The neo zones C + D were cut out jointly, and their separation was achieved by developing with a 3:1 benzene–hexane mixture on alumina (the original sequence was then inverted).

The photochemically established ratios appear in Table IV.

TABLE IV

COMPOSITION OF STEREOISOMERIC MIXTURES OBTAINED FROM DEHYDRO-β-CAROTENES BY IODINE CATALYSIS, IN LIGHT (% OF STARTING MATERIAL)

	Starting material		
	All-*trans*	Neo-A	Neo-D
All-*trans*	17	18	17
Neo-A	28	30	30
Neo-B	4	5	3
Neo-C	10	11	10
Neo-D	12	15	15
Neo-E	4	6	5
Neo-F	6	6	3
Neo-G	3	2	1
Neo-H	1	1	1
Unaccounted for	15	7	16

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES, JOSEPH E. SEAGRAM AND SONS, INC.]

The Chromatographic Separation of Hardwood Extractive Components Giving Color Reactions with Phloroglucinol

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By application of paper chromatography, four aromatic aldehydes giving phloroglucinol color reactions have been isolated in the ethanol extracts of oak hardwood. They have been identified as vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde. In general, both coniferaldehyde and sinapaldehyde were found to exist in other hardwoods examined, while only coniferaldehyde was found in soft woods. The lignin color reaction with phloroglucinol may thus be associated with sinapaldehyde as well as coniferaldehyde.

Introduction

Plant physiologists and botanists have long known that lignified membranes can be identified by the characteristic colors which are formed with certain organic reagents. As early as 1834, Runge[1] showed that a blue color is formed when sprucewood is treated with phenol in the presence of hydrochloric acid. Since that time, a number of organic and inorganic reagents have been shown to undergo color reactions with wood, and many investigators have sought an explanation for the color formation. In 1913, Czapek[2] extracted a fraction from sprucewood—hadromal—which he thought to be the color-forming substance in wood. The composition of this extract was not determined until Adler and Ellmer[3] isolated both vanillin and coniferaldehyde from a (spruce) hadromal preparation. It now seems evident that the characteristic color tests for lignin are dependent upon the presence of coniferaldehyde groups.[4,5] An important reaction of wood is the formation of a magenta color with phloroglucinol in the presence of hydrochloric acid. At the present time, coniferaldehyde and its methyl ether[6] are the only pure compounds which have been shown to give colors with phloroglucinol comparable to those obtained with wood and certain isolated lignin preparations.

Klason[7] and Freudenberg[8] found evidence of the presence of low molecular weight lignin in the extractives of wood; however, owing to the small quantity present an investigation of the identity of this lignin-like material was not carried out. By the application of paper chromatography it was possible to study the trace lignin-like substances in hardwood extractives. In addition to vanillin and syringaldehyde—the usual oxidation products of hardwood—two other aldehydes which give purple colorations with phloroglucinol were shown to be present. The magenta color formation of one spot suggested the presence of coniferaldehyde as one aldehyde. The presence of the sinapaldehyde grouping may be suspected also from the known chemistry of hardwood. In addition, a sinapaldehyde grouping is indicated from work of Pew.[9] In this work a part of the resorcinol color with spruce and aspen wood corresponds to the condensation product of resorcinol with coniferaldehyde substituted in the 5-position with a methoxy or propenyl group.

Experimental

Preparation of Wood Extracts.—(a) White oak chips were ground into a coarse meal. A 500-g. portion of the ground wood was covered with 95% ethyl alcohol in a glass-stoppered flask, and the mixture was allowed to stand for two days. The solvent was decanted and the process was repeated similarly three times. The ethyl alcohol extract was concentrated to 30 ml. by distillation at 30° under reduced pressure in a stream of nitrogen. The concentrate was extracted repeatedly with ethyl ether, and the ether extract was taken nearly to dryness *in vacuo*. The residue

(1) F. F. Runge, *J. prakt. Chem.*, **1**, 24 (1834).

(2) F. Czapek, "Biochemie der Pflanzen," Vol. I, Jena, 1913, p. 689.

(3) E. Adler and L. Ellmer, *Acta Chem. Scand.*, **2**, 839 (1948).

(4) For a discussion and bibliography of the relation of coniferaldehyde to the lignin color reaction, *cf.* F. E. Brauns, "The Chemistry of Lignin," Academic Press., Inc., New York, N. Y., 1952, chapter 4.

(5) Also *cf.* E. Hagglund, "Chemistry of Wood," Academic Press., Inc., New York, N. Y., 1951, chapter 4.

(6) E. Adler, K. J. Bjorkqvist and S. Häggroth, *Acta Chem. Scand.*, **2**, 93 (1948).

