

THE SEPARATION AND IDENTIFICATION OF "HADROMAL"

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INTRODUCTION

This thesis was undertaken in an effort to identify the color producing material in wood known as "Hadromal". It was desired to harmonize the divergent results of Grafe (1) and Czapek (2) who differed regarding the chemical nature of "Hadromal".

The hope was that some light might be thrown on the relationship between lignin and "hadromal". Finally, it was desired to see whether or not the material was present in sufficient quantity to be commercially practicable as a source for vanillin and coniferin.

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1. Botan Centrallblatt 38,753,(1889)
 2. Z. Physiol Chem., 27, 141,(1899)

REVIEW OF LITERATURE

Many of the early workers in the field of wood lignin such as Schulze, Czapek, Erlich, Nickel, Grandmougin, Sclivanow, Grafe, and others, were impressed by the many color reactions which woody tissues gave with certain reagents.

Nearly 100 years ago, Runge (1) found that pine wood, treated with phenol and hydrochloric acid, assumed a greenish blue coloration, and with aniline sulfate, a yellow color was produced. Since that time, many reagents have been used in both a theoretical and practical way in making these color tests. For example, the Maule reaction may be used to distinguish hard woods from soft ones according to Crocker (2).

The question, as to whether these reactions can be attributed to some minor constituent or to some group in the lignin complex, has been a controversial subject for many years.

Singer (3) considered these colorations due to vanillin or coniferin, and Nickel (4) showed that wood behaves like an aldehyde toward Schiff's reagent. He later showed that wood treated with bisulfite then failed to give these color tests.

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1. Phillips, Chemical Reviews, Feb. (1934)
 2. Crocker, Ind Eng. Chem., 13, 625, (1921)
 3. Monatsh, 3, 395, (1882)
 4. Botan. Centrallblatt, 38, 753, (1889)

Of these workers, one of the most important was Czapek (1). By digesting wood with SnCl_2 for a long period of time, subsequently treating the residue with benzene, and after the solvent was distilled off, crystallizing from ligroin, he obtained an impure brownish product M. P. 70-80. He called his product "Hadromal".

Czapek's hadromal was partially purified as the bisulfite compound. It reacted with concentrated sulfuric acid to give a strong red violet color, reduced silver nitrate and Fehling's solution, gave a red color with ferric chloride, and red color with Millon's reagent. The hadromal solution gives exactly the same color tests as did the untreated wood. When warmed, the product had a vanillin like odor. It was partially soluble in water, very soluble in alcohol and ether, and fairly insoluble in hot ligroin. For this reason, ligroin was used as a fractional crystallizing medium. Dilute alkali affected good solubility to a yellow color, although Czapek reported that the aqueous solution was a neutral one.

When completely divested of its hadromal, wood gives no color tests. However, due to the large amounts of impurities and to the exceedingly small quantity of product, Czapek was never able to positively identify his product, but proposed

1. Z. Physiol Chem., 27, 141,(1899)

coniferyl aldehyde as the most probable compound.

Grafe (1) violently disagreed with Czapek regarding the chemical nature of hadromal. He proposed that it consisted of a mixture of methyl furfural, vanillin, and pyrocatechin, though Czapek later showed that no such combination could duplicate the properties of hadromal.

The work of Crocker (2) and Hoffmeister (3) supported the work of Czapek. By using a continuous extraction apparatus by which he was able to heat the wood meal ~~stannous chloride~~ reaction mixture, Hoffmeister was able to prevent super-heating effects and, so he claimed, prevented decomposition of the Hadromal as fast as it was formed. He reported obtaining two grams of hadromal in this way, though its existence was doubted by a number of investigators.

By using a hydrogen atmosphere when distilling off his benzene, he prevented possible oxidation at this point. He found that his compound had a molecular weight of 184. This checked that of coniferyl aldehyde and not that of vanillin, 149. By oxidizing it with dilute acids and potassium dichromate, he was able to oxidize it to vanillin and isolate the product thus formed. Even air oxidation over long periods of time would do the same.

