AN ABSTRACT OF THE THESIS OF

Harry John Kiefer for the Ph. D. in Chemistry

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Abstract approved _____ (Major Professor)

The purpose of this investigation was to determine the composition of Douglas fir bark bast fibers to obtain information that might lead to more complete utilization of Douglas fir timber resources. Almost 1.5 million tons of bark are destroyed or burned as hog fuel each year. It was found that the bast fibers compose 36 to 47 per cent of the air dried bark as received in the mills. Structural examinations of the fibers in bark sections by means of a microscope revealed the presence of a ring type or layer structure. The fiber is surrounded by a wall or matrix of reddish-brown lignified material which by microscopic examination appeared to be removed by dilute aqueous alkali solutions. This lignified matrix seems to be responsible for the relatively high lignin content of the fibers (44.8 per cent oven-dry, extractive-free basis). The presence of this lignified wall also appeared to be at least partly responsible for the difficulty experienced in isolating holocellulose from the fibers.

Extractive contents of the fibers were obtained by successive extraction with: hexane, benzene, ethyl ether, hot water and ethanol. Yields obtained on an oven dry basis were: hexane, 1.59 per cent; benzene, 0.95 per cent; ethyl ether, 0.38 per cent; hot water, 9.61 per cent; ethanol, 0.96 per cent. This relatively low sum of extractives (13.5 per cent) indicated that it would not be feasible to utilize the fibers as a source of extractives, particularly wax and dihydroquercetin. Holocellulose determinations on the extractive-free fibers by means of conventional methods revealed that degradation of the cellulose occurred when attempts were made to obtain holocellulose with less than 5.4 per cent lignin. It was found that after the bark fibers had been extracted with a dilute alkali solution, a holocellulose fraction containing from 0.1 to 0.3 per cent lignin could be obtained. This fraction could not by definition be correctly called "holocellulose" since 9.3 per cent carbohydrate material was removed by the alkali along with 22.0 per cent lignin. The carbohydrate material removed by the alkali was found to consist of 1.89 per cent pentosans, 2.62 per cent uronic acid anhydride and 5.49 per cent hexosan. The "holocellulose" isolated after the alkali extraction was found to contain 77.8 per cent alpha cellulose, 1.6 per cent beta cellulose and 20.6 per cent gamma cellulose. The composition of the alpha cellulose was: ash, 0.24 per cent; lignin, 0.27 per cent; mannose, 1.04 per cent; xylose, 0.51 per cent; galactose, none; glucose, 97.94 per cent. An analysis of the total carbohydrate of the extractive-free fiber was found to be: galactose, 3.54 per cent; mannose, 6.31 per cent; xylose, 11.03 per cent by fermentation or 9.90 per cent by pentosan determination; arabinose, none; and glucose, 39.85 per cent, based on the weight of extractive-free fiber. Three fractions of "lignin" materials from the fibers

were isolated and studied. The fraction of "lignin" removed by one per cent sodium hydroxide represented 49 per cent of what appeared as Klason lignin on the whole extractive-free fibers. This easily removed "lignin" material was found by infrared and chemical analysis to resemble a high molecular weight phenolic acid rather than what is generally designated lignin. A functional group analysis by methylation studies revealed that this phenolic acid contained: methoxyl, 4.3 per cent; carboxy group, 4.9-5.3 per cent; phenolic hydroxyl, 8.3 per cent; and alcoholic hydroxyl, 4.2 per cent. On this basis, one building unit of molecular weight, 850-918, would contain one carboxyl, one methoxyl, two alcoholic hydroxyl and four phenolic groups. Based upon a molecular weight of 850, functional group data, and the elementary composition (54.12 per cent carbon, 5.33 per cent hydrogen), an empirical formula was proposed for the Douglas fir bark fiber phenolic acid.

Infrared studies confirmed the presence of the carboxyl group in this phenolic acid and served to show the similarity of the acid to the phlobaphenes isolated from the Douglas fir bark bast fibers. An ultraviolet absorption spectrum of the sodium hydroxide soluble phenolic acid showed the maximum absorption peak in the 280 millimicron region. This behavior has been found to be common to many lignin, tannin and phlobaphene preparations. Oxidation of the phenolic acid from the bast fibers yielded 1.63 per cent vanillin. Evidently, the phenolic acid does not resemble lignin in respect to the presence of a vanillin nucleus. This is in accord with the infrared spectrum which shows little indication of the presence of carbonyl groups. The low methoxyl content of the bark fiber phenolic acid is comparable to the methoxyl content of the phlobaphene which was found to contain 2.77 per cent methoxyl. This relationship to phlobaphene was further displayed when the infrared spectra of the two materials were found to be almost identical. Both spectra displayed strong carboxyl or ester absorption and little or no carbonyl absorption.

The lignin extracted from Douglas fir bark fibers by means of dioxane-HCl solution was obtained in 10.8 per cent yield. An 11.8 per cent yield based on the weight of oven dry, extractive-free fibers was obtained when the fibers had been previously extracted with one per cent sodium hydroxide solution. These yields are equivalent to about 24 per cent of the total "Klason lignin" in the extractive-free fiber. Dioxane lignin was found to possess more similarity to soft wood native lignin than to the phenolic acid of the fibers. By methylation studies its functional groups were found to be: methoxyl, 14.3 per cent; alcoholic hydroxyl, 4.4 per cent; phenolic hydroxyl, 3.2 per cent; carboxyl group, 2.5 per cent. The preparation of a phenylhydrazone derivative and infrared absorption show the presence of a carbonyl group in the dioxane-HCl lignin. This dioxane lignin was the only fraction of the bark fiber lignins which exhibited decisively the presence of carbonyl groups.

The 72 per cent sulfuric acid or Klason Lignin amounted to 44.8 per cent of the extractive-free bark fibers and represented the total lignin content as it is conventionally determined. Its spectra showed it to be similar to the other two preparations but possessing decreased functional group contents. Therefore, the "lignin" in the bast fibers is not a homogeneous material and further work on its chemistry should be performed on purified homogeneous fractions.

THE CHEMICAL COMPOSITION OF THE BAST FIBERS OF DOUGLAS FIR BARK, PSEUDOTSUGA TAXIFOLIA (POIR.) BRITT.

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HARRY JOHN KIEFER

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APPROVED:

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Professor of Wood Chemistry

In Charge of Major

Redacted for privacy

Head of Department of Chemistry

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

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THE CHEMICAL COMPOSITION OF THE BAST FIBERS OF DOUGLAS FIR BARK, PSEUDOTSUGA TAXIFOLIA (POIR.) BRITT.

INTRODUCTION

The purpose of this investigation was to determine the composition of Douglas fir bark bast fibers to obtain information that might lead to more complete utilization of Douglas fir timber resources. It is estimated that an average of 6.2 billion board feet of Douglas fir timber are cut annually (46, p.3). Since there are approximately 520 pounds of bark per 1000 board feet of timber, almost 1.5 million tons of bark are destroyed in waste burners or burned as hog fuel each year (14).

In the past few years, lumber manufacturers and users have taken heed of this great waste of raw material. Encouraging potentialities for Douglas fir bark utilization have been found in the extractives removed by neutral solvents (22, 28). Useful products found consist of wax, tannin and dihydroquercetin. The wax demonstrates properties comparable to high priced, imported waxes. Douglas fir bark tannin has been used for some time to produce leather at a tannery in Dallas, Oregon. Dihydroquercetin, a "flavonoid" compound, possesses properties of pharmaceutical value; it is also an antioxidant for the prevention of rancidity of fats and oils.

