

PROXIMATE ANALYSIS OF DOUGLAS
FIR BARK AND CHARACTERIZATION
OF THE PETROLEUM ETHER EXTRACT

by

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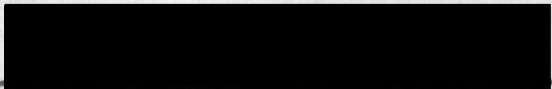
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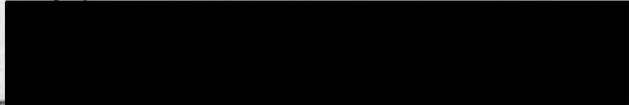


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INTRODUCTION

In recent years, arising from the need brought about by the depression of the thirties, conservation has been the watchword in the United States. Prior to that time, however, there were extensive conservation programs in other countries where the economy was in more serious difficulties than that of the United States. This originated among these countries a more vigilant period in which thriftiness of material wealth was stressed. Wood and bark utilization were important items on many of their schedules. In such industries as Pulp and Paper, Plywood, and Lumbering approximately thirty per cent of the waste in the forest and at the mills is bark. Much of this material is used as fuel and much of it is just burned to decrease its nuisance value. In the search for product improvement mechanical debarking apparatus was developed in the Pulp and Paper industry, both here and abroad. The effect of such a program was to supply the public with cheaper and whiter paper, and these processes, even though machine costs were by no means low, proved to be economically a great improvement over hand methods.

Meanwhile, in the lumbering industry, including Plywood, there was excessive waste of wood and bark, since debarking was little used and the methods that were employed

aggravated the situation by a waste of labor. Sections of a log were cut with an attempt to get as much usable lumber as possible, but slabs with bark were generally left as waste. Such saw mill refuse was sold for fuel; however, there remained, depending on the species of tree, much that could be recovered by extraction from the bark before it was sold as fuel. With the advent of cheap, efficient debarking methods in recent years the recovery of such extracts appears to be economically sound.

Adequate debarking apparatus have been developed by the Weyerhaeuser Lumber Co. and by Allis-Chalmers Inc. The former company has a system which uses water from nozzles at a pressure of 1430 pounds per square inch on a log which is revolved slowly while the nozzles move along its length (128). The latter employs a machine similar in principle, but water at only 650 pounds per square inch is played on the log in the same fashion as the above with smaller and different machinery (125). The bark can be recovered after being washed from the logs and then it can be pressed for fuel or dried for use in whatever way chosen. In this work there will be an endeavor to demonstrate that in the Pacific Northwest, where Douglas fir abounds, extraction of the bark with organic solvents yields waxes which are composed of substances of commercial value.

It was desired, that besides investigating the possibilities of commercial extraction with organic

solvents and the recovery of the waxlike extract, that the proximate analysis of bark be determined using either old methods prescribed for wood or that new methods be developed for bark such that they give reasonable values and when totaled come to 100 per cent (11.0%). It was not the aim of this study to determine the structural characteristics of the bark.

It would also be of interest to mention that several uses may be made of the extracted bark. It may be further extracted for tannins or it may be dried and used for fuel. Another use has been proposed, one in which the extracted bark is used as a thermosetting plastic powder either alone or combined with phenolic resins (39). These uses ought to be seriously considered for the exploitation of a badly neglected raw material.

HISTORICAL BACKGROUND

INTRODUCTION. The history of bark utilization is almost as old as that of wood, but this history reminds us that bark has had much more restricted attention. It was used for fuel probably simultaneously with wood, but beyond that its usefulness was due to its extraction for tannins with which the ancients cured hides. They, moreover, determined that only certain barks yielded enough tannins to be worth going through the trouble of extraction. The investigation of Douglas fir bark has proved that the tannin yields do not call for an extensive program of research, unless, due to its abundance and depletion of other sources, the Douglas fir becomes a necessary source of supply. At present, tannin supplies are adequately met by such trees as oak, pine, sumac, mangrove, quebracho and gambier.

Studies of the other constituents of bark have been carried out in order to determine how it is formed on the tree, discover its structure, and attain practical applications for its commercial use. In this work, it was pointed out previously, an attempt was made to devise methods for the proximate analysis of bark substance without paying too much attention to the structural aspects of the material and to determine the substances taken from the bark by petroleum ether extraction; therefore, the historical sketch shall include only these aspects of the

work and also a discussion of debarking and sample preparation.

DEBARKING METHODS. In the introduction of this work there were briefly mentioned two modern methods of debarking logs, and it was explained that the debarking operation was economically quite important. It has also definite importance in the sampling of bark. Prior to 1900 (131) disc barking machines of Swedish, German, and American design replaced the hand-axe, draw-knife or sap peeling spud. Drum debarking was developed in Norway by Back-Wiig and Morterud in 1891. The Thorne barker was an adaptation of the friction debarking method employed by the drum apparatus. Long logs were rotated in contact with each other either wet or dry until the bark was rubbed off. A fourth method was the cambicide procedure using certain salts in solution, which were injected into the cambium layer and the bark was stripped off after death of the tree.

Other cambicide types were developed comparatively recently. White (149) in Canada girdled trees and removed some of the bark at the girdle exposing the cambium layer and sapwood. At the bottom of the girdle about three rings deep he placed a toxicant solution consisting of three parts calcium chloride and one part sodium chlorate in a fabric container. The solution upon being absorbed destroyed the cambium layer causing a shrinkage of the bark and enabling its easy removal. Hale (41) also

discussed this type of debarking and gave some other specifications for cambicide solutions: arsenious oxide plus caustic soda, sodium chloride and zinc chloride, potassium dichromate, ferrous sulfate, and copper sulfate solutions were used.

Further comment may be made with regard to the two debarking machines mentioned in the introduction. The Weyerhaeuser machine handles much larger logs with a signalling device electronically controlled directing the operator. Each operation takes a little over 24 seconds and is not continuous, but there is only need for one operator since he is able to get logs from the mill pond or the cold deck without the aid of another. The Allis-Chalmers machine, however, uses only short sections of a log in a continuous conveyor operation. More manpower is needed to keep this machine going.

THE PROXIMATE ANALYSES. Bark has not been a subject of investigation in itself, instead, the methods which were developed for wood have, in general, been applied to it. Payen (83), Frey (34), and Thenard (136) provided the stimulus, by their early work on wood, for the later development of our knowledge of the composition of the material. Though the early analytical work was mainly concerned with elementary composition, Payen (83, 84, 85) became the first to attempt an interpretation of his analytical results. He sought to separate the woody

tissue into its component parts. "Incrustant materials" was the name he gave to the substances soluble in his purification reagents, nitric acid and caustic soda. However, not until the second decade of this century had enough knowledge been amassed so that an analytical scheme could be presented by Schorger (108) resulting from the techniques he developed at the U. S. Forest Products Laboratory. These analyses included ash, solubility in cold and hot water, ether, and one per cent sodium hydroxide solution, acetic acid by hydrolysis, pentosan, methyl pentosan, cellulose and methoxyl. It is interesting to note that a lignin determination was not included in the original scheme, and it was not until 1922 that other workers at the same laboratory (90, 98, 99, 126) employing Schorger's original procedures included the lignin determination together with the alpha, beta, and gamma celluloses. These procedures indicate the types of components present in an individual wood, but they overlap to a certain extent and do not give determinations whose sum approximates one hundred per cent.

Schwalbe and Becker (116) proposed an analytical procedure which is quite similar to that of Schorger. Their analyses included solubility in ether and alcohol, lignin, ash, nitrogen (calculated as protein), pentosans, methyl pentosans, pectins, acetic acid by hydrolysis, and methyl (CH_3) number. In this scheme the values for methyl

pentosan and pectin might be seriously questioned, but their other data is comparable to Schorger, et al.

Dore (26) developed a proximate analysis scheme for soft woods which had to be supplemented, however, when applied to hard woods. This system accounted for substantially one hundred per cent of the wood under examination, but cold water and five per cent sodium hydroxide solubilities had to be added for the hard woods. The fractional separations Dore made were derived by successive operations on the same sample as follows: (a) volatile fraction, (b) fraction soluble in benzene, (c) fraction (from b) soluble in alcohol, (d) fraction (from c) soluble in water, and (e) fraction (from d) soluble in caustic soda. On the completely extracted material lignin and cellulose were determined.

Schorger (108) and Hawley and Wise (42) pointed out the following errors in Dore's procedure: (a) the digestion with alkali lowers the yields of cellulose and lignin, (b) information on the total pentosan content is lacking, and (c) the ash content should be included for a true summation.