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1. Botan. Centrallblatt, 38, 753, (1889)
 2. Ind Eng. Chem. 13, 625, (1921)
 3. Ser. 60, 2062, (1927)

The hadromal showed the usual aldehyde reactions and adds two atoms of iodine, showing the presence of a double bond. Mild oxidation with dilute $K_3Fe(CN)_6$ gave $4,3 OH(CH_3O)C_6H_3CH = CHCOOH$ and reactions with para bromo benzoyl chloride indicate that only one OH group is present, thus discounting Grafe's suggestion that hadromal contained pyro-catechin. These facts point to hadromal as being coniferyl aldehyde. Hoffmeister, like Czapek, believed it to be present in woody tissues as the cellulose ester to the amount of about three per cent.

In his study of the color reactions of wood, Croker (1) prepared hadromal by the Czapek method. By studying the spectrum of some of the colors produced and comparing them with a number of known solutions of aldehydes which possibly could be in the wood, he came to the following conclusions:

- (1) There is only one aldehyde in the wood which is responsible for color tests.
- (2) This aldehyde is present only in very small quantity; probably about a hundredth of a per cent.
- (3) This material is the same regardless of the ligno-cellulose source; oil of sassafras and oil of cloves behave the same as wood.
- (4) Order of intensity of color tests are given as:
Phloroglucinol - orcinol - resorcinol - pyrogallol
- (5) Acid amides do not react. No tertiary amine has been found which does so. Essentially all of Croker's work

1. Crocker, Ind Eng. Chem., 13, 625, (1921)

agrees with that of Czapek.

Pauly and Feuerstein (1) took sides with Grafe in criticizing the work of Czapek and Hoffmeister. They completely agree with Grafe. They point out that mixed melting points of Hadromal with coniferyl aldehyde lower its melting point more than twenty degrees.

Klason (2) believed his alpha lignohydrosulfonic acid obtained by treating wood with seventy-two percent sulfuric acid, was an SO_3H derivative of coniferyl-paraldehyde, but was contested by Pauly and Feuerstein, who claim that neither Hoffmeister nor Klason had the least trace of coniferyl aldehyde present, due to certain modifications which they made in the Pauly and Wascher (3) method of synthesis of this compound. Pauly also adds that coniferyl aldehyde cannot add iodine under the conditions set forth by Hoffmeister.

In his method, Hoffmeister claims to have produced coniferyl aldehyde by condensation of vanillin with acetaldehyde. He should have protected the phenolic OH group with an ester residue. Repeated trials to synthesize coniferyl aldehyde by this method were met with failure, the vanillin being recovered in ninety-seven per cent yields. The product failed to give

1. Ber. 6213, 297-311, (1929)

2. Ber Hauptversammlung des Vereins der Zellstoff u, Papier Chemiker, pp. 52, 53, (1908)

3. Ber. 56 B, 605-610 (1923)

color tests with benzidine, a reagent sensitive enough to detect it in dilutions of 1:40,000. The melting point diagrams for mixtures of coniferyl aldehyde and vanillin indicate his method of synthesis is worthless. His failure to observe the formation of a difficultly soluble bisulfite compound indicates his product was unchanged vanillin.

In Grandmougin's (1) excellent paper presented from his polytechnic laboratories in Zurich, we find a summarization of a number of color tests into a table and a few generalizations regarding the nature of these tests, in particular, the effects of certain groupings.

As will be seen in his table, the aromatic amines in general react. Likewise so do hydroxy aromatic compounds. p nitro aniline gives an orange red color with wood but if the amine is diazotized, practically no color reaction with wood can be given. In general, the amine reactions are more sensitive than the phenolic reactions. In nearly all cases the phenolic compounds need sunlight for their action, but phloro-glucinol is an exception. Para compounds seem to have more effect than either ortho or meta ones. Para nitro aniline gives a deep color but the ortho and meta aniline gives colors which correspond more like aniline.

Primary amines in general are more effective in color

1. Z, Farben Ind 5, p. 321-23, Jan., (1906)

production than secondary ones. Thus mono methyl anilin gives scarcely any color, while anilin itself in the presence of sulfuric acid gives brilliant yellow colors. Apparently compounds with substituted halogens give weak colors. For example, p dibrom nitro aniline gives a weaker color than p nitro anilin. The benzene nucleus seems necessary for color production. Compounds of the quinoline type give no colors with wood.

