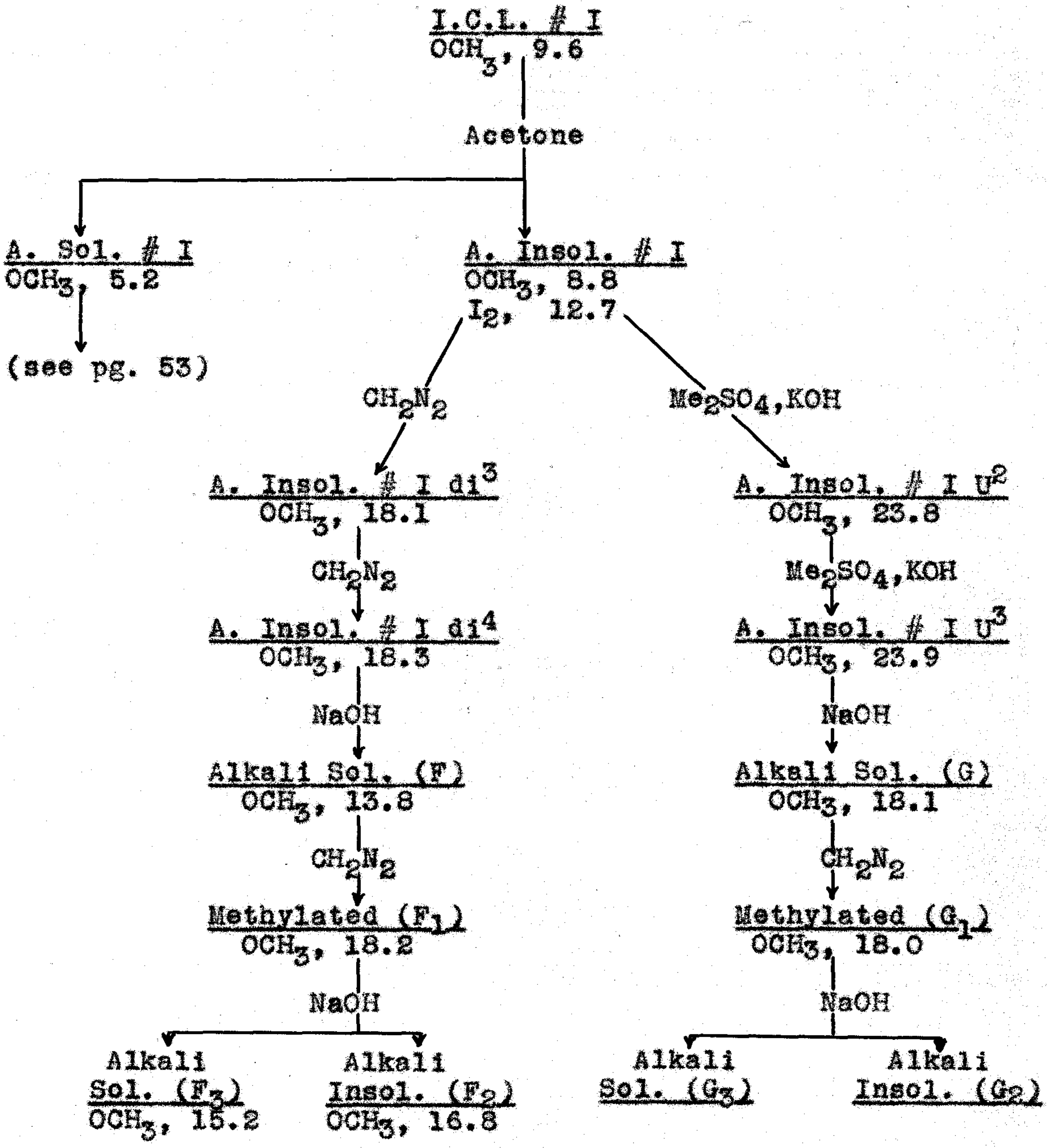


Diagram V (cont.)

The methylation and saponification of the acetone soluble and insoluble fractions of the iodo-carboxy lignin # I.