Another possibility for the utilization of Douglas fir bark rests upon a physical separation into its structural components. The Weyerhaeuser Timber Company at Longview, Washington has initiated a process whereby the bark is separated into cork, fiber and amorphous powder. These bark products are finding increasing use in plastic

compositions, adhesives, oil-well drilling muds and as a soil conditioner. Production, which began in 1947, has advanced to more than one million pounds per month, but this quantity still represents only a minor portion of that available.

A search of the literature revealed that little information is available describing the chemical composition of any of the physically separable components of Douglas fir bark. This investigation of the bast fibers was then undertaken with the thought that a more complete knowledge of the chemical composition of one of the major components may lead to greater utilization of this vast source of raw material.

SOURCE AND PREPARATION OF BARK SAMPLES

A study was made to determine the approximate fiber content present in Douglas fir bark. Four samples of 500 grams each were ground on a Gruendler hammer mill, air dried and then screened by means of a Rotap shaker. The fractions which were larger than 40 mesh were ground on a Sprout-Waldron attrition-type mill and screened again by means of the Rotap shaker to effect better fractionation. Repeated screening resulted in fractions each consisting predominately of either amorphous powder, fiber or cork.

Sample No. 1 consisted of bark taken from trees ranging from 110 to 260 years in age. They had been felled one month prior to sampling on the Crown-Zellerbach holdings in Molalla, Oregon. Equal quantities of bark were taken from top and bottom log sections. Sample No. 2 was taken from this same area but from trees ranging from 80 to 95 years in age. Sample No. 3 was a random selection taken at the Corvallis Lumber Company in Corvallis, Oregon; Sample No. 4 was also a random sample taken from the Chapman Company in Corvallis, Oregon.

The results obtained are presented in Table 1. Obviously, the values obtained are only approximate because of the impossibility of realizing absolute separation by a screening process on material of this nature. The amorphous fines passed through standard 100 mesh screen; fibers passed through 40 mesh screen and were largely retained on 60 and 100 mesh; cork particles were retained on 20, 30

TABLE 1

COMPOSITION OF AIR-DRIED BARK IN WEIGHT PER CENT

Sample	Fiber	Cork	Fines	Loss
No. 1 110-260 years	36.5	41.5	17.8	4.2
No. 2 80-95 years	47.8	33.4	15.5	3.3
No. 3 Random, Corvallis Lumber Company	35.4	32.2	18.2	14.2
No. 4 Random, Chapman Company	41.2	24.8	29.0	5.0
No. 5 Weyerhaeuser Co. Production Data	38	28	34	--

and 40 mesh screens. The losses reported were almost entirely composed of fines lost as dust.

Fibers for the chemical analysis were obtained in an almost pure state by stirring the crude fiber fraction in five times its volume of distilled water at room temperature. A small portion of wetting agent aided in the separation. By virtue of their greater specific gravity, the fibers readily sank to the bottom of the container, thereby allowing the cork and woody impurities to be skimmed from the surface. The purified fibers were then filtered off on a large Büchner funnel, air dried and placed in sealed jars for storage.

Since Sample No. 1 was believed most representative of the timber cut, fibers from this sample were used exclusively for the analysis.

I. STRUCTURAL CHARACTERISTICS

Douglas fir bast fibers are sharply-pointed, spindle-shaped particles of a red-brown color. Their largest diameter is such that they may pass through 40 or 60 mesh screens; a very small portion passed through 100 mesh and were found with the amorphous fines. Other physical properties of the fibers that are pertinent to their use as fillers and in the plastics industry have been described (10, 48).

The physiological significance of the bast fibers and their functions have not been entirely clarified. However, it is generally believed that the presence of the fibers in the bark imparts strength and protection to the inner functioning elements, particularly the sieve tubes (6, p.29).

The following photomicrographs were taken of thin bark sections to illustrate the general appearance and position of the fibers in the bark layer. The general plan for preparation of wood sections involved the removal of moisture from the sample by means of solvents and a replacement with embedding paraffin. Several procedures using amyl acetate, dioxane, and alcohols were tried but found to be unsuccessful. Apparently the cork layers were plasticized and softened to the point where they crushed under the microtome blade and would not support the harder fibers. Sections were finally made from unembedded material. The blocks were placed in the microtome, and, before each section was cut, a small amount of melted paraffin was wiped across the surface with a warm scalpel. This was followed by

a brief chilling with ice. The sections were then cut and affixed to the slide with a dilute gelatin adhesive ("Haupt's" adhesive). After drying, the paraffin was removed by placing the slide in xylol. The slides were then stained using Heidenhain's hematoxylin and safranin schedule as modified by Esau (12). The stained slides were then mounted in H. S. R. synthetic resin (Scientific Supplies). Photomicrographs were made on Eastman Super XX film, developed in D-42 contrast developer.

Views of these sections are shown in Figures 1, 2, 3 and 4. Figure 5 shows the isolated fibers.

II. EXTRACTIVE CONTENTS OF THE FIBERS

The extractives removed from Douglas fir bark by means of neutral solvents have been previously described (22, 28). It was decided, however, to make a comparison of the extractive contents of the fibers to those of the cork fraction (20) and whole bark. From these data it should be possible to determine the feasibility of utilizing the fibers for their extractive contents.

The plan of analysis was essentially that used in the extractive studies made upon the whole bark (28). Duplicate 25 gram samples of fiber, moisture content 3.80 per cent, were extracted successively with hexane, benzene, ethyl ether, hot water and ethanol. The hot water extraction was that of T.A.P.P.I. procedure T 1 m-45 (42); other solvent extractions were made by extracting for six hours in Soxhlet-type extractors. In each case except that of the hot water

