# A STUDY OF HEMLOCK BARK; SOME REACTIONS OF HEMLOCK TANNIN

Thesis for the Degree of M. S.
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# STUDY OF HEMLOCK BARK; SOME REACTIONS OF HEMLOCK TANNIN.

This thesis is respectfully submitted to the Faculty of Michigan State College in partial fulfillment of the requirements for the Degree of Master of Science.

By

Charles E. Aho

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CHARLES E. AHO

# C O N T E N T S

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# PART ONE

A. INTRODUCTION.

It is a commonly accepted fact that the forests of our country are rich in products that have merely to be extracted and converted into innumerable synthethic products that a chemist alone can visulize.

Among the most important classes of extractable constituents of forest growth are the leaf oils, wood oils, turpentine, resins, balsam, cascara, tannin, to say nothing of the various products such as wood naphthol and acetone that have been made commercially possible by destructive distillation.

Because of all these extracts and the vast number of products that can be synthesized from them, required by man, a vast chemical industry has been built around the raw products that are derived from the forests as a source.

To appreciate the vast resources of forest products attention is invited to an article (1) where it is shown that there are 798 billion board feet of wood in only the States of Idaho, Washington and Montana, available for chemical industries. It is clearly demonstrated that the northwest alone will be in a position to compete for many decades as a source or supply or forest products. These supplies, at present are being converted on a limited scale, by chemical industries into plastics, pulp and derived

products of one sort or another.

A matter of still greater importance, however, is expressed in the same article by the fact that this region alone is capable of producing annually, a huge quantity of forest raw products by nature alone with but little effort on the part of man.

Chemists of the northwest region have for a long time visualized the sizeable industries possible from forest growth insofar as the available supplies of charcoal, producer gas, acetone, methanol, ethanol, sugars, tars, or the many other products which can be totally realized from the forest growth of this particular region.

The possibilities are made still more cheerful when it is realized that in Western Oregon alone there is going to waste one and a half billion board feet of wood, predominantly coniferous, mainly because of the failure to remove them by the chemical industries. The by-products of the logging operations, so to speak, amounts to 6,000,000 cords of material representing about twenty per cent of the original stand. Waste of saw-log size material alone amounts to about one and half billion feet board measure. In addition to this, there is about 5000 feet per acre left as trees which will be windblown or destroyed in the usual slashing fires.

The Pacific Northwest, possesses a vast supply of tannin but has not as yet capitalized to any great extent on this source. The bark of western hemlock is quite as rich in tannin as the eastern hemlock. By applying a factor of bark to each cubic foot of wood, it has been calculated that there are 66 million cords of bark in this particular region of our country alone, as the offal of paper pulp operations.

From this great resource, therefore, might be imagined the huge industries necessary to convert the otherwise useless material and waste into profitable products.

Turning now to regions more familiar to us, one has only to study conditions in our own State of Michigan, where, excepting for the smaller scale, similar conditions exist.

Among the various forest products that are being devastated in our State and the one that has particularily attracted the author, is the Canadian Hemlock ( Tsuga Canadensis., Carr.). This tree is distributed throughout the State in abundant quantities. Little, however, can be said as to its value as a raw product for chemical industries. The choice of the wood is commonly converted into building lumber of a comparatively inferior grade. The needles, branches, roots, stumps and left-overs of the trunk are customarily left as waste to be burned and destroyed by the logger's slashing fire. This can be

personnaly verified by the author's experiences and cases which he has observed in the northern counties of our State.

The bark of the tree is marketed, as conditions warrant, on a more or less restricted scale. It is mainly used as a source of tannin for the leather industry. At one time it was quite widely used as a tanning agent especially in areas in which the tree was native. Since the disceveries of numerous other more preferrable tannin bearing forest growth such as the oak galls, myrobalans, divi-divi, algarobilla and valonia, the use of hemlock tannin has grown exceedingly into discredit, even in the areas in which the bark is native.

This is explained not by the fact that hemlock tannin is inferior in tanning properties, which is not the case, but by the fact that hemlock tannin imparts upon the tanned leathers a deep red color which is characteristic of the tannin extract. For this reason therefore, hemlock bark is not used quite as extensively as it should be for tanning purposes.

Ellegatannin, gallotannin and the others which have gained preferrence with the tanners because of their equal tanning properties coupled with the ease of controlling the color of the finished leather are, through necessity being imported from the other parts of the world.

So, when it is realized that rather than use a product which is so native to us, much trouble and expense is resorted-to getting a substitute from all parts of the world, can it be fully appreciated that hemlock tannin has lost its resource value as such, and that other uses must be developed for this abundant product.

Particularily with this in mind, the laboratory research to be explained in later pages was conducted.

B. DISCUSSION AND CLASSIFICATION OF TANNINS.

Of the numerous methods suggested for classifying tannins, one of the latest methods as advocated by Perkin and Everst (2) seems to have the most popular support. By their scheme, all of the tannins are divided into three groups as follows:

I - Tannins related to depsides

II - Tannins related to diphenyldimethyloid

III - Phlobaphene-producing tannins: phlobatannins.

The particular group into which a tannin will fall, is determined by boiling the tannin with a dilute mineral acid. By this treatment, the members of Group I are converted into the crystalline fission products, gallic acid and glucose. The members of group II on the other hand respond to this treatment by being converted into ellagic acid and glucose. The latter group, however, when subjected to a dilute boiling acid treatment are completely converted into dark (red or brown) colored amorphous, insoluble products called phlobaphenes.

The members of group I are found abundantly in pathological growths: those of group II being found chiefly in certain nuts and pods; whilst those of group III are found widely spread through nature in wood, bark, leaves and roots.

Of the three groups, the latter group is the most im-

portant, due to the fact that most tannins belong to this class and chiefly because only one member each of groups 1 and 11 have been established with certainty ( 3 ).

Hemlock tannin is a phlobatannin, in that, it is one of the phlobaphene producing type. For the reason that hemlock tannin is a phlobatannin, and since the work and explanations to follow center around hemlock tannin, a brief discussion of the properties of this group seems important.

Chemically, ( 4 ) the phlobaphenes are products of dehydration of the respective tannins from which they are derived, or, in other words, they are formed from the tannins by the loss of one or more molecules of water (4). In this way they are produced by the action of dilute acids on tannins and they may even be formed by pouring alcoholic or highly concentrated aqueous solutions of the tannins into cold water under which conditions the tannin seems unable to assimilate water and the phlobaphene separates as red precipitate.

Phlobaphenes exist ready formed in most tannin materials capable of producing them, and may be dissolved out of these or the dried extracts thereof, by means of alcohol.

The phlobaphenes are (4) difficultly soluble in pure or acidulated water or in pure ether, but soluble in water containing ammonia. They are freely soluble in spirit.

They are readily dissolved by dilute alkalis, alkaline carbonates and by borax. The solubility of phlobaphenes in water depends upon the degree of hydration, many tannins giving a whole series of dehydration products, of which those containing only one molecule of water less than the original tannin, are quite soluble in water, while the higher members of the series become less and less soluble in water. The soluble phlobaphenes are the coloring matters and behave like tannins themselves, precipitating gelatin and combining with hide to form leather.

Hemlock bark yields a series of such bodies (4) of which the lower members are deep red soluble tannins and the higher members form the red sediment which occurs in hemlock extract. It is not possible to decolorize hemlock extract without at the same time reducing its tanning powers, though by preparing and concentrating it at low temperatures, the proportion of insoluble higher anhydrides formed may be kept at a minimum.

