cellulosic incrustation is evidenced by increased yields of this lignin by the action of *Daedalea quercina* on oak and birch. Therefore, further decay of the "guaiacyl lignin"-free woods should liberate the syringyl containing fraction or fractions either as "guaiacyl-syringyl lignin" and/or as "syringyl lignin."

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Investigations on Lignin and Lignification. VIII.[1] Isolation and Characterization of Bagasse Native Lignin

BY GEORGE DE STEVENS AND F. F. NORD

Bagasse native lignin has been isolated and characterized with respect to chemical composition, solubilities in various solvents, color tests, the acetate and phenylhydrazone derivatives and ultraviolet and infrared absorption spectra. Lignin liberated by the action of the cellulolytic mold, *Poria vaillantii*, was also isolated and found to be identical with the native lignin in all respects examined. Enzymatic decay increased the yield of native lignin by eight times. Ultraviolet spectroscopic studies reveal that bagasse native lignin probably contains a carbonyl or ethylenic double bond conjugated with a dioxy phenyl ring. The oxidation of this lignin yielded vanillin and syringaldehyde in a ratio of 1:0.75. Bagasse native lignin is classified as a guaiacyl-syringyl lignin.

Wood-destroying molds are often classified as either "brown rot" or "white rot" fungi, the former assimilating mainly the carbohydrate portion of the wood, while the latter utilize lignin as their substrate. Consequently, a degradation of cellulose by "brown rot" molds should liberate the incrusted lignin, thus making it more accessible to isolation by the use of an inert solvent. That this is actually the case was recently shown when molds of this type grew on white Scots pine. The native lignins isolated before and after enzymatic attack were found to be identical.[2]

Previous investigations on lignin from bagasse, the supporting fiber of the annual plant *Saccharum officinarum*, employed specimens which had been extracted with solvents containing either alkali[3] or dilute nitric acid.[4] In consequence, studies on such specimens would be only of limited value in determining the chemical nature of the lignin as it exists *in situ*. The present report characterizes bagasse "native lignin" and the additional lignin liberated from the incrustants by the action of the "brown rot" fungus *Poria vaillantii*. Since the lignins were extracted with neutral ethanol at room temperature, they probably suffered no chemical change during isolation. Nord and co-workers[5a,b] gave references to similar isolations of lignin fractions from various woods and discussed the significance of certain intermediates in the mechanism of lignification.

Experimental

Isolation of Native Lignin.—The lignin employed for comparative purposes was obtained essentially by the method of Brauns.[6] Air-dry virgin bagasse, ground to 40 mesh, was extracted thoroughly with cold water and with ether. It was then extracted at room temperature in a percolator-type extractor[5b] with 95% ethyl alcohol until the extract no longer gave the phloroglucinol–hydrochloric acid color reaction. Upon removal of the alcohol by distillation at reduced pressure, a resinous material remained. This was washed well with cold water and with ether. The resulting powder was dried, dissolved in dioxane, centrifuged, filtered and precipitated into thirty times its volume of ice-cold distilled water. The precipitate was dried, redissolved in dioxane and reprecipitated into thirty times its volume of ether. This procedure was repeated until a constant methoxyl value was obtained. The lignin so isolated and purified was a light tan colored, electrostatic powder. The yield amounted to 0.4% on a moisture-free basis.

The acetate and phenylhydrazone derivatives were also prepared.[6]

Sterilization and Inoculation of Bagasse Samples.—Ten-gram samples of ground bagasse were weighed into five Fernbach-type culture flasks, and to each was added 30 ml. of the following nutrient medium

Neopeptone	1.0 g.
KH_2PO_4	1.5 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.
Thiamine hydrochloride	2.0 mg.
Tap water to	1 liter

The flasks were plugged with cotton and sterilized by Tyndallization. After cooling, each flask was inoculated with a 5-ml. spore-mycelial suspension of *Poria vaillantii*[7] which had been previously sown on a medium containing the above nutrients plus glucose and agar. This organism was used because preliminary experiments indicated that it assimilated the carbohydrates in bagasse at a faster rate than some other wood-destroying molds.

The inoculated flasks were incubated in the dark at 27–28° for eight months. The decayed bagasse was analyzed periodically for its relative cellulose and lignin content.

