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Structural Aspects of the Color Reaction of Lignin with Strong Acid[2]

BY JOHN C. PEW

The well-known green color produced by the action of strong acids on spruce wood was examined spectroscopically and found to be composed of a yellow and a blue component. The yellow color, which develops first, resembles that given by substituted cinnamaldehydes with the acid reagent. Among several of such aldehydes investigated, only 5-propenylconiferylaldehyde gave a yellow color with an absorption maximum at identical wave length to that of the spruce wood. The blue color appears to be the result of a phenol–aldehyde condensation between the substituted-coniferylaldehyde group and a phenylpropane unit in the lignin structure, a position ortho to the side chain being attacked. Two model compounds of this type were synthesized, the blue colors of which resembled that produced in wood. The "hidden maximum" in the ultraviolet absorption spectra of untreated wood sections was resolved by using, as a reference material, a section which had been bleached with sodium peroxide. The resulting curve has a maximum absorption nearly identical to that of coniferylaldehyde substituted in the 5-position with a carbon side chain. The bleaching of spruce groundwood would appear to be due, in part at least, to the destruction of this grouping.

Though it is an old observation[3] that wood and other lignified materials are colored green by strong acids, the chemistry involved has never been explained. Recently, Abadie[4] indicated that the reaction occurs only with lignin–cellulose complexes and not with lignin alone, a view originally advanced by Hägglund and Björkmann.[5] This does not seem likely since the carbohydrate-free "native lignin" of Brauns[6] gives an intense coloration, and Adler, Björkqvist and Haggroth[7] pointed out that a similar reaction is produced with coniferylaldehyde.

The typical course of the color change may be observed by immersing spruce shavings in concentrated hydrochloric acid at room temperature. A pronounced yellow color develops instantly. In less than a minute the material takes on a greenish tint, changes to an emerald green in about ten minutes, becomes olive green in an hour, and finally

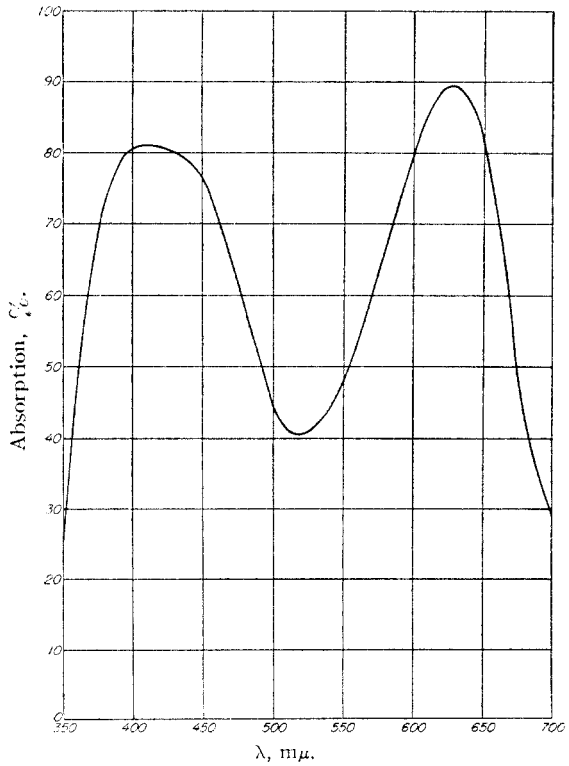


Fig. 1.—Absorption spectrum of a spruce wood section colored green by fuming hydrochloric acid and mounted in the fuming acid, in comparison with an untreated section mounted in dibutyl phthalate; temperature −10°.

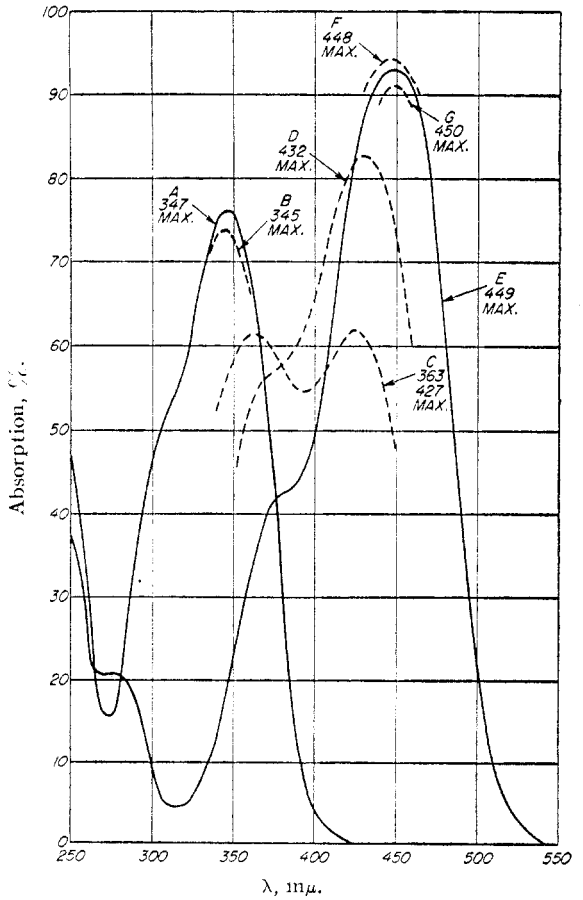


Fig. 2.—Absorption spectra of coniferylaldehyde in various solvents at temperature of −10°: A, in equal volumes of ethanol and water; B, in equal volumes of concentrated hydrochloric acid and water; C, in concentrated hydrochloric acid; D, in fuming hydrochloric acid; E, in absolute ethanol solution containing 38% of hydrogen chloride; F, in absolute ethanol solution containing 48% of hydrogen chloride; G, in absolute ethanol solution containing 28% of hydrogen chloride.

(1) Maintained at Madison, Wis., in coöperation with the University of Wisconsin. Article not copyrighted.