In 1933 Ritter and Kurth (100) described a rapid method for isolating the total carbohydrate fraction of extractive free maple wood. Freeman and Peterson (33) employed this technique in studying the composition of several American hardwoods and also proposed a summative

analysis including ash, solubility in hot water and alcohol-benzene, lignin and holocellulose (100) demonstrating that this summation, in all species studied, accounted for one hundred per cent (~~7~~1.0%) of the total wood substance. In similar manner the bark of Douglas fir has been examined, in this work, with slight changes in some of the methods in order to apply them more directly to the substance being considered.

SAMPLING. In the analysis of any material sampling is always an important item; nevertheless, there seems to be little that has been done in the sampling of bark other than to follow the methods usually used for other materials. Sharkov (120) kept bark fragments of spruce and pine in the dark until they were air-dry (most workers insist on complete air-drying prior to further handling), then he dried the material at 60°C., powdered and sifted it to 0.25 - 1.0 mm. fineness.

Wacek and Schön (145), from a ninety year old white pine cut in the fall, collected the bark after allowing the tree to weather six months before debarking. The bark was then air-dried, ground in a mortar, and finally comminuted in a drum-type mill. Other investigators generally agree that bark should be ground, but they as well as those cited in this paragraph do not mention particle size.

For wood there has been, on the contrary, excellent information for sample preparation. Mahood (69), and

Mahood and Cable (20) recommended that 80-100 mesh sawdust be used for analysis, claiming that a more representative sample could be taken; but the comparison among the results for 60-80, 80-100, and 100-120 mesh sawdusts shows little difference. The 60-80 mesh material was found to be far easier to filter and to wash. Campbell and Bryant (11) indicated that there was some degradation of the cellulose if grinding beyond 100 mesh was practiced. At the U. S. Forest Products Laboratory (7) the accepted procedure is to use the 60-80 mesh material in order to take advantage of its desirable handling properties.

MOISTURE DETERMINATION. Before any of the determination schemes outlined above may be followed the percentage of moisture held tenaciously beyond air-drying must be determined in order to base all the ensuing results on a dry weight basis. A gravimetric method of oven-drying has been accepted as reliable, but according to Schwalbe (113) drying at water bath temperatures at reduced pressure was much more rapid and accurate than oven-drying.

Marcusson (73) first determined the moisture content of materials by steam distillation using a liquid immiscible with water. Schwalbe (114) successfully adapted this for wood using xylene as the immiscible liquid. The azeotrope was caught in a graduated tube where the water separated as the lower layer and its volume could be read. Dean and Stark (23) developed a new apparatus for

this procedure whereby the azeotrope condenses in a reflux condenser and falls into a tube which has a bent sidearm an inch and a half below the mouth of the condenser. This sidearm connects to the distilling flask and allows vapors to rise and the overflow of the pure upper layer to return. The procedure is decidedly faster than oven-drying and when using a relatively large sample it is as accurate. A standard method has been proposed by the Technical Association of the Pulp and Paper Industry (134), which substitutes toluene for xylene giving certain added advantages since the temperature of reflux is not so high and carbohydrates are not decomposed (5).

The advantages in the azeotrope method for determining moisture may be enumerated as follows: (a) exclusion of volatile materials other than water, (b) more complete water removal, (c) elimination of one weighing, and (d) elimination of the possibility of oxidizing the wood by prolonged heating.

ASH. The most straightforward determination of the proximate analysis scheme is the procedure for ash and it needs little comment. Some workers sought evidence in the ash of certain tree barks of rare elements such as radium (86) vanadium, uranium, columbium, and tantalum (103). Bergström (4) determined that the phosphorus content of bark is higher than for wood, because of this it cannot be used for metallurgical charcoal, since phosphorous in

steel gives a brittle product. Other than these researches bark ash has been determined gravimetrically by a standard method as presented by Bray (7).

ORGANO-SOLUBLES. The organo-soluble portions of barks may be determined using several different solvents among which ether, methanol, ethanol, benzene, alcohol-benzene, dioxane, acetone, petroleum ether (both low and high boiling), and ethyl acetate have been used by various workers. These solvents have been used also for the extraction of the material in large amounts in order that the constituent compounds might be identified. Schorger's (108) method using ether for the solvent in a Soxhlet extractor is essentially the same as the one in common use today. It was Schorger who recommended, in the same work, that the weight of the extract should be taken and not the weight of the residue for the most reliable results.

Materials found to be soluble in the aforementioned references include volatile oils, fats, fatty acids, resin acids, resenes, phytosterols, waxes, and non-volatile hydro-carbons.

Alcohol-benzene solution is often used for the determination of extractives. Renker (96) first described this method. Dore (26) then made a comparison of the quantities of material extracted by benzene, ether, petroleum ether, carbon tetrachloride, acetone, and chloroform from representative woods of western United States

localities, and found that the differences were not marked. Bray (7) has included alcohol-benzene solubility as a standard one for wood analysis. It is also extremely useful for bark.

In such an analysis if the residue is weighed after the extraction, it has been recommended, that the material be washed with ether (33). Mease (74), Wiertelak (150), and Garbaczowna (40) claimed that cellulose adsorbs alcohols, pyridine, and alcohol-benzene solutions to such an extent that the solvents cannot be removed at 105°C. by prolonged heating. They have suggested ether washing as a remedy.

WATER-SOLUBLES. The determination of aqueous-soluble fractions was developed by Schorger (108) and very much the same methods were recommended by Bray (7). The procedures are two in number one being for cold water and the other for hot water solubility. Materials extracted by water vary a great deal depending upon the species of tree, but also depending on the region where the tree was grown. Kurth (60) has pointed out that the materials extracted are chiefly the following: mineral salts, sugars, cycloses, polysaccharides, such as mucilages, starch gums, and galactans (151), pectin-like materials, benzopyran pigments, and phlobatannins. Phlobatannins when dehydrated are converted into the phlobaphenes and are then insoluble in water (104). During the water extraction

partial hydrolysis may occur in the lignin or in the resins, thus yielding free organic acids and methanol in the aqueous extract. This action in part accounts for the greater amounts extracted by hot water and it is known that from certain woods hot water may extract the precursors of dye-stuffs (31). In the opinion of Freeman there can be no correlation of the water-soluble content of wood (bark) with definite components in the wood. He considers that the chief value of water extraction consists of the ability to remove those extraneous substances which would hinder accurate determinations of lignin and cellulose.

Schorger (108) was also responsible for the alkali-soluble materials procedure adapted as official by other investigators (7,134). The extracting solution suggested was a one per cent sodium hydroxide solution used in the cold. Attributed to this method is the removal from a sample of such substances as pentosans, hexosans, tannins, resin acids, and part of the lignin (97). The variety and indefinite nature of the alkaline extractives causes the conclusion that the significance of the determination is doubtful; however, in decayed wood (bark) the degree of fungal growth and decay can be estimated since with increased deterioration the alkali-soluble material is greater, (10,102).

VOLATILE OILS. Volatile oils have been determined

in wood and bark both as a proximate analysis and as a characterization of the oils present. Schorger (103) employed steam distillation on a known weight of sample, then he dried the residue at reduced pressure, the difference being the amount of volatile oils. For accurate results he recommended the use of the Dean and Stark apparatus (23) for the moisture determination since at 105°C., in the oven, some volatile oils would be lost.

The substances removed in this manner are essential oils, acids, and aliphatic hydrocarbons (60). Since steam may have an hydrolytic action there is a possibility of an excess of acetic and or formic acids being removed from the sample, thus the results might be questionable.

METHOXYL GROUP. A determination for the methoxyl group was described for the first time by Zeisel (153). The method depends on the formation of methyl iodide when an unknown containing methoxyl ($\text{CH}_3\text{O}-$) groups is heated with constant boiling hydriodic acid. This methyl iodide is removed from the reaction flask by carbon dioxide, which is passed through, and is absorbed in an alcoholic silver nitrate solution in an appropriate receiving flask. The silver iodide may be recovered and weighed, the methoxyl content being calculated from its weight.

This reaction, however, is not specific for methoxyl groups, but is general for all alkoxyl groups. Phillips (90) has pointed out that substances of natural origin

contain ethoxyl groups but only infrequently. Difficulty is often encountered with so simple a reagent as constant boiling hydriodic acid since some compounds will not give up their methyl groups readily; hence, the use of various reagents in conjunction with the hydriodic acid has been suggested. Herzig (43), Hewitt and Moore (44), Perkin (88) and others (27,78,24) have suggested using acetic anhydride. Glacial acetic acid was proposed by Baeyer and Villiger (1), Tröger and Müller (137), and Mannick (71). The use of phenol was suggested by Weishut (147) and recommended by Clark (14) and Phillips (90), but serious objections by Manning and Nierenstein (72), and Nierenstein (77) came out condemning the use of both acetic anhydride and phenol since both compounds decompose in the presence of hydriodic acid yielding products that form an appreciable amount of silver iodide thus introducing a serious error.

