[150TH CONTRIBUTION FROM THE COLOR AND FARM WASTE DIVISION, BUREAU OF CHEMISTRY AND SOILS, DEPARTMENT OF AGRICULTURE]

THE CHEMISTRY OF LIGNIN. II. FRACTIONAL EXTRACTION OF LIGNIN FROM CORN COBS

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In a previous communication,[1] a description was given of a lignin fraction obtained by treating corn cobs with alcoholic sodium hydroxide solution at room temperature. The analytical data presented agreed with those required for the dissected formula $C_{37}H_{33}O_9(OH)_4(OCH_3)_3$. The yield of this fraction amounted to 3.49% of the weight of the corn cobs treated. This yield represents only a part of the total lignin in corn cobs as determined for example by the Dore[2] modification of the König and Becker[3] method. The question arose whether all the lignin could be removed by prolonged and repeated extraction with alcoholic sodium hydroxide solution. If all the lignin were similarly combined with the carbohydrates, such a result might reasonably be expected. It was found, however, that only a part of the total lignin could be obtained even after exhaustive extraction with this reagent. The residual lignin could be obtained only by subjecting the extracted corn cobs to the action of sodium hydroxide solution at successively higher temperatures.

The experimental procedure employed consisted in subjecting corn cobs that had previously been extracted with a 1:1 alcohol–benzene solution to exhaustive extraction with alcoholic sodium hydroxide solution at room temperature until a test sample upon removal of the alcohol and subsequent acidulation no longer gave any precipitate of lignin. The residual material, which still contained some combined lignin, was successively extracted with a 2% aqueous sodium hydroxide solution at 100° until the extract was free from lignin. The residue from this treatment was similarly extracted with a 2% aqueous sodium hydroxide solution at 135° and finally with a 4% sodium hydroxide solution at 180°. The residue obtained from the final treatment was entirely free from lignin.

Among chemists who have accepted the view that lignin is chemically combined with the cellulose or with other carbohydrate material, much difference of opinion prevails regarding the manner in which this combination occurs. Erdmann[4] and Lange[5] assume that an ester-like union exists

[1] Phillips, THIS JOURNAL, **49**, 2037 (1927).

[2] Dore, *J. Ind. Eng. Chem.*, **12**, 984 (1920).

[3] König and Becker, "Veröffentlichungen der Landwirtschaftskammer für die Provinz Westfalen," Heft 26, **1918**, and E. Becker, *Dissertation*, Münster, **1914**, *Z. angew. Chem.*, **32**, 155 (1919).

[4] Erdmann, *Ann.*, **138**, 1 (1866); *Ann. Supplement*, **V**, 223 (1867).

[5] Lange, *Z. physiol. Chem.*, **14**, 15, 283 (1889).

between an acid group in the lignin and an hydroxyl of the carbohydrate. On the other hand Hoppe-Seyler,[6] Grafe,[7] and Mehta[8] are inclined to the view that there exists an ether-like linkage between the lignin and the cellulose or other carbohydrate. Data obtained on the fractional extraction of the lignin from corn cobs indicate that the lignin is dissimilarly combined with the carbohydrates. The assumption that *all* of the lignin is combined with the carbohydrates either as an ester or an ether is unwarranted as far as the lignin from corn cobs is concerned. It is believed that both types of linkage are present, which explanation would account for the fact that only a part of the lignin may be removed from corn cobs with alcoholic sodium hydroxide solution even after exhaustive extraction. The analyses made on the lignin fractions do not justify the conclusion that there is more than one kind of lignin present in corn cobs. There is apparently no alkali lignin present in corn cobs in the free state, for extraction with a solvent such as a 2:1 acetone–ethanol solution, which is an excellent solvent for free alkali lignin, fails to yield any lignin.

Experimental

Five hundred grams of ground corn cobs which had previously been extracted with boiling 1:1 alcohol–benzene solution was digested at room temperature with 1000 cc. of alcoholic sodium hydroxide solution (20 g. of sodium hydroxide, 400 cc. of water and 600 cc. of 95% ethanol) for forty-eight hours. The yellow liquor was decanted, the cobs were pressed and the liquor was combined with that obtained by decantation. This solution was neutralized with hydrochloric acid and the alcohol was removed by distillation under reduced pressure. After the residual solution had been made distinctly acid with hydrochloric acid, the precipitated lignin was filtered off, washed free from hydrochloric acid and dried at 80°. This digestion with alcoholic sodium hydroxide solution and subsequent isolation of the lignin was continued until no more lignin was obtained. Six extractions were required. The six lignin fractions were combined and purified by dissolving in 2:1 acetone–alcohol solution (200 cc. of acetone, 100 cc. of 95% ethanol) filtering and pouring the filtrate into 1.5 liters of boiling water containing 50 cc. of concentrated hydrochloric acid. This was allowed to cool to room temperature, filtered, washed with water until the washings gave no test for chlorine with silver nitrate and dried at 80°. A light yellow, amorphous powder was obtained (Fraction "A," Table I).

The residue from the digestion with alcoholic sodium hydroxide solution was refluxed with 2% aqueous sodium hydroxide solution for four hours. It was then filtered, the filtrate acidified with hydrochloric acid and the lignin filtered off. The refluxing with 2% aqueous sodium hydroxide solution was continued until no more lignin was obtained. The several lignin fractions were combined and purified by dissolving in acetone and alcohol as already described (Fraction "B," Table I).

The residue obtained after the extraction of lignin fraction "B" was digested with a 2% aqueous sodium hydroxide solution for four hours in an autoclave at 135°. The alkaline liquor was filtered off and the digestion with 2% sodium hydroxide was repeated

[6] Hoppe-Seyler, *Z. physiol. Chem.*, **13**, 84 (1888).