(2) Presented before the XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., Sept. 10–13, 1951.

(3) H. Warnecke, *Pharm. Z.*, **33**, 574 (1888).

(4) F. A. Abadie, *Norsk Skogindustri*, **11**, 290 (1949).

(5) E. Hägglund and C. B. Björkmann, *Biochem. Z.*, **147**, 74 (1924).

(6) F. Brauns, THIS JOURNAL, **61**, 2121 (1939).

(7) E. Adler, K. J. Björkqvist and S. Haggroth, *Acta Chem. Scand.*, **2**, 93 (1948).

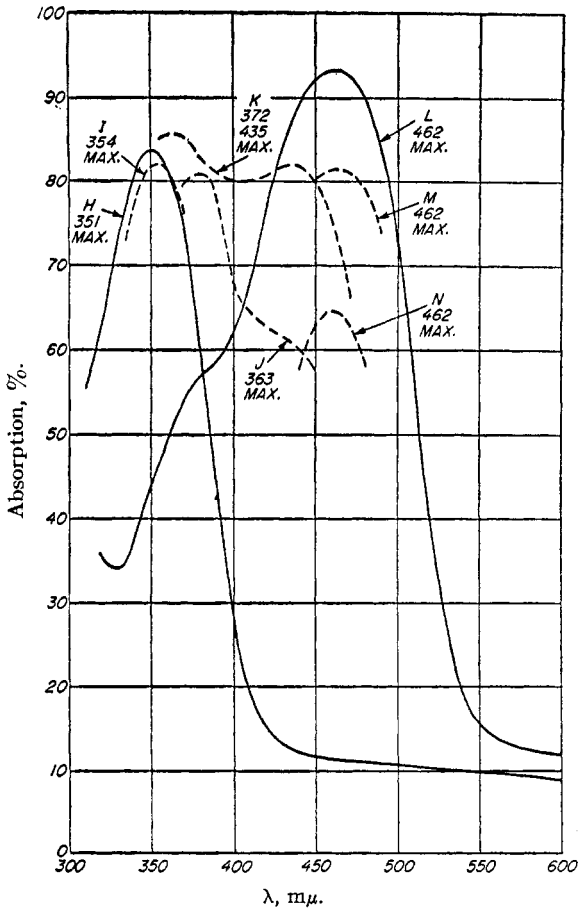


Fig. 3.—Absorption spectra of spruce wood sections mounted in various liquids in comparison with similar sections with color-reacting group destroyed, temperature −10°: H, in equal volumes of ethanol and water; I, in equal volumes of concentrated hydrochloric acid and water; J, in concentrated hydrochloric acid; K, in fuming hydrochloric acid; L, in absolute ethanol solution containing 38% of hydrogen chloride; M, in absolute ethanol solution containing 48% of hydrogen chloride; N, in absolute ethanol solution containing 28% of hydrogen chloride.

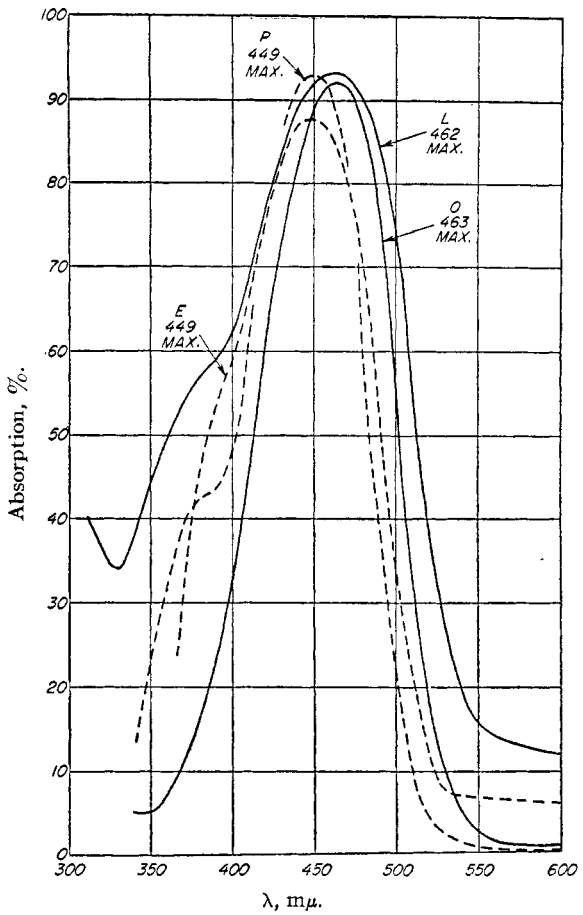


Fig. 4.—Comparison of the absorption spectrum of a spruce wood section in 38% ethanolic hydrogen chloride, L, with that of 5-propenylconiferylaldehyde in this reagent, O, and comparison of the absorption spectrum of spruce "native lignin," P, with that of coniferylaldehyde, E, in the above solvent; temperature −10°.

turns almost black after five hours. It was found that the color changes could be substantially arrested by reducing the temperature. Absorption spectra of wood sections colored green by concentrated or fuming hydrochloric acid were made at a temperature of −10° or below, using untreated sections as blanks. A typical curve is shown in Fig. 1. It is seen that the green color results from a combination of the yellow color first produced with a blue color subsequently formed on standing.

On making repeated runs, it became evident that the maximum in the yellow range varied somewhat with experimental conditions and manipulative details, and this region was more critically examined. Since, from previous work,[8] it was suspected that substituted-cinnamaldehyde groups were involved in this color reaction, the yellow colors produced by several of these aldehydes under varying conditions of acidity were studied. Figure 2 shows the results with coniferylaldehyde. With aqueous acid, the acid concentration affects the position of the maximum to a considerable extent, while with alcoholic acid solutions, the acid concentration may be varied over a considerable range without shifting the position; hence, the latter is a more desirable reagent.

