AN ATTEMPT TO SEPARATE AND IDENTIFY "HADROMAL"

A Thesis

Presented to the Faculty of the Virginia Polytechnic Institute
as a Partial Fulfillment of the Requirements for the
Degree of Master of Science
in Chemistry

by

Byron Elwood Leach

Approved:

Director of Thesis

Head of Department

Chairman, Graduate Committee

ACKNOWLEDGMENTS

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INTRODUCTION

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This investigation was undertaken to further the work of Ray (1) in an effort to isolate, and identify the color producing product of wood which is known as "Hadromal". It was hoped that the divergent results of Czapek (2), and Grafe (3) might be harmonized regarding the chemical nature of "Hadromal".

A method of separation has been worked out by means of which the impure gummy "Hadromal" extract may be divided into four fractions of varying degrees of purity. Some compounds have been obtained from the reaction mixture in a pure state, and partially identified.

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(1) M.S. Thesis, Virginia Polytechnic Institute, (1938)
(2) Z. Physiol. Chem., 27, 141, (1899)
(3) Botan. Centrallblatt, 38, 753, (1889)

REVIEW OF LITERATURE

REVIEW OF LITERATURE

There has been considerable work done in the field of wood lignin in the past half century, and some of these early workers in this field were such men as Czapek, Nickel, Grandmougin, Grafe, Schulze, and others. These men were impressed by the various color reactions that woody tissues gave when treated with certain reagents such as phloroglucinol and hydrochloric acid and with phenol and hydrochloric acid.

Runge (1) was one of the first to observe that pine wood when treated with phenol and hydrochloric acid would turn a greenish-blue color, and with aniline sulfate, a yellow color. Since that time, many reagents have been used to determine what is in wood that gives the color tests. This has been a controversial subject for many years. The question remains to be solved whether or not the color producing constituent of woody tissue could be attributed to a minor compound that is present, or to some group in the lignin complex.

Nickel (2) showed that wood behaves like an aldehyde toward Shiff's reagent, and Singer (3) believed that these colorations could be attributed to coniferin or vanillin. Nickel later showed

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(1) Phillip's, Chemical Reviews, February (1934)
(2) Botan. Centrallblatt, 38, 753, (1889)
(3) Monatsh, 3, 395, (1882)

that after wood had been treated with bisulfite, it failed to give color tests.

The important work done in an attempt to determine the compound or compounds, present in wood, responsible for the various color tests was carried out by Czapek (1). He digested wood with stannous chloride for a long period of time, and extracted the product with benzene, and then distilled off the benzene from the residue. He extracted the residue with ligroin and in this manner was able to obtain an impure brownish product melting at 70°-80° C. He named his product "Hadromal".

Czapek was never able to obtain a pure product, although he partially purified it as the bisulfite compound. Hadromal reacted with concentrated sulfuric acid to give a strong red color, gave a red color with ferric chloride, reduced silver nitrate, gave a red color with phloroglucinol and hydrochloric acid, and a red color with Millon's reagent. He showed that the hadromal gave the same color tests that the untreated wood did. Ligroin was used as a fractional crystallizing medium because the hadromal was fairly insoluble in it, but very soluble in alcohol and ether, and partly soluble in water. Dilute alkali dissolved it producing a yellow color, and Czapek reported that this solution was a neutral one.

Wood, when completely divested of its hadromal, gives no color

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

tests. Czapek (1) was never able to positively identify his product, but suggested that the compound was chiefly coniferyl aldehyde. Due to the large amount of impurity and the small amount of product a complete separation was not possible.

Hoffmeister (2) and Crocker (3) supported Czapek's work. Hoffmeister used a continuous extraction apparatus in which he was able to prevent super heating, and was able to heat the reaction mixture to the temperature of boiling benzene which, he claimed, would prevent decomposition of the hadromal as fast as it was formed. Hoffmeister claimed that he obtained two grams of hadromal, which has been doubted by several investigators.