Analytical Methods.—After separation of the fungal mycelia from the decayed bagasse, the latter was collected and dried. Cellulose was determined according to an earlier method[8] and lignin by the standard method.[9]

Isolation of Enzymatically Liberated Bagasse Lignin.—The enzymatically liberated lignin was extracted from the decayed bagasse in the same manner as indicated above under "Isolation of Native Lignin."

(1) Presented at the Lignin Round Table held during the XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., 1951.

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(3) Y. Hachihama and H. Saegusa, *J. Soc. Chem. Ind. Japan*, **37**, suppl. binding 771–2 (1934).

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(5) (a) S. F. Kudzin and F. F. Nord, *ibid.*, **73**, 680 (1951); (b) F. F. Nord and W. J. Schubert, *Holzforschung*, **5**, 1 (1951); (c) S. F. Kudzin, R. M. DeBaum and F. F. Nord, THIS JOURNAL, **73**, 4615 (1951).

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Isolation of Chemically Prepared Bagasse Lignins.—Extractive-free bagasse was used in all cases; *i.e.*, the ground material was extracted in a soxhlet apparatus first with 1:2 alcohol–benzene solution, and then with water.

Sulfuric acid lignin was isolated by the standard method[9]; the Kalb and Lieser method[10] gave good yields of fuming hydrochloric acid lignin, and alkali lignin was also obtained.[11]

Ultraviolet Absorption Spectra.—Solutions of the lignin samples were prepared for spectroscopic analysis by dissolving 1 to 2 mg. of the sample in 50 ml. of solvent (90 parts of purified dioxane to 10 parts of distilled water). A Beckman quartz spectrophotometer was used for the determination of the absorption curves.

Infrared Absorption Spectra.—For white Scots pine and bagasse native lignins a Baird Double-Beam Recording Infrared Spectrophotometer was used and a Perkin–Elmer Double Beam Recording Infrared Spectrophotometer was employed for the enzymatically liberated bagasse lignin.[12] The samples were mulled in mineral oil.

Results and Discussion

The chemical composition of bagasse native lignin and of bagasse lignins isolated by chemical means is recorded in Table I. In Table II are summarized the results of the periodic analyses of bagasse attacked by the "brown rot" wood-destroying mold, *Poria vaillantii*, and a comparison of the native and the enzymatically liberated bagasse lignin is presented in Table III.

TABLE I

COMPARISON OF BAGASSE NATIVE LIGNIN WITH LIGNINS OBTAINED BY CHEMICAL TREATMENT

Bagassel ignins	C, %	H, %	OCH_3, %
Native (N. L.)	61.5	5.7	15.3
72% H_2SO_4	60.8	5.7	15.0
10% alkali	62.9	5.7	17.0
Fuming HCl	53.4	5.7	8.8
N. L. treated with 72% H_2SO_4	63.6	5.5	15.0

TABLE II

EFFECT OF PORIA VAILLANTII ON THE COMPOSITION OF BAGASSE

Period of decay, months	Crude cellulose, %	Lignin, %
0	69.4	21.4
3	43.2	46.0
6	41.4	49.5
8	40.8	50.4

TABLE III

CHEMICAL COMPOSITION OF LIGNINS FROM SOUND AND DECAYED BAGASSE

	Sound	Decayed[a]
C, %	61.5	61.6
H, %	5.7	5.9
OCH_3, %	15.3	15.4

[a] All values corrected for ash (0.91%).

As stated previously, the yield of bagasse native lignin was 0.4%. After eight months of the cellulolytic action of the mold on bagasse, 3.3% lignin was obtained by alcohol extraction. Thus, the enzymatic decay of the lignin host, cellulose, had increased the extractable native lignin at the time of the analysis by approximately eight times.

Both samples were found to be soluble in methanol, ethanol, dioxane, acetone, dilute sodium hydroxide, pyridine and insoluble in glacial acetic acid, ether, benzene, petroleum ether and water. Each reduced Fehling solution and gave positive color tests with phloroglucinol–hydrochloric acid, aniline, diphenylamine and the phenol reagent. On treatment with 72% sulfuric acid, a lignin was recovered in 91% yield whose chemical composition was similar to the original sample and to the "Klason" lignin isolated from bagasse.

The identity of the lignin from sound and decayed bagasse is further elaborated in the infrared absorption spectra shown in Fig. 1. White Scots pine native lignin is used as a standard.