Samsel and McHard (105) pointed out that the hydriodic acid alone was highly satisfactory in all instances except for a few very insoluble compounds, and that results when using acetic anhydride or propionic anhydride and phenol were false. The standard method of the U. S. Forest Products Laboratory (7) also recommends the use of hydriodic acid alone for wood, bark and pulp.

The first men to apply Zeisel's method to plant fibers were Benedikt and Bamberger (2). Schorger (108) used the method also on several wood samples, but he applied

the Volhard titration using ferric alum indicator to estimate the silver iodide formed by titrating the excess silver nitrate. For many determinations this procedure proved to be time saving.

The absorption of volatile iodide in silver nitrate solution, in the Zeisel method, leads to the formation of $\text{AgI} \cdot 2\text{AgNO}_3$. Digestion with water is used to decompose this complex, and insufficient digestion leads to high results (30). Penniston and Hibbert (87) have made objections to the Zeisel determination because (a) it is tedious and time consuming, (b) it requires rigorously purified reagents especially hydrogen sulfide free hydriodic acid and alcoholic silver nitrate, (c) it is difficult for an inexperienced analyst to get accurate results.

Viebock and Schwappach (143) and Viebock and Brecher (142) have proposed a method which is the recommended one at present. It consists of distilling the methyl iodide from the reaction flask, which contains only the sample and the hydriodic acid, by passing carbon dioxide through as in the old method. The methyl iodide is caught in each of two receivers containing a little bromine dissolved in potassium acetate acetic acid solution. The iodide is converted to iodate by the bromine and it can be titrated with standard sodium thiosulfate solution after addition of potassium iodide which releases free iodine by reacting with the iodate. This method is an adaptation of Leipert's (64)

volumetric iodine determination. The advantages of this procedure have been noted (87) and are (1) the high titration factor of an iodate-iodide titration, (2) the relative insensitiveness to impurities in either the sample or the reagents.

An adaptation of the aforementioned methods has been developed by Christensen, et al (13). A modified Pregl apparatus was used, but the receiving flask was evacuated by a water pump and the stopcock was lubricated with glycerol. The iodic acid formed in this vessel condensed and precipitated on the walls. In the reaction flask they placed a five milligram sample, a few crystals of phenol, and four or five drops of acetic anhydride. Two milliliters of constant boiling hydriodic acid were introduced through the capillary stopcock into the reaction flask after the sample was dissolved. These workers at first attached a Kipp generator for carbon dioxide to force the methyl iodide through, but later Christensen and Friedman (12) allowed the evacuated receiver to pull the methyl iodide through leaving the capillary stopcock open. They found that air slowly being pulled through the reaction mixture did not cause any detectable errors. A U-tube, charged with 0.5 ml. each of five per cent cadmium sulfate and five per cent sodium thiosulfate served as a bubble counter and the solution removed contaminating gases from the vapors passing through such as hydrogen sulfide

(present due to the method by which the constant boiling hydriodic acid was prepared) and iodine that might be free. Heat was supplied by a micro-burner to an 85 per cent phosphoric acid bath and the temperature was kept between 120° - 130°C.

Blank runs were always made and a little practice proved helpful. This determination has several advantages, (1) the purity of the hydriodic acid need not be so rigidly controlled, (2) the time factor is considerably shortened, (3) the amount of sample needed is much smaller, (4) the phosphoric acid bath is easy to control for the proper temperature, and (5) no carbon dioxide has to be used. This method also has the important advantages of the Viebock and Schwappach procedure.

HOLOCELLULOSE. In bark the determination of a holocellulose fraction is much more significant than any individual determination for the various types of cellulose since this fraction of the bark gives relatively low yields and thus has little commercial value. However, it is instructive to note what the early methods for the determination of cellulose were, since other workers, who investigated bark, have used many of them.

The earlier methods dealt with the oxidation of the non-cellulosic constituents of wood. Schulze, (111), and Schulze and Henneberg (112) treated wood with a mixture of aqueous potassium chlorate and nitric acid. Hoffmeister

(47) used a mixture of nitric and hydrochloric acids, and Lifschutz (68) oxidized the non-cellulosic constituents with a mixture of nitric and sulfuric acids. Zeisel and Stritar (154) and Cross and Bevan (19) used a mixture of dilute nitric acid and potassium permanganate. Yields were necessarily low in such procedures due to the severity of the conditions of oxidation and hydrolysis.

Treatment of wood with phenol was used with little success by Bühler (8), since the treatment of wood with phenolic compounds produces a badly degraded cellulose of low yield. Hillmer (46) compared various substituted phenols as delignifying agents finding that only those derivatives with a nitro, halogen or an aldehyde group in a meta or para position to the hydroxyl were suited for action on lignin in the absence of a catalyst.

Halogenation methods have proved to be the most successful for cellulose determinations. Müller (76) proposed a procedure to be performed on extractive free wood using 1.25 per cent aqueous bromine and ammonia alternately until the reaction was complete. A good yield was obtained, but the method was too time consuming.

Fremy and Terreil (35) described in detail a determination for the cellulose of wood by the use of chlorine water, followed by treatment with alkali. This discovery of theirs was neglected, however, and Cross and Bevan (20) some years later independently published their chlorination

method for isolating cellulose.

From comparative data Renker (97) postulated necessary requisites of a procedure for cellulose, (a) it must yield a cellulose of maximum purity, free from lignin and colored compounds, or products of decomposition - e.g. oxycellulose and hydrocellulose, (b) the method must be rapid and simple for technical use, (c) the most desirable process is the one that produces the least change in the cellulose.

Recently, a promising new procedure for the determination of cellulose has been introduced. It involves the use of certain amino compounds for extracting the chlorinated non-cellulosic constituents of wood, thus for obtaining the holocellulose (total carbohydrate content) Ritter and Kurth (100) repeated alternate treatments of extractive free wood with chlorine and alcohol-pyridine solution. This proved to be faster and comparable to Schmidt's determination of "Skelettsubstanzen" (106). Later, due to the inability to get good results on spruce wood Kurth and Ritter (61) increased their pyridine concentration from fifteen to fifty per cent and also left out a bleaching step of washing with water.

Van Beckum and Ritter (139) found that the best solvent was three per cent by volume of ethanolamine in 95 per cent ethanol and it had no action on the polysaccharides in the carbohydrate fraction. The recommended procedure is given both by Bray and by TAPPI (7,135); it is quick and

with a minimum of experience an investigator or an analyst can get duplicate results in three hours (140).

Sharkov (120) employed the method for cellulose of Kürschner and Hoffer (58) on the bark of aspen and birch. This procedure consisted of taking a 20 per cent solution of concentrated nitric acid in 95 per cent ethanol and treating a sample until the phloroglucinol test for lignin was negative (21).

Lewis and Laughlin (67) made a comparative study of this method with the Cross and Bevan determination and concluded that the Kürschner and Hoffer yield never represented the true cellulose. Instead, the Cross and Bevan cellulose was always higher, but never by the same or a proportional amount. They also determined that by the alcohol-nitric procedure that the chain length of the cellulose was altered since the viscosities in Schwizer's reagent (cuprammonium solution) were much lower than that of the Cross and Bevan cellulose. Other workers have also confirmed this.

LIGNIN. Methods for the determination of lignin have never been considered satisfactory since it cannot, as far as is known, be isolated in an unchanged state (91). In other words, it is not known exactly what it is that there is to determine, and until the true nature of lignin is discovered no rational method can be developed. Among the approximate determinations known are direct and

indirect procedures. The latter determinations consider certain groups common to lignin or some color reaction with which the substance can be estimated. Suitable factors enable a calculation for apparent lignin. Most of these methods are only of historical interest and are rarely used today.

The procedure of Schulze (111) is the oldest. He oxidized lignified plant material with nitric acid and potassium chlorate and considered that the loss in weight represented the lignin. As was pointed out under the description of cellulose determinations, this procedure gave high results for not only was the lignin completely oxidized, but the hemicelluloses and perhaps some of the cellulose were also destroyed.

Benedikt and Bamberger (2) determined the percentage of the methoxyl and demonstrated the close relationship between the apparent lignin as determined by Schulze (111) and the percentage of methoxyl. This of course depends on the assumption that in lignified plants lignin is the only substance containing methoxyl groups. However, it is generally known that pectic substances also contain methoxyl groups.

There is a color reaction procedure that was developed by Cross, Bevan, and Briggs (21) in which a phloroglucinol solution in hydrochloric acid is shaken with the sample and then allowed to stand overnight whereupon it is

titrated with a furfural or formaldehyde solution in hydrochloric acid. After each addition of the latter solution the reaction mixture is allowed to stand for two minutes at 70°C. If a red spot appears when a drop of the reaction mixture is placed on paper and held over a small bunsen flame for one minute without burning the paper then the reaction is incomplete, for otherwise the spot will be colorless and the reaction complete. A blank is run on the phloroglucinol and from this data the lignin may be calculated.