After refluxing the stannous chloride reaction mixture with benzene for a period of about twenty hours, and then distilling the benzene off over a hydrogen atmosphere he was able to prevent possible decomposition at this point. His isolated product had a molecular weight of 184. This molecular weight checks with coniferyl aldehyde and not with vanillin, 149.

Hoffmeister's hadromal by oxidation went to vanillin. The vanillin thus formed was identified. Hadromal showed the usual aldehyde reactions such as oxidation with dilute $K_4Fe(CN)_6$ giving $4(OH),3(CH_3O)C_6H_3CH=CHCOOH$. The reaction with para bromo benzoyl chloride indicates the presence of only one OH group. This pointed toward the fact that it was probably coniferyl

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(1) Z. Physiol. Chem., 27, 141, (1899)
(2) Ber., 60, 2062, (1927)
(3) Ind. Eng. Chem., 13, 625, (1921)

aldehyde. Hoffmeister (1) like Czapek (2), believed that hadromal was present in woody tissue to the extent of about three per cent. However, this was only an estimation as to the actual amount of hadromal in the wood.

Crocker (3) in his study of wood and the study of the color reactions in which he used the Maule reaction to determine soft wood from hard wood, agreed with Czapek in that the color producing properties could be attributed to a minor constituent in the wood. After studying the spectrum of some of the colors produced and comparing them with a number of known solutions of aldehydes that might be present in wood, Crocker 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 sassafrass 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.

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(1) Ber., 60, 2062, (1927)
(2) Z. Physiol. Chem., 27, 141, (1899)
(3) Ind. Eng. Chem., 13, 625, (1921)

Some of the workers that violently disagreed with Czapek (1) are Grafe (2), Pauly and Feurstein (3). Grafe regarded the chemical nature of hadromal not as coniferyl aldehyde, but as a mixture of methyl furfural, vanillin, and pyrocatechin, but Czapek later showed that no combination of the three mentioned compounds could duplicate the properties of hadromal. Pauley and Feurstein agree with Grafe, and point out that the mixed melting points of coniferyl aldehyde and hadromal lowers the melting point about twenty degrees.

Klason (4) seemed to think that his alpha-ligno-hydro-sulfonic acid was the SO_3H derivative of coniferyl-paraldehyde; but Pauly and Feurstein disagreed with him on that point. They claimed, that neither Klason nor Hoffmeister had a trace of coniferyl aldehyde because of the method used by Pauly and Wascher (5) in the synthesis of the compound.

Risi and Labrie (6) in their work on the aromatic materials of maple syrup attribute the aroma to the formation of hadromal in the course of cooking the syrup. In regard to hadromal there can be very slight quantities of other aromatic materials present. The destructive sublimation of hadromal gives vanillin, guaiacol, and vanillic acid, as does the

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(1) Z. Physiol. Chem., 27, 141, (1899)
(2) Botan. Centrallblatt., 38, 753, (1889)
(3) Ber., 6213, 297-311, (1929)
(4) Ber Hauptversammlung des Vereins der Zellstoff u, Papier Chemiker, pp. 52,53 (1908)
(5) Ber., 56 B, 603-610, (1923)
(6) Can. J. Research, 13 B, 175-184, (1935)

aromatic extract of maple syrup. They seem to think that Czapek's (1) hadromal is formed by catalytic synthesis beginning with the fragments called lignin.

Their investigation of the flavor of maple syrup showed that it depended to a great extent on a phenolic substance which is associated with a crystalline aldehyde melting at 74°-76° C., and similar in odor and properties to vanillin. This aldehydic substance is the principle one, and the extract may contain other aldehydic substances, but in minute quantities.