The band at 3400 $cm.^{-1}$ is due to the presence of bonded hydroxyl groups in the lignin complex. However, the intensity of the absorption band in this region is less pronounced in the case of bagasse native lignin than in the lignin of the standard. This is in accordance with the observed difference in the methoxyl content of the acetates of the two samples (see Table IV). Absorption from 1700 to 1670 $cm.^{-1}$ is characteristic for the presence of an aldehyde or ketone carbonyl grouping. Phenyl ring skeletal vibrations with possible para substitution are evidenced at 1600 and 1512 $cm.^{-1}$. The 1430 $cm.^{-1}$ band establishes the presence of aliphatic groupings in the molecule. Since absorption below 1400 $cm.^{-1}$ is attributed to the vibration of the molecule as a whole, specific interpretation of bands arising in this spectral region is limited. However, the bands at 1325 and 1170 $cm.^{-1}$, absent in the spectrum of the softwood native lignin, are indicative of a difference in the molecular structure of the two lignins. Absorptions at 1269 and 1224 $cm.^{-1}$ are in the spectral region of aromatic or unsaturated C–O groupings. The bands at 2945, 1460, 1380 and 725 $cm.^{-1}$ are due to mineral oil absorption and thus are of no interpretative value. Finally, absorption at 835 $cm.^{-1}$ can be assigned to the presence of a trisubstituted phenyl ring. Therefore, a comparative evaluation of the absorption spectra of the white Scots pine and bagasse lignins reveals that there is a general similarity between the two, but noticeable differences are observed in the relative intensities of the bands, suggesting some difference in the arrangement of the groups present therein.

Ultraviolet spectroscopic studies demonstrated firstly, that native and enzymatically liberated bagasse lignins are identical, and secondly, that it has a molecular constitution somewhat different from other lignins heretofore studied. The absorption curves are shown in Fig. 2.

Unlike other lignins,[2,5a,13] our preparation gives a plateau from 283 to 295 mμ and maximum absorption at 315 mμ. It is well known that absorption within the region 250 to 290 mμ is due to the electronic oscillations of the oxygen-substituted phenyl ring in lignin. Several investigators,[14,15,16] working with aromatic compounds which could be related

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(11) W. J. Powell and H. Whittaker, *J. Chem. Soc.*, **127**, 132 (1925).

(12) We wish to thank Drs. Carl C. Clark and James D. Hardy of the Department of Physiology, Cornell University Medical School, and Miss C. Vitiello of the Schering Corporation, Bloomfield, N. J., for the facilities placed at our disposal and their courteous coöperation.

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

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(15) R. A. Morton and A. L. Stubbs, *J. Chem. Soc.*, 1947 (1940).

(16) H. W. Lemon, THIS JOURNAL, **69**, 2998 (1947).