Spruce wood sections were examined under varying acid conditions. In this case, sections in which the color-forming properties had been destroyed were used as the reference material, reasons for which will be discussed later. Figure 3 shows the results. A similarity between the wood and coniferylaldehyde is evident, but the maxima with alcoholic hydrogen chloride do not coincide in the two materials. The first column in Table I compares the maxima obtained with a number of substituted cinnamaldehydes[9] with those of spruce wood, spruce "native lignin" and Freudenberg's enzymatic coniferyl alcohol polymer,[10] using the ethanolic hydrogen chloride reagent. The entire curves for some of these substances are shown in Fig. 4.

The curve for spruce wood (L) has its maximum at nearly identical wave length as that of 5-propenylconiferylaldehyde, curve 0; and curve P for

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

(9) The substituted benzaldehydes appear to form a parallel series, except that the maxima occur at lower wave length.

(10) K. Freudenberg and W. Heimberger, *Ber.*, 83, 519 (1950).

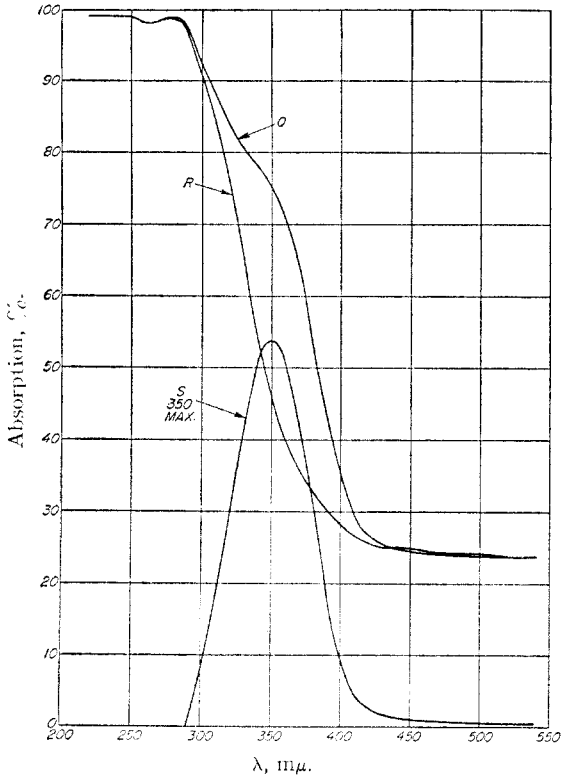


Fig. 5.—Absorption spectra: Q, spruce wood section mounted in nujol in comparison with a ground-surface quartz plate as reference; R, same except spruce section first bleached in buffered sodium peroxide; S, the unbleached section using the bleached section for reference, temperature −10°.

spruce "native lignin" coincides exactly in this respect with that of coniferylaldehyde, curve E. The absorption spectrum of the Freudenberg polymer (not plotted), on the other hand, has its maximum slightly lower than that of 5-propenylconiferylaldehyde but well above that of coniferylaldehyde.

TABLE I

COMPARISON OF THE ABSORPTION SPECTRA OF CERTAIN SUBSTITUTED CINNAMALDEHYDES WITH THOSE OF SPRUCE WOOD, SPRUCE "NATIVE LIGNIN" AND AN ENZYMATIC CONIFERYL ALCOHOL POLYMER (FREUDENBERG POLYMER)

Substance	Absorption spectra maxima, mμ: In hydrogen chloride–ethanol solution (38% of acid)	In ethanol–water solution (1:1)
Spruce wood	462	351
Spruce "native lignin"	449	...
Freudenberg polymer	457	...
p-Methoxycinnamaldehyde	425	326
o-Coniferylaldehyde	335	305
Coniferylaldehyde	449	347
Coniferylaldehydemethyl ether	451	343
Coniferylaldehyde isopropyl ether	457	347
5-Methoxyconiferylaldehyde	470	354
5-Propylconiferylaldehyde	452	352
5-Propenylconiferylaldehyde	463	352

Since the substituted cinnamaldehyde group was shown to be present in spruce wood in the presence of strong acids, it became of interest to discover if the group was present, as such, in the untreated wood or was generated by the reagent. In Fig. 5, curve Q shows the absorption spectrum of a spruce wood section mounted in nujol, using a ground surface of the quartz slide (in order to partially compensate for the light scattering of the wood structure) as a blank. The curve has the usual maximum at about 280 mμ and shows a distinct bulge on the right-hand side. This bulge was noted in some lignin preparations by other investigators[11] who spoke of it as a "hidden" or "masked" maximum. The bulge was eliminated in curve R, which was produced with a section first bleached in buffered sodium peroxide solution. Such sections gave only weak colors with phloroglucinol reagent or with hydrochloric acid. On the other hand, peroxide bleaching does not produce profound chemical changes in the wood.[12] Bleached sections had substantially all the light-absorbing and light-scattering properties of the unbleached section, except those contributed by the aldehydic color-producing portion and, therefore, formed ideal blank or reference material for studying the substituted-cinnamaldehyde group. The bleached sections were used, as previously stated, in measuring the yellow color produced by acids. When the untreated wood section was run against the bleached section, curve S was produced, which corresponds to the bulge in curve Q. The position of the maximum matches closely that of coniferylaldehyde and its 5-propyl- or propenyl-substituted derivatives as indicated in the second column of Table I. Thus, substituted-coniferylaldehyde groups appear to be present in untreated spruce wood and are probably largely responsible for the yellowish color.[13]

It was noted that substituted cinnamaldehydes gave an intense yellow color with aqueous alkaline solutions, including even sodium bicarbonate, provided a free hydroxyl was present in the benzene nucleus. Spruce wood sections, on the other hand, gave scarcely perceptible color with sodium bicarbonate solution and only a weak color with sodium hydroxide. Considering the intensity of the color yielded by spruce wood with acids, much stronger colors would be expected with alkali if any substantial portion of the substituted-cinnamaldehyde groups contained free hydroxyls. These alkali colors are under further investigation.

