[CONTRIBUTION FROM THE NEW JERSEY AGRICULTURAL EXPERIMENT STATION]

On the Nature of Lignin[1]

BY SELMAN A. WAKSMAN AND T. C. CORDON

Among the most obscure plant constituents, which have so far baffled the ingenuity of the investigator, lignin occupies a prominent place. This chemical complex or group of complexes is characterized largely by the method of preparation, as well as by the presence of certain characteristic chemical 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 acid and 42% hydrochloric acid and (c) its combination with certain organic compounds, such as phenol, methoxy glycol, etc. The presence of a definite percentage of methoxyl groups (OCH_3) has usually been considered as one of the most important characteristics of the lignin molecule.

Recently, there appeared certain contributions by Hilpert and associates[2] which tend to question the validity of our present ideas on the distribution and properties of lignin. The fact that various sugars (fructose, pentoses) give, on prolonged boiling with acids, dark colored compounds which have properties similar to lignin,[3] especially their non-solubility in concentrated acids, was interpreted 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[4] 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[5] to lead to the formation of "synthetic lignin." The more than a century old conception of preparations obtained from carbohydrates by means of acids, alkalies and heat, and usually designated as "artificial humic acids" has thereby been renewed.

Hilpert further suggested[6] that his proof of the non-existence of lignins in plant materials is substantiated by the fact that on treatment of straw and wood with acids at very low temperatures (−12°), practically all the organic matter was brought into solution. On diluting the extract with water, a flaky 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 resulted which contained 61% carbon and 17.7% OCH_3, namely, a complex characteristic 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. However, Storch[7] 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 solution, in pyridine and in aqueous acetone. A similar amount of lignin could be dissolved out directly from the wood by treatment with 5% sodium hydroxide. Wedekind[8] treated wood flour with Schweitzer's reagent and obtained a lignin preparation which was not different in appearance and chemical properties from lignin prepared by treatment with acids. This led him to conclude that the existence of beech wood lignin is beyond any question.

In the various recent investigations on the decomposition of plant residues by microörganisms[9] it was found that lignin is one of the most resistant plant constituents and, therefore, tends to accumulate. This phenomenon largely explains the formation and accumulation of humus, the major part of which consists of lignin transformation

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[1] Journal Series Paper of the Department of Soil Microbiology, N. J. Agr. Expt. Station.

[2] R. S. Hilpert and E. Littmann, *Ber.*, **68**, 16–18 (1935).

[3] R. S. Hilpert and E. Littmann, *ibid.*, **67**, 1551–1556 (1934).

[4] G. J. Ritter, R. M. Seborg and R. L. Mitchell, *Ind. Eng. Chem., Anal. Ed.*, **4**, 202 (1932).

[5] L. F. Hawley and E. E. Harris, *ibid.*, **24**, 873–875 (1932).

[6] R. S. Hilpert and H. Hellwage, *Ber.*, **68**, 380–383 (1935).

[7] K. Storch, *ibid.*, **68**, 2367–2374 (1935).

[8] E. Wedekind, *Die Zellstoff-Faser*, **33**, 14–15 (1936).

[9] S. A. Waksman and F. G. Tenney, *Soil Sci.*, **24**, 317–333 (1927); **28**, 55–84 (1929).

TABLE I

INFLUENCE OF TEMPERATURE UPON THE YIELD[a] AND CHEMICAL NATURE OF ACID LIGNIN

Preliminary acid (2% HCl) treatment	Temp. of treatment with 80% H_2SO_4, °C.	Lignin yield, %	Sugar in filtrate, as glucose, %	Methoxyl in lignin, %	Carbon, %	Ash, %	Total methoxyl in lignin on the basis of 100 g. of original dry material, g.
None	2.0	27.07	60.6	10.28	55.87	6.84	2.78
None	8.5	21.38	65.0	13.21	58.50	8.88	2.82
None	18–20	21.86	63.4	12.67	59.55	7.94	2.77
Treated	2.0	21.03	61.3[b]	12.30	57.40	7.62	2.59
Treated	9.0	18.71	63.0[b]	13.45	59.38	8.59	2.52
Treated	18–20	18.52	63.6[b]	13.33	60.55	8.09	2.47

[a] On total dry basis. [b] Including sugar in dilute acid extract.

products.[10] It was also found[11,12] that the decomposition of wood by certain fungi which attack the cellulose constituents gives rise to a residue greatly enriched in lignin. These facts speak also against Hilpert's ideas.