(7) P. Klason, "Beitrage zur Kenntnis der Chemischen Zusammensetzung des Fichtenholzes," Berlin, 1911, p. 34.

(8) K. Freudenberg, A. Janson, E. Knopf and A. Hagg, *Ber.*, **69**, 1415 (1936).

(9) J. C. Pew, THIS JOURNAL, **73**, 1678 (1951).

was extracted repeatedly with benzene, and the benzene was concentrated to 30 ml. *in vacuo* for application in paper chromatography.

(b) Charred white oak was extracted with 55% ethyl alcohol for 48 months. Ten liters of the extract was concentrated *in vacuo* under nitrogen to one liter. The aqueous concentrate was extracted repeatedly with benzene, and the benzene was concentrated to one liter. The resulting concentrate was extracted repeatedly with water, which in turn was concentrated to one liter. The water was extracted three times with 250-ml. portions of ethyl ether; the ether was removed by evaporation in a stream of nitrogen, leaving a red-brown oil with a sweet maple-like odor. The oil was dissolved in a few ml. of ethyl alcohol.

(c) Samples of different woods were obtained from various lumber and cabinet wood suppliers and the woods identified only by their trade designations. Each wood was ground into a coarse meal. A 100-g. portion of each ground wood was extracted in a Soxhlet apparatus with 500 ml. of 95% ethyl alcohol for five hours. The extracts were evaporated nearly to dryness *in vacuo*. Each residue was subjected to solvent partition as previously described for charred oak extract. The ether residues were dissolved in a few ml. of ethyl alcohol for application in paper chromatography.

Chromatographic Procedure.—The chromatographic method of Stone and Blundell[10] was adapted to the separation of the aldehydes in the wood extractives. The solvent for the upper phase was modified from a 6:1:1 mixture of ligroin, *n*-butyl ether, and water to a 6:2:2 mixture. The ligroin,[11] as received, was purified by distillation to remove an ultraviolet absorbing impurity. The fraction boiling at 100–106° was used.

Whatman No. 1 filter paper was used for the descending chromatographs. For qualitative examinations, 7 in. by 18 in. sheets were run in a 10 in. glass cylinder. For the separation of larger quantities, 16 in. by 22 in. sheets were employed in a Chromatocab.[12] Equilibration of the sheets was accomplished by placing both the aqueous and organic phases in the base of the containers. The best results were obtained by overnight equilibration of the samples. After equilibration the solvent was added to the empty troughs and the chromatographs were allowed to run for six hours.

After drying the sheets the spots were located by ultraviolet light and the following indicator sprays: (a) 2,4-dinitrophenylhydrazine, 0.1% in 2 *N* HCl; (b) Folin–Denis reagent[13] and (c) phloroglucinol,[13] 2.5% in 3 *N* HCl.

Preliminary chromatographs were performed comparing the oak extracts and known mixtures of vanillin, syringaldehyde and *p*-hydroxybenzaldehyde. The oak extracts were found to separate into one indistinct and four distinct spots with R_f values of 0.02, 0.07, 0.15, 0.26 and 0.42 (designate spots 1, 2, 3, 4 and 5, respectively). Spots 5 and 3 matched the R_f values for known vanillin and syringaldehyde. Spots 2 and 4 showed a blue-green and yellow-green fluorescence, respectively; and spot 1 developed a blue-white fluorescence under ultraviolet light. With the phloroglucinol spray vanillin and syringaldehyde gave peach-colored spots, spots 2 and 4 gave purple colors characteristic of the lignin test, and spot 1 formed an orange color.

Since the formation of the purple colorations with phloroglucinol suggested the presence of coniferaldehyde and sinapaldehyde, each of these compounds was synthesized. With paper chromatography the synthetic coniferaldehyde gave the same R_f value as spot 4 and developed an identical red-purple color with phloroglucinol. The synthetic sinapaldehyde matched spot 2 in R_f value and formed the same blue-purple color with phloroglucinol.

Identification of Vanillin, Syringaldehyde, Coniferaldehyde and Sinapaldehyde.—As paper chromatography showed no difference in the composition of the two oak extracts, and a large quantity of the char extract was available, it was employed to recover the aldehydes for identification. The char extract was applied in a continuous line across a number of large paper sheets and chromatographed for six hours. After development and drying, the individual zones were located by spraying a narrow vertical strip, cut from the paper sheet, with dinitrophenylhydrazine. Solutions of each aldehyde were prepared by extracting the paper strips with ethyl alcohol. Vanillin and syringaldehyde were eluted in a Soxhlet apparatus, and coniferaldehyde and sinapaldehyde were eluted by cold washing. The ultraviolet and visible spectra of the extract aldehydes matched the values obtained on known vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde. The spectral data are summarized in Table I. The spectra were determined in 95% ethyl alcohol in 1-cm. cells on a Beckman DU spectrophotometer. Alkaline curves were obtained by the addition of 0.5 ml. of 10% aqueous $(CH_3)_4NOH$ per 2.5 ml. of sample.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF NEUTRAL AND ALKALINE VANILLIN, SYRINGALDEHYDE, CONIFERALDEHYDE AND SINAPALDEHYDE