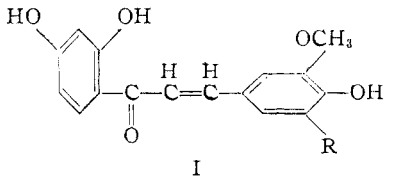
The blue color generated in wood in the presence of strong acids during standing was next investigated. Figure 1 shows the maximum absorption of this color to be located at 628 mμ. Work on the color reaction of spruce wood with phenols indicated that a substituted coniferylaldehyde group in the wood condensed, in the presence of acids, with the added phenol, as illustrated in a previous publication.[8] A color with maximum absorption at identical wave length as the color produced in the reaction of spruce wood with resorcinol was obtained

(11) E. J. Jones, *Tappi*, **32**, 311 (1949).

(12) G. W. Jones, *ibid.*, **33**, 149 (1950).

(13) Such aldehydes may be only slightly yellow or even colorless in the crystalline state or in some solvents, but yellow in other solvents. Thus, a 0.0005 *M* solution of sinapaldehyde in absolute ethanol is intensely yellow.

by the acidification of the lithium aluminum hydride reduction product of the chalcone I where

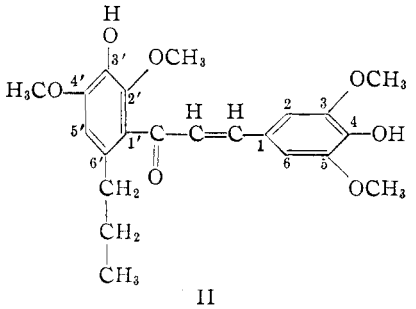


I

R is either a methoxyl or propenyl group. In the absence of added phenols, it is reasonable to suppose the reaction would occur with phenolic nuclei in the lignin.

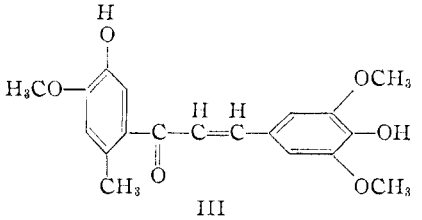
Using an appropriate substituted-coniferylaldehyde grouping to represent the attacking group and a suitable phenylpropane group to represent the phenolic nuclei attacked, the model chalcone II was synthesized.

The methoxyls in the 2′- and 5-positions represent C linkages (syringyl groups being substantially absent in spruce). When the chalcone is reduced with lithium aluminum hydride and the product



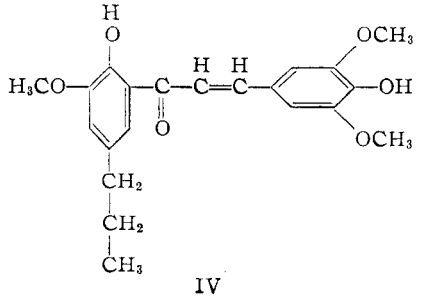
II

dissolved in fuming hydrochloric acid, a blue color is produced, which is shown in Fig. 6, curve U. Curve T represents the color developed by wood in the fuming acid. The maximum of the absorption spectrum of reduced chalcone occurs at 11 mμ lower wave length than that of the wood, but the two curves are similar. Chalcone III, under similar



III

conditions, gave a maximum at 620 mμ (curve V), only 8 mμ below that of wood. On the other hand, with the chalcone IV, the color of the acidified reduction product was violet (curve W). The



IV

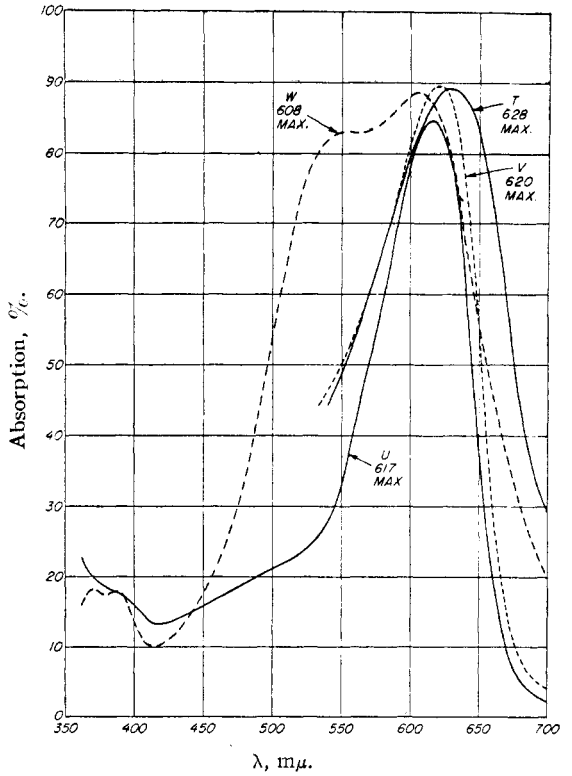


Fig. 6.—Absorption spectrum of the blue component of the green color produced in a spruce wood section with fuming hydrochloric acid, T, compared with that of reduced 3′,4-dihydroxy-2′,3,4′,5-tetramethoxy-6′-propylchalcone, U, of reduced 3′,4-dihydroxy-3,4′,5-trimethoxy-6′-methylchalcone, V, and of reduced 2′,4-dihydroxy-3,3′,5-trimethoxy-5′-propylchalcone, W, in fuming hydrochloric acid; temperature −10°.

maximum occurred at 608 mμ, and the shape of the curve was decidedly different from that obtained by the action of the acid on spruce wood.