Venkastewaren (141) compared results of this method with those of the direct methods using 72 per cent sulfuric acid and fuming hydrochloric acid. It was found to give much higher results than the other two. The author's belief was that the phloroglucinol was absorbed by the aldehydes giving complexes which simulated an endpoint.

A method based on the fact that nitric acid, when added to lignin, causes oxides of nitrogen to be given off was developed by Seidel (118). A special apparatus is required and the quantity of the oxides of nitrogen is determined by titration with permanganate. An empirical factor is used to calculate the percentage of lignin.

From the fact that lignin takes up a considerable quantity of chlorine Waentig and Gierisch (146) have devised a method whereby the amount absorbed is used to estimate lignin. Chlorine is passed through the sample and

air is finally used as a wash to remove excess reagent, until no further weight increase takes place. Pringsheim and Semmler (119) applied this method to lignin in straws and fodder plants, finding that the results agreed reasonably with those obtained by direct procedures.

Among these indirect determinations there is one micro-method. Mehta (75) utilized the fact that phosphomolybdic and phosphotungstic acids in phosphoric acid are sensitive reagents for detecting minute amounts of aromatic substances containing hydroxyl groups. This is a colorimetric procedure and standard lignin solutions must be prepared which are then compared to unknown solutions. Such a determination must indeed be doubtful since there is not enough known about lignin to be able to prepare a standard solution as specified.

Another halogen method developed by Kirschner and Wittenberger (59) utilizes bromine absorption under proper conditions. The amount of the halogen absorbed is directly related to the lignin content. The ratio between these two values was designated by these men as the "proportional number". They found the average number for several lignin containing materials. Rossmann's method of determining the excess bromine as iodine with potassium iodide and sodium thiosulfate is used. The iodine number thus determined when multiplied by the "proportional number" gives the per cent of lignin.

The procedures most commonly used for lignin are those of the direct approach. Among these either the lignin is left as a residue or it is removed and the carbohydrate material is left.

After the extraction of the so-called extraneous matter from wood or bark those procedures most commonly used to determine lignin may be performed. Among these methods are those that depend on the removal of cellulose and associated carbohydrates by means of strong mineral acids. In these methods the residue lignin, is weighed, but according to Phillips (92) weighing a residue cannot be satisfactory. There are certain sources of error (1) the possibility of incomplete hydrolysis of the carbohydrates, (2) the formation of reversion or insoluble condensation products from the carbohydrates or other substances which might be present, (3) the possibility of removing some portion of the lignin as the result of preliminary treatment with alcohol-benzene solution (this will be explained later), hot water and dilute acids, (4) contamination of the lignin residue with nitrogenous complexes. In bark analysis workers have only very rarely made determinations for nitrogen. Schwalbe (115) reported only traces of proteins in the bark of some hardwoods. Lewis, et al (66) do not even mention nitrogen at all in their discussion of redwood bark.

Payen (84,85) was the first to use strong sulfuric

acid as a hydrolytic agent for the removal of the carbohydrates from wood. That cellulose could be hydrolyzed in the cold with a reagent such as 72 per cent sulfuric acid was shown by Flechsig (28). This fact was confirmed by Ost and Wilkening (79) and applied by Klason (52). König and Rump (54) treated the plant material with a preliminary extraction of alcohol-benzene and then used the 72 per cent sulfuric acid for the hydrolysis. They employed iodine and sulfuric acid in order to make sure the reaction was complete. A sample was taken out periodically until finally no blue coloration showed under the microscope.

Schwalbe and Becker (117) first subjected their materials to a hydrochloric (sp. gr. 1.19) acid hydrolysis and an extraction with hot water before using the 72 per cent sulfuric acid. This method has been used by many other investigators in slightly modified form (93).

Klason (53) modified his original procedure recommending that 64 or 66 per cent sulfuric acid be substituted for the 72 per cent acid. The length of time for the reaction was extended to two days and then after dilution with water, filtration and washing, a 10.5 per cent hydrochloric acid solution was added to the sample and the mixture digested on a steam bath for twelve hours. The solution was then removed by filtration and the lignin washed with water until both acids were absent in the washings.

A procedure that has been accepted by the U. S. Forest Products Laboratory (7) was one modified from earlier methods by Ritter, Seborg and Mitchell (101). It included diluting the 72 per cent sulfuric acid-lignin mixture with water to a three per cent concentration of the acid and refluxing for four hours before filtering and washing with hot water. The residue was calculated as lignin, but it might also be ashed and the ash subtracted from the lignin for more accurate values.

Peterson, Walde and Hixon (89) have warned that the temperature of the 72 per cent sulfuric acid should be kept at between 4° - 15°C. so that high values of lignin would not be obtained, since at higher temperatures some of the carbohydrates might be charred. Bray (7) does not call for such rigorous control, but he does recommend that the temperature not be allowed to exceed 20°C.

The fuming hydrochloric acid method was indirectly discovered by Willstätter and Zechmeister (151) when they discovered that the acid (sp. gr. 1.212 to 1.223 at 15°C.) would disintegrate and dissolve cellulose in the cold completely. Krull (57) applied this fact to the determination of lignin. Phillips (94) gives several other applications and modifications of this procedure as devised by others.

There is another method that has been used by investigators of bark and that is the method of Freudenberg

et al (36, 37). It consists of hydrolyzing the material alternately (previously degummed by digestion with cold five per cent aqueous sodium hydroxide solution) with boiling one per cent sulfuric acid and cupprammonium solution. The latter reagent and, moreover, the preliminary treatment dissolves some lignin (138,50,127), so that the yield by this method is always low and never represents the entire lignin fraction. This lignin is called the "Cuproxam-lignin". The material obtained is light brown in color and possesses a high methoxyl content.

APPLICATIONS, COMPARISONS IN BARK DETERMINATIONS.

Many workers analyzing the bark of various species of trees have employed the methods described above. Sharkov (124) used the procedure of Kirschnner and Hoffer (58) on the bark of birch and aspen in order to separate the cellulose. Later working with pine bark (122) he found four types of lignin. A was soluble in alcohol while B was not. C was obtained from the alkali insoluble residue by heating with sodium sulfate solution and D was obtained by chlorinating the residual fraction. He determined the methoxyl values of these lignins and concluded that those with higher $\text{CH}_3\text{O}-$ were more resistant to chemical action. In further work on the bark of pine bark, Sharkov (120) determined lignin by König's (54) method. He did not check his results with other more acceptable procedures.

Clotofski, et al in several papers on pine and beech

bark determined the various constituents in several ways. They (15) used acetone for the organic extraction and both the Willstätter and the Klason methods for the lignin of pine bark. Two years later while working on beech (18) they found that methanol gave the best yield of extract and used Klason's procedure for lignin as previously. In another paper (17) on beech bark they tried several methods for lignin and determined the methoxyl content of the resulting lignin. Among the procedures were Klason (53) 72 per cent sulfuric acid, 30 - 32.5 per cent lignin (13.0 per cent methoxyl); 72 per cent sulfuric, Hägglund, 28-30 per cent (13.2 per cent); HCl-lignin, Willstätter (151), 36.15-37.10 per cent (10.7 per cent); cuproxam-lignin, Freudenberg (37) 17.7-18.0 per cent (15.45 per cent). It is evident that the last values are for a much more enriched lignin and when this lignin was treated according to Freudenberg's (38) oxidation method for vanillin it gave a higher yield than the others.

Working on redwood bark Lewis, et al (66) used the methods of the U. S. Forest Products Laboratory (7) and found that the apparent bark lignin did not resemble spruce wood lignin since its average methoxyl content was about half. They separated the bark into fiber and dust. The fiber yielded apparent lignin in one fraction with 11.6 per cent methoxyl, in another fraction there was only 2.7 per cent methoxyl. The bark dust gave apparent lignin

yield of 60 per cent, yet the methoxyl was only 0.8 per cent. Upon treating the bark fibers in five commercial alkali cooks and one sulfite cook the pulps still contained 20-32 per cent lignin. This gave 13.3-14.6 per cent methoxyl. Their work demonstrates the complexity of the bark lignin or apparent lignin. Their comparisons were made with the lignin obtained from spruce wood (14.9 per cent methoxyl). The difficulties they encountered might somewhat explain the various results Clotofski, et al were able to obtain.

Wacek and Schön (145) working on pine bark, previous to the workers just cited, determined that the Cross and Bevan (19) cellulose method was by no means adequate for the bark. After extracting the bark with ethanol and then methanol-benzene, they used the Kürschner and Hoffer (58) procedure for cellulose and obtained a 21.5 per cent yield of which 6.5 per cent was pentosans (polysaccharides of pentoses) and 69.7 per cent α -cellulose. They also used Lifschutz' (68) method and obtained a degraded residue only having 35.3 per cent α -cellulose.