## C. BRIEF HISTORY ON THE RESEARCH ON THE CONSTITUTION OF THE HEMLOCK TANNIN MOLECULE.

In 1884, Bettinger (5) conducted an examination of hemlock tannin for the purpose of establishing its chemical formula. His work dealt with the bromine derivative of hemlock tannin which he produced by brominating the tannin extract (5). As the result of his work, Bettinger proposed the formula $C_{20}H_{18}O_{10}$ for hemlock tannin. This formula was more or less generally accepted for a period of many years.

About thirty years later, however, Manning and Nierenstein (6) conducted a rather extensive investigation of hemlock tannin and although failing to draw any definite conclusions as the result of their work, they did definitely disprove Bettinger's chemical formula of hemlock tannin. This conclusion was made (6) after a careful reinvestigation of Bettinger's work and new results obtained as follows:

(1) Bettinger's bromination method does not always yield the same product $C_{20}H_{14}O_{10}Br_4$, which requires Br = 43.60 per cent., but a series of compounds in which the bromine content varies from 40 to 49 per cent. The analyses of eleven preparations gave: Br = 41.28, 47.56, 43.88, 40.12, 43.20, 47.29, 44.56, 41.74, 48.48, 41.56 and 43.26 per cent.

(2) If Bettinger's product is obtained, it can be

fractionated into a number of compounds in which the bromine varies from 40 to 48 per cent. The fractionation method employed consisted of dissolving the brome compound in acetone and fractionally precipitating by the addition of chloroform.

Due to the amorphous character of the tannin and because of the seeming impossibility of isolating tannin in a pure state, very little work was done at this time to contribute to the knowledge of hemlock tannin.

More recently, however, by the investigations of Russell and Todd (7) (13), considerable light has been brought upon the exact constitution of hemlock tannin.

Guided by a knowledge of the fission products which result from hemlock tannin and the properties of certain known phenolic patterns, from which the fission products might possible result, coupled together with exact carbon and hydrogen determinations of pure tannin, a chemical compound, namely, bis (7,8,3',4', tetra hydroxy) flavpinacol, (fig. 1 ) was synthesized by these men, in a round about fashion, which has proved to reproduce exactly the qualitative properties of natural hemlock tannin.

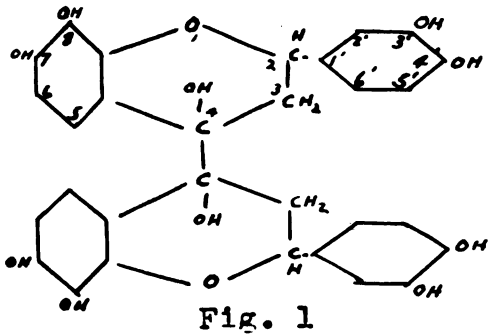
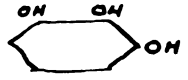
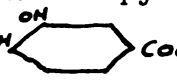


Fig. 1

To this date, however, the only known fission products of hemlock tannin are pyrogallol,  , and protocatechuic acid,  , and for this reason these investigators conclude their discussion of the synthesis with the explanation " Should another phenolic residue be found eventually amongst the decomposition products of hemlock tannin, the results just described ( qualitative comparisons) would at least indicate that flavpinacols hydroxylated on the 7,8,3',4' pattern are phlobatannins".

## D. DISCUSSION OF FISSION EXPERIMENTS PREVIOUSLY CONDUCTED ON HEMLOCK TANNIN.

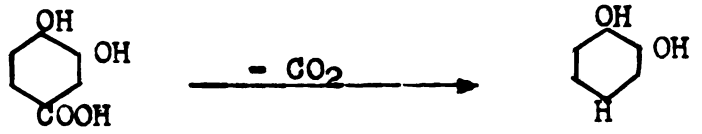
As previously stated the only known fission products of hemlock tannin are pyrogallol and protocatechuic acid. Nierenstein (6) observed this fact, partly at least, when he subjected hemlock tannin to an alcoholic potassium hydroxide hydrolysis. The only definite product obtained by him was protocatechuic acid which he observed to crystallize from water in small needles melting at 191-194°. During the hydrolysis, carbon dioxide was evolved. No pyrogallol was reported found.

He later experimented with aqueous barium hydroxide with little success. Still later, he employed a methyl-alcoholic solution of barium hydroxide which gave considerable protocatechuic acid as a hydrolytic product. The hydrolysis consisted of heating hemlock tannin with an excess of methyl-alcoholic barium hydroxide for eight to ten hours under reflux. This hydrolysis yielded protocatechuic acid and an aldehyde substance with formula $C_{10}H_8O_3$. This product reacted with phenyl hydrazine to form the phenyl hydrazone $C_{16}H_9O_2N_2$. When oxidized with potassium permanganate in alkaline suspension it formed the acid $C_9H_7O_2 \cdot CO_2H$. These tests, therefore, established the product as an aldehyde.

It is known (4) that when tannins are subjected to the action of a boiling alkaline solution for a number of hours and finally concentrated to a state of incipient fusion, they are broken up with formation of products depending upon their constitution.

Hemlock tannin when subjected to caustic alkali fusion yields protocatechuic acid and pyrogallol. When subjected to dry distillation, it yields catechol and pyrogallol.

In this case it is believed that the relationship between the product of fusion (protocatechuic acid) and the product of dry distillation (catechol) is merely the loss by the latter of carbon dioxide as follows:




**Protocatechuic Acid** **Catechol**

## E. POSSIBLE COMMERCIAL USES OF FISSION PRODUCTS AND UTILIZATION OF WASTE PRODUCTS OF HEMLOCK TANNIN.

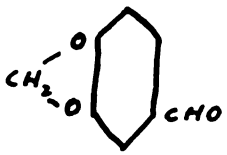
Of the two fission products of hemlock tannin, pyrogallol and protocatechuic acid, there are many commercial uses for the former. Its powerful reducing properties had found valuable uses in the precipitation of metals such as gold, silver and mercury from solutions of their salts. Its antioxidant behaviour as evidenced by the rapid absorption of oxygen by caustic aqueous solutions of pyrogallol has found extensive use as a means of oxygen determinations in gases. In addition to these it is widely used together with hydroquinone as developer in photographic work.

For protocatechuic acid, however, little can be said excepting for the many possible products that can result by chemical synthesis using protocatechuic acid as a starting substance.

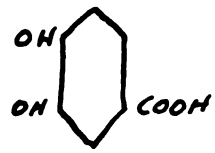
With this thought in mind and without delving too deeply into the commercial possibilities at this juncture, a study of two important commercially prepared substances, namely, piperonal and vanillin, which in structure, resemble protocatechuic acid closely, was made. In a sense, protocatechuic acid bears little relationship to these products, but their approximate similarity leads one to wonder concerning the feasibility of their synthesis from this source.

This similarity might better be appreciated by a study of the

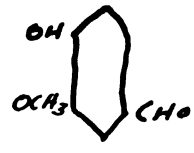
following molecular figures of the respective molecules.