In the preceding discussion, it was assumed that the substituted-coniferylaldehyde group attacks the phenolic nuclei in the main bulk of the lignin. The color-producing aldehyde unit might react only with similar units, however, so that it is interesting to note the self-condensation of these substituted coniferylaldehydes. When these aldehydes are dissolved in fuming hydrochloric acid in sufficient concentration, the deep yellow color forms immediately and gradually passes through emerald green to deep blue, and, finally, a blue-black flocculent precipitate may form. The absorption spectra, when measured in the blue stage, had an adsorption maximum at 610 mμ with coniferylaldehyde, 615 mμ with 5-propenylconiferylaldehyde and 615 mμ with 5-methoxyconiferylaldehyde. The close resemblance of the color produced from the latter to the color produced from the reduced chalcone II (max. 617) suggests a condensation of one molecule of the aldehyde with the 6-position of the second, the resulting dimer having an acrolein side chain rather than the propyl group of the chalcone product. The blue color of the aldehyde condensation product is very much more stable than that of the reduced chalcones.

Thus, it would appear that when spruce wood is

treated with strong acids a phenol–aldehyde condensation occurs, in which a substituted-coniferylaldehyde group attacks a position ortho to the side chain in a phenylpropane building unit of the lignin. Recently Richtzenhain[14] reported the isolation of metahemipinic acid from the oxidation products of methylated spruce lignin, indicating linkages in lignin not previously believed to exist. In later work,[15] however, he was able to obtain this product only from hydrochloric acid lignin, alcohol lignin or ligninsulfonic acid, that is, lignins isolated in acid media. Richtzenhain suggested the presence of lignan groups in lignin, which, under the influence of acid, undergo conversion to isolignans by nuclear condensation in the 6-position. The work reported here indicates that a side chain in the 6-position results from the phenol–aldehyde condensation previously described.

Experimental[16]

Development and Measurement of Green Color in Wood Specimens.—Sections of fresh spruce sapwood of 100-micron thickness were cut at an angle of 45° to the fiber direction and suspended in ethanol until used. The color was developed with hydrochloric acid and perchloric acid under a variety of conditions, but the method used in the example reported appeared most satisfactory. The section was allowed to air dry, was then immersed in fuming hydrochloric acid at −25° for 16 hours and, finally, mounted in the acid on a glass slide under a cover glass, the operation being conducted at −25°. For the reference materials, a duplicate section was mounted in dibutyl phthalate, which appeared to impart about the same degree of translucency to the specimen as was possessed by the one treated with acid. The slide was inserted in a holder designed for the purpose and in such manner that the annual rings of the specimens were at right angles to the slit direction of the spectrophotometer. The absorption was measured on a Beckman DU instrument adjusted to near-maximum resolution and arranged so that the cell compartment, phototube and lamp projected into a cold space, by which means the temperature in the cell compartment was maintained at −10 to −15°. In all subsequent absorption determinations, unless otherwise stated, reagents and equipment were at −25°, and measurements were made at −10 to −15°.

Development of the Yellow Color in Wood Sections.—With the aqueous solvents, sections of 100-micron thickness were immersed for 5 minutes and then mounted on quartz slides with quartz cover glasses. In alcoholic hydrogen chloride solutions, the color developed slowly and incompletely, but satisfactory results were obtained by first immersing the section in fuming hydrochloric acid for 10 minutes and then in the alcoholic acid for 1 hour. In the case of curve N, a 50-micron thick section was used to increase the amount of light transmitted. At first, a duplicate section mounted in an inert liquid and selected to approximate the light scattering effect of the treated section was used as the reference material. This was satisfactory only when the maximum absorption occurred at some distance from the point where the untreated specimen showed marked absorption. Bleached sections were more satisfactory as references and were prepared by immersing the sections in 100 ml. of an aqueous solution containing 1% of sodium peroxide, 2.5% of 40° Bé sodium silicate, 0.25% of magnesium sulfate and sulfuric acid sufficient to bring the *p*H to 10.3. The solution was maintained at 40° for 5 hours. The sections were rinsed and soaked 30 minutes in water, immersed in water made barely acid with sulfur dioxide, rinsed, soaked in water overnight, blotted and air-dried. Such sections gave a faint yellow color with concentrated hydrochloric acid and a light pink with phloroglucinol reagent, but the colors were much less intense than those produced by untreated sections. Extension of the bleaching period to 3 days did not entirely destroy the color-producing property.

Absorption Spectra of Untreated and Bleached Spruce Sections.—In making these measurements, specimens of 50-micron thickness were mounted in white mineral oil on a quartz slide, using a quartz cover glass. For reference, a second mount was prepared on the slide, but the specimen was omitted in the area covered by the spectrophotometer beam. In addition, the surface of the slide was sandblasted in this area in order to partially compensate for the scattering of light caused by the wood substance. With curve S, both the bleached and unbleached sections were mounted in mineral oil.

Absorption Spectra of Substituted Cinnamaldehydes.—To 0.5 ml. of a 0.0005-molar solution of the aldehyde in absolute ethanol (0.0025 molar in the case of *o*-coniferylaldehyde) was added sufficient of the reagent to give a volume of 10 ml. The absorption spectra were determined using 1-cm. quartz cells. All operations were carried out at low temperatures, as described above.

Development of the Yellow Color with the Freudenberg Coniferyl-Alcohol Polymer and with "Native Lignin."—A 0.002-g. portion of the enzymatic coniferyl alcohol polymer of Freudenberg was dissolved in 10 ml. of a 38% ethanolic hydrogen chloride solution and the absorption spectrum determined, using a solution of the same concentration of polymer in 95% ethanol for comparison. With the spruce "native lignin," 0.002 g. was suspended in 1 ml. of 95% ethanol and diluted to 10 ml. with the ethanolic hydrogen chloride. In this case, the reference blank contained the same amount of the lignin dissolved in 8 ml. of ethanol and 2 ml. of dioxane, the dioxane being added in order to prevent turbidity at the low temperature.