[7] Grafe, *Monatsh.*, **25**, 987 (1904).

[8] Mehta, *Biochem. J.*, **19**, 958 (1925).

until no more lignin was obtained. The lignin was isolated from the alkaline solution and purified from acetone and alcohol as already described (Fraction "C," Table I).

The residue from the foregoing treatment was heated for two hours with 4% aqueous sodium hydroxide in an autoclave at 180°. The alkaline extract was acidified with hydrochloric acid and the precipitated lignin was filtered off. The residue from this operation when similarly treated gave no lignin. The cellulose residue was nearly colorless and gave no color reaction with phloroglucinol and hydrochloric acid. When it was bleached with sodium hypochlorite, a pure white product was obtained. The yield of cellulose amounted to 35% of the weight of the moisture free corn cobs.

The results of this experiment are given in Table I.

TABLE I

LIGNIN FRACTIONS EXTRACTED FROM CORN COBS

Lignin fraction	Lignin (calcd. on corn cobs treated), %	Total lignin, %	Ash in lignin, %	Methoxy (calcd. on ash-free lignin), %	Pentosans in lignin Before purification with acetone and alcohol, %	Pentosans in lignin After purification with acetone and alcohol, %	Cellulose (calcd. on corn cobs treated). %
A[a]	4.4	48.4	0.37	16.45	0	0	35.8
B[b]	3.7	40.7	0.43	15.31	2.15	0	
C[c]	0.84	9.2	.19	15.05	0.79	0	
D[d]	.14	1.5	..	7.50[e]	..	.	

[a] "A"—Corn cobs extracted with 2% alcoholic sodium hydroxide at room temperature.

[b] "B"—Residue from "A" refluxed with 2% aqueous sodium hydroxide solution.

[c] "C"—Residue from "B" heated with 2% aqueous sodium hydroxide at 135°.

[d] "D"—Residue from "C" heated with 4% aqueous sodium hydroxide at 180°.

[e] No ash determined in this lignin fraction.

The percentage of methoxyl in the various lignin fractions was determined by the method of Zeisel and Fanto.[9] The pentosans in the lignin fractions were determined by the A. O. A. C. method.[10]

Table I shows that 48.4% of the total lignin in the corn cobs was obtained in the first fraction, 40.7% in the second, 9.2% in the third and 1.5% in the fourth. All of the lignin fractions after purification with alcohol and acetone were free from pentosans. Fraction "A" contained no pentosans or furfural yielding compounds even before purification, owing to the insolubility of the pentosans in the alcoholic sodium hydroxide solution. The statements in the literature that pentosan or some furfural yielding body forms an integral part of the lignin molecule must be considered as erroneous, especially as applied to the lignin from corn cobs. Attention is called particularly to the progressive decrease in the percentage of methoxyl in the lignin fraction with the increase in the temperature used for the extraction of the lignin.

[9] Houben-Weyl, "Die Methoden der organischen Chemie," Vol. III, Georg Thieme, Leipzig, **1923**, p. 144.

[10] "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," A. O. A. C., Washington, D. C.

Carbon and hydrogen determinations of the four lignin fractions gave the following results.

Anal. (Fraction A.) Subs., 0.1534, 0.1215: CO_2, 0.3517, 0.2783; H_2O, 0.0666, 0.0529. Found: C, 62.52, 62.46; H, 4.86, 4.87.

Anal. (Fraction B.) Subs., 0.0890, 0.0948: CO_2, 0.2043, 0.2171; H_2O, 0.0390, 0.0438. Found: C, 62.60, 62.45; H, 4.90, 5.17.

Anal. (Fraction C.) Subs., 0.1112, 0.0974: CO_2, 0.2614, 0.2298; H_2O, 0.0543, 0.0469. Found: C, 64.10, 64.34; H, 5.46, 5.39.

Anal. (Fraction D.) Subs., 0.0857, 0.1039: CO_2, 0.2139, 0.2597; H_2O, 0.0487, 0.0603. Found: C, 68.06, 68.16; H, 6.36, 6.49.

Summary

Lignin was fractionally extracted from corn cobs by a 2% alcoholic sodium hydroxide solution at room temperature, by 2% aqueous sodium hydroxide at 100° and at 135°, and finally by 4% aqueous sodium hydroxide at 180°. Each method of extraction was continued until no further lignin was obtained, before the next method in the series was employed. The results justify the conclusion that the lignin in corn cobs is unequally combined with the carbohydrates, part of it being loosely bound, possibly in the form of an ester, and the remainder being more firmly held, probably in the form of an ether-like combination.

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THE TAUTOMERISM OF BRILLIANT CRESYL BLUE

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Brilliant cresyl blue is an oxazine dye having metachromatic properties which finds important application in biological staining. The stain sold by the National Aniline and Chemical Company is the dimethyl homolog of the dye illustrated under No. 877 in the Colour Index.

With variation in concentration in aqueous solutions this dye undergoes a striking modification in color of the same type that all metachromatic dyes and the majority of aminated triphenylmethane and quinone-imide coloring matters in general undergo.[1] In relatively concentrated aqueous solutions it is present principally in a violet form, with maximum absorption at about 575mμ. The dilution of these solutions is accompanied by a progressive transition to a second dye form, which is blue, with maximum absorption at about 625mμ. Representative absorption curves are recorded in Fig. 1.

Considerable evidence has been obtained which indicates that color modification of this type does not arise from electrolytic or hydrolytic

[1] Holmes, *Ind. Eng. Chem.*, **16**, 35 (1924).