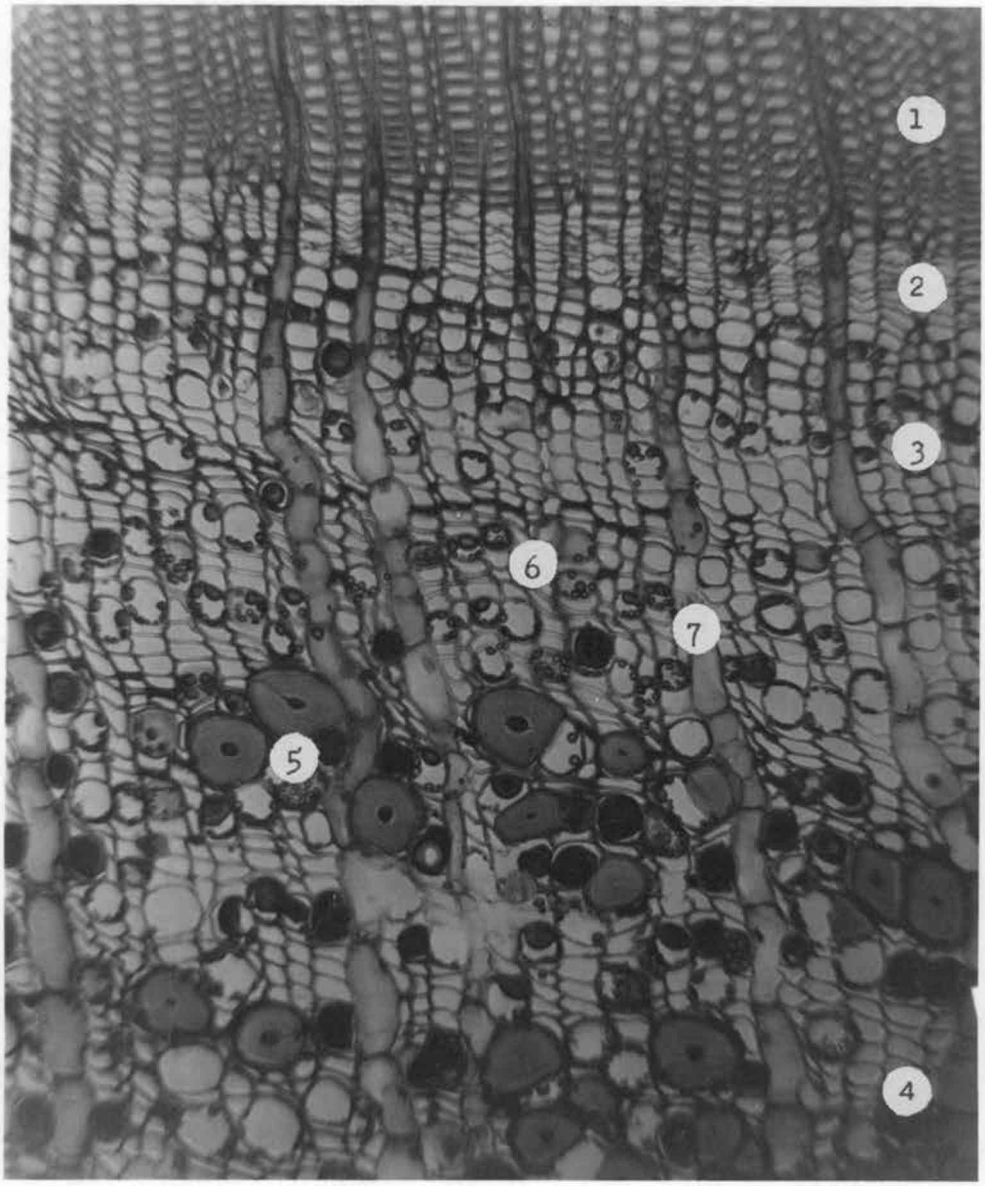


FIGURE 1

CROSS SECTION OF DOUGLAS FIR INNER BARK
SHOWING POSITION OF THE BAST FIBER (100 X)

1. Wood or xylem
2. Cambium region
3. Functioning phloem
4. Inactive phloem
5. Bast fiber
6. Phloem parenchyma cell
7. Phloem ray

FIGURE 2

TANGENTIAL SECTION OF DOUGLAS FIR BARK
SHOWING IRREGULAR, SPINDLE SHAPES (40 X)

1. Bast fiber; neighboring fiber to the right shows exposed cavity or lumen
2. Horizontal resin canal
3. Inner active phloem

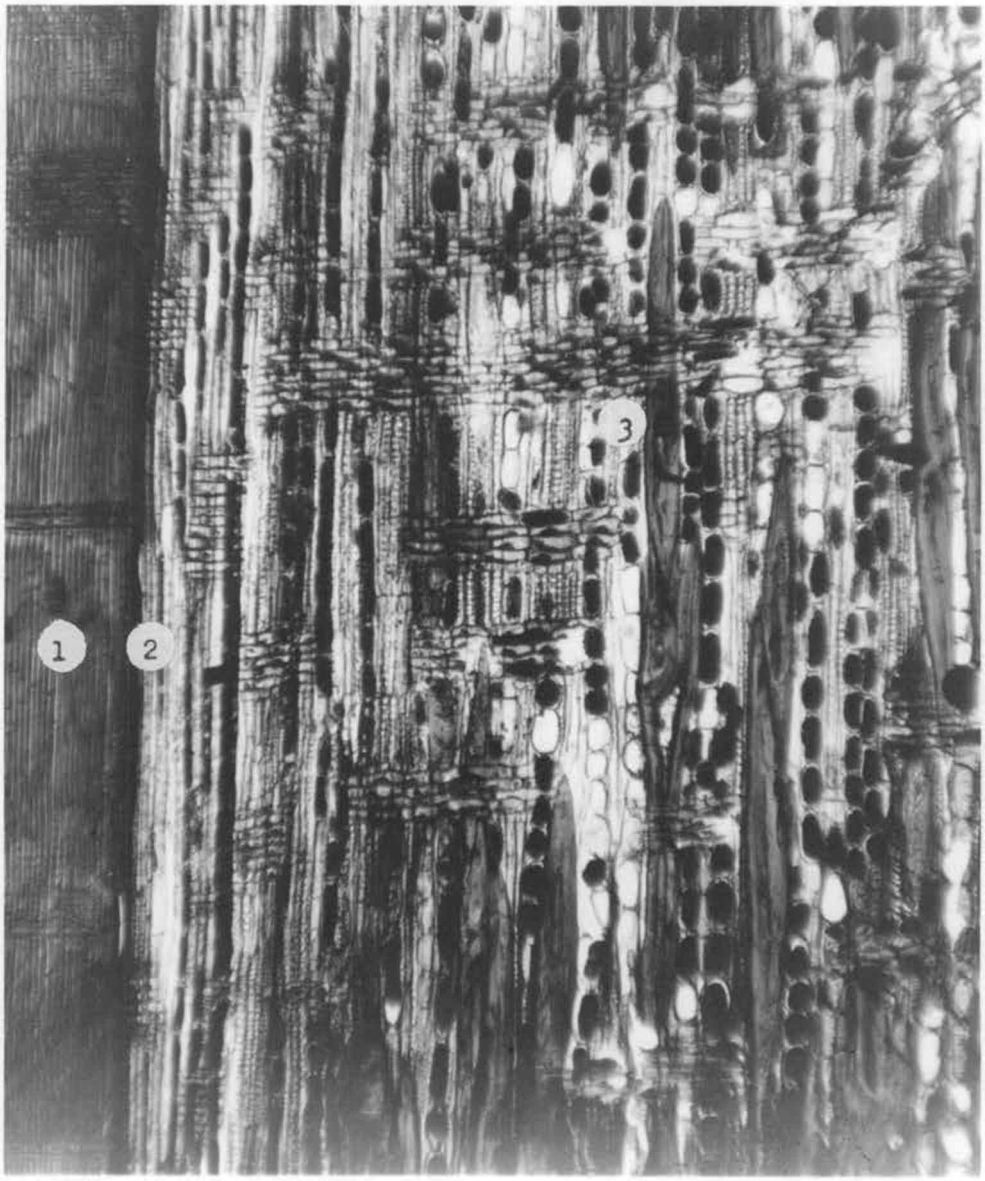


FIGURE 3

RADIAL SECTION OF DOUGLAS FIR BARK (40 X)