Methylation of the alkali soluble derivatives of the methylated acetone insoluble iodo-carboxy lignins # I. The alkali soluble fraction (F) (see Diagram V) of the acetone insoluble # I, which had been fully methylated with diazomethane, was re-methylated with CH_2N_2. The re-methylated derivative (F_1), when heated with 10% NaOH would not go completely into solution under any kind of treatment. The re-methylated product (F_1), after exhaustive treatment with hot 10% NaOH, was separated into an alkali insoluble fraction (F_2) and an alkali soluble fraction (F_3). The alkali soluble fraction (F_3) was isolated in the usual manner with concentrated HCl. The alkali insoluble portion (F_2) had a methoxyl content of 16.8%, while the alkali soluble fraction (F_3) had a methoxyl content of 15.2% and an iodine content of 10.4%.

The alkali soluble fraction (G) of the acetone insoluble # I, which had been fully methylated with dimethyl sulfate, was likewise re-methylated with CH_2N_2. The re-methylated product (G_1) had a methoxyl content of 23.7% and an iodine content of 10.3%. This latter product (G_1), however, failed to go completely into solution when exhaustively treated with 10% NaOH. No analyses were obtained on these fractions (G_2 and G_3).

Diagram V tabulates the results of the methylation of the acetone soluble fraction, and the methylation and treatment with caustic of the acetone insoluble fraction of the iodo-carboxy lignin # I. It is noteworthy that two products, A. Insol. # I di[4] and A. Insol. # I U[3], are completely soluble in 10% NaOH. The respective soluble products (F) and (G) of these two latter-named derivatives, when re-methylated with CH_2N_2, are now not completely soluble in 10% NaOH.

Methylation of the re-oxidized acetone insoluble iodo-carboxy lignin # I. The re-oxidized acetone insoluble iodo-carboxy lignin # I (OCH_3, 8.2; I_2, 17.6) (H), when completely methylated with CH_2N_2, yielded a product (H_1) (see Diagram VI) which had a methoxyl content of 17.6%. When the re-oxidized acetone insoluble fraction (OCH_3, 8.2) was methylated three times with dimethyl sulfate and caustic, as described above, and then re-methylated with CH_2N_2, a product (H_2) was obtained which had a methoxyl content of 21.6%.

Diagram VI shows the plan for the isolation of the re-oxidized acetone insoluble iodo-carboxy lignin # I and its reactions. It may be noticed that there exists a close agreement between the methoxyl content of the acetone insoluble # I and its re-oxidized derivative.

Methylation of the re-oxidized derivatives of iodo-carboxy lignin # I. The re-oxidized iodo-carboxy lignin # I (OCH_3, 8.5), when fully methylated with diazomethane (K) (see Diagram VII), gave a product (K_1) with a methoxyl

Diagram VI

The oxidation and methylation of the acetone insoluble fraction of iodo-carboxy lignin # I.