Piperonal




Protocatechuic acid




Vanillin

Inspired by the possibility, a literature study was made of the chemistry involved to effect the synthesis. Both substances are derivatices of benzaldehyde, piperonal being the 3,4 methylene dioxy derivative, and vanillin being the 3 methoxy, 4 hydroxy derivative. The basic chemistry involved therefore seems to be to convert the carboxyl group into an aldehyde group and by methylating the aldehyde so derived by some means, the product can be controlled to form into either piperonal or vanillin.

Porter (8) outlines as one of the methods of preparing an aromatic aldehyde, the application of heat to a mixture composed of the salt of an aromatic acid and a formate according to the following equation:

$$C_6H_5COONa + HCOONa \longrightarrow C_6H_5CHO + Na_2CO_3$$

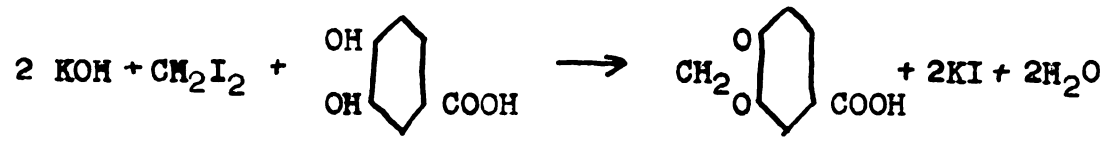
Sod. benzoate Sod. formate Benzaldehyde

The above equation might be the basis for studying the preparation of protocatechuic aldehyde:

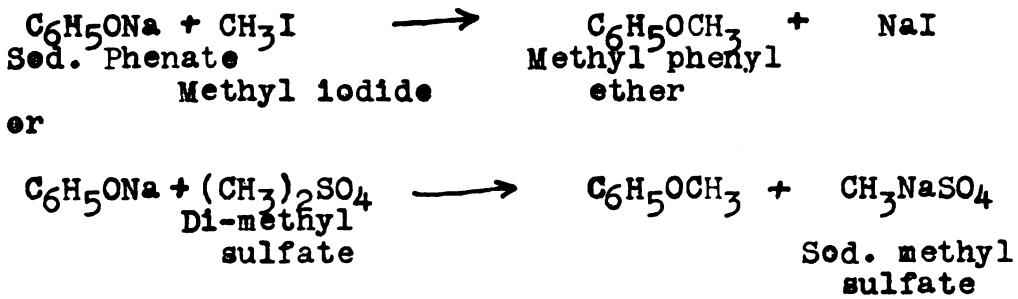
$$C_6H_3(OH)_2COONa + HCOONa \longrightarrow C_6H_3(OH)_2CHO + Na_2CO_3$$

Sod. Protocatechuate Sod. formate Protocatechuic Aldehyde

To bring about the methylation of protocatechuic acid a method outlined by Porter (9) might be used as follows:



To produce the 3, methoxy derivative of protocatechuic aldehyde, Ullmann's reaction or some modification thereof (10) might be employed. This reaction is shown by the following equation:



Having thus formulated to some extent two seemingly important uses that might be made of protocatechuic acid and having already discussed the commercial importance of pyrogallol, the other fission product of hemlock tannin, a short discussion seems necessary on the possible commercial utilization of the waste bark after removal of tannin.

The Pacific Lumber Co., of California, after discovering insulating properties in redwood bark, are converting this otherwise waste material into a useful insulating material. The bark which was formerly a nuisance and a liability is now processed and baled for fill-insulation. Uses similar to this might be devised for the waste tannin-free hemlock bark.

The waste bark might also be converted by destructive distillation into such chemicals that might result from dry

distillation. The charcoal by-product can be converted into activated carbon, a valuable product for which new uses are being found daily. Activated carbon is highly adsorptive and is becoming more and more useful in recovering solvents, in clarifying and deodorizing dry cleaning fluids, decolorizing and deodorizing edible oils and acids, in sugar refining and in purifying drinking water. These uses are being constantly increased.

Left in the bark after water extraction of tannin, are certain resins, some waxes, lignin, which cements the cellular material, and lastly of importance, the cellulose making up the foundation of the bark itself.

Lignin is a plastic material (1) which under proper conditions of manufacture and plasticization is capable of being molded or pressed in many different shapes and sizes. Lignin, also, however, forms a rather brittle resin, requiring the addition of fibrous material such as asbestos to increase the strength of the molded product considerably.

The commercial uses for resins, waxes and cellulose needs no explanation. Their value will depend upon the amounts present and the cost of obtaining them from the bark. No information could be found about the amounts of the various substances present.

# PART II

REPORT OF LABORATORY RESEARCH.

A. Discussion of work plan.

As explained in the previous pages, the main purpose of this work centers around the isolation of tannin from hemlock bark, which, by some convenient means, would be broken up into its corresponding fission products, namely, protocatechuic acid and pyrogallol. Of secondary importance is the utilization of the waste bark remaining after the extraction of the tannin.

Bearing in mind the time that would be necessary to undertake a thorough study of the entire problem, a work plan, to be followed as time would allow, was made along the lines of isolating tannin first, and then, in turn, isolating the fission products, primarily for data purposes, somewhat as follows:

(1) Water extraction (cold and hot) of hemlock bark to determine the quantity of water and bark and the most suitable time of extraction to produce a maximum yield of crude tannin.

(2) A study of hemlock bark to determine the amount of free sugar present and a study of the crude tannin to determine if depsides or glucosides are present and their amount.

(3) Preparation of chemically pure tannin to determine the feasibility of using pure tannin in the fission work to follow.

(4) Preparation of hemlock phlobaphenes to determine the feasibility of using phlobaphenes in the fission work to follow.

(5) Experiments using aqueous solutions of caustic potash to determine the effect of concentration, time and temperature on the fission of hemlock tannin.

(6) Study of the effect on hemlock bark on standing for a period of time insofar as the yield of water extractable solids are concerned.

B. Extraction experiments.

Contained within the bark and extractable with water are soluble tannin, free sugar and possibly a trace of depsides. Of the total solids extracted, however, and making up the greater part of the whole, is soluble tannin.

Without considering the possible botanical effect of climate, time of the year and such other conditions that might effect the tannin content of hemlock bark, the bark used in the experiments to follow was removed from freshly felled trees in the Northern Michigan during the month of February. The whole bark was allowed to dry at room temperature over night to remove for the greater part the frozen moisture natural for this period of the year. Before drying the bark consisted of 20% moisture and considerable difficulty was experienced in grinding, thus necessitating the removal of the excess moisture.

After drying over night, the bark was broken-up in a shredding mill until it was the size of ordinary sawdust. In this condition it was allowed to air-dry for several days so as to bring the moisture content into equilibrium with the atmosphere. It was next collected and placed in stock bottles and tightly corked. The material was used from these bottles as needed.

The cold water extractions were carried out in a

six liter Erlenmeyer flask equipped with an efficient stirring rod.

The cold water extraction experiments consisted of nine experiments using, each time, equal amounts of water and bark, varying only the time of extraction. After each extraction the liquid was hurriedly filtered from the solids by means of two-eight inch suction funnels each attached to a four liter suction flask. Due to the presence of a large amount of finely divided solids and a consequent hinderance in filtering, it was necessary that two such units be used concurrently to insure adequate speed of filtration.

At the end of each extraction, a 500 ml sample of the filtrate was taken and evaporated to dryness on a steam bath and from the weight of the residue, the total solids in the filtrate was computed.

The following cold extraction data were recorded:

**Table 1 - Effect of time upon the extraction of water-soluble constituents from hemlock bark.**

Wt. crushed air-dried bark used in each experiment-200g.