	Neutral			
	Max. (1), mμ	(2), mμ	(3), mμ	Alkaline Max.
Syringaldehyde	304.5	229[a]		
	308.0	[b]		370
	307	230.5		
	307	(oak)		371
Vanillin	308	278	232[a]	
	310	275	[b]	353
	308.5	278.5	231.5	
	308	279	229 (oak)	353.5
Coniferaldehyde	342	242		421
	341	(oak)		421
Sinapaldehyde	347	245.5		443
	345	(oak)		443

[a] R. F. Patterson and H. Hibbert, THIS JOURNAL, 65, 1862 (1943). [b] H. W. Lemon, *ibid.*, 69, 2998 (1947).

The syringaldehyde fraction was evaporated to dryness and when recrystallized from benzene plus heptane melted at 107–108.5°. A mixed melting point with an authentic sample (m.p. 111–112°) showed no depression.

The vanillin fraction was converted to the 2,4-dinitrophenylhydrazone which when recrystallized from dioxane plus petroleum ether melted at 264–265° dec. A synthetic sample of the derivative melted at 265–266° dec., lit. 271°[14] dec. The spectra of the DNPH's[15] in neutral and alkaline ethyl alcohol were similar as shown in Table II.

TABLE II

ULTRAVIOLET AND VISIBLE ABSORPTION SPECTRA OF DNPH'S OF VANILLIN, CONIFERALDEHYDE AND SINAPALDEHYDE

	Neutral Max., mμ	Alkaline Max., mμ
	Oak	
Vanillin	397	493
Coniferaldehyde	408.5	510
Sinapaldehyde	410	515
	Synthetic	
Vanillin	398	493
Coniferaldehyde	408.5[a]	510
Sinapaldehyde	408.5[b]	515

[a] M.p. 266° dec., lit. 265–266° (4). [b] M.p. 273° dec.

The coniferaldehyde and sinapaldehyde also were converted to their DNPH's and purified by chromatography on silicic acid–Celite[16] columns. The coniferaldehyde DNPH was eluted with benzene, and the sinapaldehyde DNPH was eluted with 0.05% ethyl alcohol in benzene.

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

(11) Eastman Kodak Co., pract., boiling range 90–120°.

(12) Mfd. by Chromatography Division, University Apparatus Co., Berkeley, Calif.

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

(14) N. R. Campbell, *Analyst*, **61**, 392 (1936).

(15) Hereafter in this paper 2,4-dinitrophenylhydrazone will be abbreviated DNPH.

(16) A. A. Rosen, K. Y. V. Sundstrom and W. F. Vogel, *Anal. Chem.*, **24**, 412 (1952).

Mixed chromatographs of the eluted and known DNPH's gave no separation. The spectra of the corresponding pairs of derivatives were identical, as shown in Table II.

Infrared spectra on the coniferaldehyde and the sinapaldehyde gave further evidence of their identity. A Baird double beam infrared spectrophotometer was used. The samples, dissolved in $HCCl_3$ to about 6% concentration, were run in 0.05 mm. NaCl cells, with the solvent in a matched compensating cell. The coniferaldehyde samples exhibited the same bands at 6.0, 6.2, 6.6, 6.8, 7.0, 7.3, 7.8, 8.8, 9.6 and 10.3 μ; and the sinapaldehyde samples gave the same bands at 6.0, 6.2, 6.6, 6.8, 7.0, 7.3, 7.5, 8.2, 9.6, 10.3, 11.0 and 12.2 μ. The CH bands at 3.4 and 6.8 were intensified in both wood components, and the OH band at 2.8 μ showed pronounced broadening, characteristic of hydrogen bonding.

Because of the similarities between the recovered fractions and the synthetic samples of the same compounds with relation to their chromatographic behavior, ultraviolet spectra, infrared spectra and the properties of the corresponding DNPH's, the identities of the two components of oakwood extract which give lignin-like colorations with phloroglucinol are therefore established as coniferaldehyde and sinapaldehyde.

Sinapaldehyde and Coniferaldehyde in Hardwoods and Soft Woods.—Descending paper chromatographs were run on the extracts of the different woods, as previously described for oak extracts. The developed chromatographs were examined by spraying the sheets with phloroglucinol reagent. A comparison of the sinapaldehyde and coniferaldehyde content of the woods, as indicated by the appearance of the purple colorations, is summarized in Table III.