1. Wood or xylem
2. Cambium
3. Bast fiber

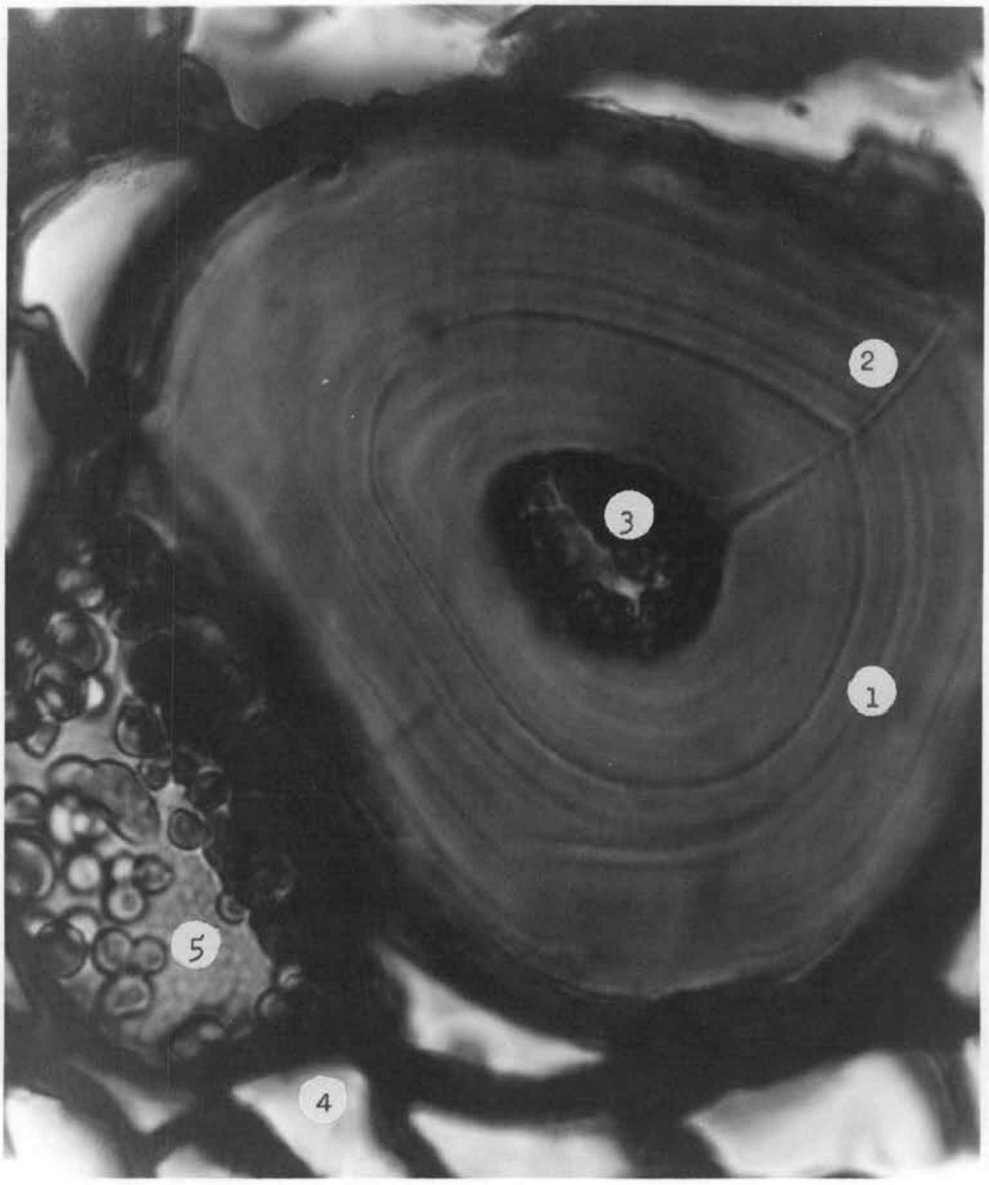


FIGURE 4

CROSS SECTION OF DOUGLAS FIR BARK BAST FIBER (1000 X)

1. Ring structure
2. Pit
3. Cell cavity or lumen showing living protoplast
4. Crushed sieve cells
5. Phloem parenchyma

FIGURE 5

ISOLATED DOUGLAS FIR BARK BAST FIBERS (8 X)

extraction, the solvent containing the extractives was filtered into a tared, glass evaporating dish and evaporated to near dryness on a hot water bath. Drying period was completed by means of an eight hour period in a vacuum oven at 60° C.

Results obtained together with the results of previous investigations are shown in Table 2. The results obtained indicate that the bast fibers are relatively poor in extractives, particularly the waxes and dihydroquercetin. It would therefore not be advisable to utilize the fiber fraction of the bark as a source of these substances.

TABLE 2

COMPARATIVE EXTRACTIVE CONTENTS OF DOUGLAS FIR BARK FRACTIONS

Per Cent Oven Dry Weight of Unextracted Sample

Solvent	Fiber	Cork[1]	Whole Bark	
Hexane	1.59	5.84	5.47	Light yellow wax
Benzene	0.95	2.50	2.52	Brown wax
Ethyl Ether	0.38	19.00	5.95	Dihydroquercetin
Hot Water	9.61	7.10	6.68	Tannin, Carbohydrates
Ethanol	0.96	6.91	7.70	Phlobaphene
Sum	13.49	41.35	28.32	

Hergert, Herbert L. The chemical nature of Douglas fir cork. M.S. Thesis, Oregon State College, 1951.

EXPERIMENTAL PROCEDURE

An overall percentage composition of the bast fibers as determined by T.A.P.P.I. Standard Methods of Wood Analysis (42) is presented in Table 3. Values in the literature for Douglas fir wood are presented as a means of comparison.

An examination of the values in Table 3 revealed that the greatest difference between the bark fibers and the wood exists in the 14 per cent higher lignin content of the bark fibers. The methoxyl content of the Klason lignin from the fibers was found to be approximately one-half that of the wood. This indicated that the fiber lignin is different or contains a fraction differing from that of the wood. The ash content of the fiber was also found to be considerably higher than that reported for the wood.

The plan for more complete analysis of the fibers in this investigation was first an examination of the carbohydrate portion followed by a characterization of lignin fractions separated by various means.

I. CARBOHYDRATE PORTION OF THE FIBERS

Holocellulose. Investigations by Wacek and Schon on spruce bark (44), Lewis et al. (30) on redwood bark, and Kurth (26) have demonstrated the difficulties encountered in separating the cellulose portion of barks from lignin. Similar difficulties were also experienced in the course of this investigation of the bast fibers from Douglas fir bark.

TABLE 3

COMPARISON OF DOUGLAS FIR BARK BAST FIBERS AND WOOD

Per Cent Oven Dry Weight of Materials

	Fiber	Wood[2]
Ether Solubles	2.92	1.32
Alcohol Solubles	8.65	5.46
Hot Water Solubles	2.58	2.82
Sum of Extractives	14.15	9.60
Ash	0.601	0.175
Lignin	44.80	30.15
Holocellulose (Chlorite)	54.58	71.40
Pentosans (Uncorrected)	8.62	10.11
Methoxyl	3.89	4.75
Methoxyl on lignin	7.16	15.20[3]
Acetyl	2.39	0.59[3]
Uronic Acid Anhydride	4.62	2.80[3]

[2] Graham, Harold M. and Ervin F. Kurth. Constituents of extractives from Douglas fir. Industrial and engineering chemistry, 41: 409-414. 1949.

[3] Lewis, Harry F. The significant chemical components of western hemlock, Douglas fir, western red cedar, loblolly pine and black spruce. Tappi 33:299. 1950.

The solubilities in Table 3 are reported on the basis of the oven dry unextracted materials and were determined successively in the order ether, alcohol and hot water. All other analyses are reported on the basis of the oven dry extracted material and were determined on the extracted sample prepared in accordance with T.A.P.P.I. procedure T 12 m-45.

Attempts to prepare holocellulose by means of the monoethanolamine and chlorination procedure T 9 m-45 (42) proved unsuccessful because of filtration difficulties. Therefore, an attempt was made to prepare holocellulose by a modified method of Jayme, employing sodium chlorite and acetic acid (50). To determine conditions which might prove satisfactory for isolation of the holocellulose, a series of determinations were made on 2.5 gram samples of the fibers. Duplicate samples were subjected to a different number of treatments with sodium chlorite and acetic acid in the following manner: The sample was placed in a 250 milliliter Erlenmeyer flask with 160 milliliters of water, 10 drops of glacial acetic acid and 1.5 grams of sodium chlorite. The flask was then placed in a hot water bath maintained at 70 to 80° C. for one hour. At the end of the one hour period another addition of the same amount of sodium chlorite and acetic acid was made. These additions were repeated each hour for the number of times indicated in Table 4. Upon completion of the treatments, the flask was removed from the water bath and cooled to room temperature before filtering on a sintered glass crucible. The holocellulose was washed with ice water and finally acetone to facilitate drying in a vacuum oven at 50° C. to constant weight. This method of drying was used because it was found that when the bark fiber holocellulose was prepared in the foregoing manner and dried in an oven at 105° C., it became hardened to such an extent that subsequent lignin determinations on the holocellulose were difficult to check.