Risi and Labrie came to the conclusion that hadromal is composed of furfural, vanillin, and guaiacol, and not of methyl furfural, vanillin, and pyrocatechin as thought by Grafe (2). Risi and Labrie (3) were only able to obtain traces of hadromal. It was obtained by extraction of the syrup with ether, then the dried residue extracted with petroleum ether. In this manner they obtained needle-like crystals in a yellow oil which was dried on a porous plate, and recrystallized from petroleum ether. These crystals gave several color reactions; with concentrated sulfuric acid it gives a yellow to chocolate brown color, ferric chloride, a light blue, aniline a pale yellow, and with phloroglucinol and hydrochloric acid a rose color.

Hoffmeister (4) in his method, claimed to have produced coniferyl

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(1)Z. Physiol. Chem., 27, 141, (1899)
(2)Botan. Centrallblatt., 38, 753, (1889)
(3)Can. J. Research, 13B, 175-184, (1935)
(4)Ber., 60, 2062, (1927)

aldehyde by the condensation of acetaldehyde with vanillin, but he failed to protect the OH group with an ester residue. Repeated trials to prepare coniferyl aldehyde by this method have met with failure, ninety per cent of the vanillin being recovered. The melting point diagram of coniferyl aldehyde with vanillin shows that this method of preparation is worthless.

Grandmougin (1) in his paper presented from the Polytechnic Laboratories in Zurich, gives a good summation of a number of color tests, and a few generalizations in regard to the nature of these tests, and the effects of certain groupings. In general, he shows the aromatic amines, and the hydroxy aromatic compounds react. In general, the amines are more reactive than the phenolic reactions.

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(1) Z. Farben Ind. 5, p. 321-323, Jan., (1906)

THEORETICAL DISCUSSION

THEORETICAL DISCUSSION

Because of the uncertainty of the structure of lignin, a lengthy discussion of this substance "Hadromal" would be worthless. All that is known about lignin is that there are certain groups present such as a hydroxyl, and methoxy groups. Lignin will undergo acetylation, which has been used in a crude way to determine the number of hydroxyl groups. Different kinds of wood all give a different amount of lignin, and the combustion methods show that the chemical constitution of the different lignin extracts is nonhomogeneous. Whether hadromal is a part of the lignin complex, or present in the wood not chemically combined with lignin is still unknowm.

In the study of this substance, hadromal, it is evident that there are only minute quantities of it present in wood. From a 400 gram sample of wood it is possible to obtain only traces of crystals. This agrees with the results obtained by Risi and Labrie (1).

The white solid that was isolated in this investigation by extracting the complex hadromal residue with ligroin, seemed to be pure. The melting point was sharp at 78° C. This compound was proven not to be a color producing compound of wood, when treated with phloroglucinol and hydrochloric acid. This compound gave the usual aldehyde reactions,

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(1) Can. J. Research, 13B, 175-184 (1935)

such as a positive test with Shiff's reagent, and precipitate with phenylhydrazine. It gave a slight greenish-blue color with ferric chloride showing that it contains a hydroxyl group. No definite structural formula can be given yet because of the minute quantities of this material isolated, and more work will have to be done to establish the formula.

Hoffmeister (1) claimed to have obtained two grams of hadromal which has been doubted by several investigators, and could not be duplicated in this investigation.

Because of the large amount of impurities, which it was impossible to eliminate by the method proposed by Czapek (2) in his work on this product, a new method was worked out which it is believed will eliminate some of the difficulties. This method makes it possible to separate the extract into four distinct parts. It consists of extracting the benzene solution of hadromal with solutions of 5% $NaHCO_3$, Na_2CO_3, and NaOH respectively, which divides the benzene solution into four groups of varying degrees of acidity. No references could be found in literature that used this method of separation.

The sodium bicarbonate extract removes the more acidic compounds. The sodium carbonate extract contains a less acidic group. These two extracts are the color producing fractions, when treated with such reagents as phloroglucinol and hydrochloric acid, while the other two fractions do not give that test. By extracting the bicarbonate and mostly the carbon-

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(1) Ber., 60, 2062, (1927)
(2) Z. Physiol Chem., 27, 141, (1899)

ate extracts each with ligroin it was possible to obtain minute crystals that are almost free from oils. Because of the minute quantities of the crystals isolated and the oil coating on them it was only possible to classify the compounds in a general way.