Reduction and Development of Color with the Chalcones.—The chalcones, in amounts of 0.02 millimole, were reduced with lithium aluminum hydride as previously described,[8] except that at the conclusion of the reduction, the the mixture was acidified with dilute acetic rather than hydrochloric acid. The reduction product was dissolved in 20 ml. of ethanol. To develop the color, 1 ml. of the solution of reduced chalcone II, 0.3 ml. of the solution of reduced chalcone III and 0.35 ml. of the solution of reduced chalcone IV were each diluted to 10 ml. with fuming hydrochloric acid.

Absorption Spectra of the Products of Self-condensation of Substituted Cinnamaldehydes.—With coniferylaldehyde and 5-methoxyconiferylaldehyde, 0.005 millimole was dissolved in 10 ml. of cold fuming hydrochloric acid and the solutions allowed to stand 1 hour at room temperature to develop the blue color. With the 5-propenyl compound, precipitation occurred under this procedure, and, therefore, 2 ml. of a 0.0005 molar ethanol solution of this aldehyde was diluted to 10 ml. with the fuming acid and the solution allowed to stand 3 hours at room temperature. In all cases, the colored solutions were next cooled to −25° and the absorption spectra measured at −10 to −15°.

5-Propenylvanillin Methoxymethyl Ether.—To 2.9 g. (0.015 mole) of 5-propenylvanillin[8] in ethanol was added an ethanol solution of potassium hydroxide (0.015 mole). The ethanol was evaporated and the residue ground and dried at 120°. This residue was refluxed with 10 ml. of absolute ether and 4 ml. (0.053 mole) of chloromethyl ether for 48 hours, water added, and the ether layer removed. The ether solution was thoroughly extracted with 2% aqueous sodium hydroxide, dried with anhydrous sodium sulfate, concentrated, and cooled to −30°. Yellowish prisms separated. The yield was 1.90 g. (54%). Recrystallization from cold ether after light treatment with charcoal gave colorless prisms, m.p. 29–30°.

Anal. Calcd. for $C_{13}H_{16}O_4$: C, 66.08; H, 6.83. Found: C, 66.20; H, 6.91.

5-Propenylconiferylaldehyde Methoxymethyl Ether.—To 1.80 g. of 5-propenylvanillin methoxymethyl ether, dissolved in 10 ml. of methanol and 7 ml. of water, twice the calculated quantity of acetaldehyde as a 20% aqueous solution was added portionwise over a 4-hour period. The reaction mixture was stirred vigorously and maintained at a temperature of 70°; the solution was kept just distinctly alkaline to litmus by addition of 10% aqueous potassium hydroxide. Additional methanol was added from time to time to prevent excessive turbidity. The reaction was continued for 1 hour after completing the addition of the acetaldehyde. The mixture was then cooled, diluted with water, extracted with ether, and the ether extract dried and cooled to −30°. The crystals were recrystallized from cold

(14) H. Richtzenhain, *Acta Chem. Scand.*, **4**, 589 (1950).
(15) H. Richtzenhain, *Svensk Papperstidning*, **53**, 644 (1950).
(16) All melting points were corrected.

ether (−30°) several times to yield 0.53 g. (27%) of colorless needles, m.p. 87–88°.

Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.68; H, 6.76.

5-Propenylconiferylaldehyde.—The methoxymethyl ether of this compound (0.40 g.) was dissolved in 5 ml. of 50% aqueous acetic acid containing 0.3% of sulfuric acid and the solution heated on the steam-bath for 20 minutes. The mixture was cooled and the glistening pale yellow scales filtered and washed with water. The yield was 0.31 g. (93%), m.p. 111–113°. Recrystallization from aqueous ethanol gave cream-colored needles, m.p. 112–113°.

Anal. Calcd. for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.61; H, 6.35.

5-Propylvanillin Methoxymethyl Ether.—The potassium salt prepared from 11.7 g. (0.06 mole) of 5-propylvanillin was refluxed with 50 ml. of absolute ether and 9.1 ml. (0.12 mole) of chloromethyl ether for 48 hours, two additional 4.5-ml. portions of chloromethyl ether being added during that period. The mixture was cooled, dissolved in water, and the ether layer separated, extracted with 2% aqueous sodium hydroxide, and dried. The ether was distilled off, the residual oil taken up with 25 ml. of petroleum ether, and the solution cooled to −30°. The solvent was decanted from the crystals, which were recrystallized from petroleum ether with a yield of 10.8 g. (76%). Repeated recrystallization yielded nearly-colorless crystals, m.p. 19–20°.

Anal. Calcd. for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61. Found: C, 65.18; H, 7.69.

5-Propylconiferylaldehyde Methoxymethyl Ether.—To a stirred solution maintained at 70° and containing 10.8 g. (0.46 mole) of 5-propylvanillin methoxymethyl ether in 80 ml. of methanol and 60 ml. of water, twice the calculated amount of acetaldehyde as a 50% aqueous solution was added over a 4-hour period. The solution was kept just distinctly alkaline with a 10% potassium hydroxide solution. The reaction was continued 1 hour after all acetaldehyde had been added and the mixture heated on the steam-bath until most of the methanol was driven off. The residue was dissolved in water, extracted with a mixture of ether and petroleum ether, and the ether solution dried. The solution was cooled to −30° and 2.65 g. (22%) of product obtained. Repeated crystallization gave glistening, flat, nearly-colorless prisms, m.p. 74–75°.

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.25; H, 7.65.

5-Propylconiferylaldehyde.—The methoxymethyl ether (1.5 g.) was hydrolyzed as described under the corresponding propenyl compound. The yield was 1.10 g. Recrystallization from aqueous ethanol after charcoal treatment gave light-cream-colored, microscopic needles, m.p. 84–85°.

Anal. Calcd. for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 71.09; H, 7.41.