These men also studied spruce bark and extracted it at first with methanol-benzene. They isolated the carbohydrates as with pine bark and also obtained 14.1 per cent tannins with a water extraction, but their yield of lignin depended on the previous extraction of the sample, thus when the sample was extracted with only methanol-benzene,

the Klason (59) lignin was 30.3 per cent; however, when water, methanol and benzene were used in succession only 19.0-19.55 per cent (depending on the acid strength 67 or 72 per cent) was obtained. The methoxyl for this was only 5.6 per cent, but when acids of lower concentration were used higher percentages of lignin with lower methoxyl values were the result.

The Willstätter (181) method was applied to this extracted bark and 20.1 per cent lignin with 5.05 per cent methoxyl resulted. If the bark was extracted only with ether a higher yield of lignin was obtained, 38.5- 40.3 per cent. They concluded that the lignin of the bark is much more difficult to handle, has a lower methoxyl content, and that the tannins interfere, and upon their removal some of the lignin is also taken out.

PETROLEUM ETHER EXTRACTION AND OTHER ORGANIC SOLVENT EXTRACTIONS. Early investigators sought to find valuable substances in the extracts of certain tree barks. Kähler and Tillberg (55) received a patent in Sweden for the extraction of birch bark with nitrobenzene or mixtures of nitrobenzene and low boiling alcohols. They recovered the betulin from the bark. Dischendorf (25) also worked with birch bark and after washing the bark with two per cent sodium carbonate solution and two times with hot water, he extracted betulin in 23 per cent yield with ethanol.

In Germany, Zellner, et al, during the twenties

extracted many tree barks with petroleum ether in the search for valuable substances. From field maple (158), hazel, and "grauerle" they isolated octadecyl alcohol; $C_{34}H_{60}O_2$; and alnuresinol $C_{34}H_{58}O_3$. Later working with English elm (148) they isolated phytosterol finding it hydrated with one molecule of water. About the same time two more papers came out (156) giving a long series of compounds that were isolated from various trees. These compounds were alnulin (alnuresinol), ceryl alcohol, phytosterol, plantanolic acid, sativinic, dihydrostearic, palmitic, arachidic, behenic, and $C_{20}H_{40}O_2$ acids, $C_{13}H_{34}O_2$, $C_{20}H_{40}O_2$, $C_{27}H_{48}O$ alcohols. The last paper on bark (157) came out soon after the others and included substances isolated from the sycamore: ceryl alcohol and phytosterol; hawthorn: stearic acid, palmitic acid, ceryl alcohol and $C_{31}H_{51}O$ or $C_{31}H_{50}O$; horse chestnut: a hydro-carbon and an alcohol resembling ceryl, also from an ethanol extraction, sitosterol; from spruce: ceryl alcohol, stearic and palmitic acids.

A number of years passed before any significant work was done on the extraction of the bark of commercially important trees. Takahasi (132) working with fir bark used ethyl acetate as solvent and recovered a compound $C_{30}H_{44}O_3$ which he attempted to characterize and presented some tentative structures.

Sharkov in his long series of papers on the barks of various trees of Russia determined, by extraction with

95 per cent ethanol (120) in a Soxhlet apparatus, that the inner bark or bast of such trees as spruce, birch and aspen contain no suberin, but that the dead outer portion of the bark contains this substance. The next year working with pine, fir, oak, plane, and sequoia (123) he obtained suberin and the following acids from it: phellonic, eicosane-dicarboxylic, suberic and suberolic employing the procedure worked out by Zetzsche and Sonderegger (159).

Again in Germany, Lehmann and Eisenhuth (63) treated pine bark with alcohol and isolated a fatty wax. The part of this was soluble in ether and the other not. This latter fraction was determined to be phlobaphenes. The ether soluble part was separated by sodium carbonate solution into a non-acid and an acid part. The acid part contained resin acids and arachidic acid, but no acids volatile with steam, there was also present a large resin acid of an indetermined formula. The non-acid part contained a volatile fraction (pine oil) of terpenoid nature. The remainder soluble in ether was treated with petroleum ether and a crystalline fat was recovered. It yielded chiefly arachidic acid upon saponification. A neutral compound was separated from this solution of ether-petroleum ether after it was evaporated down somewhat. It was $C_{22}H_{38}O_2$ and gave the Liebermann-Burchardt reaction for sterols.

That same year in answer to Lehmann's work Clotofski, et al (15) admitted that pine bark had been the

subject of research for a number of years at the Technische Hochschulen, Berlin, and Braunschweig. They employed acetone as the extracting solvent and obtained an average yield of ten per cent. The yield they claimed depended on where the bark was obtained, in other words, at the base of the trees and from very old trees the yield was low, but from the upper parts and from younger trees the opposite was true. Fractionation of the acetone extract with ether, petroleum ether and water resulted in the isolation of a major fraction which showed the presence of mixed fatty acids and esters. Several acids, saturated and unsaturated, were recovered, but they did not at all resemble those substances which were isolated by Lehmann.

In other works on beech bark Clotofski, et al (18) used several solvents including ethanol, dioxane, acetone, methanol and petroleum ether. The yields of extract in the first paper were 9.2 per cent, 12.8 per cent, 7.6 per cent, and 12.2 per cent respectively. When they employed successive extraction on the first extract from a methanol extraction using water, petroleum ether and finally methanol, their results were not satisfactory. They used a complicated procedure to accomplish their work and methanol was the extracting solvent that they finally used in their pilot plant extraction. The methanol solution was cooled and a precipitate formed which was filtered. This portion of the extract consisted of a paraffin and a wax.

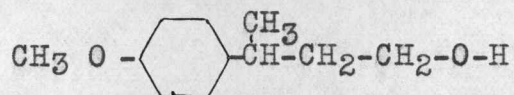
The latter was saponified and distilled at reduced pressure. An alcohol $C_{20}H_{41}OH$, M.P. $73^{\circ}C$. and an acid $C_{20}H_{40}O_2$, M. P. $56 - 57^{\circ}C$. were obtained. The material remaining in the methanol was divided into water soluble and water insoluble parts. Sugars and tannins were in the water soluble portion, but in the water insoluble material there was some that was soluble in sodium carbonate. This was a mixture of aliphatic and some resin acids. Saponification of this fraction showed the presence of some paraffin and some waxy alcohols. The acids were a mixture M.P. $69-72^{\circ}C$. The material insoluble in the sodium carbonate was saponified and yielded an alcohol presumed to be arachic (but it would appear to have too high a melting point to be that compound, its melting point is far closer to that of lignoceryl alcohol). Phytosterol was recovered from this portion and the acidified acids when purified proved to be like carnaubic acid and some resin acids.

In the second paper a large amount of beech bark was extracted with a low-boiling petroleum ether. Isolated from this extract were two compounds other than found previously. They were a $C_{29}H_{48}O_2$ and a sterol $C_{24}H_{40}O$. The former gave a Salkowski reaction suggesting that it too was a sterol.

In 1940 Voss (144) received a German patent for the isolation of the fatty acids from bark. The raw material was finely ground and then hydrolyzed in aqueous or

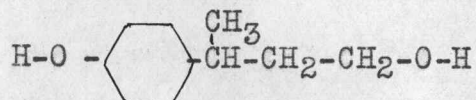
alcoholic alkali (very dilute) solution under pressure in an autoclave. The fats were then extracted with an appropriate solvent when the reaction in the autoclave was complete.

In this review there have been several references to a compound or mixture of compounds known as betulin. Sosa (129) solved this problem of identification and found that the substance could be separated into (1) betulinol,



(2) 3-p-hydroxyphenyl-1-butanol- β -d-glucopyranoside,

(3) betuligenol,



Spirk (130) recommended a preliminary extraction of bark with hot water in a commercial extraction to remove not only the tannins but also the sugars, and thus enable easier extraction and separation of the fats, etc.

Pajari (80) used petroleum ether for the extraction of a green oil from Scotch pine. As much as 4.1 - 8.5 per cent of the bark was free fatty acids isolated by distilling the methyl esters. Found were lignoceric and palmitic acids and by steam distillation caproic acid was obtained from the crude extract. By oxidizing the fatty acids mildly hydroxyl compounds were isolated. Among them were dihydrostearic, α and β sativic, linusic and isolinusic acids. These were obtained from oleic, linoleic, and

linolenic acids. Only 4.7 - 9.5 per cent of the bark oil was found to be resin acids. Also found were 10.0 - 11.9 per cent of unsaponifiable matter and 0.5 per cent of this was crystallized. Sitosterol and dihydrosterol were isolated and identified. Lignoceryl alcohol was recovered from the mother liquors and identified, the name of the solvent was not given. The last two neutral compounds to be isolated and identified were arachic alcohol and nonacosane.