EXPERIMENTAL DATA

In obtaining hadromal by Grafe's method, about 150 gms. of wood were treated with distilled water in an autoclave at 180° centigrade for one hour. The resulting mixture was extracted with benzene, preferably by shaking at intervals in a large five liter bottle for several hours. The benzene solution becomes either amber or orange, depending on the completeness of removal of gums by previously letting the wood stand overnight in an alcohol-benzene solution.

Several runs of 150 gms. were extracted and the benzene solutions united. After distilling off the benzene in vacuum, a yellow gum was left. On extraction with ligroin (b. p. 50-70) yellow crystals were observed under a microscope. Efforts were made to crystallize this gum. Carbon disulfide, carbon tetrachloride, ether, acetone, chloroform, dioxane, and a number of other solvents were tried in an effort to crystallize it. Extremely good solubility was noted in the case of dioxane, chloroform, and acetone; practically complete insolubility resulted in the other solvents. No crystallization occurred in any case. A great number of combinations of these solvents, using variable proportions of each was tried on the gum in an effort to find a crystallizing medium. None was found.

Despite the practically unsuccessful efforts to obtain

Grafe's hadromal in quantity in a crystalline form, the benzene solution diluted with alcohol gives reactions. These are listed in the following table and will be discussed later in comparison with that of Hoffmeister and Czapek.

In addition to the color reactions, it was noted that the gum goes into solution in alkali giving a yellow solution. Bicarbonate solutions dissolved the gum. Such a solution was evaporated to dryness and excess bicarbonate was neutralized to procure a crystalline product. But still no such result was forthcoming. The aqueous solution has a strong odor of furfural.

Twenty-eight organic dyes such as methylene blue, Corallin, Fluorescein, para red, alpha nitroso beta naphthol, Eosin etc. were tried in an effort to get still further color tests. Essentially, they all failed.

Both the aqueous and benzene solutions of Grafe's method gave aldehyde tests with Schiff's and Rosaniline reagents if allowed to stand several hours.

More success in obtaining a compound of crystalline nature was obtained by using Hoffmeister's method. A glass container was placed inside an ordinary Soxhlet jacket. In it was placed a paste of wood meal and SnCl_2 . A round bottomed, one-liter flask filled two-thirds with benzene was placed at the bottom of the Soxhlet and a reflux

condenser was attached at the top. Inside the Soxhlet, a thistle tube was arranged so that the hot benzene coming from the condenser bubbled through the paste and extracted the hadromal. Essentially it was the same apparatus as Hoffmeister used.

After each tube of wood paste was exhausted as shown by tests with phloroglucin, a fresh charge was put in. The benzene solution containing hadromal was finally withdrawn and distilled in vacuum. A heavy precipitate which appeared in the bottom of the flask proved to be only a hydrolysis product of the Stannous chloride, some of which had been swept over from the reaction tube. The aqueous solution gave aldehyde tests with rosaniline. The benzene solution behaved with color reagents like Czapek's hadromal which will be tabulated later. On evaporating the solvent in vacuum, distinct needle crystals were observed under a microscope. As with other workers, Hoffmeister's claimed yield of two per cent could not be duplicated.

Zinc chloride was tried in place of Stannous chloride as possibly being more productive than the stannous salt but behaved similarly to the latter.

As in the case of extraction by Grafe's method, care

must be taken to remove gums and resins by allowing the wood to stand overnight in an alcohol-benzene solution.

The author found Czapek's method to be most productive in obtaining crystalline material. 350 grams of sawdust previously exhausted with an alcohol-benzene mixture was placed in each of several ordinary zinc cooking pails with porcelain lining and covers. About 100 grams of stannous chloride was added to each and enough distilled water mixed in to make a thin paste. This was then slowly cooked until the wood assumed a flesh color and no longer reacted with phloroglucinol. Water was added from time to time as it was evaporated off. The solution gradually assumed an amber color. The time necessary for complete exhaustion of the wood varies. Around fifteen hours cooking was necessary.