TABLE 4

CHLORITE HOLOCELLULOSE DETERMINATIONS

Per Cent Oven Dry Weight of Extractive-free Fibers

Test No.	Treatment	Holocellulose	Lignin on Holocellulose	Holocellulose Corrected for Lignin	Total Lignin plus Holocellulose
1 A	4 treatments, cooled to room temperature and filtered	61.42	7.35	56.90	100.98
2 A	4 treatments, held at room temperature 24 hrs. before filtering	59.16	6.66	56.08	100.88
3 A	4 treatments, held at room temperature 48 hrs. before filtering	57.78	5.55	54.58	99.38
1 B	6 treatments, cooled to room temperature and filtered	55.26	5.05	52.47	97.27
2 B	6 treatments, held at room temperature 24 hrs. before filtering	52.35	2.42	51.07	95.87
1 C	2 treatments, cooled to room temperature, filtered, followed by 2 more treatments, cooled to room temperature and filtered	58.33	5.42	55.15	99.95

Table 4 shows the results of holocellulose determinations carried out on the fibers under various conditions.

The total lignin value on the extractive-free bast fibers was found to be 44.80 per cent by the 72 per cent sulfuric acid method T 13 m-45 (42). This value was used in making the calculations in Table 4. Data shown in the table indicate that if an attempt is made by this method to reduce the lignin content of the holocellulose to less than six per cent, degradation occurs. An attempt was made to determine if some of the degradation could be avoided by filtering off the holocellulose after two treatments and then continuing the treatment with fresh water and reagents. In this manner an excess of the hot reagents and reaction products would not be in contact with the holocellulose for as long a period of time. The results obtained (Test 1 C, Table 4) indicated that some advantage was gained by this method, since one per cent more lignin could be removed without appreciably degrading the holocellulose. Other determinations were made employing a similar procedure, but none was more satisfactory than 1 C.

One per cent sodium hydroxide extraction of the extractive-free fibers. It has been shown that with materials in which the lignin is difficult to remove, an extraction with dilute alkali may be advantageous in obtaining a cellulose fraction suitable for analysis (41). This procedure was investigated using the T.A.P.P.I. method T 4 m-44 (42), whereby a one gram sample of extractive-free fibers

in 150 milliliters of one per cent sodium hydroxide solution is placed in a boiling water bath for one hour. It was found that 31.35 per cent of the oven dry weight of fiber was extracted by the alkali. A lignin determination on the residue indicated a 33.21 per cent lignin content. This figure shows that 22.0 per cent lignin and 9.35 per cent carbohydrate material of the extractive-free fibers were removed by an extraction conducted in this manner.

Holocellulose determinations on the alkali extracted fibers. Both the monoethanolamine holocellulose determination, T 9 m-45 (42) and the chlorine dioxide holocellulose determination (50) were attempted on the alkali extracted fibers. In the case of the monoethanolamine procedure, a chlorination of the material is followed by an extraction of the chlorinated lignin with a monoethanolamine-ethanol solution in which the chlorinated lignin is soluble. The chlorination-extraction procedure is repeated until the lignin is removed as evidenced by no further color change in the material being treated. Eight chlorination cycles were used in treating the fibers; filtration became difficult in the last two cycles, but an end point was indicated at this point.

In the case of the chlorine dioxide method described in the foregoing section, it was found that only three treatments with sodium chlorite and acetic acid were necessary to reduce the lignin content to a point where the cellulose could be used for further investigation.

Results of holocellulose determinations are shown in Table 5.

TABLE 5

HOLOCELLULOSE DETERMINATIONS*

Per Cent Oven Dry Weight of Extractive-free Fibers
Treated with One Per Cent Sodium Hydroxide

Test No.	Treatment	Holocellulose*	Lignin on Holocellulose	Holocellulose Corrected for Lignin	Total Lignin plus Holocellulose
1	8 chlorination cycles followed by monoethanol-amine extractions	66.45	0.10	66.39	99.60
2	3 treatments with sodium chlorite and acetic acid	68.01	0.382	67.79	101.00

* By exact definition this is not true holocellulose because 9.35 per cent carbohydrate material was also removed with 22.01 per cent "lignin" by the prior one per cent sodium hydroxide extraction.

These results indicate that either method may be employed for preparing a cellulose fraction suitable for further analysis. This fraction could not by definition be correctly called holocellulose since 9.35 per cent carbohydrate material was removed by the one per cent sodium hydroxide extraction along with the 22.01 per cent "lignin". To determine the nature of the soluble cellulosic material, pentosan and uronic acid determinations were performed upon the fibers before and after the alkali extraction. The soluble portion which appeared as Klason lignin was characterized in the section of this investigation dealing with lignin.

Pentosans were determined by Tollen's method, T 223 m-43, whereby the pentosans are decomposed by 12 per cent hydrochloric acid, and the furfural evolved is weighed as the phloroglucinide (42). Uronic acid anhydride content was determined by the method of Whistler, Martin and Harris (49), whereby the CO_2 evolved on decomposition of the polyuronides is absorbed on ascarite and weighed. Pentosan values were corrected for furfural evolved from the polyuronides (36).

Results of these determinations are shown in Table 6. The data given in Table 6 indicated that 25.0 per cent of the pentosans and 56.7 per cent of the polyuronide material were removed under the conditions of the one per cent sodium hydroxide extraction employed.

TABLE 6

PENTOSAN AND POLYURONIDE DETERMINATION ON FIBERS

Per Cent Oven Dry Weight of Extractive-free Fibers

	Extractive Free Fibers	NaOH Treated	Removed by NaOH
Pentosans (Corrected)	7.57	10.05	1.89
Uronic Acid Anhydride	4.62	2.92	2.62
Hexosans (Difference)			4.84
Total Removed by 1% NaOH			9.35

Alpha, beta, and gamma cellulose. A sample of holocellulose was prepared from the sodium hydroxide extracted fibers by the chlorine dioxide method employing three treatments with sodium chlorite. This holocellulose was then examined for alpha, beta, and gamma cellulose by the method of Launer (29). This technique is based upon the oxidation of the cellulose fractions with excess potassium dichromate; the excess dichromate is then determined by titration with Mohr's salt. Results obtained are shown in Table 7.

TABLE 7

PER CENT OVEN DRY WEIGHT OF HOLOCELLULOSE

Alpha Cellulose	77.8
Beta Cellulose	1.6
Gamma Cellulose	20.6

Sugar components of the holocellulose. A qualitative paper-chromatographic analysis was applied upon the hydrolyzate from Klason lignin determinations on extractive-free fiber prepared according to T.A.P.P.I. method T 12 m-45 (42). This method of separation, applied to sugar mixtures by Gordon (9, 18), depends upon the difference in partition coefficients of the sugars between the stationary water phase and the moving solvents on filter paper. The apparatus employed consisted of a glazed sewer tile eight inches in diameter and approximately three feet in length which had been ground flat on the bell end to facilitate sealing the top by means of a glass plate. The tile was then placed in an upright position in a pan two inches deep. The pan was filled with water which had previously been saturated with an 80 per cent butanol-20 per cent ethanol mixture.

