are in agreement with the observed data reported by Rietz.[4]

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Investigations on Lignin and Lignification. V. Lignin of Cork[1]

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Two procedures have been successfully applied to the isolation of authentic native lignin preparations from wood.[2,3a,b,4] Although several lignins have been isolated by chemical means from cork and bark,[5–11] no native product has been obtained from these sources. We wish to report on the isolation and fractionation of the native lignin of cork.

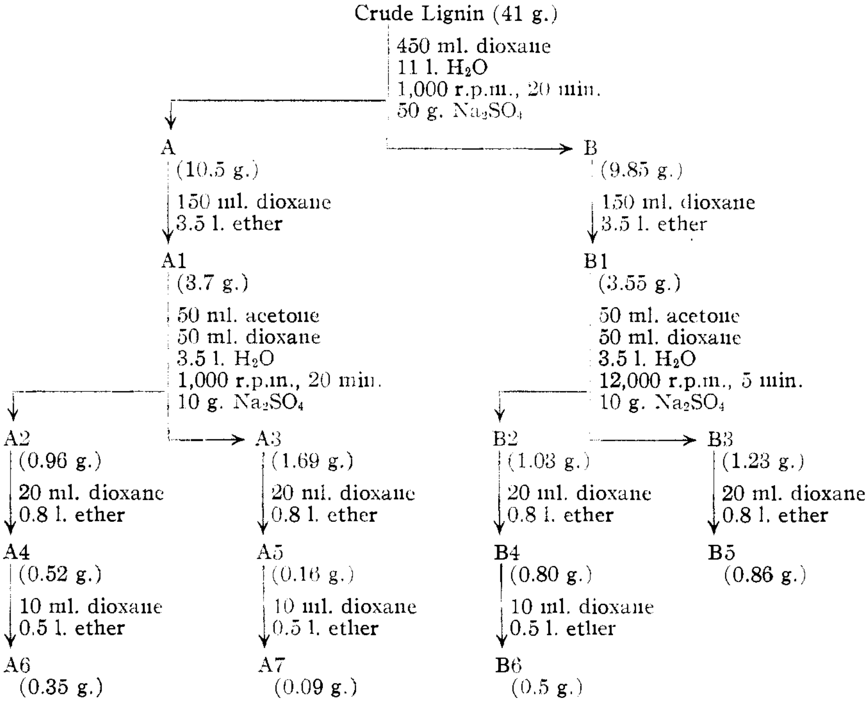

Fig. 1.—Fractionation of the cork native lignin.

This material was fractionated according to the scheme summarized in Fig. 1, and the various fractions were studied with regard to their composition, ultraviolet and infrared absorption spectra, their reaction with the "phenol" reagent,[12,13] and with the phloroglucinol-HCl test.[14,15,16]

An alkali lignin of cork was also prepared.

The dissimilarity of the composition of our native lignin from the alkali lignin, and from the other cork lignins[10,11] suggested to us that our product might be admixed with phenolic extractive materials such as tannin and/or phlobaphene. This supposition was verified by paper chromatography.

Experimental

Native Lignin.—One and one-half kilograms of "männlicher Kork",[10] the bark of *Quercus suber*, ground to 40–60 mesh, was extracted with alcohol in a percolator-type extractor[17] until the extract no longer gave the phloroglucinol-HCl test. The solution was evaporated to dryness *in vacuo* at 40°. The residue (120 g.) was washed with ether several times, and again with water by centrifugation and resuspension with fresh solvent until the supernatant became opalescent. The residual material (41 g.) was then treated as outlined in Fig. 1. Solutions in purified dioxane[18] were added to the ice-cold non-solvents, slowly with stirring. Anhydrous sodium sulfate was used as the salting agent throughout.

Alkali Lignin.—Ether- and then methanol-preextracted cork was autoclaved for 8 hours at 23 pounds pressure in the presence of ten times its amount of 3% sodium hydroxide. The filtered hot mixture was acidified with hydrochloric acid, and the resulting precipitate was redissolved in base and reprecipitated with acid. It was then reprecipitated from acetone solution with ether until a constant methoxyl value (12.40%) was obtained.

Spectra.—The ultraviolet absorption spectra were obtained with a Beckman model DU quartz ultraviolet spectrophotometer, absolute ethanol being used as the solvent. The infrared absorption spectra were determined in an experimental model of the Baird double beam recording spectrophotometer. The wave length accuracy of the instrument was 0.04 μ. The samples were run as mulls in mineral oil.

Colorimetry.—The colorimetric readings were taken in an Evelyn Photoelectric Colorimeter. A 550 mμ filter was used in the phloroglucinol-HCl test. Isolated native white Scots pine lignin was used as the standard, and the reaction is accurate to $\pm 3\ \gamma$ in the range 20–120 γ/ml. The production of color is reported to be due to the presence of coniferyl aldehyde groups.[16,19a,b,c] The cork native lignin on contact with phloroglucinol and HCl gives a color similar in spectrum to that obtained with the softwood lignin. The results of the phloroglucinol-HCl test are expressed as per cent. of lignin, the white Scots pine native lignin being taken as 100%. A 600 mμ filter was used for the study with the Mehta reaction. The cork alkali lignin was applied as the standard, and the reaction could be conducted with an accuracy of $\pm 5\ \gamma$ in the range 50–200 γ/ml. Addition of 15% Na_2CO_3 is necessary for full development of the color.

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The results with the phenol reagent are expressed in terms of optical density after 3 minutes × 1,000 divided by the γ of lignin taken.