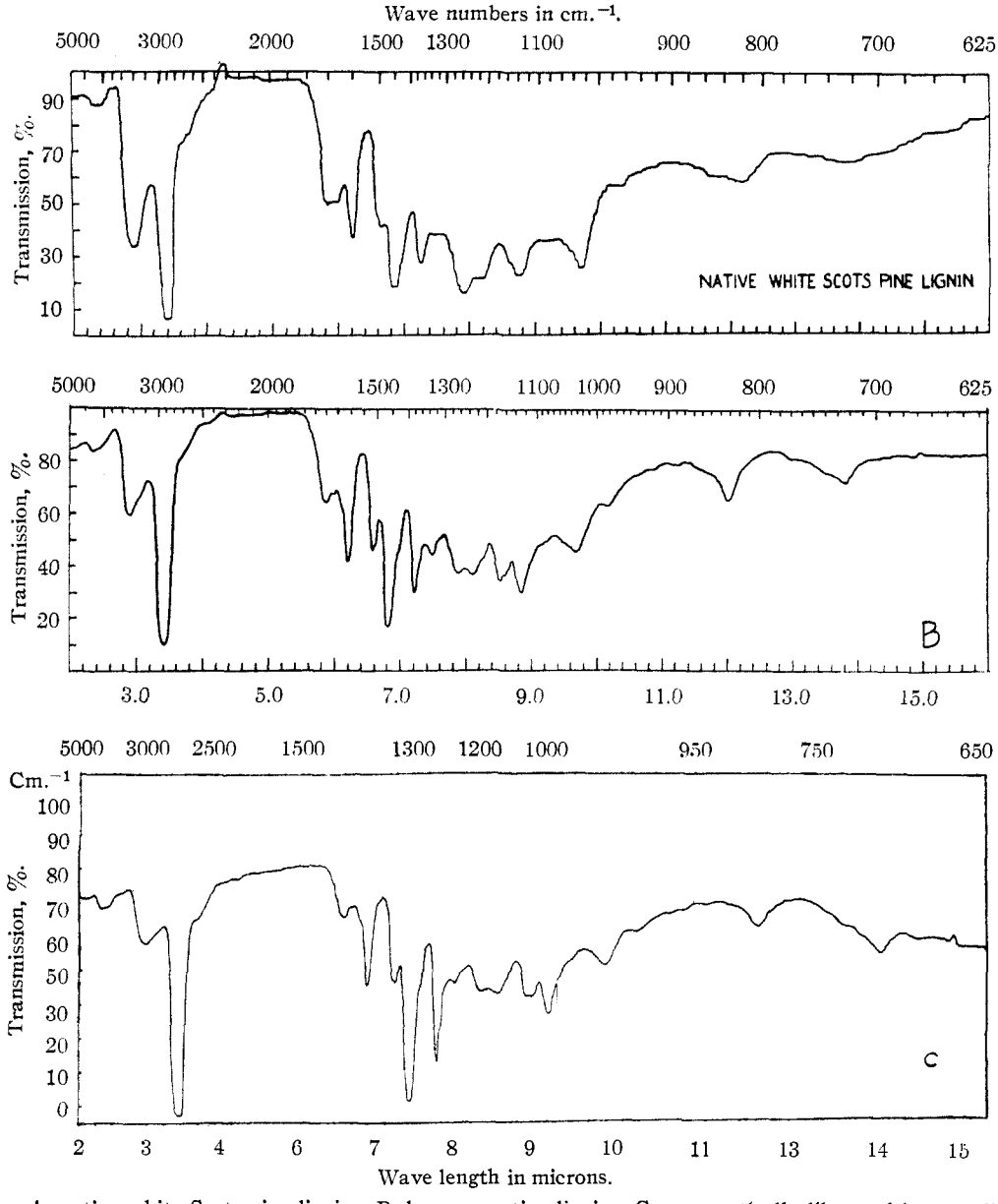
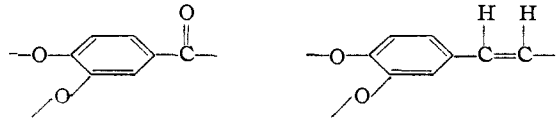


Fig. 1.—A, native white Scots pine lignin; B, bagasse native lignin; C, enzymatically liberated bagasse lignin.

to lignin, have shown that absorption in the spectral region 300 to 350 mμ can be assigned to the influence of a carbonyl or ethylenic double bond in conjugation with a phenoxy ring. In a recent report[17] the ultraviolet absorption spectra of the ethyl esters and parent aldehydes of various ethers of 3,4-dihydroxycinnamic acid were presented. Their spectra are very similar to the one given by our lignin sample. Therefore, it can, at least, be assumed that bagasse native lignin contains one or both of the following building stones



This further corroborates findings[18] concerning the presence of a double bond conjugated with the phenyl ring in the lignin polymer.

However, the above considerations did not necessarily exclude the possibility of the presence of a syringyl type nucleus in our lignin sample. Since bagasse native lignin gave a cerise color when treated according to the Mäule color reaction,[19] qualitative evidence for its presence[20] was established. In order to ascertain quantitatively the extent to which the syringyl group was present, a micro-analytical method[21] was employed. In Table IV are recorded the results of this analysis along with a general comparison of the chemical

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(18) Ralph E. Glading, *Paper Trade J.*, [23] **111**, 32 (1940).

(19) C. Mäule, *Beiträge wiss. Bot.*, **4**, 166 (1900).

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composition of the native lignins of bagasse, white Scots pine and maple.

TABLE IV

COMPARISON OF THE NATIVE LIGNINS FROM BAGASSE, WHITE SCOTS PINE AND MAPLE

	Bagasse	Native lignins White Scots pine	Maple
C, %	61.5	64.0	61.0
H, %	5.7	6.3	5.6
OCH_3, %	15.3	14.5	17.4
OCH_3, % of acetate	13.3	10.1	12.8
OCH_3, % of phenylhydrazone	14.1	13.3	15.5
Oxidation products:			
Vanillin, %	17.8	19.5	17.2
Syringaldehyde, %	13.3	nil	4.5

Two possible considerations can be advanced from the above data: (1) Bagasse native lignin is a "guaiacyl-syringyl" lignin,[5c] the two building stones accounting for 31.1% of the polymer and only 8.2% of the total methoxyl content. Determination of the remaining 7.1% of the methoxyl group and isolation of other oxidation products will be of great importance for the elucidation of its structure. (2) Besides the "guaiacyl-syringyl" lignin, the presence of a "syringyl" lignin is also possible.[4c] Such fractionation studies are now in progress.