% moisture in bark - - 8

Volume water used in each extraction - -5000 ml

Extraction temperature ( room temp.) - - 22.5° C.

| Extraction Time | Weight Residue | Total solids Extracted | % Solids extracted (Dry basis) |
|---|---|---|---|
| 7 min. | 1.98 g | 19.8 g | 10.8 |
| 15 min. | 2.01 g | 20.1 g | 10.9 |
| 30 min. | 2.02 g | 20.2 g | 10.9 |

( data continued )

| Extraction time | Weight Residue | Total solids Extracted | % Solids extracted ( Dry basis ) |
|---|---|---|---|
| 1 Hour | 2.05 g | 20.5 g | 11.1 |
| 2 Hours | 2.09 g | 20.9 g | 11.4 |
| 4 Hours | 2.17 g | 21.7 g | 11.8 |
| 8 Hours | 2.31 g | 23.1 g | 12.6 |
| 24 Hours | 2.32 g | 23.2 g | 12.6 |
| 72 Hours | 2.45 g | 24.5 g | 13.3 |

As can be seen from the above figures, the solids extracted by water, are for the greater part extracted almost immediately.

A large volume of water compared with the weight of sample was purposely taken to insure an adequate amount of solvent for the purpose of these tests. To insure the adequacy of solvent, several extractions were carried out on the side, in identically the same manner as explained above, varying only the amount of water, that is, using both lesser and larger amounts than that used in these tests and there was found to be no change in the amount of extractable solids per given weight of bark. Having thus been assured of the adequacy of water, the amount used was therefore adopted.

As the bark was mixed with water and the stirring started, an immediate coloration in the water was observed indicating that extraction had begun. Hemlock extract poss-

esses a deep wine red color and clear when fresh, but when allowed to stand for a day or longer, a gradual clouding is observed. From all appearances a precipitation takes place as evidenced by the gradual clouding to a darker dirty brown. In the meantime filtering failed to completely separate the liquid from the fine suspension of solids.

The solids remaining after evaporation of the extract were of a uniform dark brown color. The solids deposited alond the sides of the evaporating dish, as the water was removed, in a hard glassy mass and the more concentrated substance at the bottom was left in a thick hard cake.

Removing from the dish, the solids so obtained, with a spatula and grinding, left a dark brownish-red powder possessing little or no odor compared with the characteristic odor of hemlock bark.

One of the peculiarities of hemlock tannin might be mentioned at this time, in that, when an attempt was made to clean the evaporating dish from the solids using water, the supposedly soluble solids were extremely slow in dissolving. The solids no longer seemed to possess the rapid dissolution in water as was the case in the original extraction. Soaking the dish with water for upwards of an hour was necessary before the solids could be entirely freed from the sides of the dish, although there seemed to be every indication that the solids were dissolving, except at an extremely slow rate.

Having obtained the desired data relative to cold water extractions, a series of hot water extractions were next conducted to obtain similar data and ascertain the effect of temperature on the extraction.

Having established and explained previously that the quantity, above a certain limit, of water used, for the extraction, has no bearing upon the amount of extractable solids obtained, to facilitate ease of handling and to avoid the use of overly large equipment, the volume of water used in the tests to follow was cut to 2500 ml. The same weight of bark was used as heretofore. Each test was carried out in a five liter flask attached to a reflux condenser. Four experiments were conducted for varying lengths of time from thirty minutes to three hours. The mixture was allowed to reflux for the specified time and then hurriedly filtered while hot. As was done before, a 500 ml sample was evaporated to dryness and the total solids computed. When the filtrate reached room temperature it changed to a brown cloudy solution with no signs of precipitation.

The following results were recorded:

Table 2 - Effect of temperature on hot extraction of soluble constituents from hemlock bark.

| | | |
|---|---|---|
| Weight air-dried bark used in each experiment | - | 200 g |
| % Moisture in bark | - | 10 |
| Volume of water used | - | 2500 ml |

| Reflux Time | Weight Residue | Total solids Extracted | % Solids extracted (Dry basis) |
|---|---|---|---|
| 30 min. | 9.6 g | 48.00 g | 26.7 |
| 1 Hour | 9.62g | 48.10 g | 26.71 |
| 2 Hours | 9.621g | 48.12 g | 26.72 |
| 3 Hours | 9.63g | 48.15 g | 26.73 |

Again complete extraction had taken place, practically speaking, before the end of the first interval. The solids obtained upon evaporation appeared identical with these derived from the cold water extraction.

After separating the bark residue from the refluxed mixture and allowing it to dry, a marked difference could be seen in it compared with the bark residue remaining after cold extraction. The bark residue was much lighter in color and appeared "more spent" in that it seemed to contain only the fibers making up the body of the bark, where after the cold extraction, very little change in color and appearance took place.

No effort was made to determine the decided increase in the amount of solids resulting from hot water extraction compared with the cold.

C. GLUCOSE DETERMINATIONS TO DETERMINE AMOUNT OF FREE GLUCOSE AND DEPSIDES IN THE BARK.

Often found along with certain phlobatannins (7) are free sugars and traces of foreign glucosides which in structure are closely related to depsides. The depisides when hydrolyzed with dilute mineral acids yield gallic acid and glucose and if other means of decomposition are employed, pyrogallol, presumeably by decarboxylation of gallic acid, and glucose are formed. This reasoning has been applied therefore to explain the increase in glucose content which often accompanies the acid hydrolysis of some phlobatannins.

With this in mind therefore, and to check the possible presence of sufficient free sugar and glucose forming bodies in the bark that might ultimately contaminate the crude tannin for the experiments to follow, it was believed necessary at this point to experiment along these lines to acquire data bearing upon the problem.

To promote this end, a fresh water extract of hemlock tannin was prepared by mixing 100 grams of bark with 2500 ml of water and throughly stirring the mixture for fifteen minutes.

To determine the presence and extent of free glucose, as such, in the freshly prepared untreated extract, a 50 ml sample was removed and the glucose determined according to the Defrens-O'Sullivan method (12) for sugar analysis.

To the sample was added a sufficient quantity of basic lead acetate to precipitate all traces of tannin which would seriously effect the determination to follow. The solution was filtered free from the lead tannate and then treated with anhydrous sodium carbonate to remove any excess lead from the solution.

15 cc of freshly prepared Fehling's solution A was mixed with 15cc of Fehling's solution B in a 250 cc flask together with 50 cc distilled water and placed in a boiling water bath for 5 minutes. Then 25 cc of the tannin-free liquid was run rapidly into the solution. The mixture was allowed to remain in the bath for 15 minutes and then rapidly filtered through a Gooch crucible containing a layer of asbestos fiber 1 cm thick. The crucible had been prepared previously and checked for constant weight. The $Cu_2O$ that was formed in the reduction was left in the crucible and it was throughly washed with water to remove alkaline impurities. The Gooch crucible was dried and heated to a dull red over a Meaker flame for fifteen minutes. The red $Cu_2O$ was now oxidized to black CuO. The Gooch crucible was transferred to a desiccator, and after allowing to stand for a sufficient time, quickly weighed.

The following data was recorded:

| | |
|---|---|
| Weight of air-dried bark used | 100 g |
| % Moisture | 7 |
| Volume water used | 2500 ml |
| Extraction time | 15 min. |

Total weight solids extracted ---------- 10.05 g

% Solids extracted (dry basis) ---------- 10.8

Weight glucose in the extract ----------310.0 mgs.