The contents of each pan are added to a large five-liter bottle and the entire mixture shaken with 300 cc. of benzene though ether will suffice. This is allowed to stand for several days. The benzene solution becomes light amber, similar in color to the hadromal-benzene solution obtained by Grafe. A heavy emulsion appears between the water and benzene layers. It is fine wood particles and hydrolyzed stannous chloride. Care must be taken to avoid withdrawing this with the benzene. A large pipette is useful here. The benzene is now ready to be distilled in vacuum. A gum is

left which may be partially crystallized by using several successive hot portions of hot ligroin (B. P. 50-70°). Colorless needles were obtained in sufficient quantity to take a micro melting point, which due to gum present was not sharp, being 75-79. Czapek had reported 70-80 for his hadromal.

Taking five cc. of the benzene solution and diluting with three cc. of alcohol, we get a number of color reactions which in general agree with those described by Czapek.

Table of Hadromal Color Tests

Obtained by Hoffmeister and Czapek's Methods

Reagent	Without HCl		With HCl	
	Immedi- ately	After 3 hrs.	Immediately	After 3 hrs.
Phenol	---	---	---	red (3days)
Resorcinol	---	pale violet	---	pale violet
Hydroquinone	---	---	---	---
Beta Naphthol	---	---	---	---
Phloroglucin	---	red	red	red
Pyrogallol	---	---	---	---
2,4 dinitrophenyl hydrazine	---	---	yellow	yellow
Aniline	---	yellow	yellow	yellow
*Benzidine	red violet	red violet	---	---
Pyrole	---	red	red	black
Ferric chloride	red	red	---	---
Con. Sulfuric acid	red violet	red violet	---	---

* Carried out in acetic acid

Color Reactions of Hadromal Obtained by

Grafe's Method

In Sunlight

Reagent	Without HCl		With HCl	
	Immed- iately	After 3 hrs.	Immed- iately	After 3 hrs.
Phenol	---	---	---	pale orange
Resorcinol	---	pale violet	---	strong violet
Hydroquinine	---	---	---	---
Beta Naphthol	---	---	---	---
Phloroglucin	orange	olive green	light green	almost black
Pyrogallol	---	pale violet	---	pale violet
2,4 dinitro phenyl hydrazine	light orange	deep orange	deep orange	deep orange
Pyrogallol	---	pale violet	---	pale violet
*Aniline	---	yellow	yellow	yellow
**Benzidine	deep red	deep red	deep red	deep red
Pyrrole	red	black	red	black
Ferric chloride	---	---	---	---
Con. Sulfuric acid	green	green	---	---

* In sulfuric acid

** In acetic acid

Color Reactions of Hadromal Obtained by Grafe's Method

In Diffused Light

Reagent	Without HCl		With HCl	
	Immediately	After 3 hrs.	Immediately	After 3 hrs.
Phenol	---	pale red	---	pale red
Pyrogallol	---	---	---	---
Phloroglucin	---	---	red to olive green	to black
p nitro phenol	---	---	pale violet	deep purple
Mono methyl anilin	---	---	weak yellow	weak yellow
p toluidine	yellow	orange	lemon yellow	yellow
Quinoline	---	---	---	yellow
Naphthol	---	---	---	pale violet
Hydroxylamine	---	---	---	---
Hydroquinone	---	---	---	---
Methyl orange	slightly acid			

Reagent	Vanillin	Coniferyl aldehyde
	m.p. 82.5° b.p. 2.7 mm. 131° 2.8 mm. 132°	82.5° 2.5 mm. 157° 3.0 mm. 162° 5.0 mm. 175°
Conc. Sulfuric acid	yellow	red
Ferric chloride	blue green	no color in cold
Anilin	yellow	red yellow
Benzidine in Acetic acid	yellow	red violet (1:40,000)

Some interesting comparisons may be made by using the tables given so far, and by using a table comparing vanillin and coniferyl aldehyde which was prepared by Pauly and Feuerstein. It will easily be seen that Grafe and Czapek were working with different compounds and, therefore, should differ in their opinion as to the chemical nature of "hadromal".