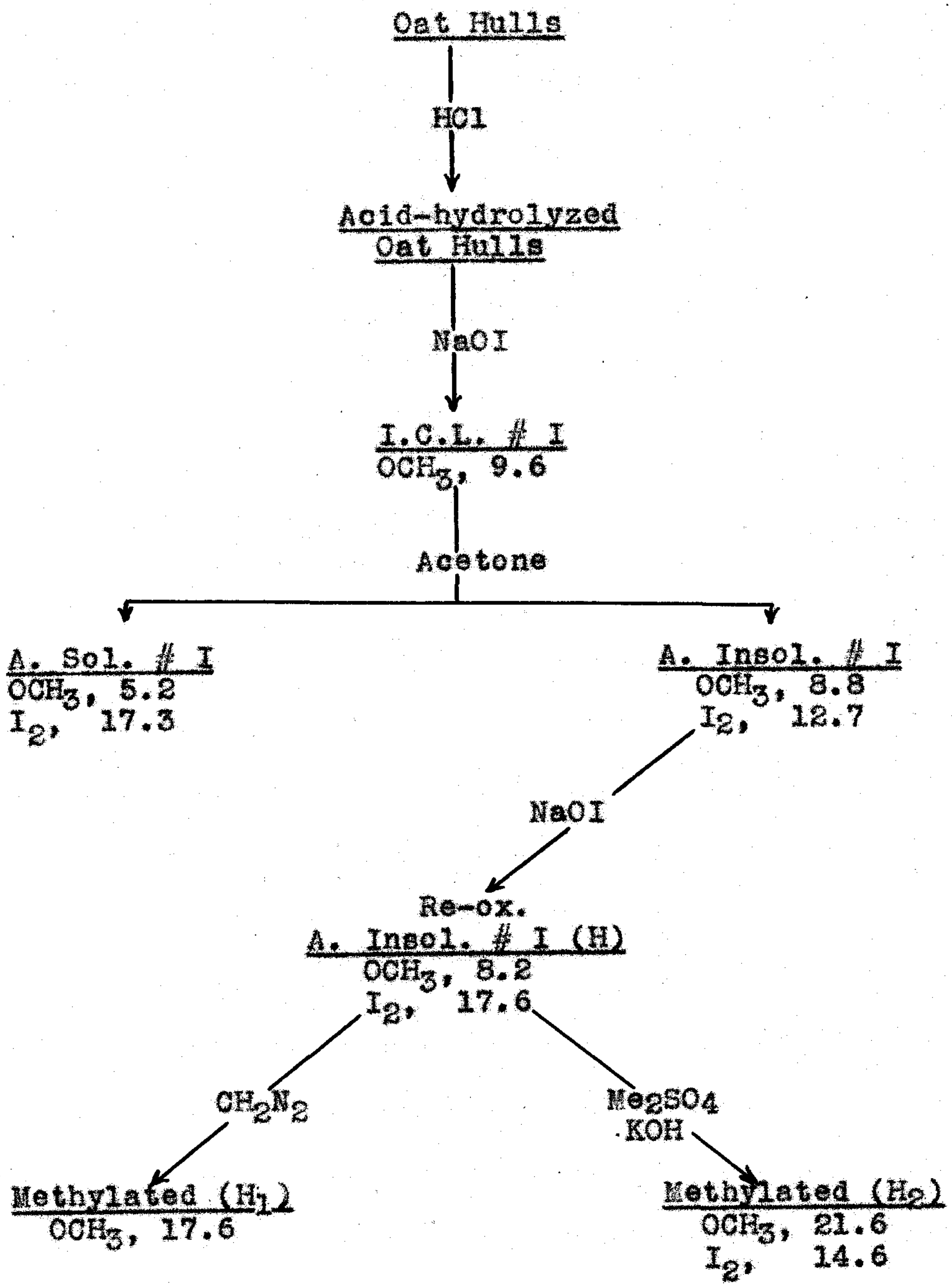
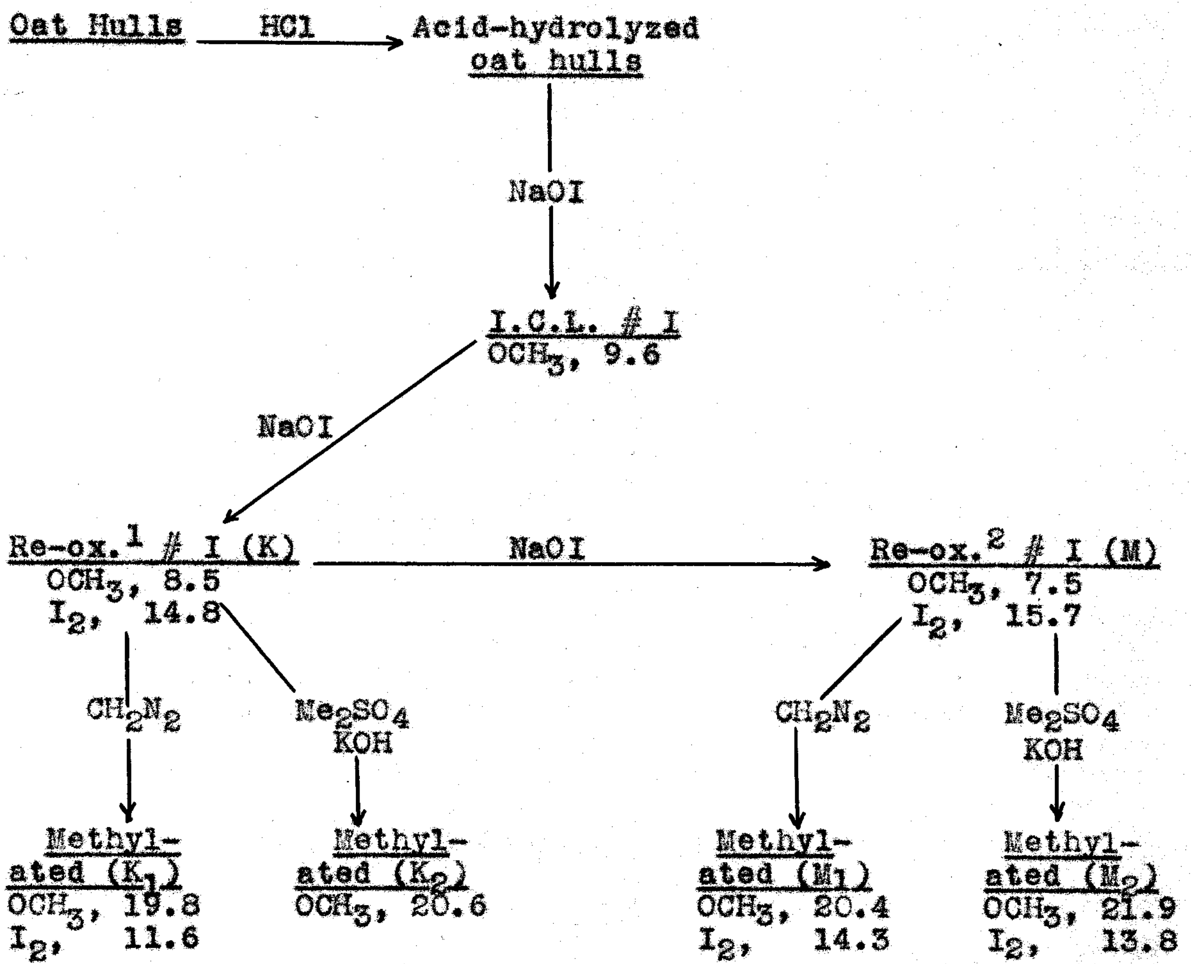