Later Pajari (81) using petroleum ether for the extraction isolated lignoceric, palmitic, oleic, linoleic, and linolenic acids and presented the exact yields obtained. These acids were isolated as a liquid mixture.

In his latest paper on pine bark oil, Pajari (82) isolated the same acids again including dehydroabietic acid and several compounds only identified by their empirical formulae.

In these works, as cited, there is noticed no extraordinary descriptive material on the isolation and characterization; however, Hibbert and Phillips (45) working on the nature of the resins of Jack pine wood described procedures that could be easily applied to the isolation and identification of the fats and waxes of bark. These have been referred to liberally in this work.

EXTRACTED BARK. The extracted bark need not be treated as solely a waste material or as a material that

has its only utility as a fuel. The use of the bark fibers of redwood for cushions, mats and pipe coverings served as the basis for a United States patent claim for Weiss (148) as early as 1920. The bark was softened under water and was beaten to separate the fibers. Such fibers are not destroyed by the extractions and although there are certainly not enough extractives in redwood bark (63) to warrant attention, surely other bark fibers may be handled in a similar fashion even after extraction with organic solvents. Before this time Kress (56) suggested that spent tanning bark should be used for mixing with rag stock to produce roofing felt, wall paper and fiber boards or conduit. More recently redwood bark was the subject of another patent (22) which described the treatment of the material for the separation of its fibers by boiling in a solution of aluminum sulfate. The product was said to be suitable for making sheets or boards. Lattemann, in a German patent (62), described the process of softening elm bark in water, and in this way freeing it from slime. It was then compressed to remove as much of the water solution as possible. The residue was then heated with a dilute solution of potassium hydroxide and sodium hydroxide at 80°C. under pressure. The product was rinsed, finally, and was then used as a textile fiber. Another patent was given to Schnell (107) in Germany, for devising a machine which would transform the bark into spinnable

fibers.

In the introduction there was a brief notation concerning molding products from extracted bark. In 1941 R. O. Phillips (95) patented a process which utilized comminuted bark for the preparation of moldable plastics. Work along these lines is now being carried forward at Oregon State by Friedman (36) using extracted Douglas fir bark, finely ground, as a molding material.

There is also a step beyond simple burning which might be mentioned here. According to Bergström (3), bark may be carbonized as wood is even though there is somewhat added difficulty in doing so; however, bark gives a larger charcoal yield but less acetic acid and methanol.

In the interests of conservation the last thing that should be done with bark is to burn it. It must, however, be realized that for certain companies the only economic use for bark must be as a fuel. If, nevertheless, they are of ample enough resources to handle the recovery of the extractable substances or else can collect and sell the material beyond what they require for fuel such a program of conservation seems indicative. Apparatus for efficient use of bark as a fuel has been developed.

Le Sueur (65) has designed a special drying oven for the material and others have developed efficient furnaces adapted especially for the burning of bark. Hulsart (48), Calnan and Franklin (9) and Branford (6) are among these.

THE SAMPLE

DISCUSSION. Logs are brought to the mills of the Pacific Northwest in several ways. Operations giving the most economical results are practised, therefore, rivers and ponds are used as much as possible to bring the logs to the mills and to store them. A "Cold Deck" (storage pile of logs) may be maintained so that continuous operation can be sustained in the off season when logging ceases. Railroads and trucks help to supplement the cheaper transportation facilities.

A log may be allowed to lie in the forest on an average of three to four months and as much as six months to a year after being felled and bucked. These logs and also those from the cold deck and from the rivers and ponds (immersed for long periods therein) do not give as much extractives as do fresh logs. The latter may be defined as logs which have lain only for a month or less in the forest. Cold deck logs besides being subjected to severe weathering conditions also suffer from mold infection. This condition cuts the amount of extractives that the bark would normally yield.

Samples of Douglas fir bark were taken from both cold deck and fresh logs. The latter had lain in the forest about one month prior to removal to the mill. It was decided to take both types in order to determine definitely

that for extraction the fresh material was far superior. Both types were obtained from the Corvallis Lumber Company, ground to pass a 20 mesh screen and then air-dried in thin layers for several days.

Previous work with bark in this laboratory had demonstrated that use of 60-80 mesh samples led to such difficulties in filtering and washing as to make the analyses tedious and in some instances impossible. For this reason, it was decided that 20 mesh material might give better results even though extraction time would have to be lengthened.

It is true that although a particle size of 20 mesh was desired at this point, there was about ten per cent which could have passed the 60 mesh screen, however the advantages of the 20 mesh material were not lost since these smaller particles cling to the larger ones especially when wet. The sample that has a particle size of 20 mesh has the advantage of being easy to extract while no small particles are lost through the pores of the coarse alundum crucibles. Therefore, the determination is more accurate with the larger particles especially when the residues are weighed.

After the completion of the water solubles and the alcohol-benzene solubles determinations, the samples were divided into two approximately equal parts and they were pulverized in preparation for both the lignin and the

holocellulose determinations to about 60-100 mesh. It was important not to throw away any of the material that would pass 100 mesh since errors would result. Lignin would have been too low and holocellulose too high since the bark dust containing a greater percentage of lignin is more easily pulverized. This was prescribed since in both these determinations the reaction was much slower and hardly complete when a large particle size was allowed. Filtering becomes a simple problem even with such relatively fine particles if proper precautions are taken.

EXPERIMENTAL. The samples of each type were handled in the same manner. First they were ground in the hog mill and everything passing 20 mesh was saved. The residue was further ground in a Wiley mill through an 8 mm. screen and again passed through a 20 mesh sieve. The last grinding was done in the Wiley mill through a 2 mm. screen after which all of the material passed the 20 mesh sieve.

The bark was spread thinly on newspapers and allowed to air-dry for several days. Then it was put into drums in which the material was thoroughly mixed and stored. Samples were removed from the top, middle and bottom of each drum.

The moisture determination following the Dean-Stark procedure but using toluene instead of xylene was performed on the air-dry material, and for the cold deck bark it was 11.37 per cent, for the fresh bark it was 10.80

per cent. The procedure recommended by the Technical Association of the Pulp and Paper Industry was used. From these data the succeeding determinations of the proximate analysis scheme were calculated to a dry basis.

Before the lignin and the holocellulose determinations, the sample was pulverized by grinding it in a mortar until all of it would pass at least the 60 mesh sieve.

THE PROXIMATE ANALYSES

DISCUSSION. The scheme of analysis suggested by Freeman and Peterson (33) for use on wood was adapted for this investigation of bark. This scheme was selected because the constituents should total 100 ($\pm 1.0\%$) per cent. All of the values were based on the dry weight of the sample. To reiterate, such a scheme could in no way aid in the elucidation of the structure of bark, but could supply a worker complete information as to the type of constituents the material is composed of and a quantitative estimation of each type. To accomplish this the methods presented by Bray (7) for ash, hot water and alcohol-benzene solubles were applied almost without revision, and satisfactory results were obtained. It is to be noted that many workers have found that alcohol-benzene solution dissolves some lignin as well as usual organic substances. In the latter two determinations the drying of the residue was altered since it was felt that at 105°C . some changes would occur that would make the ensuing determinations doubtful (5). In fact, for all of the other drying operations after the lignin and the holocellulose determinations the 105°C . drying operation has been deleted. Instead, a vacuum oven was employed holding it at $60^{\circ} - 65^{\circ}\text{C}$. at a reduced pressure of 25 to 45 mm. of mercury.

The lignin of the bark was isolated with more difficulty than has been noted for wood. At first the standard

procedure outlined by Bray (7) was attempted, but very high results were obtained. The procedure was modified to subject the sample to constant stirring for five hours with the 72 per cent sulfuric acid reagent and the temperature of the reaction was kept below 20°C. using a cool water bath. Stirring was efficient but not too rapid. The rate was about 200 revolutions per minute. After this treatment the sample did not react further and excellent checks were obtained.

The holocellulose determination was carried out in a manner similar to that given by Bray (7), however, the bleaching step was omitted until the very end since the water if added earlier caused the cellulose to swell badly and thus hindered further treatment. An alcohol wash would remedy such a situation if need be. The Sieber-Walter apparatus described by Schorger(110) was used; nevertheless, it was found necessary to lead the chlorine in from above the sample and also that the system need not be sealed. In fact if it were kept sealed an explosion might occur and ruin the sample.

The methoxyl determination was performed according to Christensen, et al (12,13). In some of the runs there was an attempt to use phenol alone as the solvent. There was little difference in the results. This was used in order to have more solvent action than when using acetic anhydride.

EXPERIMENTAL. Ash, hot water and alcohol-benzene (1 to 2 by volume) determinations were carried out as described by Bray (7). The residue from the hot water extraction was dried at reduced pressure at 60° - 65°C. until constant weight was obtained. An RA 98 alundum crucible was employed to catch the residue. This porosity was the most efficient one for the next determination of alcohol-benzene solubles.