In particular, it may be noted that benzidine dissolved in 50% acetic acid yields a red violet color with both Czapek's hadromal and with known coniferyl aldehyde but gives a brilliant scarlet with Grafe's hadromal. Pauly's table (1) indicates the probable dilution for a red violet color is 1:40,000. Known solutions of vanillin and furfural with benzidine give a scarlet like that given with Grafe's hadromal. Known coniferyl aldehyde gives a red color with ferric chloride and concentrated sulfuric acid. So does Czapek's hadromal. Grafe's hadromal gives no reaction with ferric chloride and gives a green with sulfuric acid.

With phloroglucinol both known coniferin and vanillin give red colors. Czapek's hadromal gives an orange color which quickly changes to olive green and finally to black. This follows the color changes described by Crocker for furfural.

1. Ber. 62, 308, (1929)

By allowing the hadromal obtained by Czapek's method to be oxidized five days in the air, the benzene solutions of it fail to give a red violet color with benzidine but gave a yellowish orange, which according to Pauly's table is the color given by vanillin with this reagent. The coniferin apparently has been oxidized to vanillin. The order of intensity of these tests agree with the order given by Crocker.

Of the new reagents tried 2,4 dinitro-phenylhydrazine was significant. With known furfural, it failed to react, but gave a yellow-orange with Grafe's hadromal, known coniferin and Czapek's hadromal.

Methyl furfural, as reported by Grafe, was detected by color tests given by Mulliken (1). Furfural, not proposed by Grafe, was detected by odor and precipitation with bromine in carbon tetrachloride as directed by Mulliken (2).

Pyrocatechin, proposed by Grafe, could not be detected by the following sensitive tests:

- (1) One tenth per cent formaldehyde † sulfuric acid (conc.)
† pyrocatechin gives a red violet ring (3).

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1. Mulliken Identification of Organic Compounds, Vol. 1.
 2. Mulliken Identification of Organic Compounds, Vol. 1, p. 24.
 3. Artero Ross, Bac. Chim. Far., 58, 265-70, (1919).

(2) Two cc. of water + sodium nitroprusside gives a green ring with pyrocatechin (1).

This is not definite proof that no pyrocatechin was present however. Due to the extremely small quantity of material present in the hadromal mixture, a negative test may mean that the test is not sensitive enough, as for example the ferric chloride test for vanillin is not given by Grafe's hadromal though all investigators grant that he has it in his mixture.

An attempt was made to run an absorption spectrum on the various hadromals but the results were worthless due to experimental difficulties and to the impurities of the compounds.

By running successively oak, spruce, and maple sawdust, it was found that apparently the differences in wood used caused no change in the type of product obtained. This was done because Grafe used spruce while both Hoffmeister and Czapek used oak. It was thought at first by the author that their divergent results might have been due to differences in method of obtaining it.

1. Lad Ekkert Pharm Centrollblatt, 67, 566, (1926)

As has been previously mentioned, ordinary methods of crystallization could not be used due to the extremely small quantity of material obtained by the different methods of extracting the wood. Therefore, this color test data the author has presented, served as an alternative. Now, the compound which apparently is identical with the hadromal obtained by Czapek's method is coniferyl aldehyde, but we must have some means of checking the unknown hadromal solutions obtained by the different methods, with a standard.

In order to have a reference compound with which to compare the unknown hadromals, coniferyl aldehyde was synthesized according to the directions of Pauly and Wascher (1).

The CH_3OCH_2 - ethers have been found to be an excellent means of protecting phenolic Hydroxyl groups which diminish

1. Ber. 56, 13, 603-10, (1923)

reactivity of the aldehyde group in vanillin and related aldehydes. These ethers are quite stable toward alkalis but are very sensitive to acids, so that after the aldehyde group has undergone the desired reaction, the phenolic group may be replaced by acid treatment. This is especially useful in making the phenol acroleins.

The true difficulty of the preparation of these aldehydes by the method of Pauly and Wascher lies in the condensations of p methoxy methylo vanillin with acetaldehyde. This compound was prepared thus: In 100 grams of toluene 8 grams of sodium were suspended, 20 grams of alcohol added and heated with mechanical stirring until ethyl formation. 33 grams of vanillin were added. An immediate conversion to sodium vanillate took place. This solid was dried, ground fine and suspended in 150 grams of toluene. 18 grams of chloromethyl ether was added and warmed at 60 degrees for four days. Excess of two per cent sodium hydroxide was added and allowed to stand several hours to remove unreacted vanillin.