% Free glucose in bark (dry basis)---------- .3

Having determined the amount of free sugar in the bark, a series of hydrolysis experiments were next conducted to determine the effect of acid hydrolysis on the water extract and thereby determine the extent, if any, of glucosides present.

The original filtrate, from which 50 cc had been removed for free sugar analysis, was treated with 200 ml of 1.05 N HCl. The mixture was gently refluxed for one hour and another 50 cc sample was removed and a sugar determination made of it. To the remainder of the solution was added another 200 ml of 1.05 N HCl and refluxed again for one hour and another sugar determination was made. This was repeated until the original extract had been refluxed for five hours with increasing concentrations of HCl as shown by the following data:

| | | |
|---|---|---|
| Volume of extract (Start) | --- | 2450 ml.* |
| Total solids in solution (Previously computed) | --- | 9.849 gr.* |
| Glucose content (To start) | --- | 295 mgs.* |

* These amounts remained after removal of the 50 cc used for free sugar determination.

Table - 3 Effect of time of acid treatment upon the glucose content of hemlock bark extract.

| Time Reflux (Hours) | HCl Added cc 1.05 N | Wt. glucose in solution ( mgs. ) | Wt. increase of glucose ( mgs. ) | % increase in wt. from original |
|---|---|---|---|---|
| 1 | 200 | 432 | 137 | .2 |
| 2 | 200 | 420 | --- | .2 |
| 3 | 200 | 417 | --- | .2 |
| 4 | 200 | 410 | --- | .2 |
| 5 | 200 | 408 | --- | .2 |

## Calculations

On the basis of 432 milligrams of glucose in the 2450 cc of original extract after the first hour of hydrolysis, the original 2500 cc would have contained :

2450 / 2500 = 432 / X X = 480 mgs.

Increase in glucose (based on original 2500 cc) =

480 - 310 = 170 mgs.

% by weight increase in glucose (based upon weight dry bark) =

.170 / 94 = .0018 or approximately .2%.

These determinations seemed to indicate that there was no increase in the glucose content after the first hour of reflux, and based upon this content the sugar had increased about .2 per cent. This slight increase seems difficult to accept as indicative of the presence of depsides or other glucosides, the difference being so slight especially in light of the low concentration of glucose, that it could almost be taken as a limit of error in the glucose analysis.

It was not considered that the sugar in the amount present would affect in any manner the course of the reaction to be studied and hence no attempt was made to remove it.

## D. PREPARATION OF PURE HEMLOCK TANNIN TO DETERMINE FEASIBILITY OF USING PURE TANNIN FOR FISSION WORK.

Of the two best known methods for preparing pure hemlock tannin, namely, that of Nierenstein (6) and Russell (7), the method employed by Russell was used chiefly bevause of its simplicity and the fact that water is used in the initial stage as an extracting agent.

The method consists of extracting freshly ground bark with water. The crude tannin is separated from the solution by salting out with sodium chloride. The tannin precipitates as an amorphous solid. This is collected and dried in a vacuum and the tannin separated from traces of salt and other impurities by extraction with acetone in a Soxhlet apparatus. The acetone tannin mixture is removed to a steam bath and heated till the extract becomes very viscous. It is then transferred to a vacuum desiccator where the tannin rapidly puffs up and dries. By washing the dried material with ether, a moderately homogenous product results. The product is dried in an oven for 24 hours at 100 - 120° C. Tannin retains traces of solvents obstinately requiring these conditions to free it from the solvents.

A freshly prepared solution of hemlock tannin in water was prepared and the procedure followed carefully. The following results were obtained:

Weight air dried bark used (dry basis) -------- 300 gr.

Volume water used for extraction ---------- 5000 ml.
Solids extracted (by analysis) ---------- 33 gr.
Weight pure tannin recovered ---------- 5 gr.
% by weight pure tannin recovered from bark ------- 1.7.

At the point in the procedure where salting out is carried out, it was observed after adding two portions of 200 grams salt each, a large amount of tannin still remained in the solution. Believing the amount of salt to be inadequate, increasing amounts of salt were added until the saturation point of the solution was reached. After each increase, more and more tannin could be seen salting out of solution, but even at the saturation point, the solution still possessed a deep red coloration indicating that not all of the tannin had been salted out.

The filtrate after separation of the solids, was permitted to stand for two days during which time a precipitate seemed to be gradually separating out and the solution changing simultaneously from the typical red color to a lemon yellow.

From this it was gathered that if tannin can be completely separated out by salting, it was a very long process and without a doubt, not a method feasible for commercial practices.

The precipitate resulting from salting out, while still in the liquid was of a pleasing light pink color, which, immediately after separation from the liquid and in con-

tact with the air, changed to a buff color. Upon drying the color changed to a deep maroon.

## E. PREPARATION OF HEMLOCK PHLOBAPHENE TO DETERMINE FEASIBILITY OF USING PHLOBAPHENES IN FISSION WORK.

It has been demonstrated by Allen (4) that the hemlock phlobaphenes can be as conveniently converted into the fission products of hemlock tannin as can the pure tannin. Furthurmore, the lead salt of tannin can be used for the fission work or any combination or mixture of the three. With this in mind and to alleviate the fear that the crude tannin might still be contaminated with foreign materials in quantities to affect the fission work, it seemed important at this point to conduct experimentation on the preparation of phlobaphenes to determine the technique involved in their isolation.

Phlobaphenes as explained before, are produced by the boiling treatment of tannin with a dilute mineral acid. A fresh water extract of tannin was prepared by treating 200 grams of air dried bark with 5000 ml of water and stirring for fifteen minutes. Analysis of the filtrate showed that there was a total solid content of 20.001 grams.

Because of the difficulty of handling such a large amount of solution, 2500 cc of the whole was taken for these tests.

The tannin extract containing roughly 10 grams of solids, was placed in a 5 liter flask together with 500 cc 1.05N HCl and refluxed for an hour. During the reflux period, the solution remained a deep red in color with no indication of precipitation during boiling. The solution was cooled and

when room temperature was reached, a slow but gradual clouding was observed with still no signs of a precipitate settling to the bottom. After the solution was allowed to set over night, a red amorphous precipitate had settled to the bottom. The precipitate was separated and upon drying it turned into a pink powdery substance weighing 4.915 grams, roughly 50% of the total solids in the solution.

The filtrate on the other hand, remained a clear deep red solution indicating a marked concentration of tannin remaining.

A series of refluxes were carried out with increasing amounts of hydrochloric acid to determine the effect of HCl concentration on the precipitation of phlobaphenes. The entire solution was refluxed on five different occasions for one hour, each time increasing the HCl content by 200 ml 1.05 N HCl. In each instance, the solution was allowed to set over night to enable the phlobaphenes to separate out.

The following results were obtained:

Table (4) - Effect of time and amount of acid upon the yield of phlobaphenes.