To prepare the paper chromatogram, large sheets of Whatman No. 1 filter paper were cut into strips 43 by 12 centimeters in size. One per cent aqueous solutions of reagent grade glucose, galactose, mannose, xylose and arabinose were prepared; a small amount of each of these sugar solutions was then introduced as a circular spot five millimeters in diameter and one centimeter apart on an horizontal line drawn 7.5 centimeters from the top of the paper strip. The end of the strip containing these sugar spots was then placed in a glass trough suspended across the ledge formed by the bell on the inside of the glazed tile. The trough was filled with a mixture of 80 per cent butanol and 20 per cent ethanol which had previously been saturated with water. The entire system was allowed to stand from

18 to 24 hours in a constant temperature room at 24° C.

After drying the paper strip, the sugar spots were located by spraying the paper with a mixture containing equal parts of 0.1 N silver nitrate and 5 N ammonium hydroxide solution. The strip was then dried in an oven at 105° C. for five to ten minutes; the sugars appeared as dark spots on a light background. By comparing the position of the known sugars with that of the unknown, a qualitative estimation was made of the sugars in the unknown hydrolyzate.

The determinations were made upon the filtrate from the lignin determinations made on the extractive-free Douglas fir bast fibers. This filtrate contained the carbohydrate fraction of the fiber hydrolyzed by the acid to simple sugars. The solution used for the test was prepared by neutralizing with barium carbonate, filtering off the precipitated barium sulfate, concentrating the solution of barium uronate and sugars, treating with alcohol to precipitate the insoluble barium uronate, filtering and distilling off the alcohol from the sugar-containing filtrate. The residue of sugars was dissolved in a small amount of water, and the solution obtained was used for the tests.

Glucose, galactose, xylose and mannose or arabinose or both were found to be present. The latter two sugars were found at approximately the same positions on the developed chromatogram in both the known and unknown determinations. Wise has shown that mannose, arabinose and certain other sugar combinations cannot be readily differentiated by present paper chromatographic methods (52).

However, mannose was easily determined in the presence of the other sugars in wood by its characteristic phenylhydrazone (51). No arabinose was found by fermentation analysis in the following quantitative assay.

Microbiological assay of sugars in holocellulose. A two gram sample of extractive-free bast fibers was hydrolyzed, according to the T.A.P.P.I. lignin determination, T 13 m-45 (42). After hydrolysis, 38 grams of calcium carbonate were added slowly to the hot solution to neutralize the sulfuric acid present. The solution was then heated until the pH had reached 6-7 as indicated by Alkacid test paper; it was then allowed to settle overnight before making up the solution to 1400 milliliters and filtering. The precipitate of calcium sulfate was not washed; instead, the volume was measured and 40 milliliters was allowed for the bulkage of the calcium sulfate. The ratio of the volume before filtering to that after filtration gives a measure of the amount of the original sample in the hydrolyzate to be analyzed (51).

The filtrate was evaporated to a thin syrup (about 25 milliliters) under reduced pressure at 80° C., filtered and poured into 125 cubic centimeters of 95 per cent ethanol. This caused the calcium uronates to precipitate out. After standing overnight, the calcium uronates were removed by filtration and the alcohol was evaporated off on a steam bath. The thin syrup was diluted to approximately 75 milliliters and clarified with 10 milliliters of 10 per

cent zinc sulfate solution and an equivalent amount of sodium hydroxide solution according to the method of Browne and Zerban (8, p.846).

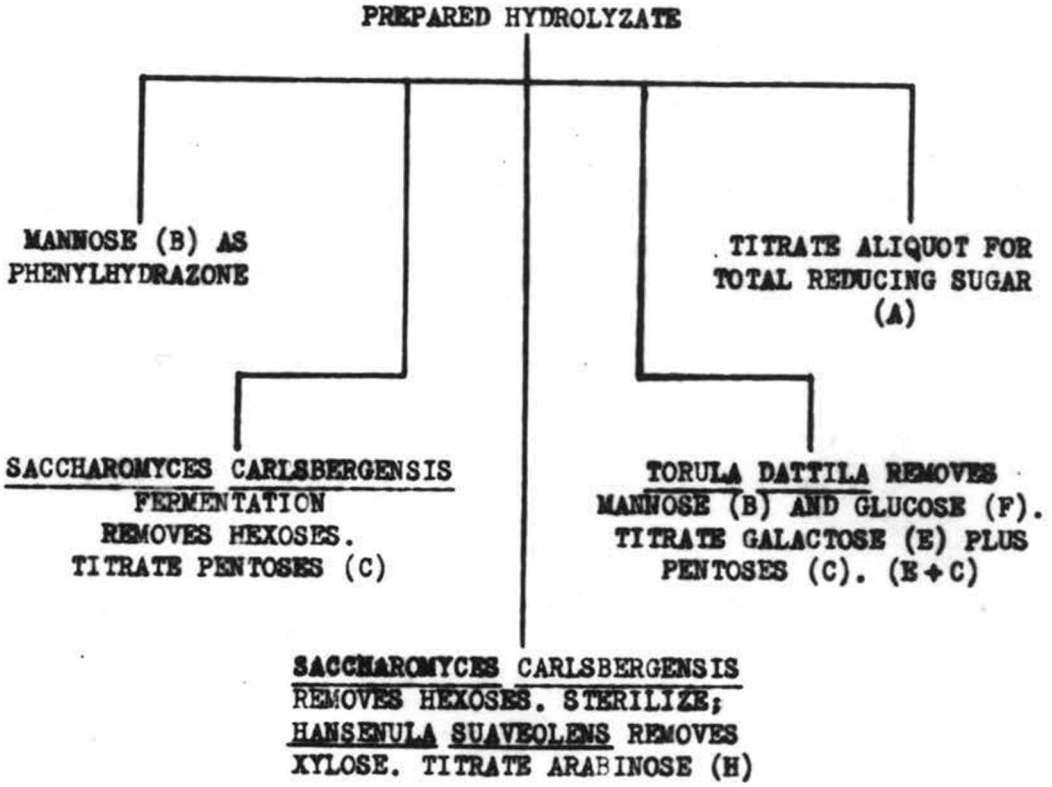
After clarification the solution was made up to 100 milliliters in a volumetric flask and 25 milliliter aliquots were inoculated with the proper organisms for the sugar determination as shown in Figure 6.

Fermentation procedures. Fermentations were made in much the same manner as those of Wise and Appling (53). Standard suspensions of yeasts were prepared from bottle slants of 48 hour cultures. In all cases fermentations were carried out in an agitator at 30° C. for 24 hours. Aliquot portions of the fermented solutions were diluted to within the range of the Somogyi reagent used for reducing value determinations (40).

Standard reducing sugar curves were prepared using reagent grade glucose, xylose, galactose and arabinose. Reducing values were determined by Somogyi's method (40). Paper chromatographic methods employed on the fiber hydrolyzate had previously indicated the presence of glucose, galactose, xylose, and mannose and/or arabinose. Mannose was determined by means of its phenylhydrazone (51). No arabinose was found to be present by fermentation analysis.

Results of the sugar analysis are shown in Table 8.