The toluene was distilled at reduced pressure and the remaining solid distills at 140-43 degrees at two millimeters. A ten-gram yield was obtained on the first run and twenty-two grams on the second giving 25% and 58% yields respectively.

In 110 cc. of methyl alcohol and 170 cc. of water were added twenty grams of the product obtained above in a one-liter round bottom flask fitted with two dropping funnels, thermometer,

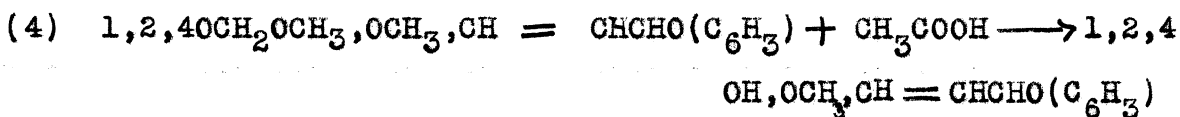
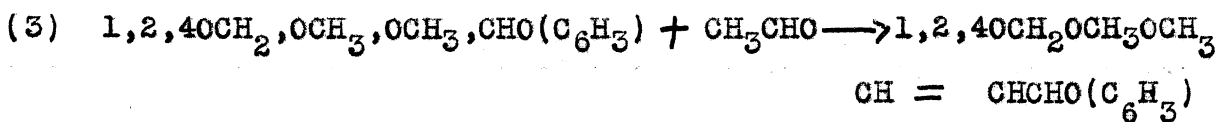
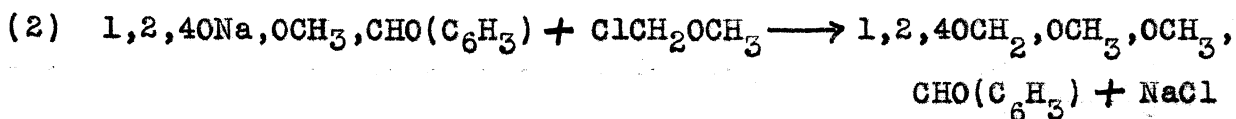
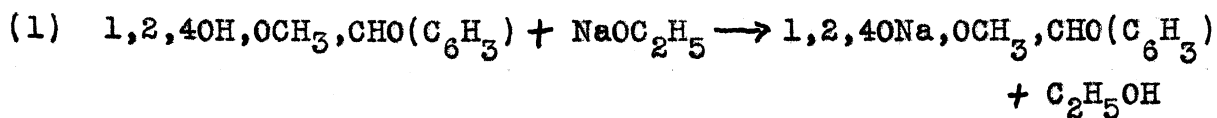
and reflux condenser. Eight drops of five per cent potassium hydroxide were added. It is important that the mixture react alkaline, but excess alkali must be avoided. This condition must continue throughout the entire reaction time while the condensation gradually uses up alkali.

About four or five drops of alkali are added hourly according to directions but it was found that more was needed to keep the solution alkaline. Every 15-20 minutes, five then three cc. of aqueous six percent acetaldehyde was added. A sure sign of reaction occurs when the solution becomes gold yellow without decomposing to a brown sludge. On the average, total consumption of alkali is eighty drops and of the acetaldehyde 150 cc.

After ten hours, the solution is allowed to cool and the alkali neutralized by a few drops of acetic acid, and the solution with a hundred. This solution is then boiled for one-half hour. Shake two times with fifty cc. of cold benzene, after drying with calcium chloride the benzene is distilled in vacuum. The remaining yellow oil distills. At 130-140 degrees, unchanged vanillin ester comes over, using two to four millimeters pressure. The coniferyl aldehyde distills at about 160-165 degrees. It solidifies in the receiver. Yield: seven and one-half grams or 35%. M. p. 78-80 degrees.

Charring occurs in the distillation despite the low pressure. However, the carbon may be removed by crystallizing in toluene.

To summarize the reactions:



THEORETICAL DISCUSSION

The specific mechanism whereby "Hadromal" is formed by the various methods is not known, but probably a hydrolysis of the pentosans and cellulose esters in the wood takes place. Supposedly, if the wood pentosans are hydrolyzed to furfural methyl furfural would be produced by hydrolysis of methyl pentosans. Whether vanillin and coniferin are produced by hydrolysis or are present in minute traces has been a subject for some discussion.

