Vacuum distillation of the crude alcohol yielded fraction I (b.p. 33–36°, 0.6 mm.) and fraction II (b.p. 97–130°, 0.4 mm.). Fraction I was redistilled at atmospheric pressure, b.p. 169–173°, n^{23}D 1.4790 (*o*-chloroethylbenzene boils at 180°, n^{25}D 1.5187; 2-methylcyclohexanone boils at 166°, n^{20}D 1.4487). Fraction II was seeded with 1,4-di-(*o*-chlorophenyl)-butane and allowed to stand in the refrigerator until recrystallization was complete. The liquid product (7.8 g., 22%) remaining after filtration of the crystals was mainly alcohol V and the corresponding olefin. Cyclization of this material with 90% sulfuric acid (23 ml.) by the procedure used in Part A yielded 6.95 g. (96% based on partially purified alcohol) of VI which boiled at 115–117° (0.22 mm.), n^{24}D 1.5666.

1,4-Di-(*o*-chlorophenyl)-butane. A.—This compound was synthesized in a manner analogous to that used for the preparation of 1,4-diphenylbutane.[10] From β-(2-chlorophenyl)-ethyl bromide (5.53 g.), magnesium (0.69 g.), anhydrous cupric chloride (4.67 g.) and anhydrous ether (40 ml.) there was obtained 0.75 g. (21%) of 1,4-di-(*o*-chlorophenyl)-butane which melted at 51–52° (b.p. 156–162°, 0.5 mm.).

Anal. Calcd. for $C_{16}H_{16}Cl_2$: C, 68.82; H, 5.78. Found: C, 68.87; H, 5.89.

B.—The crystals obtained as a by-product in the preparation of alcohol V were recrystallized from ethanol and had a melting point of 51–52° alone or when mixed with the sample prepared in part A.

4a-Methyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene.—8-Chloro-4a-methyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene (2.6 g.) was dissolved in absolute ethanol (120 ml.) and to this refluxing solution small pieces of sodium (6.0 g.) were added during 3 hours. The excess alcohol was distilled and the residue treated with ice-water and sufficient acetic acid to make the solution acid. The hydrocarbon was extracted with petroleum ether and after removal of the solvent was vacuum distilled; b.p. 100–103° (0.5 mm.), n^{25}D 1.5453.

Anal. Calcd. for $C_{15}H_{20}$: C, 89.93; H, 10.07. Found: C, 90.09; H, 10.09.

A sample of this hydrocarbon (0.4 g.) was dehydrogenated using palladium-on-charcoal (0.08 g.) at 280–320°. White crystals sublimed onto the condenser of the dehydrogenator during the process. After recrystallization from methanol the dehydrogenated product melted at 97–98° alone or when mixed with an authentic sample of phenanthrene.

(10) E. E. Turner, *J. Chem. Soc.*, **115**, 559 (1915); E. E. Turner and F. W. Bury, *ibid.*, **123**, 2490 (1923).

9-Keto-4a-methyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene (VII).—4a-Methyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene (1.3 g.) was dissolved in glacial acetic acid (14 ml.) and cooled to 0°. Chromic anhydride (1.7 g.) dissolved in 80% acetic acid was added and the reaction mixture stirred at 0° for 10 hours and then allowed to stand for three days at room temperature. The reaction mixture was diluted with water (175 ml.) and extracted with ether. The ether solution was washed with sodium bicarbonate solution, dried and concentrated. Vacuum distillation of the residue produced 0.5 g. of ketone VII, b.p. 130–132° (0.5 mm.), n^{25}D 1.5675.[11] This was purified by conversion to the 2,4-dinitrophenylhydrazone which was recrystallized from ethanol and ethyl acetate, m.p. 209.5–210.5°.

Anal. Calcd. for $C_{21}H_{22}O_4N_4$: C, 63.94; H, 5.62. Found: C, 64.17; H, 5.70.