Volume original solution ---------- 2500 cc

Solids in original solution ---------- 10.001 g.

| Time of reflux | 1.05 Norm. HCl added | Wt. phlobaphenes precipitated | Solids Remaining |
|---|---|---|---|
| 1 Hour | 200 ml | 4.9150 g | 5.0860 g |
| 2 Hours | 200 ml | 1.3100 g | 3.7760 g |
| 3 Hours | 200 ml | .7730 g | 3.0030 g |

Data continued -

| Time reflux | 1.05 Norm. HCl added | Wt. phlobaphenes precipitated. | Solids Remaining |
|---|---|---|---|
| 4 hours | 200 ml | .2607 g | 2.7423 g |
| 5 hours | 200 ml | .1105 g | 2.6318 g |

The solution at this point was still a deep red in color indicating that the tannin had not been entirely precipitated. Of the total solids only 74% had been recovered by conversion into phlobaphenes.

E. FISSION OF CRUDE TANNIN BY ALKALI (AQUEOUS) HYDROLYSIS.

As explained in previous pages, hemlock tannin has been demonstrated to break into its fission products when hydrolyzed with methyl-alcoholic potassium hydroxide. Better success has been claimed using methyl-alcoholic barium hydroxide and refluxing for eight to ten hours. When fused with potassium hydroxide, similar products are obtained, but no quantitative figures are given by the investigators.

Believing that a fission of hemlock tannin might result using less drastic means than fusion with potassium hydroxide and without having to resort to hydrolysis with methyl-alcoholic solutions of potassium and barium hydroxides, a series of experiments using aqueous potassium hydroxide were conducted to determine the effect of KOH concentrations, temperature and time of reflux on the fission of hemlock tannin.

As stated before, previous investigators had observed that when fission is taking place, carbon dioxide is evolved presumeably by the decarboxylization of protocatechuic acid into catechol. Hence, an apparatus was set up (fig. 2), consisting of a reflux condenser and a stirrer ( mercury seal) attached to a three liter three necked flask. To the end of the condenser was attached an absorption train consisting of a tube containg commercial " Dehydrite " to remove the moisture from any carbon dioxide which escapes from the reaction mixture and then in turn a tube containing commercial " Ascarite " to absorb any carbon dioxide es-

caping from the reaction mixture. To the ascarite tube was attached a calcium chloride tube to prevent the ascarite from absorbing atmospheric moisture. A thermometer was placed in the remaining neck of the flask to indicate the reflux temperature.

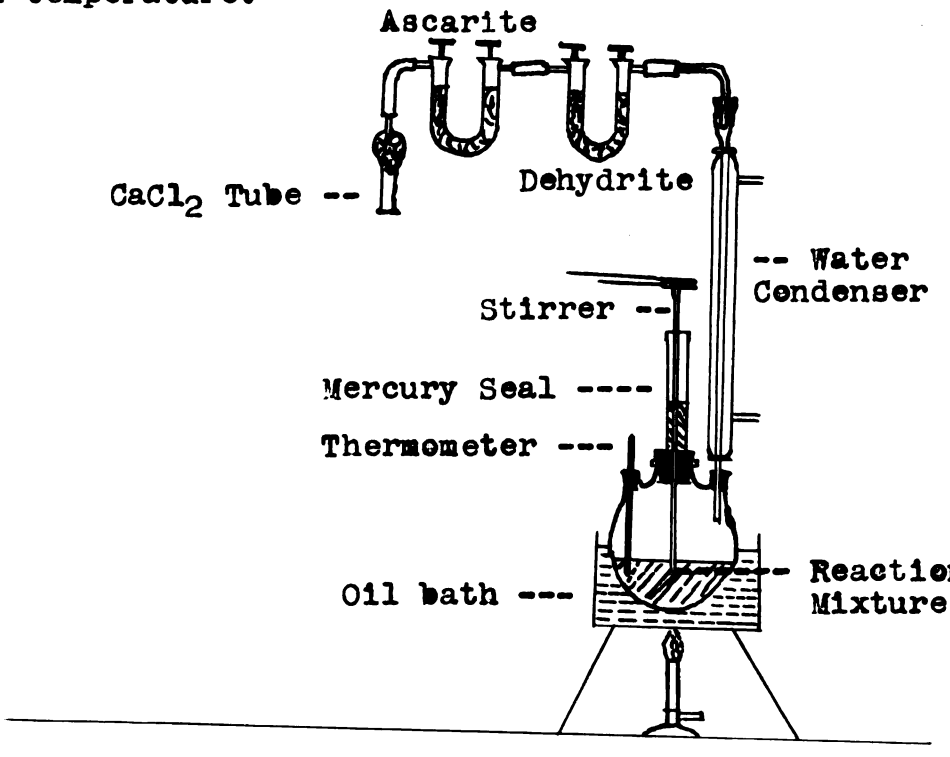



Fig. 2

Hemlock tannin was freshly prepared by cold extraction and treated with varying concentrations of aqueous potassium hydroxide. For each concentration the time was varied from two hours of reflux to five hours of reflux.

The following results were obtained:

Table 5 - Effect of potassium hydroxide concentration and time upon the reaction.

Volume aqueous KOH solution used in each reflux - 150 cc

Wt. crude tannin used in each experiment - 20 g.

| KOH Conc. | Wt. * KOH | Reflux time | Reflux Temp.°C | Substance Formed | Material balance (Organic) |
|---|---|---|---|---|---|
| 10% | 16 g | 2 hrs | 108 | Black tarry mass. | 19.5 g |
| 10% | 16 g | 5 hrs | 108 | " | 19.5 g |
| 20% | 36 g | 2 hrs | 121 | " | 19.5 g |
| 20% | 36 g | 5 hrs | 121 | " | 19.5 g |
| 40% | 84 g | 2 hrs | 142 | " | 19.5 g |
| 40% | 84 g | 5 hrs | 142 | " | 19.5 g |
| 60% | 147 g | 2 hrs | 160 | " | 19.5 g |
| 60% | 147 g | 5 hrs | 160 | " | 19.5 g |
| 80% | 217 g | 4 hrs | 182 | " | 19.5 g |
| 85% | 235 g | 4 hrs | 204 | " | 19.5 g |

* These weights taken from " Handbook of Chemistry and Physics" by Chemical Rubber Publishing Co., 22nd Edition, page 1137.

After each trial, the reaction mixture was treated with distilled carbon-dioxide-free water and the solution then removed from the flask. Enough water was then added to enable the caustic solution to be filtered free from such insoluble materials as might be present. The filtrate was then neutralized with dilute hydrochloric acid until slightly acidic as indicated by litmus. The solids resulting from the neutralization were next separated from the liquid by filtration. The resulting filtrate was next treated with activated carbon and boiled to dispel the dark coloration. This treatment was repeated until the filtrate was water clear.

The filtrate was evaporated to dryness and the organic

materials separated from the potassium chloride by repeated washings with ether. The combined ether washings were evaporated to dryness to determine the amount of organic materials extracted.

The activated carbon residue was likewise washed with ether and the ether washings evaporated to determine the amount of organic materials extracted.

The solids resulting from neutralization were in like manner treated with repeated washings with ether and the ether evaporated to determine whether any of the desired product was absorbed or mixed with it.

By this method of treating the reaction mixture, the desired products, protocatechuic acid and pyrogallol, if present, would be freed from the inorganic salts and other impurities, and would show up after evaporation of the ether washings. Protocatechuic acid exists in the diluted caustic solution as potassium protocatechuate. After neutralization and slight acidification, the potassium protocatechuate is converted into the acid. Pyrogallol is unaffected.