Diagram VII

The methylations of the re-oxidized iodo-carboxy lignins # I.



content of 19.8%. The product (K), when completely methylated with dimethyl sulfate and caustic, as described above, gave a product (K_2) with a methoxyl content of 20.6%.

The re-oxidized iodo-carboxy lignin # I (K), when treated with NaOI, gave another re-oxidized product, re-ox.^{2} # I (M) (see Table I for abbreviations). When the product, re-ox.^{2} # I (M) (see Diagram VII), was completely methylated with diazomethane, a derivative (M_1) was obtained which had a methoxyl content of 20.4%. The re-ox.^{2} # I (M), when completely methylated with dimethyl sulfate and caustic, gave a derivative (M_2) which had a methoxyl content of 21.9%.

Diagram VII shows these above described reactions with the respective analyses of the various derivatives. The proximity of the analyses of the re-ox.^{1} # I and the re-ox.^{2} # I are noteworthy.

DISCUSSION OF RESULTS

The difficulty of repeating another investigator's research on lignin has been a well known fact for many years. Two different investigators working on the same lignin-containing tissue and using the same methods have often been unable to reproduce each other's work. Extensive discussions on the difficulty of repeating another investigator's work have been well reported on by Abderhalden (1), Fuchs (9), Freudenberg (10), and Phillips (28).

The ammonia lignins isolated by the same method but by two different investigators in this Laboratory have been found to be in remarkably close agreement with each other. The carbon and hydrogen analyses and the oxidation values have been found to be in excellent agreement, whereas the methoxyl content of the two prepared lignins differed by 1.6%.

The fact that the ammonia lignin prepared from oat hulls could be fractionated into an acetone soluble and an acetone insoluble fraction indicated that the ammonia lignin, as previously reported, was not a homogeneous product. These two fractions differed considerably. From the products methylated with diazomethane and dimethyl sulfate the number of hydroxyl and methoxyl groups could be readily calculated. For the calculations it was assumed that the methylation with suggested the previously mentioned conditions as being the most favorable for the methylation of lignin products. They still, however, suggest extreme caution in drawing conclusions regarding the structure of lignin based on this type of methylation.