Whether coniferyl aldehyde is alone responsible for the various color tests that woody tissue gives when treated with certain reagents could not be definitely proved. Coniferyl aldehyde as prepared by Ray (1) gives the same color tests with phloroglucinol and hydrochloric acid, and similar odor to the small crystals that were isolated.

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(1) M.S. Thesis, Virginia Polytechnic Institute, (1938)

CONCLUSIONS

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(1) It has been shown that hadromal obtained by Czapek's method (1), as he proposed, does contain coniferyl aldehyde.

(2) A hydroxy aromatic aldehyde which does not give the characteristic color test of woody tissue with phloroglucinol and hydrochloric acid was isolated from the hadromal mixture.

(3) A new method of separation was found, namely, extracting the benzene solution of hadromal with 5% solutions of $NaHCO_3$, Na_2CO_3, and NaOH respectively.

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

EXPERIMENTAL DATA

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This method of obtaining hadromal made use of a continuous extraction apparatus, essentially the same as Hoffmeister's (1). He used an ordinary Soxhlet jacket with a reflux condenser connected to the top and a two liter flask underneath. The flask was filled two-thirds full with benzene, then in the Soxhlet jacket he placed the wood meal mixed with stannous chloride, and the benzene boiled up, and was allowed to drip through the mixture. The method used in this experiment was essentially the same as the above, only instead of the Soxhlet tube a large flask was used which would hold a charge of sawdust of approximately 400 grams. By using this large flask more sawdust could be used and more hadromal obtained from each run.

During the course of this investigation, a total of 30 runs were made. The following discussion of the various products isolated was a combination of two or more runs. Because of the minute quantities of the substances present micro methods were employed in obtaining the data on the different compounds.

The sawdust (A) was first degreased, so as to remove some of the gum that was present in the wood, and this was done by allowing the sawdust to stand overnight in an alcohol-benzene solution. Then 400 grams of this degreased sawdust (B) that has been filtered to remove the

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(1) Ber., 60, 2062, (1927)

