g. of sulfuric acid and 70 cc. of water, keeping the temperature at about 50°. Stirring was continued for thirty minutes after all of the alcohol had been added, and the mixture then distilled until no more oil passed over. The oil was separated, dried with calcium chloride and distilled. The boiling point, density and refractive index were the same as those of the saturated ketone obtained from homomesityl oxide.

The semicarbazone melted at 96°.

3,4-Dimethyl-2-hexanone.—This was prepared by hydrolyzing ethyl *sec.*-butylmethylacetoacetate by the regular procedure. Ninety grams of ethyl acetoacetate yielded 90 g. of crude ethyl methylacetoacetate, which in turn gave 62 g. of pure ethyl *sec.*-butylmethylacetoacetate. The ketone was obtained by refluxing the ester with 300 cc. of 15% aqueous sodium hydroxide; b. p. 158°, d_4^{22} 0.8295, n_D^{22} 1.412.

The semicarbazone melted at 124–126°.

Summary

1. An improved method for the preparation of homomesityl oxide has been described.

2. The structure of homomesityl oxide is definitely established as $C_2H_5(CH_3)C{=}CHCOC_2H_5$.

SEATTLE, WASHINGTON

[188TH CONTRIBUTION FROM THE COLOR AND FARM WASTE DIVISION, BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

THE CHEMISTRY OF LIGNIN. V. THE DISTILLATION OF ALKALI LIGNIN WITH ZINC DUST IN AN ATMOSPHERE OF HYDROGEN

BY MAX PHILLIPS

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Considerable difference of opinion prevails among investigators of the chemistry of lignin as to whether this substance belongs to the aliphatic, aromatic, hydroaromatic or heterocyclic series.[1] Among the earliest constitutional formulas for lignin may be mentioned the Keto-R-hexene formula of Cross and Bevan[2] and that proposed by Green.[3]

Willstätter and Kalb,[4] as the result of their work on the reduction of lignin with hydriodic acid and red phosphorus, conclude that lignin is structurally related to the carbohydrates. However, none of the products which they obtained were homogeneous, and they were unable to identify any definite chemical substance.

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[1] An excellent résumé of the literature up to 1926 dealing with the constitution of lignin is given by K. Kürschner in his monograph, "Zur Chemie der Ligninkörper," p. 141, published in F. B. Ahrens' "Sammlung Chemischer und Chemischtechnischer Vorträge," Vol. 28, Ferd. Enke, Stuttgart, **1926**, and in the monograph by W. Fuchs, "Die Chemie des Lignins," Julius Springer, Berlin, **1926**, p. 277.

[2] Cross and Bevan, "Cellulose," Longmans, Green and Co., London, 2d ed., **1895**, p. 137; Vol. III, 1912, p. 104.

[3] Green, *Z. Farben Textilchemie*, **3**, 97 (1904).

[4] Willstätter and Kalb, *Ber.*, **55**, 2637 (1922).

Jonas[5] and Marcusson[6] contend that lignin is not aromatic but is made up of furan nuclei.

Schrauth[7] assumes that the fundamental unit of the lignin molecule is formed by the condensation of three molecules of 5-hydroxymethylfurfural. The unit so formed consists of a compact condensed ring system, of which three of the outer rings are furan nuclei and the other three outer rings and the central ring benzene nuclei.

The constitutional formulas for lignin, which at various times have been advanced by Klason,[8] all contain at least one aromatic nucleus, generally some coniferyl derivative coupled with a heterocyclic nucleus.

Kürschner,[9] as the result of his investigations on the sublimation of lignin, advanced the hypothesis that lignin is a polymer of the glucoside coniferin. Freudenberg, Belz and Niemann[10] have recently come out in favor of an aromatic structure for lignin. In a more recent publication Freudenberg, Zocher and Dürr[11] advanced the hypothesis that lignin is a condensation product of α-vanillyl-glycerine and α-piperonyl-glycerine.

The present investigation was undertaken with the hope of obtaining degradation products of lignin that would help to clear up even in part the structure of this substance. It was felt that a zinc dust distillation of lignin in an atmosphere of hydrogen might lead to definite degradation products which would throw some light on the chemistry of this compound.

The only work reported in the literature on the zinc dust distillation of lignin is that by Karrer and Bodding–Wiger.[12] They heated lignin with zinc dust at red heat and obtained an oil which they fractionated. None of the fractions were oxygen free, nor did they appear to be homogeneous, nor did these authors identify any definite chemical substance in their distillation product.