Paper Chromatography.—Fractions A6, A7 and B6 were chromatographed on Schleicher and Schuell Paper No. 696, with water as the developing solvent. If the paper were sprayed with phloroglucinol–HCl, pink spots were obtained on the original base line. If an identical strip were sprayed with phenol reagent, and then with 15% Na_2CO_3, blue spots appeared, both on the original base line and at the liquid front. The intensities of the blue spots were of the same order of magnitude.

TABLE I

COMPOSITION OF CORK LIGNIN FRACTIONS

Fraction	C, %	H, %	OMe, %	Lignin, %	Phenol reagent
A1	58.86	5.66	5.15	15, 17.5	33.8, 34.1
A2[a]	59.6	5.14	3.7	21	38.5
A3	58.62	4.94	3.5	12	18.7
A4[b]	59.8	5.8	4.8	14	58.6
A5	58.61	5.86	5.4	12	24.2
A6	59.2	5.5	4.6	..	..
A7[c]	57.2	5.7	5.1	..	..
B1[d]	59.13	5.1	5.34	13	24.8
B2	58.77	4.93	4.26	..	..
B3[e]	58.3	5.2	4.21	6	34.4
B4[f]	58.18	5.29	4.75	14	12.1
B5[g]	...	..	4.68	..	..
Alkali lignin	...	..	12.40	..	58.6, 59.5

Corrected for: [a] 1.5% ash; [b] 2.3% ash; [c] 1.0% ash; [d] 1.8% ash; [e] 11.97% ash; [f] 2.1% ash; [g] 10.22% ash.

Discussion

Our lignin appears to be contaminated with other extractable material such as tannin and/or phlobaphene. This is shown by its low methoxyl content. Although low methoxyl lignins have been obtained from barks,[5,6] it is not certain whether they were free of non-lignin matter. Also, phlobaphene has been obtained, admixed with native lignin,[20,21] and lignin of redwood bark was reported[22] to be contaminated with low methoxyl containing components.

While it appears that our fractions possess varying total composition, as evidenced by their spectra (Figs. 2, 3), the fact that the lignin content does not rise upon successive reprecipitation indicates that a pure lignin cannot be obtained from this mixture by the Brauns method for isolating and purifying native lignin.

Although the ultraviolet spectra are generally similar, the infrared spectra differ, particularly in the regions of 865 and 722 cm.$^{-1}$. Their incomplete resolution, as compared to those spectra of wood lignins previously obtained,[3b,4,23] suggest the complexity and inhomogeneity of our lignins. The complexity of bark over wood[24] is thus borne out by these experiments.

Acknowledgments.—We wish to thank the Armstrong Cork Company, Lancaster, Pa., for generous samples of virgin and refugo cork and Drs. J. D. Hardy and Carl C. Clark of Cornell University Medical College for obtaining the infrared spectra for us. The microanalyses were done by Mr. A. A. Sirotenko of this department. This work was carried out under the auspices of the Office of Naval Research.

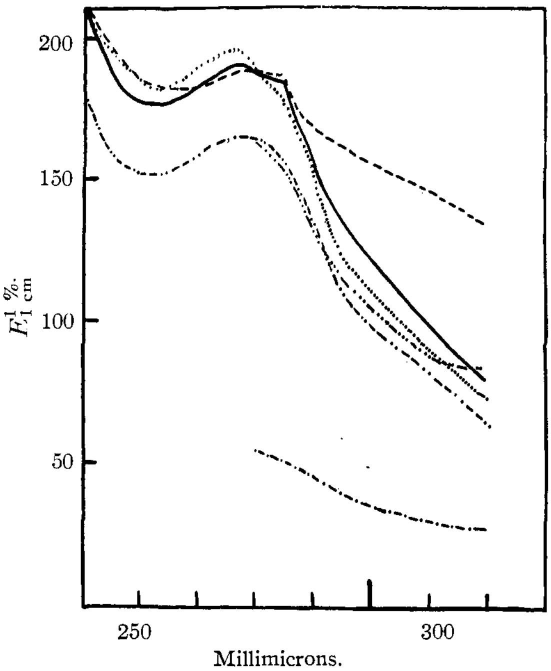

Fig. 2.—Ultraviolet spectra of cork lignin fractions: native cork lignin A6, ————————; A7, ...; B6, —··—··—··—··; A1, —·—·—·—·—·—·—; B1, —···—···—···; cork alkali lignin, - - - - - - - - - -.

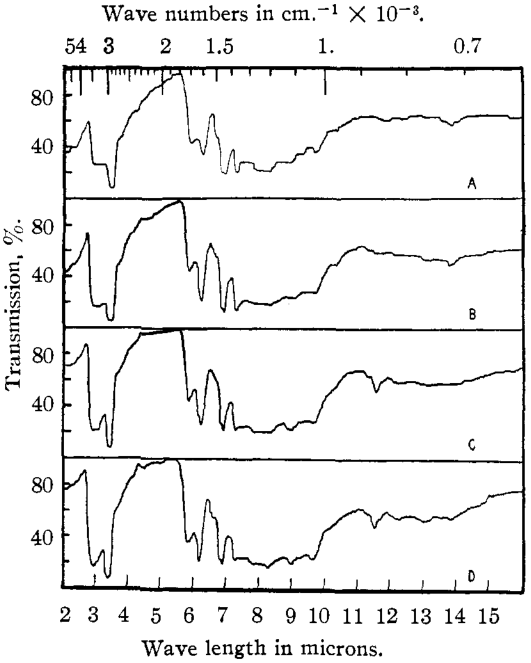

Fig. 3.—Infrared spectra of cork lignin fractions: Cork alkali lignin, A; cork native lignin: A6, B; A7, C; B6, D.

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