Hibbert[17] has shown that the lignin of hardwoods is characterized by the presence of syringyl groups in addition to the guaiacyl groups of softwood lignins. In view of the structural similarities between coniferaldehyde and vanillin, on the one hand, and sinapaldehyde and syringaldehyde, on the other, it seems reasonable to conclude that sinapaldehyde is a characteristic of hardwood extractives in the same manner that syringaldehyde is a characteristic oxidation product of hardwood lignins. In general, this idea has been borne out by the chromatographic comparison of several hardwoods and softwoods. The softwoods clearly contained coniferaldehyde, while the hardwoods showed the presence of sinapaldehyde as well as coniferaldehyde. Only two woods in the group examined failed to show evidence of extractable phloroglucinol-reacting aldehydes. Apparently some woods do not contain these aldehydes in a free state. To evaluate the degree of correlation, more species of both hardwoods and softwoods will have to be examined.

TABLE III

	Coniferaldehyde	Sinapaldehyde
Post oak (*Quercus stellata*)	+	+
Forked leaf oak (*Quercus alba*)	+	+
White maple (*Acer saccharum*)	+	+
Butternut (*Juglans cinera*)	+	+
Black walnut (*Juglans nigra*)	+	+
Avodiré	+	+
Philippine mahogany	+	+
Black cherry (*Prunus serotina*)	Nil	Nil
Yellow pine (*Pinus sp.*)	+	Nil
Douglas fir (*Pseudosuga taxifolia*)	+	Nil
Cypress (*Taxodium distichum*)	+	Nil
Redwood (*Sequoia sp.*)	Nil	Nil

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(17) H. Hibbert and M. J. Hunter, THIS JOURNAL, **61**, 2190 (1939); H. Hibbert, J. J. Pyle and L. Brickman, *ibid.*, **61**, 2198 (1939); H. Hibbert, J. L. McCarthy and R. H. S. Creighton, *ibid.*, **63**, 3049 (1941).

[CONTRIBUTION NO. 325 FROM THE CHEMICAL DEPARTMENT, EXPERIMENTAL STATION, E. I. DU PONT DE NEMOURS & CO.]

Rapid Acetylation of Impregnated Cellulose*

BY JOHN C. THOMAS

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Soluble, high-molecular-weight cellulose triacetates have been prepared in extremely short reaction times by the action of acetic anhydride, at temperatures above 90°, on cellulose impregnated with certain combinations of compounds from aqueous solution and dried. For example, cotton linters impregnated with 38% of their weight of urea and 2% of their weight of ammonium sulfate reacted with excess acetic anhydride in one minute at 138° to give a soluble triacetate with a DP (degree of polymerization) of 585. In the same system at 95°, acetylation was complete after 13 minutes, and a product with a DP of 720 was formed. Other compounds could be used in place of urea and ammonium sulfate. The presence of both types of impregnants was necessary, and structural requirements for active impregnants of each type were determined.

Although there is a large amount of published work, especially in the patent literature, on the use of various types of compounds for treating cellulose to make it more active toward esterification, one technique for achieving such activation has apparently received little attention. This is the impregnation of cellulose with an activating agent from aqueous solution, followed by drying. Haller and Ruperti[1] found that cellulose impregnated in this way with the potassium salt of a weak acid, *e.g.*, potassium acetate, was active toward acetylation with acetic anhydride. Marschall[2] used for this purpose the ammonium salt of a strong acid, *e.g.*, ammonium sulfate or ammonium perchlorate. The present paper describes a new method of activation and esterification, which involves impregnating cellulose with certain *combinations* of compounds and esterifying with acid anhydrides at elevated temperatures.

It was shown in this Laboratory, in confirmation of the work of Haller and Ruperti, that cellulose impregnated from aqueous solution with 40% of its weight of potassium acetate could be acetylated beyond the diacetate stage with excess boiling acetic anhydride. The cellulose retained its original form and had the same DP (degree of polymerization) as the starting material (*ca.* 1850), but the reaction was slow, requiring about 20 hours, and the product was insoluble in organic liquids. It also was found that cellulose impregnated with 40% ammonium sulfate could be completely acetylated under the same conditions in 30 minutes. However, the product, although soluble in organic solvents, was degraded to a DP of about 50.

In searching for an activating agent that would have the advantages of both potassium acetate and

* Presented before the Division of Cellulose Chemistry at The A.C.S. Meeting in Chicago, Ill., September 9, 1953.

(1) R. Haller and A. Ruperti, U. S. Patent 1,930,895 (1933).

(2) A. Marschall, U. S. Patent 2,172,447 (1939).