Chromatographic Separation of 2,4-Dinitrophenylhydrazones (VIII) and (IX).—The mixture of 2,4-dinitrophenylhydrazones of VII[6] (98 mg., m.p. 166–184°) was placed on a 30-cm. column of acid-washed alumina (10 g.) in a benzene–petroleum ether solution. Fifty fractions were eluted each with 125 ml. of 30% benzene in petroleum ether. The last ten fractions were combined and recrystallized from ethanol–ethyl acetate. The sample of VIII obtained in this way melted at 208–210°.[12] The melting point of a mixture of VIII with the 2,4-dinitrophenylhydrazones prepared from either sample of VI also melted at 208–210°. The ultraviolet absorption spectra of VIII and IX in 95% ethanol were identical with λ_{max} at 385 mμ and λ_{min} at 320 mμ. The infrared absorption curves of the sample of VIII obtained by chromatography and from VI were identical; there were significant differences particularly in the region 8–10 μ between the curves of VIII and IX. The X-ray lattice spacings (d/n) for the two samples of VIII were identical and different from that of IX.[5]

(11) M. S. Newman and M. D. Farbman, ref. 6, report the mixture of isomeric ketones to boil at 125–138° (0.5 to 1 mm.), n^{23}D 1.5673.

(12) In the previous separation of these 2,4-dinitrophenylhydrazones ref. 5, the last four fractions of thirty-four yielded a product which melted at 186–190°.

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[CONTRIBUTION NO. 268 FROM THE DEPARTMENT OF ORGANIC CHEMISTRY AND ENZYMOLOGY, FORDHAM UNIVERSITY]

Investigations on Lignin and Lignification. XI. Structural Studies on Bagasse Native Lignin[1]

BY GEORGE DE STEVENS AND F. F. NORD

RECEIVED AUGUST 12, 1952

The native lignin from bagasse and the lignin liberated through the action of cellulolytic enzymes present in the wood-destroying mold, *Poria vaillantii*, have been found to be identical in all respects examined. Besides vanillin and syringaldehyde we obtained equivalent amounts of *p*-hydroxybenzaldehyde from each of these lignin preparations on oxidation with nitrobenzene and alkali. Chemical evidence in conjunction with ultraviolet spectroscopic examination indicates that a *p*-hydroxyphenyl ketone is prevalent in bagasse native lignin molecule. The possible presence of a flavanone-type structure in this preparation is considered.

In previous studies on bagasse native lignin it was established that its native lignin fraction is identical with the lignin liberated by the action of enzymes present in wood-destroying molds of the "brown rot" type.[2] The subject matter of this report deals, in part, with the further comparison of these lignin fractions and also with chemical, chromatographical and spectroscopical studies on bagasse native lignin for the purpose of elucidating its structure.

The reactions utilized in the chemical investigations were methylation, acetylation, mercaptolysis, oxidation and hydrogenation. The analytical methods applied were paper chromatography as well as ultraviolet and infrared absorption spectroscopy. Finally, model compounds served to correlate findings pertaining to a possible lignin structure with its chemical and physical behavior.

(1) Presented before the Cellulose Division of the American Chemical Society, Atlantic City, N. J., September, 1952. The data recorded are taken from a part of the dissertation of G. de St. submitted to the Graduate School of Fordham University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(2) (a) G. de Stevens and F. F. Nord, THIS JOURNAL, **73**, 4622 (1951); (b) **74**, 3326 (1952).

Experimental

Isolation of Bagasse Lignins.—Bagasse native lignin was obtained by applying the method outlined by Brauns[3] while the isolation and properties of the enzymatically liberated lignin have been previously described.[2,4]

Methylations. (a) With Diazomethane.—The diazomethane was prepared by dissolving 0.5 g. of nitrosomethylurea[5] in a cooled mixture of dioxane (7 ml.) and 45% potassium hydroxide (1 ml.). The dioxane solution was then decanted and allowed to stand over pellets of potassium hydroxide overnight. This solution was added to 0.5 g. of native lignin dissolved in 5 ml. of dioxane. After standing for 24 hours, the reaction mixture was centrifuged and precipitated into ether. This methylation and purification procedure was repeated several times until a constant methoxyl value was obtained.