In the present investigation three phenolic substances were isolated, two of which were identified as catechol and guaiacol. The third phenol gave a crystalline dinitrobenzoyl derivative melting at 110° and contained the methoxyl group but was not further identified. It was probably a homolog of guaiacol. It is believed that the results obtained in this investigation tend to give support to the hypothesis that at least one aro-

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[5] Jonas, *Wochenbl. Papierfabr.*, **56**, 24A, 83 (1925).

[6] Marcusson, *Z. angew. Chem.*, **34**, 437 (1921); **35**, 165 (1922); **36**, 42 (1923); *Ber.*, **58**, 869 (1925).

[7] Schrauth, *Z. angew. Chem.*, **36**, 149 (1923).

[8] Klason, *Svensk. Kem. Tidskrift*, **1897**, 135; *Arkiv. Kem. Min. Geol.*, **6**, No. 15, 1 (1917); *Ber.*, **53**, 706 (1920); **56**, 300 (1923); **58**, 375, 1761 (1925); **62**, 2523 (1929); **63**, 792, 1548, 1983 (1930).

[9] Kürschner, Ref. 1, p. 164.

[10] Freudenberg, Belz and Niemann, *Ber.*, **62**, 1554 (1929).

[11] Freudenberg, Zocher and Dürr, *ibid.*, **62**, 1814 (1929).

[12] Karrer and Bodding-Wiger, *Helv. Chim. Acta*, **6**, 817 (1923).

matic nucleus exists in the lignin molecule.[13] The presence of other nuclei in addition to the aromatic nucleus is however not excluded.

In all of the experiments, considerable amounts of carbon dioxide were evolved during the distillation. This may be explained on the assumption that lignin contains either a lactone, carboxyl or esterified carboxyl group. Van der Haar[14] has recently shown that only those substances which contain two oxygen atoms attached to the same carbon atom, that is to say, carboxylic acids and their esters and lactones give off carbon dioxide when subjected to a zinc dust distillation in a current of hydrogen. Ketones, aldehydes, alcohols, phenols, quinones, purine derivatives and pyrones do not give off carbon dioxide under these conditions.

Experimental

The apparatus used in these experiments is illustrated in Fig. 1. It consisted of a glass tube, B, 3.5 cm. outside diameter and 45 cm. long. This tube was constricted at one end and bent at a right angle in the form of an adapter. This was attached to a vertical condenser F, to which, in turn, was attached a 300-cc. Erlenmeyer flask, G, the inner tube of the condenser reaching to within about one centimeter of the bottom of this flask. The latter was connected in series to two other 300-cc. Erlenmeyer flasks, G′ and H. Flasks G, G′, which served as receivers for the distillate, were immersed in an ice-bath. Flask H contained barium hydroxide solution to collect any carbon dioxide that might come over. The glass tube was inserted into an asbestos-covered metal tube (A) 8.5 cm. in diameter, which served as an air-bath and was held in place by transite end pieces C. This tube was heated by means of three fishtail burners J. The temperature of the bath was determined by means of thermometer D. Dry hydrogen was led into the system through tube E.

The glass tube (B) was filled as follows. A plug of glass wool was placed at the constricted end of the tube. This was followed by a 10-cm. layer of pumice about the size of peas which had been impregnated with zinc dust. The coating of the pumice with zinc dust was accomplished as follows. A paste was prepared by rubbing up 100 g. of zinc dust with 30 cc. of water. The pumice was added to this and the mixture stirred until the pumice was well covered with the zinc dust paste. The pumice was then removed from the paste and dried.

After the pumice–zinc dust layer was placed a mixture consisting of 50 g. of alkali lignin[15] and 150 g. of zinc dust to which had been added some pumice impregnated with zinc dust. Finally a 5-cm. layer of pumice–zinc dust was added.

The distillation experiment was begun by passing through the system a fairly rapid stream of dry hydrogen until all of the air was replaced. The current of gas was then diminished so that only about three bubbles per second passed through. Heat was

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[13] It may be of interest to point out in this connection that phenol ethers had previously been found in the oil obtained by the destructive distillation of lignin (W. Fuchs, "Die Chemie des Lignins," Julius Springer, Berlin, **1926**, p. 151).

[14] Van der Haar, *Rec. trav. chim.*, **48**, 1170 (1929).