The dried residue was then extracted for 24 hours by alcohol-benzene (1 - 2 by volume) and the extracted material was then washed with about 50 ml. of ether. The crucible and its contents were dried at 60° - 65°C. and at reduced pressure.

For the following two determinations the samples were divided into two approximately equal parts and handled as described previously.

The lignin procedure of Bray (7) was followed, but the time of the reaction was lengthened to five hours with continuous stirring and the temperature kept at 15° - 20°C. by a cool water bath. After thorough washing of the residue it was dried at reduced pressure and at 60° - 65°C.

The holocellulose determination (7) was performed without the water washing after the extraction of the chlorinated lignin until after the tenth or twelfth chlorination. Then a final chlorination and washing finished the extraction. The holocellulose residue was dried to

constant weight as the other residues had been.

The methoxyl determination was performed on the isolated lignin from the fresh bark sample employing about a five mg. sample. The procedure of Christensen, et al (12, 13) was followed except for two of the determinations which were done using one gram of phenol as the solvent.

The results appear in table one.

TABLE I
PROXIMATE ANALYSIS DETERMINATIONS

DETERMINATION	COLD DECK BARK	FRESH BARK
ASH	1.20 %	1.19 %
HOT WATER SOLUBLES	6.13	9.85
ALCOHOL-BENZENE SOLUBLES	7.16	12.75
LIGNIN	45.82	51.86
HOLOCELLULOSE	39.02	24.75
TOTAL	99.33	100.40
METHOXYL (in lignin)		7.18

PETROLEUM ETHER EXTRACT

EXTRACTION. The extraction was performed, at first with low boiling petroleum ether (B.P. 35° - 65° C.), but later this was changed by replacing the low boiling material with high boiling petroleum ether (B.P. 90° - 127° C.). An extraction apparatus was fashioned from a 20 gallon steel drum. A copper coil condenser was placed within and just below the cover and fixed into it. Hose connections also were fitted to a glass coil condenser placed in the cover standing vertically in a nipple welded into the cover for that purpose. This provided an opening to the atmosphere so that a pile up of pressure could not occur within the drum. Within the drum there was placed a five gallon paint can standing on an eight inch tripod. This was fixed so that in a nipple welded into the bottom of the can, on one side, a siphon could be placed. The siphon was fitted in such a way that before siphoning, the solution would cover the sample within the can. The sample was placed in the can in an open cotton bag and the cover of the drum was sealed with a section of neoprene hose cut to fit the groove in it. It was made fast with a cover clamp such as those usually found on such drums, i.e. as on drums which contain vegetable shortening. The cover was placed in a manner such that the copper coil would drain its condensate onto the sample.

Three kilograms, dry basis, of air-dried fresh bark (7 lbs. 7 ozs.) was placed in the cotton bag and five gallons of high boiling petroleum ether in the drum. The drum was heated by three electric hot plates and the bark was extracted for forty eight hours. In order to obtain more efficient heat exchange the drum was insulated with Lo - K (fireproofed cotton manufactured by Lockport Cotton Batting Company, Lockport, New York). The efficiency of the extraction apparatus was decidedly improved by this insulation.

Some of the original low boiling petroleum ether extract was saved for comparison with the extract from the high boiling solvent. The change from low boiling to high boiling petroleum ether was made by mixing the former with the latter, after saving 500 ml. of the former, and distilling off the low boiling. The remainder was placed back in the drum with more high boiling solvent to complete the extraction. The low boiling material would not work in the extractor since it would not siphon.

ISOLATED FRACTIONS

Various fractions were obtained after concentrating the petroleum ether solution to a volume of two liters by the following scheme of separation. The sample of bark used for the extraction had only been air-dried and, therefore, it still contained 10.80 per cent moisture. The extract was dark colored probably due to phlobaphenes which had come out via the water in the extraction as tannins and phlobatannins and had been converted to phlobaphenes by dehydration. In an attempt to remove the phlobaphenes the two liters of extract, while still warm, were washed with 50 per cent methanol. This methanol solution yielded 1.9 g. of material, B, after evaporation of the solvent. The hot two liter petroleum ether solution was then decanted from a precipitate that would not dissolve in either solvent. This residue, however, was partially dissolved in fresh petroleum ether and washed with 50 per cent methanol since it was dark in color. The methanol dissolved nothing, but the petroleum ether yielded 0.65 g. of a yellow colored wax, C. The residue still remaining was very dark, 2.3 g., D.

Upon cooling down the two liter solution in the refrigerator 109 g. of A settled or crystallized out and was filtered off. The filtrate was concentrated to 500 ml. from which upon cooling in the refrigerator, 23.8 g. of E

was filtered. The filtrate from this was evaporated yielding an orange residue, 32.0 g. of F.

The extractor was washed with hot toluene to remove the last traces of extract and upon concentration of this 23.4 g. of a black amorphous solid, G, was obtained.

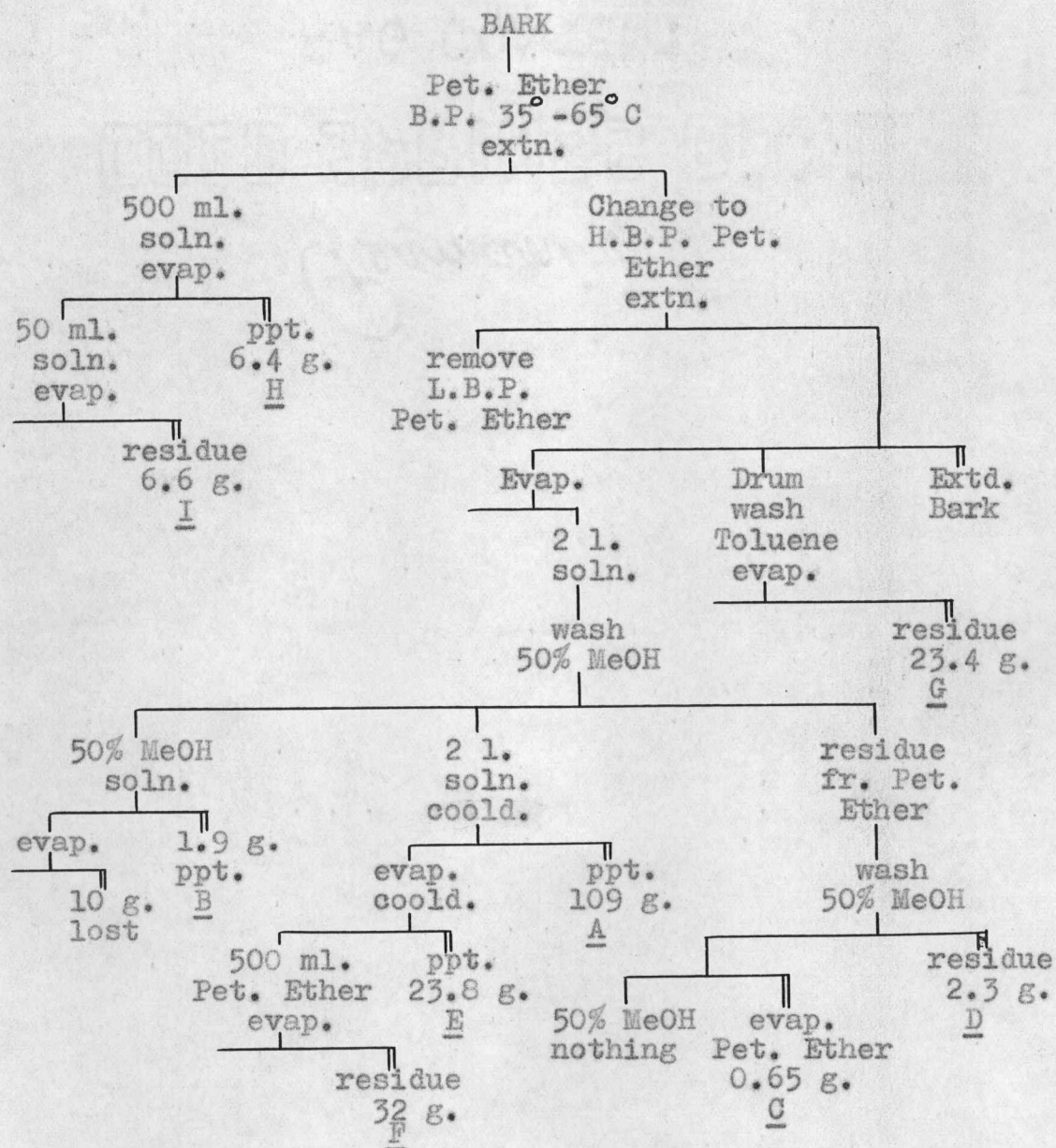
The 500 ml. of low boiling petroleum ether extract, which had been saved, yielded upon concentration to 50 ml. 6.4 g. of a light colored wax, H. From the remaining solution 6.6 g. of a wax, I, was obtained by complete evaporation of the solvent. It was also light in color.