(b) With Methanol and Hydrochloric Acid.—One-half gram of native lignin was dissolved in 10 ml. of absolute methanol. To this solution was added 2.5 ml. of a 2% methanolic hydrogen chloride solution upon which the lignin solution turned wine-red in color. After refluxing the mixture for four hours, the methanol was distilled off at reduced pressure. The residue was taken up in 5 ml. of methanol-acetone (1:1), centrifuged and precipitated into an excess of ice-cold distilled water with vigorous stirring. The precipitate was collected, dried *in vacuo*, dissolved in dioxane and precipitated into ether. The latter purification procedure was repeated several times. The yield of the methylated product was almost quantitative.

(c) With Dimethyl Sulfate.—Two and one-half grams of native lignin was dissolved in 25 ml. of acetone and to this was added with vigorous stirring 35 ml. of a 30% sodium hydroxide solution. Twenty-five ml. of dimethyl sulfate was then added slowly over a half-hour period. When the temperature of the reaction mixture began to rise, the flask was immersed in an ice-bath. Upon cooling, dimethyl sulfate was again added with stirring and the reaction was allowed to run for eight hours at room temperature. After standing overnight, the acetone was removed at reduced pressure. The residue was washed well with distilled water and dried *in vacuo* for several days. It was then dissolved in acetone and precipitated into ice-cold distilled water. The dried precipitate was dissolved in dioxane to make a 10% solution and precipitated into thirty times its volume of ether. It was collected, washed well with ether and low-boiling petroleum ether and dried. After four successive methylations and purifications a buff-colored powder was obtained.

Acetylation of Native Lignin and its Derivatives.—The lignin was dissolved in pyridine and acetic anhydride was added to the solution. After standing at room temperature for 48 hours, the acetylated product was separated and purified in the usual manner.

Mercaptolysis.[6]—0.2 gram of native lignin was dissolved in 5 ml. of thioglycolic acid and to this was added 0.5 ml. of 2 *N* hydrochloric acid. After heating the mixture for six hours at 100°, the solution was cooled, separated and purified by precipitation first into water and then into ether, dioxane being used as the dissolving agent in the latter case. The enzymatically liberated lignin and the completely methylated lignin were treated in the same way. The thioglycolic acid derivatives of native and enzymatically liberated lignins gave a positive phloroglucinol-hydrochloric acid color test.

Oxidation of Native and Enzymatically Liberated Lignins.—These lignins were oxidized with nitrobenzene and alkali at 170° for 2.5 hours. The products were chromatographically separated and quantitatively determined according to a recently described micro-method.[7] The developing solvents used were ligroin (boiling range 100 to 120°), *n*-butyl ether and water (6:1:1), respectively.

Hydrogenation.—Five grams of native lignin dissolved in 75 ml. of purified dioxane was hydrogenated in the presence of Raney nickel catalyst at 25° at a pressure of 3 atmospheres for four hours. The hydrogen uptake was nil. Increasing the temperature to 50° and the pressure to 10 atmospheres still did not bring about reduction. After completion of the hydrogenation runs, the ultraviolet absorption spectrum of the lignin was taken. In each case the absorption at 315 mμ, characteristic of bagasse native lignin, did not change. Since these reaction conditions are conducive to the reduction of side chain ethylenic bonds,[8,9] the absorption above 300 mμ for bagasse native lignin must be due to the presence of a carbonyl group conjugated with a phenoxy ring.

Paper Chromatography of Native and Enzymatically Liberated Lignins.—The radial spread technique[10] was applied to this study. The solvents employed were 95% ethyl alcohol, dioxane, methyl alcohol, *n*-butyl ether, acetone, pyridine, glacial acetic acid, 4% sodium hydroxide solution, acetone and water (1:1), and water. The lignin solvent was dioxane and phloroglucinol-hydrochloric acid served as spotting reagent. In every solvent combination employed, the purple color was developed either at the center or at the periphery of the paper (175 mm. square of Whatman No. 1 filter paper). These preliminary analyses are indicative of the chemical uniformity of each of our lignins.

Ultraviolet Absorption Spectra.—Solutions of samples were prepared for spectroscopic analysis by dissolving 1 to 2 mg. of the sample in 50 ml. of solvent and diluting the solution when necessary. A Beckman quartz spectrophotometer was used for the determination of the absorption curves.