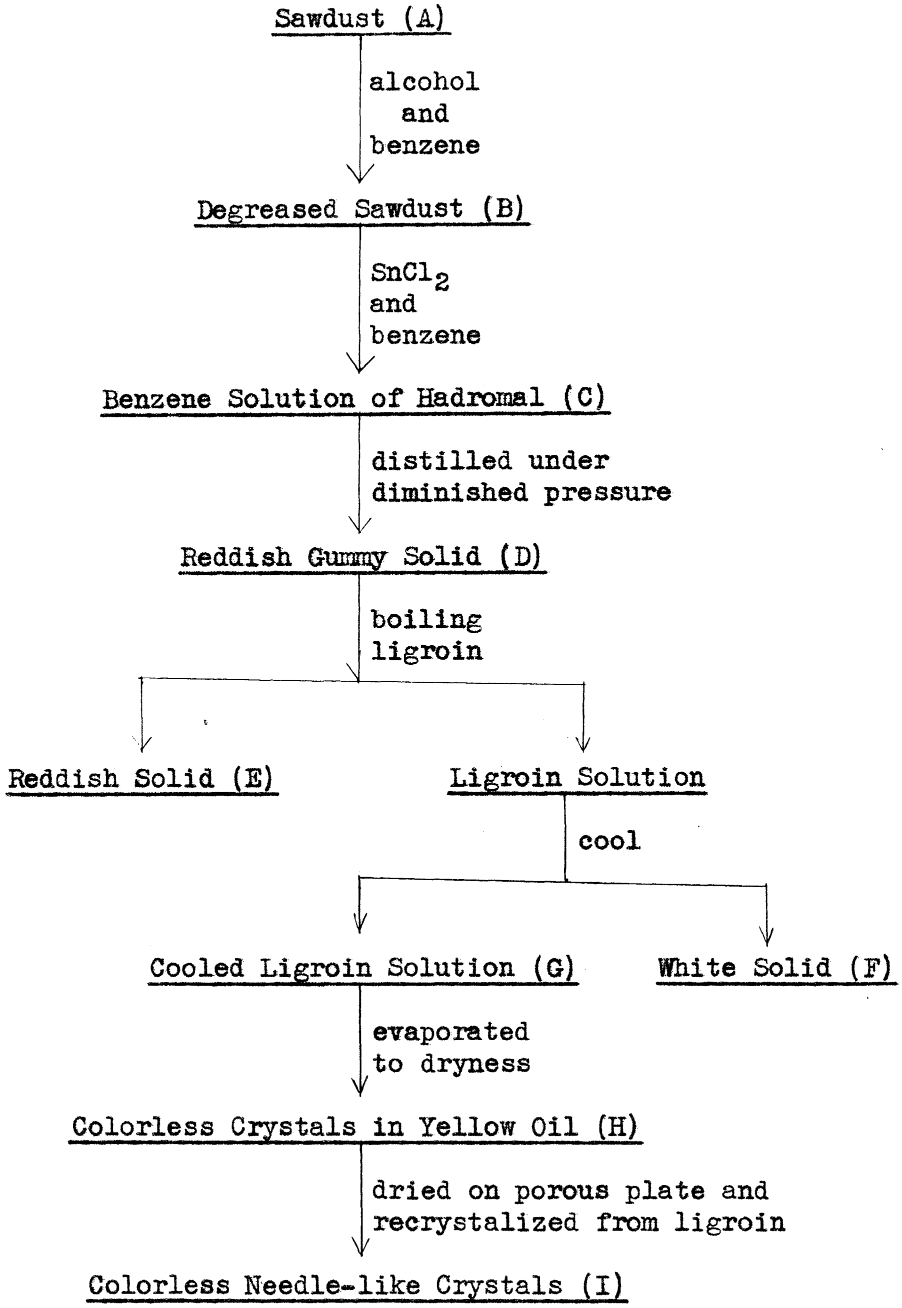
The Apparatus Used in This Investigation

A- The benzene flask which collects the hadromal
B- The heating unit for the benzene flask
C- The flask which contains the reaction mixture
D- Pneumatic trough which contains water
E- A second heater used to heat the pneumatic trough
F- Tube to return condensed benzene vapors to reaction flask
G- Return tube to benzene flask
H- Condenser

alcohol-benzene solution was mixed with 150 grams of stannous chloride, and made into a thick paste with distilled water, which was then placed in the extraction flask. The apparatus was set up as shown in the picture, and the condensed benzene vapors were allowed to bubble through the reaction mixture for a period of about 30 hours. The benzene solution gradually assumed a red color, although the color depends upon the previous treatment with alcohol-benzene solution and the time that the reaction has been allowed to proceed. The sawdust, after having been divested of its hadromal assumed a flesh color, and failed to give a color test with phloroglucinol and hydrochloric acid. Then the benzene solution (C) was removed, and distilled under diminished pressure to a small volume, which prevented the hadromal from decomposing. The remaining solution was allowed to go to dryness.

The solid (D) obtaimed was a reddish gummy product. From a 400 gram sample of sawdust it was possible to obtain about seven grams of this reddish product.