It was found, however, in this study that the acetone insoluble fraction of the ammonia lignin when repeatedly methylated with diazomethane gave a constant methoxyl content of 22.8%. Repeated methylations of the same acetone insoluble fraction with Me_2SO_4 and 45% KOH, as reported above, gave a constant methoxyl content of 23.0%. The methoxyl values obtained by these two different methods agree within the limits of experimental error. The fact that the methylation of the acetone insoluble fraction with Me_2SO_4 and caustic yielded a product having the same methoxyl content as a product methylated with diazomethane would indicate that the former method of methylation has not brought about any drastic changes in the molecule. The similarity in the methoxyl content in these two last-named products would also indicate that all of the hydroxyl groups are acidic in character and are as readily methylated with diazomethane as with dimethyl sulfate and caustic.

It has been shown by various investigators (7) (42) that the treatment of lignin with 8-10% NaOH at room temperatures brings about a distinct fractionation of the lignin.

It does not appear at all unlikely that a similar fractionation of a heterogeneous lignin product may occur during the repeated methylation processes. This possibility of fractionation during the methylation with dimethyl sulfate and caustic may partially account for the large difference in the methoxyl content of a product methylated with dimethyl sulfate and a product methylated with diazomethane. The similarity in the methoxyl content in the above-mentioned products would indicate that, in all probability, there was no fractionation taking place by the use of caustic during the methylation process. No further evidence for the fractionation of the acetone insoluble fraction of the ammonia lignin was found in any of its analyses or reactions. Whether the acetone insoluble fraction of the ammonia lignin, is a homogeneous product awaits further experimental evidence.

The iodo-carboxy lignin prepared from the ammonia lignin (I.C.L. # II) was found to differ considerably from the iodo-carboxy lignin prepared from the acid-hydrolyzed oat hulls (I.C.L. # I). A comparison of the two products is shown in Table XI. The average minimum molecular weight of the iodo-carboxy lignin # II appeared to be around 960 but was 1340 for the iodo-carboxy lignin # I.

Walde's oxidized lignins, obtained by these two methods, had an average minimum molecular weight of 1260 (38). He assumed that the oxidized lignin prepared from the acid-hydrolyzed oat hulls was identical to the oxidized lignin prepared from the ammonia lignin.

Table XI

A comparison of the analytical results of the iodo-carboxy lignin # I and the iodo-carboxy lignin # II.

	C	H	OCH_3	I_2	Av. minimum Mol. Wt.
I.C.L. # I	44.3	4.3	9.6	11.4	1340
I.C.L. # II	49.2	4.4	7.4	14.4	960

The methylation of the iodo-carboxy lignin # II (OCH_3, 7.4) with dimethyl sulfate and caustic gave a product which had a methoxyl content of 19.3%, an increase of 11.9% in the methoxyl content. The iodine content of the fully methylated product, however, was 7.6%, which amounted to a decrease four times the theoretical amount. That the high iodine content of the iodo-carboxy lignin # II was not due to an absorption phenomenon seemed to be clear from the fact that the iodine analyses were taken on products repurified by reprecipitation from warm (50-60° C.) 10% NaOH. The products were always washed free of halides before analyses were attempted. The explanation of this decrease in the iodine content cannot be adequately explained at the present time.

It was also observed that the iodo-carboxy lignin # II fully methylated with dimethyl sulfate and caustic (OCH_3, 19.3) would not go completely into solution when treated with hot 10% NaOH. This phenomenon was in contradistinction to the results obtained by Walde (38). Both the alkali soluble (A) (OCH_3, 17.2) and the alkali insoluble (B) (OCH_3, 16.7) fraction of the fully methylated iodo-carboxy lignin # II (see Diagram III, pg. 47) showed losses in methoxyl content. The alkali soluble (A) fraction, when re-methylated with diazomethane, gave a product (A_1) which had the same methoxyl content as the fully methylated product (OCH_3, 19.3). It appears from these results that the fully methylated iodo-carboxy lignin # II is a mixture which is separated into distinct fractions by treatment with hot 10% NaOH. Additional support is given to this view from the observation that the other lignin preparations were shown to be easily fractionated with suitable solvents. Further experimentation, however, is needed to establish this point.