Infrared Absorption Spectra.—A Perkin-Elmer double beam recording infrared spectrophotometer was employed.[11]

Synthesis of Model Compounds.—Acetovanillone was prepared according to an earlier method[12] and acetosyringone as in a previous procedure.[13] Commercial samples (Eastman Kodak Co.) of *p*-hydroxypropiophenone and vanillin were used. The acetates of these compounds were prepared by the usual methods.

Results and Discussion

The results of the chemical comparison of the native lignin and the enzymatically liberated lignin from bagasse are summarized in Table I. These data confirm and supplement our previous findings concerning the identity of these fractions.[2]

TABLE I

CHEMICAL COMPARISON OF THE NATIVE AND ENZYMATICALLY LIBERATED LIGNINS FROM BAGASSE

	Native, %	Enzymatically liberated, %
C	61.5	61.6
H	5.7	5.9
OCH_3	15.3	15.4
OCH_3, acetate	13.3	13.1
OCH_3, phenylhydrazone	14.1	14.0
Oxidation products[a]		
Vanillin	17.8	17.3
Syringaldehyde	13.3	12.9
p-Hydroxybenzaldehyde	9.8	10.2

[a] Values represent the average of three determinations. Percentage recovery using pure aldehydes were: vanillin, 94; syringaldehyde, 96; *p*-hydroxybenzaldehyde, 97.

In 1933 Phillips and Goss[14] oxidized methylated alkali corncob lignin with nitric acid and obtained small amounts of anisic acid as a degradation prod-

(3) F. E. Brauns, THIS JOURNAL, **61**, 2120 (1939).

(4) W. J. Schubert and F. F. Nord, *ibid.*, **72**, 977, 3835 (1950).

(5) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 462.

(6) B. Holmberg, *Arkiv Kemi Mineral. Geol.*, **24**, No. 29 (1947).

(7) J. E. Stone and M. J. Blundell, *Anal. Chem.*, **23**, 771 (1951).

(8) H. Adkins, "Reactions of Hydrogen," University of Wisconsin Press, Madison, Wis., 1937, p. 47.

(9) R. G. D. Moore and H. Hibbert, *Can. J. Research*, **14B**, 404 (1936).

(10) A. Bailey, *Paper Industry and Paper World*, **31**, 205 (1949).

(11) We are under obligation to Miss Cecelia Vitiello of the Schering Corp., Bloomfield, N. J., for obtaining the infrared absorption data and their interpretation.

(12) T. Reichstein, *Helv. Chim. Acta*, **10**, 392 (1927).

(13) F. Mauthner, *J. prakt. Chem.*, [2] **221**, 255 (1929).

(14) M. Phillips and M. J. Goss, THIS JOURNAL, **55**, 3466 (1933).

uct. Ethylated corncob lignin yielded *p*-ethoxybenzoic acid upon oxidation. Several years later *p*-hydroxybenzaldehyde was isolated[15] as one of the degradation products upon oxidizing "Klason" lignin from monocotyledons. Consequently, these authors concluded that the lignin from some angiosperm monocotyledons contained a methoxyl-free aromatic building stone.

In a recent report Stone, *et al.*,[16] maintained that this degradation product could be derived from proteinaceous material present in the lignin preparation isolated with strong chemical reagents. These authors believe that the tyrosine present in the protein is responsible for the formation of the aldehyde.

In the light of the results from our laboratory[17] the preponderance of evidence does favor the identity of the native and enzymatically liberated lignins in woody tissues. Moreover, since native lignin is representative of all the lignin in some woody tissues, *i.e.*, white Scots pine and bagasse, the use of this substance in degradative studies leaves little doubt concerning the origin of the products derived. For this reason, in our oxidation experiments we have used native lignin, which is devoid of nitrogenous material rather than a chemically isolated lignin or the woody tissue itself.[18]

Thus, our results may serve as the first unequivocal evidence that *p*-hydroxybenzaldehyde indeed has its genesis[18a] in the native lignin present in bagasse. The presence of such a non-methoxylated aromatic building stone then gives us a possible explanation for the relatively low methoxyl content of this guaiacyl–syringyl[19] lignin.