FIGURE 6



KEY

A= TOTAL REDUCING SUGAR

B= MANNOSE

C= PENTOSES (G + H)

D= HEXOSES (F + B + E)

E = GALACTOSE

F = GLUCOSE

G= XYLOSE

H= ARABINOSE

CALCULATIONS

HEXOSES (D) = A − C

GALACTOSE (E) = (E + C) − C

XYLOSE (G) = C − H

GLUCOSE (F) = D − (B + E)

SCHEME FOR MICROBIOLOGICAL ASSAY OF SUGARS

TABLE 8

CARBOHYDRATE FRACTION OF DOUGLAS FIR BARK BAST FIBERS

	Per Cent Extractive-free Fibers
Galactose	3.54
Mannose	6.31
Xylose (Fermentation)	11.03
(Xylose, calculated from Pentosans)	(9.90)
Arabinose	0.00
Glucose	39.83
Total Reducing Sugar (Fermentation Basis)	60.71

Analysis of alpha cellulose from bast fibers. Three five gram portions of extractive-free fibers were given four treatments with sodium chlorite and acetic acid to delignify them. These residues were then treated with 17.5 per cent sodium hydroxide, T 203 m-44 (42), to obtain alpha cellulose for analysis. Determinations were then made for pentosan, mannan, ash, and lignin. Galactose was found to be absent by the mucic acid test (3, p.528). Results of these determinations are shown in Table 9. Xylose was calculated from the pentosan value.

TABLE 9

COMPOSITION OF ALPHA CELLULOSE FROM BAST FIBER

	Per Cent Oven Dry Alpha Cellulose
Ash	0.24
Lignin	0.27
Mannose	1.04
Xylose (0.453 per cent Pentosans)	0.51
Galactose	0
Glucose (Difference)	97.94

LIGNIN COMPONENTS OF THE BAST FIBERS

Three fractions of lignin from the bast fibers were examined to characterize the lignin portion. The first material investigated was that lignin removed by the one per cent sodium hydroxide extraction discussed in the previous section on holocellulose. The second fraction was obtained by extraction with a solution of dioxane containing 0.4 per cent HCl catalyst. This dioxane-HCl soluble lignin was studied as obtained directly from extractive-free fibers and also as obtained from extractive-free fibers previously extracted with one per cent sodium hydroxide. A third fraction was the residual lignin of the fibers which could not be removed by one per cent NaOH and dioxane-HCl solution and was obtained as Klason or 72 per cent sulfuric acid lignin.

I. SODIUM HYDROXIDE SOLUBLE "LIGNIN"

It was shown previously in this investigation that 22.01 per cent of the materials in the extractive-free fibers which appeared as Klason lignin was removed by a one per cent sodium hydroxide extraction, T 4 m-44 (42). In the course of investigating this material it became evident that it closely resembled a high molecular weight phenolic acid rather than what is generally termed lignin. This fraction will therefore be referred to as a phenolic acid fraction rather than lignin.

Solubility in sodium hydroxide. A study was made to determine the amount of material removed from the extractive free bast fibers by means of aqueous sodium hydroxide solutions of various concentrations ranging from one to twenty per cent. Five samples weighing 1.5 grams each were extracted with 150 milliliters of alkali in 250 milliliter flasks. The flasks were maintained at the temperature of a boiling water bath for one hour. Upon removal from the water bath, each sample was filtered on a tared, sintered glass crucible, washed with distilled water, 25 milliliters of five per cent acetic acid and finally with distilled water until the washings were free of acid. The residue was then washed with acetone and ether before drying in a vacuum oven at 60° C. for four hours. Lignin determinations by the 72 per cent sulfuric acid method were made on the dry residues. Results are shown in Table 10.

It is apparent from the data in Table 10 that a one per cent sodium hydroxide solution removes almost the same amount of lignin as a ten per cent solution. A twenty per cent solution removes only 2.3 per cent more lignin than a one per cent solution. Apparently the dissolved lignin is an easily removed fraction, whereas the residual lignin is tightly bound chemically or physically in the bast fiber.

It was decided that a one per cent solution of sodium hydroxide would be used for the preparation of the soluble lignin fraction for investigation. Fifty grams of the extractive-free fibers were treated with 1500 milliliters of one per cent sodium hydroxide

TABLE 10

SODIUM HYDROXIDE EXTRACTIONS OF DOUGLAS FIR BARK FIBERS

Per Cent Oven Dry Extractive-free Fibers

Sodium Hydroxide Solution	Sodium Hydroxide Insoluble	Residual Lignin in Insoluble Portion	Dissolved Lignin (by Difference)	Dissolved Celluloses
0%	-----	44.80	-----	-----
1%	68.65	22.79	22.01	9.34
5%	64.13	21.10	23.70	12.17
10%	64.60	20.87	23.93	11.47
15%	64.97	20.48	24.32	10.71
20%	63.89	20.47	24.33	11.78

solution on a boiling water bath for one hour. The solution was then filtered on a Büchner funnel, and the filtrate was treated with sufficient dilute hydrochloric acid to render it just acid to litmus. A voluminous, brown precipitate formed and was allowed to settle overnight. The supernatent liquid was decanted from the precipitate, and the precipitate was transferred to centrifuge tubes where it was washed twice with distilled water.

The washed residues were dissolved in dioxane in which carbohydrate material is insoluble. The dioxane solutions were dried for several days over anhydrous sodium sulfate, then filtered and slowly dropped into a large volume of ether with agitation. In this way a light-brown, amorphous precipitate was obtained. The ether-dioxane solution was decanted from the precipitate, which was air dried, ground in a mortar, washed with hexane and then dried for three hours in a vacuum oven at 60° C. The yield of purified product was 6.1 grams of a calculated yield of a total of 9.8 grams. By evaporating the mother liquor and washings to 300 milliliters under vacuum, 1.9 grams more of the phenolic acid precipitate were obtained. Thus a loss of 1.8 grams was sustained. This may be attributed to difficulties in the isolation and incomplete precipitation.

Properties of the alkali soluble phenolic acid. The dried powder was found to be soluble in dilute alkali solutions forming a dark red solution. After drying, the phenolic acid was only slightly soluble in dioxane, methyl and ethyl alcohol, acetone, pyridine and

ethyl acetate, whereas the moist precipitate when first isolated could be dispersed completely in dioxane or acetone.

No color reaction was obtained with phloroglucinol and hydrochloric acid. Upon heating, the material turned dark and eventually charred. A 72 per cent sulfuric acid lignin determination on the phenolic acid gave a 79.4 per cent "lignin" content after correction for ash (0.931 per cent). Since the methoxyl content of the phenolic acid before the lignin determination (4.34 per cent) and the methoxyl content of the residue (4.28 per cent) remained substantially unchanged, it may be concluded that little or no carbohydrate material was present. The loss was evidently due to solubility of the phenolic acid in 72 per cent sulfuric acid.

Methylation studies on the phenolic acid. Diazomethane in ether methylates only the acidic hydroxyls such as carboxylic and phenolic hydroxyl groups. Dimethyl sulfate with sodium hydroxide methylates all hydroxyls except the carboxylic hydroxyls which are saponified in the alkaline medium. From the methoxyl values obtained under these various methylation procedures, it is possible to calculate the percentage of phenolic hydroxyls, alcoholic hydroxyls and carboxyl groups in the substance being investigated.

The phenolic acid from the bast fibers was methylated according to the procedures outlined in the following sections. All methoxyl determinations were made according to T.A.P.P.I. procedure, T 2 m (42).

Methylation with diazomethane. The phenolic acid was methylated to constant methoxyl content with diazomethane in the following manner: Two grams of the finely powdered material were dispersed in 20 milliliters of dioxane and two milliliters of dry methanol. Fifty milliliters of dry ether containing the diazomethane generated from six grams of nitrosomethyl urea (4, p.165, p.462) were then distilled into the reaction flask, and the flask was allowed to stand in the refrigerator overnight. This methylation procedure was carried out four times; the fifth methylation indicated that the methoxyl value was constant at 21.77 per cent.