[15] The lignin used in these experiments was isolated from corn cobs following the method described in a previous communication [This Journal, **51**, 2420 (1929)]. It was purified by dissolving in alcoholic sodium hydroxide solution, filtering, distilling off the alcohol and precipitating the lignin with hydrochloric acid. It was washed free from chlorides and dried *in vacuo* at 80°.

then applied under the front and rear ends of the tube containing the layers of pumice-zinc dust. Finally the mixture of lignin and zinc dust was gradually heated until the temperature of the air-bath was 400°. Throughout the experiment the temperature was not permitted to exceed 400°. At first a considerable amount of gas was evolved, including carbon dioxide which was indicated by the barium hydroxide solution first becoming milky and by the subsequent deposition of a precipitate of barium carbonate. Carbon dioxide was given off in all of the experiments.

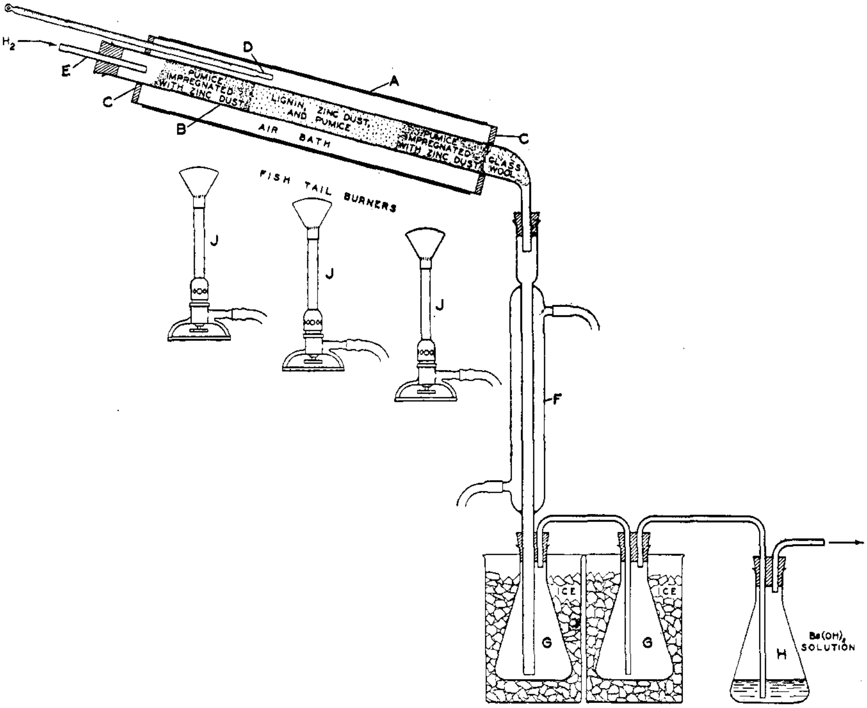

Fig. 1.—Apparatus for distillation of lignin with zinc dust in atmosphere of hydrogen.

Examination of the Distillate.—The distillate consisted of an aqueous portion together with an oil. The combined distillates from five experiments representing a total of 250 g. of lignin were combined and worked up. The aqueous portion was separated off, shaken with activated charcoal (Darco) as recommended by Stritar and Zeidler,[16] filtered and the filtrate made up to 500 cc. The oily portion was dissolved in an equal volume of ether, dried over anhydrous sodium sulfate and filtered. Upon removal of the ether by distillation, a brown oil was obtained. The yield was 40 g., equal to 16% of the weight of the lignin used.

Examination of the Aqueous Portion.—The acid in the aqueous distillate was determined by titration with standard alkali and amounted to 0.24% (calculated as acetic acid and on the basis of the weight of lignin used).

The acetone was determined by the Messenger[17] method, which was carried out as

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[16] Stritar and Zeidler, *Z. anal. Chem.*, **43,** 387 (1904).

[17] Messenger, *Ber.*, **21,** 3366 (1888).

described in a previous communication.[18] The acetone amounted to 0.36% of the weight of the lignin used.

Two 5-cc. samples were analyzed for methanol by the method of Stritar and Zeidler.[16] The weight of silver iodide obtained amounted to 0.1087 g. (mean of two determinations) which corresponds to 1.48% of methanol in the total distillate and equals 0.59% of the weight of lignin used.