Acknowledgments.—The bagasse used in these experiments was obtained through the courtesy of the Godchaux Sugars, Inc., New Orleans, La. This work was carried out under the auspices of the Office of Naval Research.

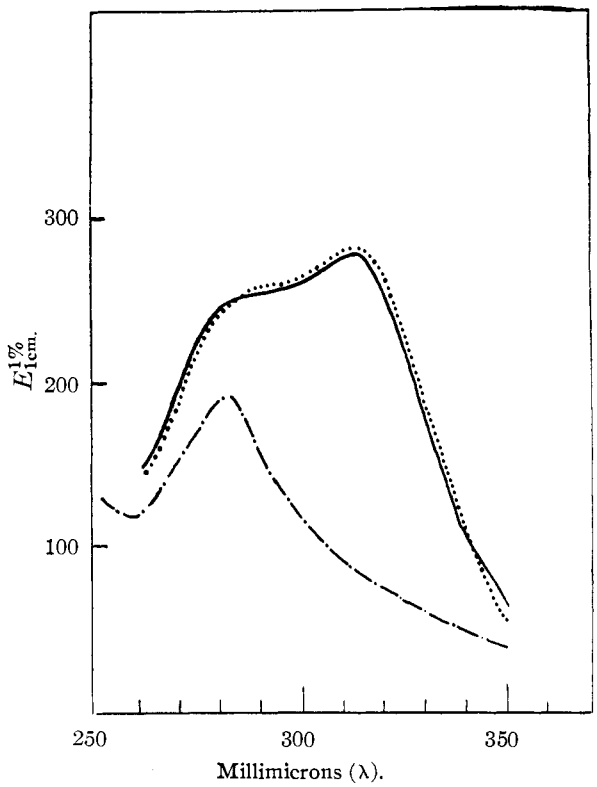

Fig. 2.—....., bagasse native lignin; ——, enzymatically liberated bagasse lignin; —·—·—, white Scots pine native lignin.

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The Synthesis of Certain Beta-Diketones Containing Perfluoromethyl and Perfluoro-*n*-propyl Groups

BY LLOYD B. BARKLEY AND ROBERT LEVINE

A number of methyl ketones have been acylated with ethyl trifluoroacetate and ethyl perfluoro-*n*-butyrate in the presence of sodium methoxide to give the corresponding beta-diketones in high yields.

While considerable work has been done on the synthesis of β-diketones of the general formula $RCOCH_2COR'$, for the most part the radicals R and R′ have been aliphatic and aromatic moieties. In this Laboratory, we have prepared a variety of β-diketones in which at least one of these radicals is a heterocyclic nucleus.[1,2,3] Recently, Reid and Calvin,[4] have reported the synthesis of a number of acyltrifluoroacetones by the reaction of ketones with ethyl trifluoroacetate in the presence of sodium methoxide. With the exception of two compounds, 2-thenoyl and 2-furoyltrifluoroacetone, the β-diketones prepared by these workers[4] contained aliphatic and aromatic acyl groups.

The present paper reports our results on the synthesis of a number of β-diketones in which R is a thiophene (or substituted thiophene), a furan (or substituted furan), a thianaphthene, or benzofuran radical and R′ is the perfluoromethyl or perfluoro-*n*-propyl group. Mesityl oxide has also been converted to the corresponding 1,3-dicarbonyl compounds.

The heterocyclic β-diketones were prepared by the acylation of the appropriate methyl ketones with ethyl trifluoroacetate or heptafluoro-*n*-butyrate in the presence of commercial sodium methoxide. The required heterocyclic ketones were prepared by the methods developed in this Laboratory.[5,6,7,8] The following equation, in which *n* is 1 or 3, indicates the over-all reaction involved in the synthesis of these compounds.

$$RCOCH_3 + C_nF_{2n+1}CO_2C_2H_5 \xrightarrow{NaOCH_3} RCOCH_2COC_nF_{2n+1} + C_2H_5OH$$

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