The results of the determination of the chemical reactivity of bagasse native lignin are summarized in Table II.

By means of acetylation the presence of three hydroxyl groups was established in this preparation.

Methylation of native lignin with diazomethane yields a product possessing two new methoxyl groups which are not split off on treatment with 72% sulfuric acid.[20] This methylated compound is insoluble in dilute alkali suggesting that one of the groups methylated is of the phenolic type. The acetylation of the diazomethane methylated lignin indicates that the other two hydroxyl groups are alcoholic. Reaction of the native lignin with methanol and hydrogen chloride once again gave a product which contained two additional methoxyl groups. However, the methanol lignin was soluble in dilute alkali and treatment with 72% sulfuric acid resulted in the elimination of the added methoxyl groups. Thus, in accordance with Brauns' formulation[3] the results of the methylations serve to indicate that an enolizable carbonyl group is present in bagasse native lignin, one of the groups reacting with diazomethane being the enol, whereas the two new methoxyl groups introduced with methanol exist as the acetal. On the basis of this interpretation, the solubility of the methylated products in dilute alkali and their behavior toward 72% sulfuric acid is readily understandable.

TABLE II

METHOXYL CONTENT OF BAGASSE NATIVE LIGNIN AND ITS DERIVATIVES

	No. of entering groups	Molecular weight	Methoxyl, % Calcd.	Methoxyl, % Found
Native lignin	..	810[a]	..	15.3
Lignin derivative:				
1 Acetate	3	936	13.2	13.3
2 CH_2N_2 methylated	2	838	22.2	21.9
3 2, phenylhydrazone	0	838		22.0
4 2, acetylated	2	922	20.2	19.5
5 Methanol methylated	2	856[b]	21.7	21.5
6 Fully methylated	4	866	28.8	29.1
7 6, acetylated	~1	908	27.4	27.9
8 5, 72% H_2SO_4 treatment				16.0

[a] Molecular weight calculated on the basis of one carbonyl and four methoxyl groups per lignin unit. [b] The increase of 32 is the result of acetal formation at the carbonyl group whereby CH_3OH (molecular weight 32) must be added.

Repeated methylation of the isolated native lignin with dimethyl sulfate yielded an amorphous, electrostatic powder with a 29.1% methoxyl content. In the infrared spectrum of this sample there was still noticeable a small absorption band at 2.84 μ, the region characteristic for bonded hydroxyl groups (Fig. 1). Thus, it appeared that at least one hydroxyl group could not be methylated. However, treatment of this methylated lignin with acetic anhydride in pyridine gave an acetylated product which did not exhibit an absorption band at 2.84 μ (Fig. 1). Earlier[21] it was suggested that a *t*-hydroxyl group is present in lignin which can be acetylated but not methylated. Although it was expected that three hydroxyl groups would be methylated with dimethyl sulfate, in reality four such groups reacted. One of these was phenolic, another was alcoholic and the remaining two could be formed by the splitting of a cyclic ether ring under the conditions of the experiment. The phenolic, alcoholic and the suggested *t*-hydroxyl groups, however, are those which are acetylated during the treatment of the isolated native lignin with acetic anhydride.

According to earlier investigations[22] the action of thioglycolic acid on lignin is analogous to that of the bisulfites. Moreover, the evidence indicates that this reaction is a relatively mild one. The lignothioglycolic acids of the native and enzymatically liberated lignins from bagasse were prepared and

(15) R. H. J. Creighton and H. Hibbert, THIS JOURNAL, **66**, 37 (1944).

(16) J. E. Stone, M. J. Blundell and K. G. Tanner, *Can. J. Chem.*, **29**, 734 (1951).

(17) F. F. Nord and G. de Stevens, *Trans. N. Y. Acad. Sci.*, [2] **14**, 97 (1951).

(18) More recently B. Leopold (*Acta Chem. Scand.*, **6**, 38, (1952)) in Erdtman's laboratory reported the isolation of *p*-hydroxybenzaldehyde in small yields on oxidizing spruce wood. He was led to believe that lignin was the source of this compound. However, it must be kept in mind that in his case the whole wood with its total protein content and other non-lignin materials were degraded and not the native lignin *per se*. His findings and considerations must be evaluated accordingly.