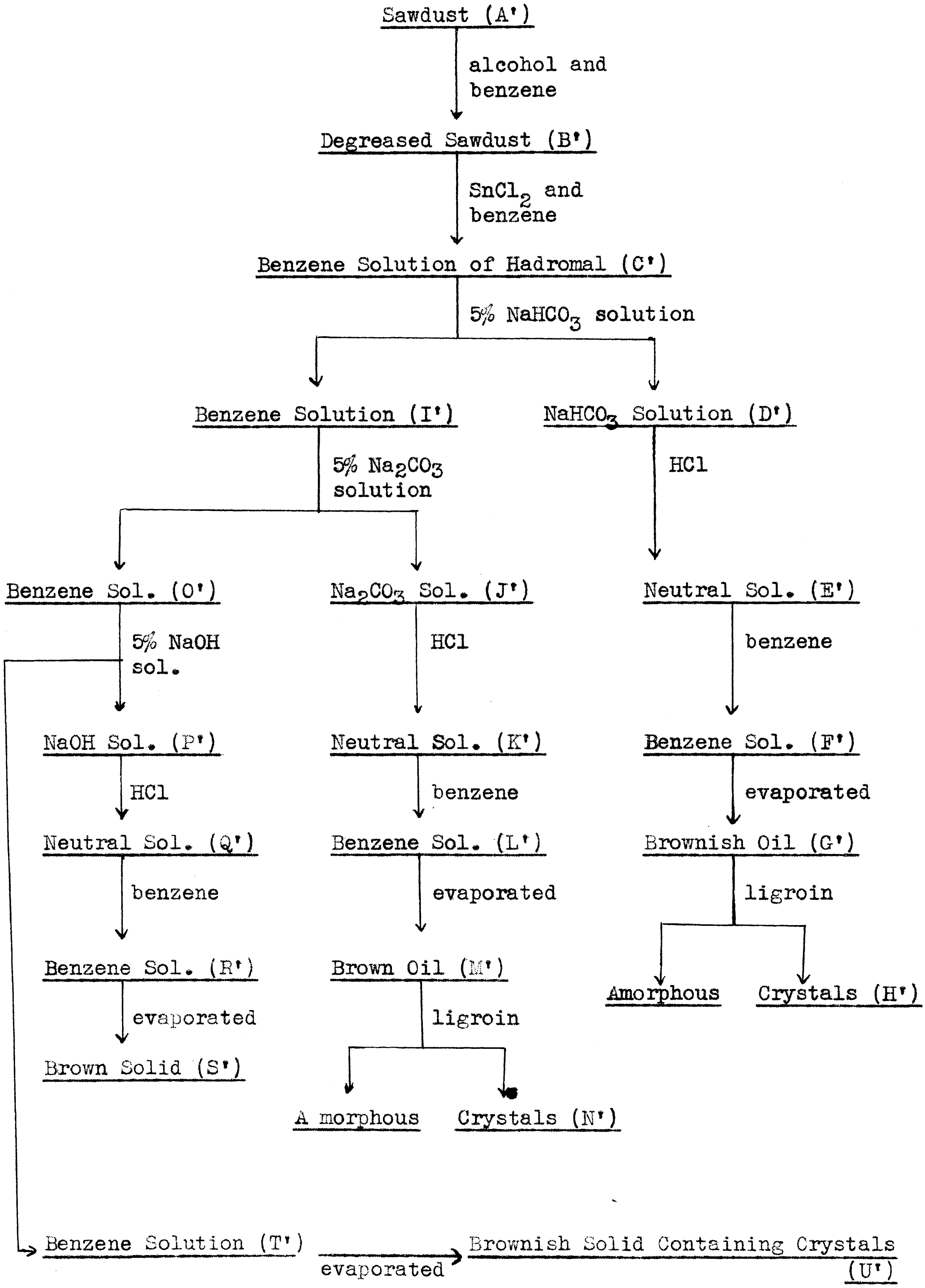
Ligroin (boiling point 50-70° C.) was used as a fractionsl solvent in an effort to find a means of separating this reddish gum (D). The gum was refluxed for a few minutes with boiling ligroin, and of the seven grams obtained two grams dissolved, leaving a reddish-brown solid (E), which did not dissolve on further treatment with that solvent. This solid (E), evidently a mixture of phenolic compounds was soluble in dilute NaOH producing a yellow color. It gave a red color with ferric chloride, rose color with phloroglucinol and hydrochloric acid, a reddish color with fuming sulfuric acid, and decolorized bromine water. Nc