The fact that the iodo-carboxy lignin # I could be quantitatively oxidized with NaOI resulting in the formation of re-oxidized lignins was of note. The once oxidized iodo-carboxy lignin # I (re-ox.^{1} # I) had four hydroxyl groups and two methoxyl groups; the same number as the twice-oxidized iodo-carboxy lignin # I (re-ox.^{2} # I). Iodoform was the product isolated from the oxidation in each case. Table XII shows the similarity in the analyses of these two products and their derivatives. It would appear that the temperatures for several hours. It was probable that condensation of the acetone with the oxidized lignin had taken place during the extraction process.

A comparison of the acetone soluble and acetone insoluble fraction of the iodo-carboxy lignin # I revealed a close relationship to the parent product. From the results of the methylation reaction, both with diazomethane and dimethyl sulfate, the number of hydroxyl and methoxyl groups can readily be calculated for these two fractions of the iodo-carboxy lignin # I. It was assumed that the methylation with dimethyl sulfate and caustic of these two acetone fractions with the above prescribed precautions, gave products that were completely methylated. On such an assumption it was found that the acetone soluble fraction had five hydroxyl groups and one methoxyl group, while the acetone insoluble had five hydroxyl and three methoxyl groups. Of the five hydroxyl groups in the acetone insoluble fraction, two were found to be carboxylic in character. Table XIII shows the relationship of the analytical results of these two fraction with the parent iodo-carboxy lignin # I. It may be readily observed that there is a wide divergence in the results of the two fractions when compared to the parent product.

The complete methylation of the iodo-carboxy lignin # I with diazomethane gave a product (OCH_3, 19.2) which failed to go completely into solution when treated with alcoholic

NaOH. An alkali soluble (D) and an alkali insoluble fraction (C) were isolated. (See Diagram IV, pg. 50). Both the alkali soluble (D) (OCH_3, 14.7) and the alkali insoluble (C) (OCH_3, 4.0) showed decided losses in methoxyl content. In view of the fact that the iodo-carboxy lignin # I is a mixture readily fractionated with acetone, it was not surprising to find this separation of the diazomethane methylated product into an alkali soluble and insoluble fraction.

Table XIII

A comparison of the analyses of the iodo-carboxy lignin # I and its acetone fractions.

	Parent product				CH_2N_2 Methyd.		Me_2SO_4 Methyd.	
	C	H	OCH_3	I_2	OCH_3	I_2	OCH_3	I_2
I.C.L. # I	44.3	4.3	9.6	11.4	19.2	9.3	23.0	9.3
A. Sol. # I	52.5	4.8	5.1	17.3	16.4	12.6	21.5	—
A. In. # I	51.6	4.8	9.7	12.8	18.2	10.8	23.9	13.2

The iodo-carboxy lignin # I fully methylated with dimethyl sulfate and caustic gave a product (OCH_3, 23.0), which when treated with alcoholic NaOH, went completely into solution. (See Diagram IV, pg. 50). The soluble lignin derivative (E) showed a loss of 2.6% methoxyl. The presence of carboxylic groups in the alkali soluble fraction (E) was indicated by the loss in methoxyl content, complete solubility in 0.1 N NaOH, and the liberation of CO_2 from a $NaHCO_3$ solution.

The importance of technique in regard to treating these methylated derivatives with caustic cannot be over-emphasized. Products which seemingly fail to go into solution in a test tube will go completely into solution when carefully pulverized and treated with water and caustic prior to the refluxing process. It is also highly desirable to have all the products as free from ash as possible.

The acetone insoluble fraction of the iodo-carboxy lignin # I fully methylated with diazomethane (OCH_3, 18.3) was readily soluble in hot 10% aqueous NaOH. The soluble product (F) (see Diagram V, pg. 54), when isolated, was re-methylated with CH_2N_2. The methoxyl content of the remethylated product (F_1) went back to the original methoxyl value (18.2). This re-methylated fraction (F_1), after treating with hot caustic, failed to go completely into solution under any set of circumstances. The same phenomenon was observed in exactly the analogous way with the acetone insoluble fraction fully methylated with dimethyl sulfate (OCH_3, 23.9). The explanation of this phenomenon remains conjectural at the present time.