(18a) F. F. Nord and G. de Stevens, *Naturwissenschaften*, **39**, 479 (1952).

(19) S. F. Kudzin, R. M. DeBaun and F. F. Nord, THIS JOURNAL, **73**, 4615 (1951).

(20) Forest Products Laboratory, Method for Chemical Analysis of Pulps and Pulpwoods, Madison, Wis., 1939.

(21) K. Freudenberg, F. Sohns and A. Janson, *Ann.*, **518**, 62 (1935).

(22) B. Holmberg, *Svensk Papperstidn.*, **39**, Special No. 113 (1936).

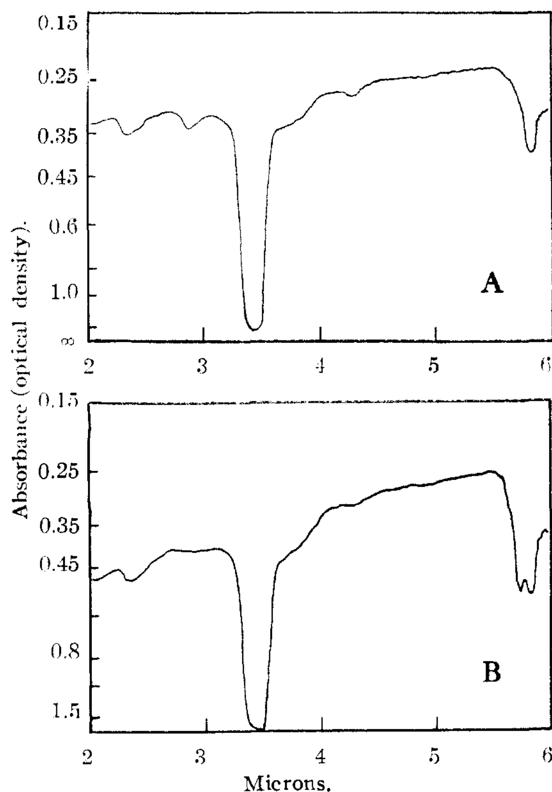


Fig. 1.—A, dimethyl sulfate methylated bagasse native lignin; B, acetate of dimethyl sulfate methylated bagasse native lignin.

found to be analytically identical (Table III) each giving positive phloroglucinol–hydrochloric acid color tests. Their behavior with respect to ultraviolet absorption is also the same (Fig. 2).

Table III

Analytical Data of Bagasse Lignothioglycolic Acids

	Lignin	No. entering groups	Molecular weight	S, % Calcd.	S, % Found	OCH_3, % Calcd.	OCH_3, % Found
1	Native	3	1086	8.86	8.96	11.4	11.6
2	Enzymatically liberated	3	1086	8.86	8.60	11.4	11.4
3	Acetate of 1	2	1172			10.6	10.7
4	Fully methylated native	2	1050			23.6	23.0

The dimethyl sulfate methylated bagasse native lignin was insoluble in dilute alkali. However, when it was heated with thioglycolic acid in 2 N hydrochloric acid, a dithioglycolic acid derivative was obtained. This derivative was now soluble in alkali, indicating the presence of free carboxyl groups.

Interpretation of the Ultraviolet Spectroscopic Data.—It had been reported previously[2a] that bagasse native lignin gives a plateau from 282 to 295 mμ and a maximum absorption peak at 315 mμ. The maximum above 300 mμ was attributed to the presence of a chromophoric group (ethylenic or carbonyl) conjugated with a phenoxy ring.[23,24] Our hydrogenation experiments indicate that the chromophore is a carbonyl group.

Gladding[25] has shown that the ultraviolet absorption spectra of the derivatives of spruce native lignin are very similar to the spectrum of the native lignin. Due to the unusual absorption of bagasse native lignin above 300 mμ it was of interest to determine the ultraviolet spectroscopic behavior of its derivatives in this region.

(23) Edward J. Jones, Jr., *TAPPI*, **32**, 311 (1949).