Experimental

In the following investigations wheat straw was used. The proximate chemical composition of the straw[13] was as follows:

	Dry material, %
Ether soluble	1.85
Cold water soluble	3.36
Hot water soluble	2.17
Hemicelluloses	23.28
Cellulose	33.82
Lignins	15.20
Water insoluble proteins	2.12
Ash	3.68
Total accounted for	85.48
Methoxyl content	3.32

Twelve 10-g. portions of the straw (7% moisture) were placed in beakers. Six were treated with 100-cc. portions of 2% hydrochloric acid, for two hours at 100°, then filtered, washed and dried. The insoluble residue amounted to 59.08% of the original material on a dry basis; the acid treatment removed all the starches, practically all the hemicelluloses and other readily hydrolyzable carbohydrates; the glucose content of the filtrate was 24.7% of the original material. The untreated and acid treated portions of straw were now triturated with 50-cc. portions of 80% sulfuric acid, at three different temperatures, namely, at 2.0, 8.5–9 and 18–20°, for two and one-half hours. The preparations were then diluted with 15 volumes of water and heated, at 100°, for four hours. The residues were filtered, washed thoroughly, dried, weighed and used for analysis. The yields and chemical composition of the variously treated preparations are given in Table I.

The "lignin yield" of the straw treated at the lowest temperature (2°) was higher, both in the original and in the acid pretreated samples, than at the higher temperatures. The lignin obtained at the lower temperatures was also characterized by a lower methoxyl, carbon and ash content and a lower amount of sugar in the filtrate. The higher "lignin yield" at 2° was thus simply due to an insufficient hydrolysis of the complex carbohydrates in the straw.

In order that the higher yield of lignin at the lower temperature should not be interpreted as "lignin synthesis," all the lignin preparations were again treated with 80% sulfuric acid but now at room temperature. The results presented in Table II show conclusively that the higher yield of lignin at the lowest temperature was due to an insufficient hydrolysis of some of the complex carbohydrates. The final calculation of the yields of lignin at the different temperatures shows that they are practically the same. Only the pretreatment of the straw with dilute mineral acid has reduced the lignin yield by about 2%. This is probably due to the fact that certain hemicelluloses give a small amount of residue not acted upon by concentrated acid; this residue will come down with the lignin, unless previously removed by treatment with hot dilute acid. This is largely the reason why the pretreatment of plant material with dilute mineral acid has been made as a part of the routine analysis for lignin determination, as in the proximate method of analysis proposed by Waksman and Stevens[13] and recommended more recently by Norman.[14] It has been shown, for example, that poly-mannuronic acid, commonly known as alginic acid, contains a certain fraction which is not hydrolyzed by concentrated acids.[15]

TABLE II

INFLUENCE OF SECOND ACID TREATMENT, AT ROOM TEMPERATURE, OF THE VARIOUS LIGNIN PREPARATIONS

Lignin obtained at a temp. of °C.	Lignin, yield,[a] %	Sugar in filtrate,[a] %	Final lignin yield of, on basis of original material, %
2.0	73.5	23.3	19.90
8.5	91.1	4.1	19.48
18–20	94.8	1.5	20.75
2.0[b]	84.5	12.3	17.77
9.0[b]	95.0	1.8	17.77
18–20[b]	96.4	0.7	17.85

[a] Yields reported on basis of lignin preparations used.
[b] Previously treated with dilute acid.

The yields of lignin reported in Table II are higher than those found in the original straw. This is due to the fact

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[10] H. G. Byers and I. C. Feustel, *Soil Sci.*, **42** (1936).

[11] R. Falck, *Ber.*, **60**, 225–232 (1920).

[12] S. A. Waksman and K. R. Stevens, THIS JOURNAL, **51**, 1187–1196 (1929).

[13] S. A. Waksman and K. R. Stevens, *Ind. Eng. Chem., Anal. Ed.*, **2**, 167 (1930).

[14] A. G. Norman and S. H. Jenkins, *Biochem. J.*, **28**, 2147–2159 (1934).

[15] E. Schmidt and F. Vocke, *Ber.*, **59**, 1585 (1926).

