

[166TH CONTRIBUTION FROM THE COLOR AND FARM WASTE DIVISION, BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

## THE CHEMISTRY OF LIGNIN. IV. LIGNIN FROM OAT HULLS

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Several investigators have reported on the composition of lignin obtained from annual crop plants. Beckmann, Liesche and Lehmann<sup>1</sup> investigated a lignin fraction isolated from winter rye straw and concluded that the composition of the lignin from that source may be represented either by the formula  $C_{40}H_{44}O_{15}$  or  $C_{42}H_{46}O_{16}$ . Powell and Whittaker<sup>2</sup> isolated the lignin from flax shives by the alkali method. Their lignin preparation appeared to be a fairly homogeneous substance and its composition agreed with the formula  $C_{45}H_{48}O_{16}$ . In a recent communication<sup>3</sup> the writer reported on a lignin fraction isolated from corn cobs by the alcoholic sodium hydroxide method. All the results obtained agreed closely with the formula  $C_{40}H_{46}O_{16}$ . The presence of four hydroxyl groups capable of being acetylated and three methoxyl groups was shown.

The two lignin fractions described in this paper were isolated from oat hulls by extracting them successively and exhaustively, first with alcoholic sodium hydroxide solution at room temperature, and then by refluxing with 4% aqueous sodium hydroxide solution. 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 groups and three methoxyl groups were shown to be present, whereas the results on the lignin fraction isolated from oat hulls indicate the presence of three hydroxyl and four methoxyl groups. It would appear then, that in the lignin fraction from oat hulls one more hydroxyl group is methylated, the total number of hydroxyl and methoxyl groups being the same in both cases.

The analytical results on the residual lignin fraction isolated by means of aqueous sodium hydroxide solution are more in agreement with that represented by the formula  $C_{42}H_{40}O_{15}$ . In this lignin fraction three hydroxyl and four methoxyl groups were also shown to be present.

Most investigators have assumed that the alkoxy groups present in lignin are methoxyls, although few have actually proved this. The fact that a precipitate of silver iodide is obtained when lignin is distilled with hydriodic acid, as in the Zeisel method, in no way implies, of course, that a methoxyl group is present, as other alkoxy groups would react in a similar manner. In both lignin fractions from oat hulls methoxyl

<sup>1</sup> Beckmann, Liesche and Lehmann, *Z. angew. Chem.*, **34**, 285 (1921).

<sup>2</sup> (a) Powell and Whittaker, *J. Chem. Soc.*, **125**, 357 (1924); (b) **127**, 132 (1925).

<sup>3</sup> Phillips, *This Journal*, **49**, 2037 (1927).

groups only were definitely shown to be present. The alkyl iodide obtained when the lignin was distilled with hydriodic acid was identified as methyl iodide by the method of Feist.<sup>4</sup> Furthermore, by determining the percentage methoxyl of the lignin preparations by the Zeisel<sup>5</sup> method and by the method of Kirpal and Bühn,<sup>6</sup> identical results were obtained. This would necessarily exclude the presence of other alkoxy groups such as, for example, ethoxyl groups, as the Kirpal and Bühn method is known to give low results when alkoxy groups other than methoxyl are present.

### Experimental

Five hundred grams (464.3 g. of moisture-free material) of oat hulls was treated with 2.5 liters of 2% alcoholic sodium hydroxide solution (prepared by dissolving 50 g. of sodium hydroxide in 1 liter of water and adding sufficient 95% ethanol to make a volume of 2500 cc.) and allowed to stand at room temperature for twenty-four hours. The liquid was then poured off, neutralized with hydrochloric acid and the alcohol was distilled off under reduced pressure. To the aqueous residue, 10 cc. of concentrated hydrochloric acid was added and the precipitated lignin filtered off. The process of extraction with alcoholic sodium hydroxide was repeated until no more lignin was extracted. Five such extractions were required and 2 liters of alcoholic sodium hydroxide solution was used for each extraction. The lignin fractions thus obtained were combined and washed with distilled water until the wash water no longer gave a test for the chloride ion. The crude lignin thus obtained, after being dried in a desiccator over sulfuric acid, amounted to 34.5 g. (6.9% yield calculated on the weight of hulls taken). This was treated with 500 cc. of acetone-alcohol solution (2 volumes of acetone to 1 volume of 95% ethanol), filtered and the alcohol and acetone were removed by distillation under reduced pressure. The lignin thus obtained was washed with water and dried at 56° over sulfuric acid in the vacuum Abderhalden drier. The yield of this purified lignin amounted to 26 g. (5.6% of the weight of the dry hulls taken). That portion of the oat hulls remaining after the treatment with the alcoholic sodium hydroxide solution was washed with water and dried at 100°. The weight amounted to 356 g. (71.2% of the weight of the hulls). A carbon and hydrogen determination made upon the lignin gave the following results.