After a lapse of several years, a sample of Walde's methylated iodo-carboxy lignin (OCH_3, 22.2), which went completely into solution in hot 10% NaOH to give him a soluble lignin with a methoxyl content of 17.4%, would not go completely into solution under any set of circumstances. It is most unlikely that a variance in technique can account for this phenomenon. Many variations in the procedure were tried without any apparent success. Later Walde (39) was also able to substantiate the above results. A more extended investigation of the repeated methylation and treatment with caustic of any one of these fractions would be highly desirable and would, in all probability, throw considerable light on this phase of the constitution of lignin.

The NaOI oxidation of the acetone insoluble fraction of the iodo-carboxy lignin # I also yielded a product which had four hydroxyl groups and two methoxyl groups. The analyses of this latter-named product, and its derivatives, agree fairly well with the analyses of the re-oxidized iodo-carboxy lignins # I and their derivatives. Table XIV shows the similarities in the analyses of these three products. (See Table I for the meaning of the abbreviations).

It would appear that by repeated oxidations of the iodo-carboxy lignin # I and its acetone insoluble fraction, an oxidized product which is common to the lignin molecule is obtained. It would be of interest to see how much farther this step-wise oxidation does occur and whether the re-oxidized products agree in number of methoxyl and hydroxyl groups as do the above named products.

Table XIV

Similarities in the analyses of the re-oxidized iodo-carboxy lignins # I and the re-oxidized acetone insoluble # I.

	Parent product		CH_2N_2 methylated deriv.		Me_2SO_4 methylated deriv.	
	OCH_3	I_2	OCH_3	I_2	OCH_3	I_2
Re-ox.[1] # I	8.5	14.8	19.8	11.6	20.6	11.0
Re-ox.[2] # I	7.5	15.7	20.4	14.3	21.9	13.8
Re-ox. A. Insol. # I	8.2	17.6	17.6	--	21.6	14.6

SUMMARY

1. The properties of the ammonia lignin prepared from oat hulls agreed remarkably well with the ammonia lignin prepared by Walde.

2. Ammonia lignin was fractionated with acetone. The acetone soluble fraction had five hydroxyl groups and three methoxyl groups as compared to three methoxyl and three hydroxyl groups found in the acetone insoluble fraction.

3. The NaOI oxidation value of the acetone soluble fraction of the ammonia lignin was 212 as compared to an oxidation value of 163 for the acetone insoluble fraction. The non-fractionated ammonia lignin had an oxidation value of 188.

4. Iodo-carboxy lignin prepared from the ammonia lignin has a minimum molecular weight of 960. It was, in all probability, a mixture as indicated by the results obtained from the methylation reaction with dimethyl sulfate and caustic.

5. Iodo-carboxy lignin prepared from acid-hydrolyzed oat hulls was not the same as the iodo-carboxy lignin prepared from the ammonia lignin. The former had a minimum molecular weight of 1340.

6. Two successive oxidations with NaOI have been performed on the iodo-carboxy lignin prepared from the acid-hydrolyzed oat hulls. Each isolated product had four hydroxyl and two methoxyl groups. Iodoform was identified in each of the alkaline oxidizing solutions.

7. Iodo-carboxy lignin prepared from the acid-hydrolyzed oat hulls was fractionated by the use of acetone. The acetone soluble fraction had one methoxyl and five hydroxyl groups, whereas the acetone insoluble fraction had five hydroxyl and three methoxyl groups.

8. The acetone insoluble fraction of the iodo-carboxy lignin prepared from the acid-hydrolyzed oat hulls was re-oxidized with NaOI to give an oxidized lignin having four hydroxyl and two methoxyl groups. The same number of hydroxyl and methoxyl groups was observed on the re-oxidized iodo-carboxy lignins.

9. An unidentified crystalline product has been obtained in the oxidation of the acetone insoluble fraction of the iodo-carboxy lignin prepared from the acid-hydrolyzed oat hulls.

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