TABLE III

ALKALI SOLUBLE LIGNIN FROM STRAW

	Per cent. of total dry material					
	Cold alkali treatment			Hot alkali treatment		
Constituents	Insoluble residue	Soluble lignin	Sugar in filtrate	Insoluble residue	Soluble lignin	Sugar in filtrate
Total yield	67.6	9.4	21.1	47.6	12.4	23.0
Acid lignin in	10.6[a]	...	..	3.8[b]	...	..
Methoxyl in lignin	11.73	15.35	..	11.13	15.44	..
Total methoxyl recovered	1.24	1.44	..	0.42	1.92	..
Sugar in concd. acid hydrolysate	...	...	49.0	...	...	42.8
Total sugar	...	...	70.1	...	...	65.8

[a] Ash content, 7.86%. [b] Ash content, 1.12%.

that the nitrogen content of the lignin (0.56–0.58%), because of some admixture of protein, and the ash content (7.94–8.88%) have not been accounted for in the table. When these are accounted for, the lignin yield of acid pretreated material will be reduced to 15.56%, which compares favorably with the amount found in the proximate analysis, 15.20%. The somewhat higher figure for the former is no doubt due to the fact that fats and waxes were not removed.

The methoxyl yields for the lignin preparations obtained at the different temperatures were exactly the same. However, in the samples treated with dilute acid the methoxyl yield was lower than in the untreated samples. All are lower than the methoxyl content of the original straw. This is due to the fact that the latter contains complexes (pectins) in which the methoxyl is in an ester-combination, which is destroyed by the acid treatment. The preliminary extraction with dilute acid results in the removal of these complexes.

In order to emphasize further that the determination of lignin in the plant material by treatment with concentrated acids is influenced only to a limited extent by synthetic processes, as a result of the action of the acid on the carbohydrates, the following experiments dealing with alkali lignin may also be reported here.

Fifty-gram portions of straw were treated with 750 cc. of a 5% solution of sodium hydroxide for twenty-four hours at room temperature or for four hours at 100°. The extracts were neutralized with hydrochloric acid, made acid (2% HCl), and heated for two hours at 100°, to hydrolyze those hemicelluloses which were dissolved by the alkali. The precipitates were washed, dried and weighed. The results (Table III) show that the total lignin yield obtained from straw by alkali treatment which has been followed by treatment with concentrated acid is practically the same as that obtained by the acid treatment alone. The cold alkali solution extracted less lignin than the hot alkali. The lower recovery of the lignin in the hot alkali treatment is either due entirely or partly to losses during the colloidal dispersion with the hot alkali solution, or to the non-recovery of that part of the hemicellulose complex which ordinarily comes down with the acid lignin. The methoxyl content was higher in the alkali soluble portion of the lignin than in the alkali insoluble; however, it was the same in both the cold and in the hot alkali soluble portions. When the total methoxyl yield is calculated, it is found that the cold alkali treatment gave in the alkali soluble and alkali insoluble portions of the lignin exactly the same amount of methoxyl (1.24 + 1.44 = 2.68) as the acid treatment, which was 2.66, on the average of the results given in Table I. The hot alkali treatment gave a somewhat lower methoxyl recovery, namely, 2.34 g. per 100 g. of dry straw, which points again to the destruction of some of the lignin by the hot alkali treatment, or to its change into a state in which it is no longer precipitated by acid.

In order to compare the yields of alkali lignin obtained directly from straw with those obtained from the acid lignin of the straw, some of the latter prepared by two successive treatments with 80% sulfuric acid at room temperature, was extracted with cold and hot 5% sodium hydroxide solution, in a manner similar to the extraction of the straw itself (Table IV). Here as well, the hot alkali treatment removed a larger amount of the lignin than the cold; the methoxyl content of both was here also practically the same. The methoxyl content of the alkali-soluble portion was higher than of the alkali-insoluble portion. The previous treatment of the straw with an acid tended to destroy some of the methoxyl, thus reducing its content in alkali lignin. This phenomenon was emphasized in a previous report.[16]

TABLE IV

ALKALI SOLUBLE PORTION OF ACID LIGNIN

	Per cent. of total dry acid lignin			
	Cold alkali treatment		Hot alkali treatment	
Constituents	Insoluble residue	Soluble lignin	Insoluble residue	Soluble lignin
Yield	75.0	15.4	31.9	50.4
Methoxyl	13.77	14.29	12.76	14.43

Summary

A study has been made 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. The hydrolysis of the carbohydrates was incomplete at the lower temperature; this tended to mask the characteristic properties of the lignin. When lignin obtained from straw at 2° 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.

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(16) S. A. Waksman and H. W. Smith, THIS JOURNAL, **56**, 1225–1229 (1934).