3-Acetoxy-2,4-dimethoxy-6-propylacetophenone.—Five grams (0.021 mole) of the acetate of 2,6-dimethoxy-4-propylphenol, prepared by acetylation with acetic anhydride and pyridine of a hardwood distillate composed largely of 2,6-dimethoxy-4-propylphenol, was refluxed 4 hours with 10 ml. of acetic anhydride and 2 drops of sulfuric acid. The mixture was poured into water with stirring, warmed, and then let stand in a refrigerator overnight. The solution was decanted from the semi-crystalline mass, dissolved in ether, the ether solution washed with 2% aqueous sodium bicarbonate, dried, and the ether evaporated. The residue was taken up in petroleum ether, the solution treated with charcoal, and then allowed to stand overnight at −30°. The solvent was decanted from the separated resin and the resin crystallized from cold ethanol. The yield was 2.54 g. (43%). Repeated crystallization gave colorless prisms, m.p. 68–69.5°.

Anal. Calcd. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.41; H, 7.23.

3′,4-Diacetoxy-2′,3,4′,5-tetramethoxy-6′-propylchalcone.—To 1.82 g. (0.01 mole) of syringaldehyde and 2.80 g. (0.01 mole) of 3-acetoxy-2,4-dimethoxy-6-propylacetophenone dissolved in 2ml. of ethanol was added 8 ml. of potassium hydroxide solution (40 g. of pellets, 60 g. of water), and the mixture heated in a closed flask at 70° for 48 hours, with frequent shaking during the first 8 hours and occasional shaking thereafter. The orange solution containing some crystals was dissolved in 150 ml. of cold water and the solution acidified with dilute hydrochloric acid. The yellowish resin that separated was dissolved in ether, the ether solution shaken with several portions of 30% aqueous sodium bisulfite solution and dried, and the ether evaporated. All attempts to crystallize the residue were unsuccessful. The residue was acetylated with 10 ml. of acetic anhydride and 5 ml. of pyridine for 5 hours, and the mixture poured into water. The crystalline crust that formed overnight was recrystallized from ethanol. The yield was 2.86 g. (59%). Recrystallization of the diacetate from ethanol gave pale yellow crystals, m.p. 171–173°.

Anal. Calcd. for $C_{26}H_{30}O_9$: C, 64.19; H, 6.22. Found: C, 64.19; H, 6.22.

3′,4-Dihydroxy-2′,3,4′,5-tetramethoxy-6′-propylchalcone.—To 0.972 g. (0.002 mole) of the corresponding 3′,4-diacetoxy compound dissolved in 15 ml. of methanol, 10 ml. of *N* sodium hydroxide was added and the mixture refluxed 15 minutes. The solution was cooled, diluted to 100 ml. with water, and acidified with dilute hydrochloric acid. When coagulation of the resin was complete, the aqueous solution was decanted, the resin taken up in ether, and the ether solution washed with sodium bicarbonate solution. The ether solution was dried, the ether evaporated, and the resin dried at 50° under vacuum. Attempts to crystallize the resin failed.

3′,4-Dihydroxy-3,4′,5-trimethoxy-6′-methylchalcone.—To a solution of 3.64 g. (0.02 mole) of syringaldehyde and 3.60 g. (0.02 mole) of 3-hydroxy-4-methoxy-6-methylacetophenone[17] dissolved in 4 ml. of ethanol was added 16 ml. of potassium hydroxide solution (40 g. of pellets, 60 g. of water) and the mixture heated in a closed flask at 70° for 20 hours with occasional shaking. The solution was diluted to 800 ml. with water, acidified with dilute hydrochloric acid and the mixture allowed to stand until crystallization of the orange, resinous chalcone was complete. The chalcone was recrystallized from aqueous ethanol to yield a hydrate in the form of bright yellow needles, which on drying at 120° yielded 4.10 g. (60%) of a pale yellow powder, m.p. 162–163°. Recrystallization from benzene gave a pale yellow crystalline powder, m.p. 163–164°.

Anal. Calcd. for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85. Found: C, 66.34; H, 5.93.

Allyl Ether of 2-Hydroxy-3-methoxyacetophenone.—After refluxing 8.3 g. (0.05 mole) of 2-hydroxy-3-methoxyacetophenone,[18] 6.05 g. (0.055 mole) of allyl bromide, 9.00 g. of anhydrous potassium carbonate and 20 ml. of anhydrous acetone for 8 hours, the mixture was diluted with water and the solution extracted with ether. The ether solution was thoroughly extracted with 2% aqueous sodium hydroxide, dried with anhydrous sodium sulfate, and the ether distilled. The oily residue was distilled yielding 9.4 g. (78%) of a colorless oil, b.p. 101–103° (0.6 mm.).

Anal. Calcd. for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 69.51; H, 6.61.

2-Hydroxy-3-methoxy-5-allylacetophenone.—Nine grams of the allyl ether of 2-hydroxy-3-methoxyacetophenone was heated to 210° in a metal-bath until rearrangement started, when the source of heat was removed. After boiling stopped, the liquid was refluxed at 75 mm. for 2 hours and distilled at 13 mm., collecting the fraction boiling between 154 and 189°. The yield was 6.9 g. of an oil partially solidifying at −30°. Recrystallization several times from ether (−30°) yielded 3.7 g. of pale yellow crystals, m.p. 43–44°.

Anal. Calcd. for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 70.18; H, 6.87.

2-Hydroxy-3-methoxy-5-propylacetophenone.—Three grams of the corresponding allyl compound in 25 ml. of acetic acid was hydrogenated at room temperature and pressure, using 0.3 g. of a catalyst consisting of 5% of palladium-on-barium sulfate. The yield was 2.95 g. of yellow crystals, m.p. 18–19°.

Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.21; H, 7.75. Found: C, 69.52; H, 7.66.

2′,4-Dihydroxy-3′,3,5-trimethoxy-5′-propylchalcone.—In 3 ml. of ethanol were dissolved 0.73 g. (0.004 mole) of syr-

(17) R. H. F. Manske and A. E. Ledingham, *Can. J. Research*, **22B**, 115 (1944).