(24) H. W. Lemon, This Journal, **69**, 2998 (1947).

(25) Ralph E. Gladding, *Paper Trade J.*, **111**, 32 (1940).

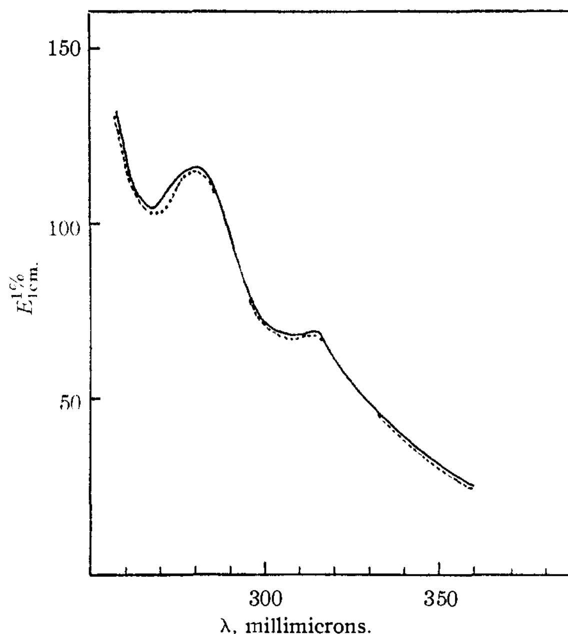


Fig. 2.——, bagasse native lignothioglycolic acid; ......, bagasse enzymatically liberated lignothioglycolic acid.

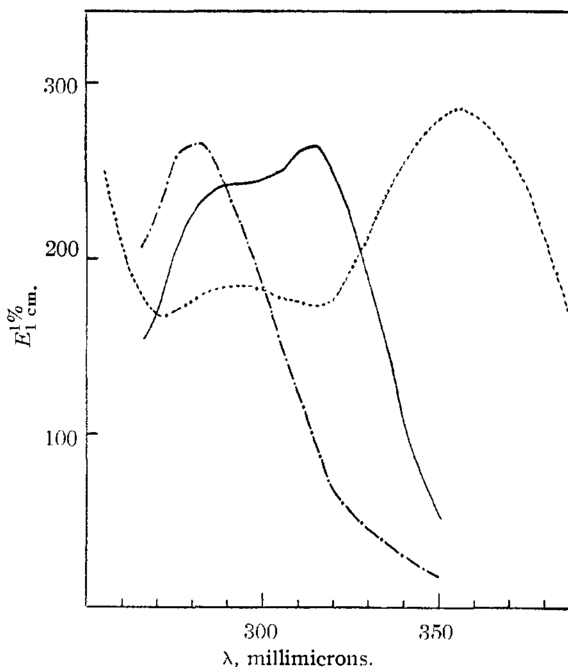
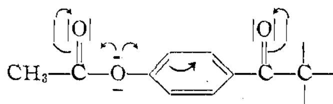


Fig. 3.——, bagasse native lignin in 95% ethyl alcohol; ...., bagasse native lignin in 0.02 N alkali; -.-.-.-, acetate of bagasse native lignin in 95% ethyl alcohol.

In 0.02 N alkali the maximum absorption peak has been shifted to 355 mμ (see Fig. 3). However, the acetate derivative in a neutral solvent gave no absorption at 315 mμ, but only one maximum at 280 mμ. This hypsochromic shift could be due to

either a change in the lignin complex on acetylation or to a crossed conjugation phenomenon



In order to correlate these shifts in absorption with the theory, the spectroscopic behavior of model compounds closely related to the three degradation products of bagasse native lignin, namely, *p*-hydroxybenzaldehyde, vanillin and syringaldehyde was determined.

The absorption spectra of the model compounds and their acetate derivatives were run in neutral and in alkaline solvents. The results of these analyses are listed in Table IV.