A study has also been made of the alkali lignin of the same straw. Cold alkali treatment removed less lignin than hot alkali. The sum total of the lignin extracted by cold alkali and that left in the residue was practically the same as the lignin obtained by acid treatment alone; the same was true of the methoxyl recovery. Hot alkali treatment reduced both the recovery of the lignin as a whole and of the methoxyl as well.

Preliminary treatment of the straw with dilute acid reduced the lignin yield. This was possibly due to the destruction of some hemicelluloses which would otherwise remain with the acid lignin residue. This interfering effect of hemicelluloses upon the yield of acid lignin has long been recognized, and pretreatment with dilute acid in the determination of lignin in plant materials has been utilized in the standard methods of analysis.

These results lead to the conclusion that the hypothesis proposed by Hilpert, that lignin does not exist in plant materials but is a result of the action of the acid upon certain carbohydrates, has no foundation. One cannot, of course, deny the fact, long recognized, however, by every investigator of lignin, that the present methods of lignin extraction and lignin determination modify considerably its chemical nature.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

Hydrophenanthrenes and Related Ring Systems from Dieneynes[1]

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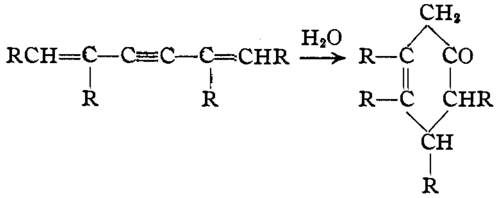
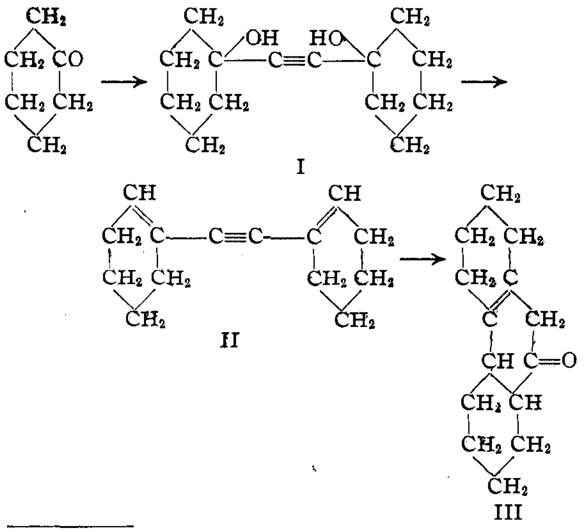
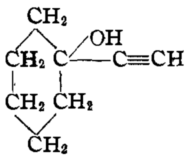
Previous work in this Laboratory has shown that a tetrasubstituted open-chain dieneyne can be hydrated and cyclized by the action of sulfuric acid in acetic acid. This reaction seemed to offer a novel method for the production of Δ^{11}-dodecahydrophenanthrone-9 (III) from cyclohexanone and acetylene through the preceding series of reactions.

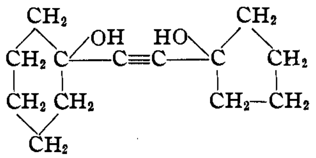




The acetylenic glycol has been prepared by DuPont[3] by treating the dimagnesium halide of acetylene with cyclohexanone. The yields by this procedure are only about 35–37%, and a better method of preparation was sought. Gould and Thompson[4] found that potassium tertiary butylate was a good catalyst for condensing acetylene with β-ionone, and this method was tried on cyclohexanone. This gave an excellent yield (81%) of 1-ethynylcyclohexanol (IV), but the method could not be adapted to making the glycol in one operation. By treating this acetylenic alcohol with an excess of ethylmagnesium bromide, and then with cyclohexanone, the glycol could be obtained in 48% yields, but this is not a practical procedure, as the over-all yield is lower than can be obtained by the DuPont method.[3] A mixed acetylenic glycol (V) having one cyclohexane and one cyclopentane ring was prepared by condensing 1-ethynylcyclohexanol with cyclopentanone.



IV



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[1] This is the third paper on cyclization of dieneynes. For previous papers, see THIS JOURNAL, **55**, 1655, 4276 (1933).

[2] Chemical Foundation Fellow in Organic Chemistry.

[3] DuPont, *Ann. chim.*, [8] **30**, 485 (1913).

[4] Gould and Thompson, THIS JOURNAL, **57**, 340 (1935).