During the ensuing course of the work it became apparent that the extraction with methanol had accomplished little since it effected no distinct separation of the phlobaphenes that were doubtlessly present.

The total yield including ten grams which were lost in the isolation of B amounted to 217.1 g., 7.25 per cent of the bark on a dry basis.

The accompanying figure shows the scheme of separation.

FIGURE I
DOUGLAS FIR BARK
EXTRACTION SCHEME



SAPONIFICATION, IODINE AND ACID VALUES

Each fraction wherever possible was analyzed for its saponification equivalent, acid number, and iodine number. Such data are essential for the true picture of any fat or wax.

The saponification equivalents were determined using the Reiman (95) double indicator method as adapted to semi-micro methods by Ketchum (51), although at first the usual macro procedure was employed for samples A and E. The acid values for these latter samples were determined by the usual methods, but for the others a semi-micro method was employed in which thymol blue was used as indicator. The sample was dissolved in only 10 ml. of hot ethanol and was titrated carefully with 0.2 N potassium hydroxide solution to first shade of greenish yellow. This titration was performed using a micro burette so that the titration data were as accurate as the weight of sample data. The classical Wijs iodine number procedure was employed for all of the iodine number data.

The results are listed in TABLE II.

TABLE II

SAMPLE	SAPONIFICATION EQUIVALENT	ACID NUMBER	IODINE NUMBER
<u>A</u>	389.0	7.04	105.1
<u>B</u>	333.5	-	114.9
<u>D</u>	281.9	-	101.0
<u>E</u>	413.0	11.79	83.65
<u>F</u>	420.0	42.50	158.1
<u>G</u>	330.0	-	-
<u>H</u>	348.6	36.40	74.9
<u>I</u>	411.0	43.30	144.6

IDENTIFICATION OF CONSTITUENTS

The largest two portions of extract were examined according to procedures similar to those given by Hibbert and Phillips (45). Fifty grams of A were dissolved in 500 ml. of chloroform and extracted with a slight excess of five per cent sodium hydroxide. An emulsion in the chloroform layer was broken by filtration. Following the sodium hydroxide extraction an oily insoluble material was observed that was suspended in the chloroform layer. The basic water solution was made acid and was extracted with ether, which was then washed and dried (anhydrous Na_2SO_4 was used for all drying). The ether was evaporated and 0.8 g, (1.6 per cent of A) of free acids were obtained.

The chloroform layer with the material suspended in it was washed with saturated salt solution to remove any base. After isolating the dissolved and insoluble materials by evaporation of the chloroform in a flask, water was added and a steam distillation was performed to isolate the essential oils known to be present in Douglas fir bark (49) and which have already been characterized as terpenes. The distillate was extracted with ether and upon drying and evaporation of the ether 0.5 g. (1.0 per cent of A) was obtained.

The material remaining in the flask was taken up with chloroform and the chloroform was evaporated completely. The residue was dissolved in 250 ml. of dioxane

and 250 ml. of approximately 4-N sodium hydroxide was then added. The material was saponified by refluxing this mixture for several hours. When at the boiling point the material was all in one phase, an advantageous condition for any saponification. The dioxane was removed by steam distillation and then the mixture remaining was extracted with chloroform. An emulsion formed which contained quite a bit of insoluble material and was broken by centrifuging. Between the two liquid layers resulting there was a large amount of this solid material which would not dissolve. The separation was made and the solid was washed thoroughly with water and then chloroform. After this there still remained 11.1 g. that did not dissolve. The chloroform layer and washings were combined and upon evaporation yielded 10.5 g. of neutral compounds. The acids isolated from the basic solution in the usual way yielded 24.15 g. Also isolated were 2.3 g. of phlobaphenes from the acidified water solution by heating and thus precipitating them and from between the layers when the acidified soap solution was extracted with ether. The acids were steam distilled after being isolated and 0.21 g., (0.42 per cent of A) of volatile acids were obtained by extracting the distillate with ether and evaporating the dry ether solution completely.

The 11.1 g. of solid was resaponified and 0.6 g. of acids were recovered (total acid yield 24.75 g., 49.5

per cent of A). The remainder was difficultly soluble in the usual solvents except hot glacial acetic acid from which it could be recrystallized. In 600 ml. each of benzene and high boiling petroleum ether only gel formation took place at both low and high temperatures. From the recrystallization in glacial acetic acid and then another recrystallization from petroleum ether, lignoceryl alcohol M. P. 76° - 77°C . was obtained, acetate M.P. 56° - 57°C . The yield of the alcohol was 10.5 g., (21.0 per cent of A). Only part of it was isolated by the recrystallizations, but from the mother liquors essentially all of the remainder was isolated. The phenyl urethane of lignoceryl alcohol was prepared as a new derivative, since this derivative is far easier to prepare than the acetate and is isolated in appreciable yield even when only working with 100 mg. of compound while the acetate must usually be made with a larger weight sample and the yields are low. The melting point was quite sharp, M.P. 80.5° - 81.5°C . The 0.6 g. of acid obtained gave a neutral equivalent of 203.0 and an iodine number of 33.0.

The combined acids were treated with lead acetate to perform the lead salt - ether separation of the saturated from the unsaturated acids, but no separation was accomplished perhaps due to the large percentage of solid acids present. The neutral equivalent of the combined acids was 341.0 by potentiometric titration and the

iodine number was 93.21.

The 10.5 g., 21.0 per cent, of neutrals isolated from the chloroform were dissolved with difficulty in methanol after making a Liebermann test for sterols on the dry wax. The test was positive, but weak, and no sterols were isolated. This solution was extracted with petroleum ether in an effort to separate the sterols from the waxy alcohols. From the petroleum ether only docosanol-1 was isolated and after recrystallizing from petroleum ether had a M.P. 71.5° - 72.5°C. , the phenyl urethane M.P. 86°C. was made as derivative. From the methanol, also only docosanol-1 could be isolated. This accounted for practically all of the neutrals except the small amount of sterol not isolated.

From F (25 g.), in the same manner as for A, acids and neutrals were isolated. There were obtained 1.0 g. of essential oils (4 per cent of F) refractive index 1.4513, and 0.95 g. of free acids (3.8 per cent) neutral equivalent 258.0 and iodine number 69.0. The acids from the saponification of F yielded 12.0 g. (48.0 per cent) neutral equivalent 271.0 and iodine number 160.0. The neutral alcohol isolated was docosanol-1 5.2 g. (20.8 per cent) and accounted for the rest of the neutrals.

SUMMARY AND CONCLUSIONS

Previous work on the proximate analysis of barks and characterization of various extracts from the barks has been critically reviewed.

The proximate analysis of Douglas fir bark has been performed consisting of the following analyses: ash, hot water and alcohol-benzene solubles, lignin and holocellulose. The values obtained total 100.0 (\pm 1.0%) per cent, indicating that the procedures used gave satisfactory results.

The procedures commonly used in the analysis of wood have been modified in the following manner:

1. Twenty mesh samples were substituted for 60-80 mesh in certain procedures.
2. Products were dried at 60-65°C. at reduced pressure instead of at 105°C.
3. In the determination of lignin using the accepted 72 per cent sulfuric acid method, the reaction was allowed to proceed for five hours with constant agitation, instead of for two hours with intermittent agitation.
4. In the holocellulose determination of Ritter and Kurth (100) the bleaching step was deleted until before the last chlorination in order to avoid swelling the cellulose and thus preventing easy filtration and passage for the chlorine.

through the sample.

A bulk sample of the bark was extracted with high boiling petroleum ether yielding 217.1 g., 7.25 per cent on a dry basis.

Several fractions of the petroleum ether extract were isolated for comparison; saponification, acid and iodine values were determined on these fractions.

The largest fractions were characterized in a systematic manner to determine, where possible, the constituent compounds or at least mixtures of compounds, such as acids, by their neutralization equivalents and iodine numbers. Two alcohols were found and identified, docosanol-1 and lignoceryl (tetracosanol-1) alcohol, the former being reported for the first time as extracted from bark. A new derivative was prepared for the latter, the phenylurethane having a M.P. 80.5° - 81.5°C . Yields for the various fractions of compounds were given, and included, other than those mentioned, essential oils, free acids, volatile acids, and a small amount of phlobaphenes (in one of the fraction). Sterols were detected by the Liebermann test, but none could be isolated because of their minute content in the material.

It is hoped that with this work some inspiration for taking advantage of a badly neglected raw material is encouraged and that this investigation may serve as a guide to further work which will lead to the economical

utilization of Douglas fir bark.

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