TABLE IV

	Compound	Non-alkaline λ_{max}	$E^{1\%}_{1\,cm.}$	λ_{max}	$E^{1\%}_{1\,cm.}$	Alkaline λ_{max}	$E^{1\%}_{1\,cm.}$
1	Native lignin			315	268	355	285
2	Acetate of 1	282	266				
3	*p*-Hydroxypropiophenone	276	945			326	1554
4	*p*-Acetoxypropiophenone	243	747				
5	Vanillin	280	680	310	685	348	1850
6	*p*-Acetoxyvanillin	257	584	305	226		
7	Acetovanillone	275	596	303	515	343	1420
8	*p*-Acetoxyacetovanillone	255	563	303	215		
9	Acetosyringone			302	557	362	1192
10	*p*-Acetoxyacetosyringone	270	344				

In all cases the absorption in alkaline solution is shifted to a longer wave length. This is probably due to the electronic interaction of the sodium salt of the phenol group conjugated with the carbonyl group. The acetates of these compounds like the acetate of native lignin exhibit a hypsochromic shift. This seems to lend support to the crossed conjugation effect of the acetate of bagasse native lignin.

The ultraviolet absorption spectra of the methylated bagasse lignins are presented in Fig. 4. Once again the absorption at 282 mμ has been magnified relative to that in the region above 300 mμ. Furthermore, the completely methylated lignin exhibits an absorption maximum at 310 mμ rather than at 315 mμ. It is significant that the behavior of these lignin derivatives in the region above 300 mμ is qualitatively similar to that found[26] for the 3-4-dimethoxyphenyl-1-propanone compounds.

Skarzynski[27] has shown that flavanones have a characteristic absorption peak at 320 mμ. It has

(26) R. F. Patterson and H. Hibbert, THIS JOURNAL, **65**, 1862 (1943).

(27) B. Skarzynski, *Biochem. Z.*, **301**, 150 (1939).

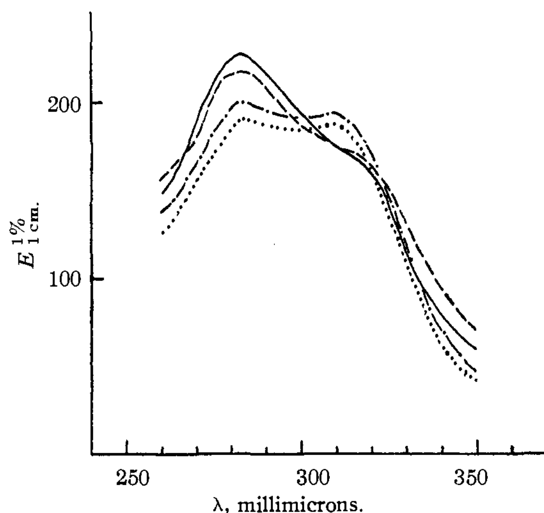
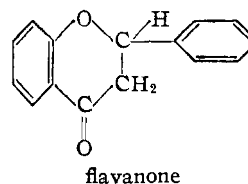


Fig. 4.——, diazomethane methylated bagasse native lignin; - - - -, methanol methylated bagasse native lignin; -.-.-., dimethyl sulfate methylated bagasse native lignin; . . . ., acetate of dimethyl sulfate methylated bagasse native lignin.

also been reported[28] that flavanones containing free phenolic groups have a similar absorption maximum. However, the methylated and acetylated derivatives of hydroxy-substituted flavanones[27] gives a maximum at a lower wave length. The behavior of hydroxy-substituted flavanones in alkaline medium is also similar to the bagasse native lignin. In the light of the work of these authors and our chemical and physical investigations it appears that a flavanone type moiety could reasonably be considered as a part of the structure of bagasse lignin.



flavanone

The increase in the number of hydroxyl groups on methylation with dimethyl sulfate and alkali (splitting of a cyclic ether linkage) lends support to this suggestion.

Acknowledgment.—This work was carried out under the auspices of the Office of Naval Research. The bagasse under study was received through the courtesy of the Godchaux Sugars, Inc., New Orleans, La. The mold culture used was obtained through the courtesy of Dr. W. J. Robbins, Director of the New York Botanical Gardens. The analyses reported were carried out by Mr. A. A. Sirotenko of this Department.

NEW YORK 58, N. Y.

(28) F. Tayeau and J. Masquelier, *Bull. soc. chim. France*, 1172 (1948).