(18) W. Baker, N. C. Brown and J. A. Scott, *J. Chem. Soc.*, 1922 (1939).

ingaldehyde and 0.83 g. (0.004 mole) of 2-hydroxy-3-methoxy-5-propylacetophenone, and 6 ml. of an aqueous potassium hydroxide solution (25 g. of pellets, 75 g. of water) added. The mixture was heated in a closed flask for 24 hours at 70°, with frequent shaking at first and occasional shaking during the remaining time. The reaction mixture was dissolved in water, acidified with dilute hydrochloric acid, shaken with petroleum ether to remove the bulk of the unreacted phenone, and the aqueous solution drained from the precipitated orange resin. The resin was dissolved in ether, the ether solution shaken with several portions of 30% sodium bisulfite solution, and the ether solution dried. The ether was evaporated and the residue dissolved by boiling with several portions of petroleum naphtha. The combined naphtha extracts were cooled in a refrigerator and the solvent, containing some residual phenone, was decanted. The resinous residue was crystallized from 50% aqueous ethanol, yielding 0.20 g. of orange crystals sintering at 68° and fusing to a viscous resin at about 73°. The product, apparently a hydrate, was dried at 105° and recrystallized from petroleum naphtha to give orange needles, m.p. sinters 110°, melts 114–116°.

Anal. Calcd. for $C_{21}H_{24}O_5$: C, 67.73; H, 6.50. Found: C, 68.03; H, 6.58.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Vitamin B_{12}. XVIII. The Degradation of Vitamin B_{12} to 1-α-D-Ribofuranosyl-5,6-dimethylbenzimidazole

BY NORMAN G. BRINK AND KARL FOLKERS

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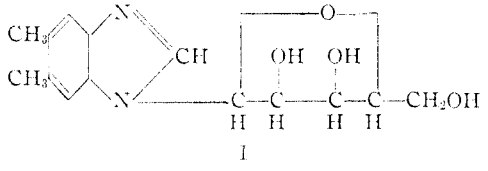
Acid hydrolysis of vitamin B_{12} yielded a product which has been identified as 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole. It was characterized as a 1-pentofuranosyl-5,6-dimethylbenzimidazole by periodate oxidation studies, which led to the isolation of a crystalline α-(5,6-dimethylbenzimidazole-1)-α'-hydroxymethyldiglycolic aldehyde derivative, and which suggested the α-configuration at carbon atom one of the ribose moiety of the glycoside.

The preparations of 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole (α-ribazole) (1) from vitamin B_{12} and by synthesis have been communicated.[1] Details of the isolation of the degradation product



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from an acid hydrolysate of vitamin B_{12} and its characterization as a 1-pentofuranosyl-5,6-dimethylbenzimidazole are described herein. The final characterization of the degradation product as α-ribazole resulted from its identification with synthetic α-ribazole.[2] The assignment of the α-configuration to the degradation product followed from a comparison of the optical rotations of synthetic α- and β-ribazoles.[2] A direct correlation of β-ribazole with 1-β-D-glucopyranosyl-5,6-dimethylbenzimidazole is described below.

Hydrolysis of vitamin B_{12} in 6 *N* hydrochloric acid at 150° for 20 hours gave 5,6-dimethylbenzimidazole,[3] an observation which was shortly confirmed by others.[4] When the hydrolysis was carried out at a somewhat lower temperature, 120°, and the hydrolysate treated to yield a basic fraction isolated by continuous chloroform extraction, the absorption spectrum of the crude product suggested the presence of a different substituted benzimidazole. When a solution of the previously obtained 5,6-dimethylbenzimidazole was made alkaline, an absorption maximum appeared at 2470 Å., the magnitude of the absorption of this new peak being less, however, than those of the two principal maxima at 2810 and 2880 Å. With the basic fraction from the hydrolysis done at 120°, the new peak which appeared at 2500 Å. when the solution was made alkaline was higher than the two original absorption peaks. The crude product was only sparingly soluble in ether; and the ether-insoluble material was indicated to contain carbohydrate by the color test involving dehydration to furfural or its derivatives.[5] This same material yielded a crystalline picrate which melted at 212–214°, and hence was not identical with 5,6-dimethylbenzimidazole picrate (m.p. 273–275°). The crystalline picrate also gave a positive carbohydrate test.[5]

Subsequent hydrolyses of vitamin B_{12} done in 6 *N* hydrochloric acid at 120° afforded mixtures of the benzimidazole glycoside picrate and 5,6-dimethylbenzimidazole picrate. However, when the hydrolysis was carried out overnight at a temperature of 100°, splitting of the glycoside was negligible, and the glycoside picrate could be readily isolated in pure form. The picrate was dextrorotatory and had an ultraviolet absorption spectrum in acidic ethanol solution with maxima at 2760 Å. (E_M 10,950), 2850 Å. (E_M 10,600) and 3590 Å. (E_M 13,000). Analyses indicated that the composition of the picrate corresponded to the formula $C_{14}H_{18}N_2O_4 \cdot C_6H_3N_3O_7$. Removal of picric acid by chloroform extraction of a dilute aqueous hydrochloric acid solution of the picrate yielded the amorphous

(1) N. G. Brink, F. W. Holly, C. H. Shunk, E. W. Peel, J. J. Cahill and K. Folkers, THIS JOURNAL, **72**, 1866 (1950).

(2) F. W. Holly, C. H. Shunk, E. W. Peel, J. J. Cahill, J. B. Lavigne and K. Folkers, *ibid.*, in press.

(3) N. G. Brink and K. Folkers, *ibid.*, **72**, 4442 (1950).

(4) E. R. Holliday and V. Petrow, *J. Pharm. Pharmacol.*, **1**, 734 (1949); G. R. Beaven, E. R. Holliday, E. A. Johnson, B. Ellis, P. Mamalis, V. Petrow and B. Sturgeon, *ibid.*, **1**, 957 (1949).

(5) F. Feigl, "Qualitative Analysis by Spot Tests," Third English Edition, Elsevier, New York, N. Y., 1946, p. 410.