In summarizing the various color reactions which wood undergoes, it might be advisable to present portions of two tables of Phillips (1).

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1. The Chemistry of Wood Lignin, Chemical Reviews, Vol. 14, pp. 106-107, (1934)

Color reactions given by phenols and lignified materials

Phenol	Coloration	Investigator
Phenol	green - blue	Runge
O cresol	green - blue	Grandmougin
Thymol	green	Czapek
Catechol	blue	Wiesner
Resorcinol	blue	Wiesner
Hydroquinone	olive	Grandmougin
Phloroglucin	violet red	Wiesner
Pyrogallol	green	Wiesner
Orcinol	dark red	Lippmann
Alpha naphthol	green blue	Grandmougin

Color reactions given by amines and lignified materials

Amine	Color	Investigator
Aniline	yellow	Runge
o Toluidine	yellow	Grandmougin
p nitro aniline	orange	Bergi
Pynole	red	Ihl
Indole	cherry red	Niggl
Thalline	orange	Hagler
O phenylene diamine	orange brown	Grandmougin
alpha naphthylamine	yellow - orange	Nickel

From these tables, we see that the generalization that aromatic amines and phenols react may be made.

The nature of the color test reaction, I would suggest, is a fusing of the two benzene nuclei, namely those between the aromatic amine or phenol with the coniferyl aldehyde or vanillin for example. This would give compounds similar to the naturally occurring flavone derivatives like chrysin and quercetin. These compounds are highly colored and their hydrochloride salts are even more so. It was found that better color tests with "hadromal" were obtained in the presence of hydrochloric acid.

A lengthy theoretical discussion of the formation of "hadromal" from lignified materials would not be worth much because of the great amount of uncertainty regarding the nature of lignin itself. About all that is known is that certain groups are present which undergo characteristic reactions. The presence of methoxy groups has been used to give quantitative data of a crude sort. Lignin reacts with chlorine, and acetylation shows presence of hydroxyl groups. The presence of carbonyl and acetyl groups have been suggested but not proven.

It was hoped that the studies of the "hadromal" problem might throw some light on the chemical nature of lignin itself but no such results were obtained. It is so complex a material that much work must be done on it before we will have certainty regarding its structure.

SUMMARY AND CONCLUSION

To summarize: by the results obtained, we would say that essentially the work of Czapek was checked and slightly extended. The work of Hoffmeister could not be duplicated as to yield just as have other investigators had difficulty in doing. The existence of his two per cent yield of claimed product has yet to be proven.

The work of Grafe could not be completely checked and only two of his suggested products were found. The third was not. After some difficulty, coniferyl aldehyde was synthesized by Pauly and Wascher's method.

Much work was done in an attempt to obtain crystalline material in quantity. This failed. For this reason, color tests were made and compared. A great number of new reagents were tried for color production. A few were successful.

In view of the experimental data, we may draw the following conclusions:

- (1) "Hadromal" obtained by Czapek's method is, as he suggested, coniferyl aldehyde.
- (2) Grafe's "hadromal" at least contains furfural, methyl furfural, and vanillin.
- (3) A new color reagent was found, namely 2,4 dinitrophenylhydrazine.

- (4) The divergent results of Grafe and Czapek are due to a difference in method of extraction.
- (5) In general, the same type of product is obtained using spruce, oak, or maple sawdust.
- (6) Pauly and Wäscher's synthesis of coniferyl aldehyde will work.

Table of Special Reagents

Benzidine	2% in acetic acid
Ligroin	Commercial; b.p. 50-70
Stannous chloride	Commercial crystals
Coniferyl aldehyde	Yellow needles; for reference work
Vanillin	White needles; for coniferyl aldehyde synthesis
Wiesner's reagent	Phloroglucinol dissolved in hydrochloric acid
Chloromethyl ether	Acid free - for coniferyl aldehyde synthesis
Aromatic amine and aromatic phenol solutions	About 1/60 mole per liter of solid dissolved in alcohol for color tests.
2,4 dinitro phenylhydrazine solution	Four tenths per cent