The aqueous distillate gave with ferric chloride solution a green coloration which became red on the addition of sodium carbonate. It gave all the other characteristic tests for catechol.[19]

The aqueous distillate gave no test for furfural with aniline acetate paper and none of the tests for ω-hydroxymethylfurfural.[20] Fuchs[21] obtained ω-hydroxymethylfurfural on the distillation of technical pinewood lignin with powdered silver in an atmosphere of carbon dioxide.

Examination of the Oil.—The oil was dissolved in an equal volume of ether and the ether extracted with 5% sodium hydroxide solution. The alkaline extract was then acidified with hydrochloric acid and the oil separated mechanically from the aqueous portion. This was extracted with ether and the phenolic oil previously separated was added to the ether solution. This solution was dried with anhydrous sodium sulfate, filtered and the ether removed by distillation. A dark brown oil was obtained which amounted to 22 g. (equal to 55% of the weight of the original oil). This oil was distilled in a current of steam, and a light yellow oil was obtained which amounted to 11 g. (equal to 27.5% of the weight of the original oil). This oil was first distilled under ordinary pressure and the distillate fractionated under the same pressure. Two fractions were obtained, namely, 200–210° and 210–220°. The amount of each fraction was 4 g. and 3.5 g., respectively. Each fraction gave a greenish color with ferric chloride in alcoholic solution. A portion of fraction 200–210° (1 g.) was added to a solution of 2.3 g. (0.01 mole) of 3,5-dinitrobenzoyl chloride in 20 cc. of pyridine, and the solution heated at its boiling point under the reflux condenser. The solution after cooling was poured into cold dilute sulfuric acid. The yellow and rather viscid substance which separated out was dissolved in ether, the ether solution extracted with 5% sodium hydroxide solution and the ether removed by distillation. A solid crystalline substance was obtained. This was crystallized from 95% ethanol and recrystallized from methanol and ligroin when a substance melting constantly at 141° (corr.) was obtained. The 3,5-dinitrobenzoyl derivative of pure guaiacol was prepared, and the same melting point was obtained. When the two compounds were mixed the resulting mixture was also found to melt at 141°. Fraction 200–210°, therefore, consisted principally of guaiacol. The 3,5-dinitrobenzoyl derivative of a portion of fraction 210–220° was prepared following the method above described. The product, after several recrystallizations from ethanol, methanol and ligroin, melted at 110°. The melting point, however, was not sharp and even after three other recrystallizations it was not possible to obtain a product having a sharp melting point.

Anal. Subs., 0.0800: required 2.50 cc. of 0.2 *N* acid. Subs., 0.0374: AgI, 0.0223. Found: N, 8.75; OCH_3, 7.88.

The percentages of nitrogen and methoxyl in the dinitrobenzoyl derivatives of guaiacol and creosol are, respectively: N, 8.80, 8.43; OCH_3, 9.74, 9.34. Fraction 210–220° consisted of some undetermined phenol, possibly some homolog of guaiacol.

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[18] Phillips, This Journal, **51**, 2420 (1929).

[19] L. Rosenthaler "Der Nachweis organischer Verbindungen," Ferdinand Enke, Stuttgart, **1923**, p. 258.

[20] Ref. 19, p. 147.

[21] Fuchs, *Ber.*, **60**, 957 (1927).

The residue in the flask after the steam distillation of the phenols was a black tarry substance and amounted to 4 g. It was distilled under 15 mm. pressure, and practically all distilled over at 150–190°. Nothing definite, however, was obtained from this fraction.

The ether solution of the oil which had been extracted with 5% sodium hydroxide solution was washed with water and then dried with anhydrous sodium sulfate, filtered and the ether removed by distillation. A dark brown oil was obtained. This amounted to 10 g. (equal to 25% of the weight of the original oil). It was distilled under ordinary pressure, whereupon it all distilled over at 200–220°. The distillate was a light yellow oil.

Anal. Subs., 0.1002, 0.1460: CO_2, 0.2770, 0.4027; H_2O, 0.0705, 0.0981. Found: C, 75.38, 75.22; H, 7.87, 7.51. Subs., 0.0774; AgI, 0.0971 = 16.57% OCH_3.

This neutral substance is therefore an ether containing approximately one-half of its oxygen of the form of methoxyl. It did not react with acetic anhydride or benzoyl chloride nor with hydroxylamine or phenylhydrazine. Hydroxyl and carbonyl groups are therefore not present in this substance, and the residual oxygen exists probably in some ring combination.