Sawdust (A)
alcohol and benzene
Degreased Sawdust (B)
$SnCl_2$ and benzene
Benzene Solution of Hadromal (C)
distilled under diminished pressure
Reddish Gummy Solid (D)
boiling ligroin
Reddish Solid (E)
Ligroin Solution
cool
Cooled Ligroin Solution (G)
White Solid (F)
evaporated to dryness
Colorless Crystals in Yellow Oil (H)
dried on porous plate and recrystalized from ligroin
Colorless Needle-like Crystals (I)

solvent was found which gave a crystalline product, and it was dissolved in dilute sodium hydroxide and then neutralized with dilute hydrochloric acid in an effort to obtain a cryatalline material, but no results were forthcoming.

From the ligroin solution on cooling after refluxing with the reddish gum (D), an impure amorphous product fell out of solution, which turned red quickly on standing, but several reprecipitations from ligroin gave a white solid (F). This white solid (F) was rather stable, melting at 78° C. It dissolved with difficulty in dilute sodium hydroxide giving a yellow color, dissolved on heating in alcohol, was soluble in ether, and benzene, colored fuming sulfuric acid brown, and was insoluble in sirupy phosphoric acid. The test with ferric chloride showed that it had one or more phenolic OH groups. It gave a positive test with phenylhydrazine, and with Shiff's reagent, but a negative test with phloroglucinol and hydrochloric acid. This solid underwent acetylation easily with acetyl chloride. Experimental evidence showed that this white solid (F) was an aromatic hydroxy aldehyde, but because of the minute quantity that was isolated it was not possible to run a carbon hydrogen determination in order to determine the empirical formula.

The cooled ligroin solution (G) was concentrated in vacuum to a small volume to prevent decomposition of any compound that might have been present. Then the remaining solution was allowed to go to dryness. This left a yellow oil(H) with small colorless needle-like crystals dispersed throughout. Most of the oil was absorbed on a porous plate, and the crystals were recrystallized from ligroin. This gave needle-like crystals (I) that were optically active, and showed parallel extinction when placed


Sawdust (A')
alcohol and benzene
Degreased Sawdust (B')
$SnCl_2$ and benzene
Benzene Solution of Hadromal (C')
5% $NaHCO_3$ solution
Benzene Solution (I')
$NaHCO_3$ Solution (D')
5% Na_2CO_3 solution
HCl
Benzene Sol. (O')
Na_2CO_3 Sol. (J')
Neutral Sol. (E')
5% NaOH sol.
HCl
benzene
NaOH Sol. (P')
Neutral Sol. (K')
Benzene Sol. (F')
HCl
benzene
evaporated
Neutral Sol. (Q')
Benzene Sol. (L')
Brownish Oil (G')
benzene
evaporated
ligroin
Benzene Sol. (R')
Brown Oil (M')
Amorphous
Crystals (H')
evaporated
ligroin
Brown Solid (S')
A morphous
Crystals (N')
Benzene Solution (T')
evaporated
Brownish Solid Containing Crystals (U')

between crossed nicols of a polarizing microscope. These crystals have a similar odor and a similar crystalline structure to that of known coniferyl aldehyde. These crystals gave a positive test with Shiff's reagent, and gave the same test with phloroglucinol and hydrochloric acid that the untreated wood does. This agrees with the results that Czapek (1) obtained for his hadromal.

In an effort to obtain a more complete separation of the various products in benzene solution of hadromal, and to obtain a sufficiently pure compound to positively identify, several methods were used, However, none were sucessful in obtaining an absolutely pure compound. The best method that was found to give a separation is in outline form on the preceeding page, which will be referred to from time to time to avoid confusion during the following discussion.

The sawdust was extracted in the same manner as explained in the previous method. Then the benzene solution of hadromal (C') was extracted with a 5% solution of sodium bicarbonate in a separatory funnel until the extraction was complete. The extracts were combined and neutralized with dilute HCl, giving a neutral solution (E'), which was extracted with benzene.