*Anal.* Subs., 0.1034, 0.0974; CO<sub>2</sub>, 0.2333, 0.2203; H<sub>2</sub>O, 0.0542, 0.0507. Found: C, 61.53, 61.68; H, 5.87, 5.83.

The lignin when boiled with 12% hydrochloric acid gave no coloration with aniline acetate paper. The lignin preparation was, therefore, free from furfural yielding compounds.

A methoxyl determination made according to the Kirpal and Bühn<sup>6</sup> modification of the Zeisel<sup>5</sup> method gave the following results.

*Anal.* Methoxyl.<sup>7</sup> Subs., 0.2038, 0.2022; AgI, 0.2413, 0.2387. Found: OCH<sub>3</sub>, 15.64, 15.59; C:OCH<sub>3</sub> = 10.2 : 1.

This ratio of percentage of total carbon to the percentage of carbon present in the form of methoxyl indicates the number of carbon atoms present in the substance for each methoxyl group.

<sup>4</sup> Feist, *Ber.*, **33**, 2094 (1900).

<sup>5</sup> Houben, "Die Methoden der Organischen Chemie," Georg Thieme, Leipzig, **1923**, Vol. III, p. 144.

<sup>6</sup> Kirpal and Bühn, *Ber.*, **47**, 1084 (1914); *Monatsh.*, **36**, 853 (1915).

<sup>7</sup> The methoxyl determinations were made by Mr. Herman Weihe of this Bureau.

In order to prove definitely the nature of the alkoxy group in this lignin fraction a sample of the material was distilled with hydriodic acid, as in the Zeisel method, and the alkyl iodide absorbed in dimethylaniline, as recommended by Willstätter and Utzinger.<sup>8</sup> The crystalline compound obtained after purification with chloroform melted at 231.6° (corrected). The melting point of trimethylphenylammonium iodide is recorded by Feist as 211–212°. The above-named compound was accordingly prepared from pure methyl iodide and dimethylaniline and purified by recrystallization from hot water and from 95% ethanol. When analyzed for nitrogen (Kjeldahl) and iodine, the following results were obtained.

*Anal.* Subs., 1.0000, 1.0000: required 18.6 and 18.4 cc. of 0.2 *N* acid. Subs., 0.3180, 0.3135; AgI, 0.2826, 0.2779. Calcd. for C<sub>9</sub>H<sub>14</sub>NI: N, 5.32; I, 48.24. Found: N, 5.21, 5.16; I, 48.03, 47.91.

This compound melted at 231.6° (corrected). The melting point given by Feist is, therefore, incorrect.

The crystalline product obtained from the lignin, as above described, was mixed with some trimethylphenylammonium iodide and the melting point of the mixture taken. It was also found to melt at 231.6° (corrected).

While the above results proved fairly conclusively that the methoxyl group was present in this lignin fraction, it nevertheless could not be accepted as evidence of the absence of other alkoxy groups, although no other crystalline body could be separated from the dimethylaniline mother liquors. Willstätter and Utzinger<sup>8</sup> have described a method for the quantitative separation and estimation of the methoxyl and ethoxyl groups when both are present in the same compound. Essentially, the method consists in absorbing the alkyl iodide in a 10% solution of trimethylamine in absolute alcohol; the tetramethylammonium iodide that is formed is insoluble in absolute alcohol, whereas the trimethylethylammonium iodide is soluble. This method was found, however, to be unreliable, principally because of the fact that the tetramethylammonium iodide is not altogether insoluble in absolute alcohol. For example, when this method was applied to vanillin it was found to yield 16.3% of methoxyl and 4.6% of ethoxyl. The absence of other alkoxy groups in this lignin fraction was finally proved in an entirely different manner. The method of Kirpal and Bühn is known to give low results when alkoxy groups other than methoxyl are present,<sup>9</sup> a fact which the writer verified experimentally. The Zeisel method is, however, of more general application, and quantitative results are obtained also when applied to the determination of alkoxy groups other than methoxyl. It was accordingly only necessary to determine the percentage of alkoxy (calculated as methoxyl) by both methods in order to prove definitely the presence or absence of alkoxy groups other than methoxyl in this lignin fraction. The results of the determination of the percentage of alkoxy (calculated as methoxyl) were as follows.