Estimation of the Amount of Carbon Dioxide Given Off in the Zinc Dust Distillation of Lignin.—Although van der Haar[13] in applying the zinc dust distillation method for the quantitative determination of carboxyl groups has shown that the method invariably gives low results, it seemed nevertheless of interest to determine the approximate amount of carbon dioxide given off in the zinc dust distillation of lignin. Accordingly, three experiments were conducted in which 5 g. of lignin and 50 g. of zinc dust were used in each case. The distillation was conducted with the apparatus and under the experimental conditions previously described. The carbon dioxide given off was absorbed in a barium hydroxide solution contained in flask H (Fig. 1). The barium carbonate formed was filtered off and washed with water, free from carbon dioxide, until the wash water was no longer alkaline. The carbonate was dissolved in warm dilute hydrochloric acid and the barium determined as the sulfate. From the weight of barium sulfate obtained the quantity of carbon dioxide was calculated. The results obtained were as follows:

$BaSO_4$, g.	CO_2, g. (calcd. from $BaSO_4$)
1.0756	0.2027
1.0520	.1983
1.0807	.2037

Mean 0.2015 $\Bumpeq$ 0.2060 g.–COOH = 4.12%

Assuming that the composition of corn cob lignin may be represented by the formula $C_{40}H_{46}O_{16}$[22] and on the basis of one carboxyl group, the calculated value is 5.75% of COOH.

Summary

1. Alkali lignin, isolated from corn cobs, was distilled with zinc dust in an atmosphere of hydrogen. The distillate consisted of an aqueous portion and an oil. The aqueous distillate contained methanol, acetone and an acid (determined as acetic acid) to the extent of 1.48, 0.36 and 0.24%, respectively (calculated on the basis of the weight of lignin used). Catechol was shown to be present in the aqueous distillate. The yield of

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

oil obtained amounted to 16% of the weight of the lignin used. Fifty-five per cent. of the oil was alkali-soluble, and in this fraction guaiacol was definitely identified. A phenol containing the methoxyl group and yielding a 3,5-dinitrobenzoyl derivative melting at 110° was also obtained but was not further identified.

2. The isolation of catechol and guaiacol as degradation products of lignin is believed to lend support to the hypothesis that lignin contains an aromatic nucleus.

3. The evolution of carbon dioxide in the zinc dust distillation of lignin in an atmosphere of hydrogen indicates that, in all probability, the lignin molecule contains at least one carbon atom directly united to two oxygen atoms. This may indicate the presence of either a carboxyl group, free or esterified, or a lactone group in the lignin molecule.

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[185th Contribution from the Color and Farm Waste Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture]

THE PRODUCTION OF KOJIC ACID BY ASPERGILLUS FLAVUS

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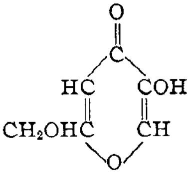
In the course of a survey of the action of a number of fungi on solutions of xylose and dextrose it was observed that the culture liquors from a strain of *A. flavus* on both sugars gave a deep red coloration upon the addition of ferric chloride solution. Continuous extraction of these cultures with ether yielded a quantity of material crystallizing as colorless needles, soluble in water, methanol, ethanol and ethyl acetate. It was identified as kojic acid by its melting point (152.6° (corr.); Yabuta, 152°; Mauer, 152°; Kinoshita, 154°), by the melting point of the monobromo derivative (161° (corr.); Yabuta, 159–160°), and by analysis of the insoluble copper salt (found, average of six determinations, 22.40% CuO. Calcd. for $Cu(C_6H_5O_4)_2 \cdot {}^1/_2H_2O$, 22.44%; Mauer found 22.12% CuO). The crystalline products obtained from the xylose and dextrose solutions were identical in all their properties.



Kojic acid was first isolated by Saito[1] from mycelia of *A. oryzae* which had been cultured on steamed rice. He thought it to be identical with β-resorcylcarboxylic acid. Shortly thereafter Yabuta[2] undertook an extensive investigation of the substance, gave it the name kojic acid and,

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[1] K. Saito, *Botan. Mag.* (Japan), **21**, 249 (1907).

[2] T. Yabuta, *J. Coll. Agr., Tokyo*, **5**, 51 (1912); *8th Int. Cong. Applied Chem., Appendix*, **25**, 455 (1912); *J. Chem. Soc. Japan*, **37**, 1185 (1916) (in Japanese); see *Chem. Abs.*, **17**, 1475 (1923); *J. Chem. Soc.*, **125**, 575 (1924).