This benzene solution (F') was distilled in partial vacuum to a small volume. The remaining solution was allowed to evaporate to dryness in the open. The residue , after all the benzene had evaporated was a brownish oil (G'), which showed no tendency to form crystals. This oil

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

gave a precipitate with phenylhydrazine, and gave a positive test with Shiff's reagent. The oil (G') was refluxed with ligroin, and the ligroin solution evaporated to dryness. Then this dried product formed minute crystals (H').

The benzene solution (I') was hand shaken as done in the previous case, but for this extraction a 5% solution of sodium carbonate was used. The benzene solution was extracted several times, and the carbonate solutions combined. This gave a dark solution (J'), which on neutralization with dilute HCl went colorless. Then this neutral solution (K') was extracted with benzene, which gave a slightly yellow benzene solution (L'). This benzene solution when evaporated under reduced pressure gave a brown oil (M') that had an aromatic odor. This oil when refluxed with ligroin went partly into solution with difficulty. The part that went into solution when evaporated forms needle-like crystals (N') in a white amorphous solid that was identical in appearence to the white solid obtained by the previous method mentioned. It was noted that these crystals and the white solid had the same odor as that of known coniferyl aldehyde. These crystals gave a rose color with phloroglucinol and hydrochloric acid, also gave a positive test with Shiff's reagent, and a precipitate with phenylhydrazine. This shows that these crystals were probably an aldehyde, which was similar in odor and in crystalline structure to coniferyl aldehyde.

The benzene solution (O') that was left from the sodium carbonate extraction, was extracted with a 5% NaOH solution as in the previous cases. The NaOH extraction was neutralized with HCl, and extracted with benzene. This gave a yellowish benzene solution (R'), which was evaporated under reduced pressure to dryness. The dried residue was a brown solid (S') that showed no tendency to form crystals, and was odorless.

The benzene solution (T') after the NaOH extraction, was evaporated to dryness under reduced pressure. This residue was a brown oil containing colorless, and needle-like crystals (U'). However, because of the lack of time it was not possible to identify these crystals.

SUMMARY

SUMMARY

The work of Czapek (1) was partially checked and slightly extended. The results of Risi and Labrie (2) were checked. Hoffmeister's (3) work was checked, all except the two per cent yield which he claimed to have obtained.

A considerable amount of work was done in an effort to obtain sufficient crystalline material to identify. This was only partially realized. It was possible to obtain crystals in a yellow oil by extracting the hadromal mixture with ligroin. These crystals were aldehydic in nature. Because of the large amount of oil present it was not possible to obtain crystals pure enough to identify positively. It is believed in view of the experimental evidence that these crystals were coniferyl aldehyde. They gave similar color tests, were identical in crystalline structure, and similar in odor to a known sample of coniferyl aldehyde.

The white solid that was isolated from the hadromal mixture by the method of Czapek seemed to be pure, as shown by the melting point which was 78°C. This compound was partially identified as a hydroxy aromatic aldehyde. This compound was not a color producing component of woody tissue which was shown by the fact that when treated with such

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(1) Z. Physiol. Chem., 27, 141, (1899)
(2) Can. J. Research 13B, 175-184, (1935)
(3) Ber., 60, 2062, (1927)

reagents as phloroglucinol and hydrochloric acid, no color was produced.

Because of the large amount of impurities obtained by the previous method, a new method of separation was worked out, which consisted of extracting the benzene solution of hadromal with 5% solutions of $NaHCO_3$, Na_2CO_3, and NaOH respectively. This separated the hadromal mixture into four distinct groups with varying degrees of purity. The first two extracts on treatment with boiling ligroin gave minute crystals almost free of oils that were identical in crystalline structure, and similar in odor to coniferyl aldehyde. These first two fractions gave the characteristic color reactions of woody tissue, while the other two fractions failed to give these tests.

The next step is to take the four fractions of the hadromal mixture, and work out a suitable method of purification. In this manner it is believed that it will be possible to isolate and identify the components of the complex mixture.