Protocatechuic acid (11) is soluble in cold water to the extent of 1.82 grams / 100 cc water and in water at $80^{o}$ to the extent of 27 grams / 100 cc. Prtogallol is soluble in cold water to the extent of 62.5 grams / 100 cc water. Catechol is soluble in cold water to the extent of 45 grams / 100 cc $H_2O$.

Therefore, because of the relatively small amount of tannin used and by using a large amount of water (3-4 liters) for dilution, both substances if present, would be, for all practical purposes, in solution from which they could be recovered.

The dark brown solution remaining after neutralization was caused by some impurity and it was therefore necessary to remove it before proceeding to recover the desired substances.

The water clear solution remaining after boiling with activated carbon should contain, for the greater part, the two substances to be recovered and catechol. Since, both substances are very soluble in ether, and since tannin and its dehydration products are insoluble in ether, this method was used to recover the fission products from the evaporated filtrate, thereby separating them from the potassium cholride, tannins and the phlobaphenes.

By washing the activated carbon residue and the solids resulting from neutralization with ether, the fission products should have been entirely extracted and recovered.

## OBSERVATIONS AND RESULTS

After each trial, a check of the ascarite tube indicated that no carbon dioxide had been absorbed, since there was no increase in weight. This therefore indicated, that if $CO_2$ had been evolved or produced by the reaction, it must

have been simultaneously absorbed by the caustic solution within the flask. As a check on this possibility, carbon dioxide-free water was used in the preparation of the dilute acid and during dilution. When the reaction mixture was neutralized, it was closely watched for an evolution of carbon dioxide which would be liberated upon acidification. In each case, however, no carbon dioxide was evolved.

After each trial, when the reaction mixture was neutralized and rendered slightly acidic, a heavy dark brown amorphous precipitate settled out of solution. Filtering left a clear dark brown solution.

Separating the precipitate from the liquid was very difficult. Several commercial filter-aids were used in an effort to ease the filtration but they proved unsuccessful. A filtering cloth was used in place of the regular filter paper but it soon clogged-up and after a short while the filtration stopping entirely. The filtration was affected by changing the filter paper after short intervals thus using one paper for only a small portion of the solution.

The brown solution upon being treated repeatedly with activated carbon gradually changed into a water clear mixture. Evaporating the water clear filtrate to dryness left a white solid mixture.

Washing this solid mixture with ether and evaporating the ether extract left no product at the low concentrations and

none until the highest concentration was reached. The amount of product was so small that a $FeCl_3$ test was necessary to determine whether the product was phenolic. The product changed a dilute ferric chloride solution into a dark green solution thus establishing the substance as a phenol.

When the activated carbon and the solids from neutralization were washed, separately, with ether and the ether evaporated, a very small amount of a black oily substance was left possessing a disagreeable odor.

Since the results of the experiments to this point were negative for the low concentrations of aqueous potassium hydroxide and only traces of phenolic material was produced at the highest concentration, it seemed important at this point to make a study of the black material with a view towards identifying it.

By examining the substance it was found that it possessed no melting point, decomposing without melting at about 207°C. It possessed the following solubility behaviour:

| | | |
|---|---|---|
| Ethyl alcohol | - | very soluble |
| Acetone | - | very soluble |
| aqueous KOH | - | very soluble |
| water | - | very difficultly soluble |
| ethyl acetate | - | slightly soluble |
| amyl acetate | - | slightly soluble |
| ether | - | slightly soluble |
| acid solution | - | flocculent precipitate. |

The substance was purified, insofar as amorphous substances

can be purified, by dissolving in alcohol and precipitating from solution by gradually diluting with water from which it separated as a flocculent precipitate. The mixture was filtered and the process repeated. The product was finally washed with water and ether and dried in an oven at 110°C. for 5 hours. The product so received was a dark brown powder. It was analyzed for carbon and hydrogen with the following results:

C - 67.45% and H - 2.33%. This leaves an oxygen content of 30.22%.

Computing an empirical formula from these figures:

67.45 / 12 - 5.621 / 1.9 - 2.96 (Carbon)

2.33 / 1.008 - 2.310 / 1.9 - 1.21 (Hydrogen)

30.22 / 16 - 1.9 / 1.9 - 1.00 (Oxygen)

of by reducing to the nearest whole numbers a resulting formula $C_{15}H_6O_5$ is obtained.

To determine whether the formula is $C_{15}H_6O_5$ or $C_{30}H_{12}O_{10}$ or some higher multiple thereof, experiments were made to determine its molecular weight by the freezing point depression method. Of the common solvents that were available for this determination, alcohol was the only one in which the substance seemed to be completely soluble. This however, was not practical because of the extremely low freezing point of alcohol.

The camphor method was next decided upon because of its wide use and relatively high melting point. Tests were made to determine the extent of solubility of the substance in

camphor. In the ratio used for the test (one part substance to twenty five parts camphor) a thoroughly uniform solution resulted as near as could be observed.

Owing to camphor's high value (40 degrees depression in freezing point resulting from one molecular weight solute in 1000 grams camphor) an ordinary thermometer was used and the test carried out in a Thiele tube. The following results were obtained:

| | |
|---|---|
| Melting point camphor alone | - 175.5°C |
| Weight camphor used | - 13.9944 g. |
| Weight substance used | - .5474 g. |
| M.P. of solution | - 172.5°C |
| Depression | - 3.0°C. |

CALCULATION OF MOLECULAR WEIGHT

| | | | |
|---|---|---|---|
| .5474 / X | = 13.9944 / 1000 | X = | 39.1178 g. solute per 1000 g. solvent |
| 39.1178 / 3 | = X / 40 | X = | 513 (approximate molecular weight of substance). |

Molecular weight of $C_{15}H_6O_5$ = 266

Molecular weight of $C_{30}H_{12}O_{10}$ = 532

Molecular weight of $C_{45}H_{18}O_{15}$ = 798

The above determination, therefore, seems to lend support to the molecular formula $C_{30}H_{12}O_{10}$, which is the formula of hemlock phlobaphene.

This and the solubility tests seemed to show that although

hemlock tannin was not broken up into its fission products to any appreciable degree under the conditions employed so far, nevertheless, there was a change in the tannin through the loss of water.

It was believed at this time that the tannin might be undergoing partial decomposition along with the loss of water. Other investigators have failed to mention whether or not such was the case leaving the possibility a matter of experimentation.

To determine whether partial decomposition was taking place, the apparatus already described, was modified so as to include at the end of the train, a receiver (fig.3) for collecting the gases, if any, over water.