*Anal.* Methoxyl (Zeisel method). Subs., 0.3358, 0.3000; AgI, 0.3931, 0.3503. Found: OCH<sub>3</sub>, 15.46, 15.42. Methoxyl (Kirpal and Bühn method). Subs., 0.2038, 0.2022; AgI, 0.2413, 0.2387. Found: OCH<sub>3</sub>, 15.64, 15.59.

It will be observed that practically the same results were obtained by the two methods. The presence of methoxyl groups only in this lignin fraction must, therefore, be assumed.

From the foregoing data the formula C<sub>40</sub>H<sub>48</sub>O<sub>16</sub> is deduced for this lignin fraction, 782.6: C, 61.35%; H, 5.92%; OCH<sub>3</sub>, 15.92%. Four OCH<sub>3</sub> groups are present = C<sub>36</sub>H<sub>34</sub>O<sub>12</sub>(OCH<sub>3</sub>)<sub>4</sub>. The empirical formula of this lignin fraction is the same as that previously<sup>8</sup> reported for the lignin from corn cobs.

<sup>8</sup> Willstätter and Utzinger, *Ann.*, **382**, 148 (1911).

<sup>9</sup> Ref. 5, p. 149.

**Preparation of Acetyl Derivative.**—To 5 g. of lignin, 10 cc. of acetic anhydride and 3 to 4 drops of concentrated sulfuric acid were added. The reaction mixture was heated on the water-bath under a reflux condenser for one hour. The reaction product was allowed to cool, poured into ice water, filtered, and washed with cold water. The product was allowed to remain in a sulfuric acid desiccator overnight and then dried for twenty-four hours at 56° in a vacuum Abderhalden drier containing sulfuric acid and solid potassium hydroxide. The percentage acetyl was determined by the Perkin<sup>10</sup> method.

*Anal.* Acetyl. Subs., 0.7011, 0.7793: required 5.50, 6.1 cc. of 0.5 *N* KOH.  
 Calcd. for  $C_{36}H_{31}O_{12}$   $\begin{cases} (OCH_3)_4 \\ (CH_3CO)_3 \end{cases}$  :  $CH_3CO$ , 16.4. Found:  $CH_3CO$ , 16.8, 16.8.

The dissected formula for this lignin fraction is, therefore,  $C_{36}H_{31}O_9(OCH_3)_4(OH)_3$ .

**Isolation of the Residual Lignin.**—Three hundred grams of the residual oat hulls which had been extracted with the alcoholic sodium hydroxide solution in the previous experiment and dried at 105° was treated with one liter of 4% sodium hydroxide and heated at 100° for four hours. This reaction mixture was filtered, and the lignin precipitated by the addition of concentrated hydrochloric acid to the filtrate. The residual oat hulls were again treated with 4% sodium hydroxide solution, and the operation was repeated until the alkaline extract when acidulated with hydrochloric acid no longer gave a precipitate of lignin. The cellulose residue obtained was washed with water and dried at 105°. The yield amounted to 151 g. (50.3% of the weight of the hulls taken). It still contained 3.4% of lignin as determined by the fuming hydrochloric acid method of Willstätter. The crude lignin obtained in the several extraction operations was combined, and when dried at 105° its weight amounted to 18 g. When boiled with 12% hydrochloric acid, this lignin gave a positive test for furfural with aniline acetate paper. It was purified by dissolving it in 500 cc. of 2% aqueous sodium hydroxide and adding to it one liter of 95% ethanol. The precipitate was filtered off, the filtrate was acidified with hydrochloric acid and the alcohol was removed by distillation. The lignin obtained was washed with water until the wash water gave no test for chloride with silver nitrate solution and dried at 105°; yield, 13 g. or 4.3% (calculated on the 300 g. of hulls used). It was an amorphous brown material. The color was darker than that of the lignin obtained by the alcoholic sodium hydroxide method. When distilled with 12% hydrochloric acid the lignin did not give a coloration with aniline acetate paper nor a precipitate with phloroglucinol.