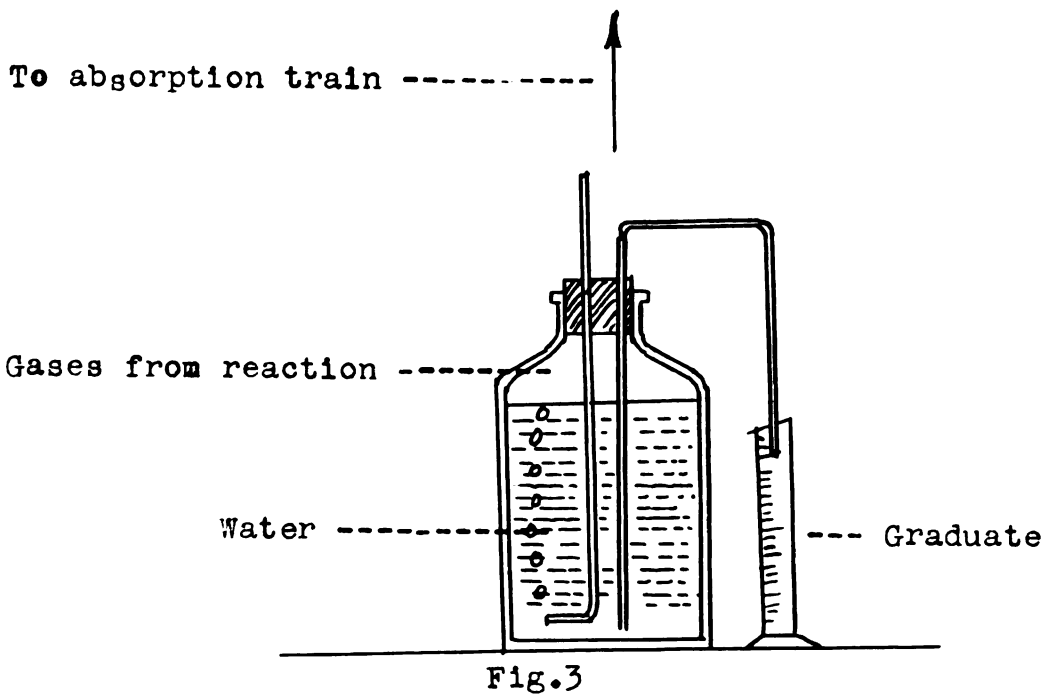



Fig.3

The experiment using 60% aqueous KOH was repeated allowing any unabsorbed gases to pass into the receiver. During

the experiment, gases could be observed passing into the receiver which continued until about 1500 cc of gases were collected.

The gas when analyzed showed the following content:

Analysis of Gas (Absorption method)

| | | | |
|---|---|---|---|
| Sample | - 102 cc | | |
| After KOH | - 102 cc | $CO_2$ | - - 0 |
| After $H_2SO_4$ (fuming) | - 102 cc | $C_2H_4$ etc | - 0 |
| After pyrogallol | - 98 cc | $O_2$ | - - 4 cc |
| After hot CuO | - 89.75 cc | $H_2$ | - - 8.25 cc |

Combustion:

| | | | |
|---|---|---|---|
| Sample | - 8 cc | | |
| Air used | - 80.9 cc | | |
| After combustion | - 88.9 cc | Contraction | - 0 |

SUMMARY OF ANALYSIS.

| | | |
|---|---|---|
| $CO_2$ | - | 0 % |
| $C_2H_4$, etc., | - | 0 % |
| $O_2$ | - | 3.92 % |
| $H_2$ | - | 8.09 % |
| $CH_4$, etc., | - | 0.0 % |
| $N_2$ | - | 87.99 % |

This determination seems to prove that hydrogen was liberated during the reaction. The nitrogen and oxygen can be accounted for as displaced air which was pushed through the apparatus as the mixture was heated. It is interesting to note, however, that there is a marked depletion in oxygen

compared with nitrogen in the usual 4 to 1 ratio of these two found in air.

During this experiment, no means was employed to separate, if possible, the displaced air from the gases liberated by the reaction mixture.

Therefore, the procedure was repeated using instead of one gas receiver, two gas receivers, so arranged, that the displaced air was collected in one receiver, which, when completed, could be shut off and the unabsorbed gases then by-passed into the other bottle. This time, however, the highest KOH concentration (85%) was used.

The following gas analysis shows the results obtained:

Total gases collected: 865 cc

ANALYSIS

| | |
|---|---|
| Oxygen - - | 5.7% |
| Hydrogen - - | 65.5% |
| $CO_2$ - - | 0.0% |
| $C_2H_4$ etc., - - | 0.0% |
| $CH_4$ etc., - - | 0.0% |
| $N_2$ - - | 28.8% |

From the above figures can be observed, therefore, that a certain amount of decomposition of tannin is taking place with the evolution of hydrogen.

Because of the shortage of time, the fission work was abandoned at this point, having shown quite clearly that up to the concentration of aqueous KOH employed, little or no

success can be expected. The tannin however is apparently undergoing the phlobatannin-phlobaphene change with some decomposition as indicated by the evolution of hydrogen. As stated before no carbon dioxide was detected.

Due to the fact that time did not permit, the work had to be abandoned at a stage which would warrant furthur investigation. Reports of other investigators fail to disclose any information relative to partial decomposition as observed in these experiments. Furthur work could be conducted to determine the point of partial decomposition on the tannin molecule and a study could be made of the extent of this partial decomposition before the tannin molecule is finally broken down into its fission products.

F. DETERMINATION OF WATER EXTRACTABLE SOLIDS IN BARK AFTER STANDING.

During the course of these experiments, and especially after the bark had stood in bottles for a little over a month, it was observed that the amount of crude tannin that was being extracted, seemingly was growing less and less as time went on. This was particularly observed when the conditions used and explained heretofore were reproduced exactly and from computations made in somewhat of a rough manner, only about half as much tannin was resulting from the extractions, than previously.

This caused a check to be made on the bark to determine if this was actually the case. At the time the bark was reanalyzed for water soluble solids, it had stood in corked bottles at room temperature in the light for three months.

A 200 gram sample of the bark was mixed with 5000 ml water and stirred for one hour. It was then hurriedly filtered and analyzed. A 50 cc sample now contained only .1129 grams representing 11.29 grams in the whole solution. The bark now had yielded only 5.64% solids in comparison to the previously shown yield of 11.1%. This fact was checked and verified.

# PART THREE

## A. CONCLUSIONS.

From the results of this work it might be concluded:

1. For all practical purposes, the crude tannin in hemlock bark is extracted in a comparatively short time when the extracting conditions used in these tests are employed.

2. There is a sizeable increase in the amount of solids extracted with hot water in comparison with cold.

3. It would be impractical from a commercial point of view to use pure tannin for fission work.

4. The amount of free sugar and foreign glucosides present in the bark is not sufficient to seriously contaminate the water extracted solids for fission work.

5. Fission of hemlock tannin does not result from aqueous alkali hydrolysis under the conditions of alkali concentration, time and temperature stated in this report. On the other hand the treatment with alkali results in decomposition with hydrogen evolution. The amount of hydrogen evolved increased with increasing concentrations of KOH. No attempt was made to isolate and indentify the decomposition products.

6. A sizeable decrease in water soluble solids in hemlock bark results from periods of long standing.

B. REFERENCES.

(1) Pacific Northwest Chemurgic Conference - March 22-23, 1937, Page 20.

(2) "The Natural Organic Colouring Matters" page 413.

(3) Chemical Reviews, 17 (1935) page 160.

(4) Allen's "Commercial Organic Synthesis" Vol. 111 pt. 1 pages 26 - 28.

(5) Ber., 1884, 17, 1041.

(6) Journal of the Chemical Society, 115, 1919, pgs 662-673.

(7) Chemical Reviews, 17, 1935, 155-185.

(8) The Carbon Compounds, page 285.

(9) The Carbon Compounds, page 362.

(10) Cohen's "Theoretical Organic Chemistry" page 453.

(11) Handbook of Chemistry and Physics 22nd Edition 1937-1938. Chemical Rubber Publishing Co.,.

(12) Sherman's "Organic Analysis " page 74.

(13) J. Chem. Society 1934, 1069.

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Study of hemlock bark; some reactions of hemlock tannin