*Anal.* Subs., 0.0900, 0.0899:  $CO_2$ , 0.2123, 0.2123;  $H_2O$ , 0.0413, 0.0423. Found: C, 64.33, 64.40, H, 5.13, 5.26.

The alkoxy groups present in this lignin fraction were shown to be methoxyls. The method previously described for the identification of the alkoxy groups was used.

*Anal.* Methoxyl (Kirpal and Bühn method). Subs., 0.3243, 0.4035: AgI, 0.3865, 0.4849. Found:  $OCH_3$ , 15.74, 15.88. Methoxyl (Zeisel method). Subs., 0.2018, 0.2130: AgI, 0.2398, 0.2521. Found:  $OCH_3$ , 15.70, 15.64.  $C:OCH_3 = 21:2$ .

Based on the above analytical data, the formula  $C_{22}H_{10}O_{15}$  or  $C_{33}H_{23}O_{11}(OCH_3)_4$  is assigned for this lignin fraction. The percentage composition for a compound of this formula is: C, 64.27; H, 5.13;  $OCH_3$ , 15.80.

**Acetyl Derivative.**—This was prepared by the method already described. The same method was used for the determination of the percentage acetyl.

*Anal.* Acetyl. Subs., 1.0575, 1.0421: required 7.99, 7.80 cc. of 0.5 *N* KOH. Calcd. for  $C_{33}H_{23}O_{11}(OCH_3)_4(COCH_3)_3$ :  $CH_3CO$ , 16.4. Found:  $CH_3CO$ , 16.2, 16.1.

<sup>10</sup> Perkin, *Proc. Chem. Soc.*, 20, 171 (1904).

The dissected formula for the lignin obtained in this fraction is, therefore,  $C_{33}H_{25}O_8(OCH_3)_4(OH)_3$ .

### Summary

1. Two lignin fractions were isolated 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 dissected formula  $C_{33}H_{31}O_9(OCH_3)_4(OH)_3$  for the first lignin fraction and  $C_{33}H_{25}O_8(OCH_3)_4(OH)_3$  for the second lignin fraction.

2. The alkoxy groups present in both lignin fractions were definitely proved to be methoxyls. A method for proving this is described.

3. The melting point of trimethylphenylammonium iodide was found to be  $231.6^\circ$  (corrected) and not  $211-212^\circ$  as recorded in the literature.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ILLINOIS]  
**THE EFFECT OF ETHYLENE UPON THE ACTIVITY OF DIASTASE  
AND INVERTASE**

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With the increasing use of ethylene for the artificial ripening of fruits and vegetables much interest is manifested in the nature and mechanism of the action. In a recent bulletin emphasizing the practical application of the use of the gas, Harvey<sup>1</sup> has appended a very complete bibliography of the literature of the subject. A difference of opinion seems to prevail as to whether the change in the fruits and vegetables induced by the ethylene is chiefly a color change or a genuine ripening in which not only color but the normal accompanying changes in composition take place. Chace and Church,<sup>2</sup> after carrying out a large number of experiments and analyses, conclude that there is no difference in the composition of the edible portion of citrus fruits subjected to the ethylene method of coloring when compared to untreated fruits under the same temperature conditions. Data on other fruits were too meager to permit definite conclusions and did not suggest any changes which would not be caused by heat alone. Harvey<sup>3</sup> insists that ethylene brings about a genuine ripening effect when applied at concentrations of 1 to 1000 of air at  $65^\circ F$ . He believes that the results obtained by Chace and Church were due to the lower concentration of ethylene used, 1 part in 5000 of air. Rosa<sup>4</sup> states that ethylene favors the rate of softening, color change and inver-

<sup>1</sup> Harvey, Bulletin 247, University of Minnesota Agr. Exp. Sta., October, 1928.

<sup>2</sup> Chace and Church, *Ind. Eng. Chem.*, 19, 1135 (1927).

<sup>3</sup> Harvey, *Science*, 67, 421 (1928).

<sup>4</sup> Rosa, *Hilgardia*, 3, 421 (1928).