Methylation with dimethyl sulfate. Two grams of phenolic acid were dissolved in five milliliters of one per cent sodium hydroxide; 35 milliliters of 30 per cent sodium hydroxide and 35 milliliters of dimethyl sulfate were added separately and dropwise with agitation over a six hour period while maintaining the mixture just alkaline. The reaction flask was maintained at 20° C. by means of a water bath. After all the dimethyl sulfate had been added, the solution was acidic and methylated lignin had precipitated from solution. The solution was centrifuged, and the precipitate was again dissolved and remethylated in the same manner. The solution was maintained slightly alkaline and allowed to stand overnight. It was then poured into 200 milliliters of water; filtered and the filtrate was made acid to litmus with dilute sulfuric acid. The precipitated lignin was centrifuged and the clear liquid was decanted off; distilled water

was added to the precipitate and allowed to stand overnight. It was again centrifuged and then dissolved in 50 milliliters of acetone. The acetone solution was dried over anhydrous sodium sulfate for 48 hours.

The filtered acetone solution was added dropwise with agitation to 500 milliliters of ether from which the methylated lignin separated as an insoluble orange colored powder. The powder was filtered off on a sintered glass crucible, washed with ether and finally petroleum ether. It was then dried in a vacuum oven at 60° C. for three hours. A value of 25.99 per cent methoxyl was obtained for this material.

Methylation with dimethyl sulfate and diazomethane. The sample of phenolic acid previously methylated with dimethyl sulfate was dissolved in dioxane and methylated to constant methoxyl with diazomethane methylation. The completely methylated material was found to be partially soluble in dioxane and acetone. Methoxyl determinations revealed a methoxyl content of 29.26 per cent. This indicated that carboxylic or enolic hydroxyl groups were not methylated in an alkaline dimethyl sulfate solution due to the saponification of the methoxyl group as soon as it formed.

A summary of the information obtained by methylation studies is presented in the following three tables.

TABLE 11

METHOXYL DETERMINATIONS ON THE METHYLATED BARK FIBER PHENOLIC ACID

	Per Cent by Weight of Materials
A - Residual lignin insoluble in 1% NaOH and 72% sulfuric acid	12.5
B - Phenolic acid soluble in 1% sodium hydroxide	4.3
C - (B) methylated with diazomethane	21.8
D - (B) methylated with dimethyl sulfate	26.0
E - (B) methylated with dimethyl sulfate and diazomethane	29.3

TABLE 12

METHOXYL GROUPS ADDED TO THE PHENOLIC ACID

	Per Cent by Weight
F - Due to phenolic and carboxylic hydroxyl groups, (C-B)	17.5
G - Due to carboxylic acid hydroxyl groups, (E-D)	3.3
H - Due to phenolic hydroxyl groups, (F-G)	14.2
I - Due to alcoholic hydroxyl groups, (D-B-H)	7.5

TABLE 13

FUNCTIONAL GROUPS IN PHENOLIC ACID BEFORE METHYLATION

	Per Cent by Weight
Methoxyl	4.3
Carboxylic Acid	4.9
Phenolic Hydroxyl	8.3
Alcoholic Hydroxyl	4.2

Calculations from the foregoing data reveal that the apparent molecular weight of the phenolic acid unit is 918 grams, assuming that it is monocarboxylic. Based on this assumption, the molecule would contain one methoxyl group, one carboxyl group, four phenolic groups and two alcoholic hydroxyl groups.

Diazomethane in ether methylates only the acidic hydroxyl groups; since phenolic acidic hydroxyl groups form ether linkages when methylated, only methylated carboxylic hydroxyls should be saponified by alkaline solutions.

This should serve as a means for confirming the presence of carboxylic acid groups. For this purpose, one gram of phenolic acid which had previously been methylated with diazomethane was saponified for one hour in ten per cent alcoholic potassium hydroxide. When cool, the saponification mixture was acidified and poured into 200 milliliters of distilled water. The precipitate was washed with water several times in centrifuge tubes, then filtered on a sintered glass crucible and dried under vacuum at 60° C. for six hours.

The methoxyl content of this sample before saponification was 22.4 per cent; after saponification it was found to be 18.8. This indicated that 3.6 per cent methoxyl was present in ester form. By the previous methylation procedure, 3.3 per cent ester methoxyl was found. These values may be considered reasonable checks for the presence of carboxylic acid groups; and they are equivalent to 5.3 and 4.9 per cent carboxylic acid groups respectively based on the weight of the phenolic acid. Calculation of the molecular weight

from 5.3 per cent carboxyl gives 850 grams and closer values for the ratio of one carboxyl group, one methoxyl group, four phenolic groups and two alcoholic hydroxyl groups. Consequently, this value was used in developing an empirical formula for the phenolic acid. This formula is presented in a following section dealing with combustion analysis.

Oxidation of the phenolic acid to vanillin. The presence of the vanillin type structural configuration is quite characteristic of most softwood lignins. Substantial yields (17-24 per cent) have been obtained from lignin when it was oxidized under the proper conditions (54, p.325). Since the sodium hydroxide soluble phenolic acid appeared as Klason lignin, it was decided to subject it to a nitrobenzene-alkali oxidation and determine quantitatively the yield of vanillin obtained. In this manner its resemblance to what is generally termed lignin would be revealed. The nitrobenzene oxidation was carried out in a manner similar to that of Freudenberg et al. (16).

Twenty grams of the phenolic acid were dissolved in 600 milliliters of two normal sodium hydroxide and placed in a steel bomb with 35 milliliters of nitrobenzene. The bomb was placed in a large rotating digester and maintained at 160° C. for 2.5 hours. After cooling to room temperature, the reaction mixture was steam distilled to remove aniline and nitrobenzene. The still residue was then acidified and exhaustively extracted with benzene in a separatory funnel. Sodium bisulfite solution was used to extract the vanillin from the

benzene; the bisulfite addition product was acidified, placed in a hot water bath and a stream of air was forced through the solution to remove sulfur dioxide.

Vanillin was quantitatively determined by the method of Hibbert and Tomlinson (21), whereby the m-nitrobenzoylhydrazone of vanillin is weighed. A yield of 1.63 per cent vanillin was obtained. This indicates that the phenolic acid portion of the bast fibers does not resemble lignin in respect to the presence of a vanillin nucleus which readily yields vanillin under these conditions.

Carbon and hyrogen combustion analysis. The phenolic acid was analyzed by the conventional, dry-combustion methods (13). Data obtained are compared to those of fir wood (54, p.296), Braun's spruce native lignin (5) and to a high molecular weight "acid lignin" from redwood bark (30). The combustion analysis of the dioxane lignin from Douglas fir bark bast fibers is also included in Table 14. The dioxane lignin more closely resembles the wood lignin and is almost identical with Braun's spruce native lignin in elementary analysis.

Both of the bark "acid lignin" materials have higher oxygen content than the wood lignin. This might be expected in view of the more acid nature of these lignin-like materials in the bark.

An empirical formula for the Douglas fir bark fiber phenolic acid was proposed from the foregoing combustion analysis data and the ratio of functional groups calculated for this material in the foregoing section dealing with the methylation studies on the phenolic acid.

This empirical formula was based upon the assumption that on a weight basis the phenolic acid contains one carboxyl group, 5.3 per cent; one methoxyl group, 4.3 per cent; two alcoholic hydroxyl groups, 4.2 per cent; and four phenolic hydroxyl groups, 8.3 per cent. The molecular weight of the monocarboxylic acid based on 5.3 per cent carboxyl content was calculated to be approximately 850.

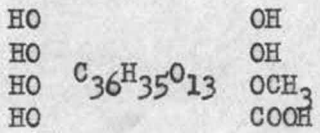


TABLE 14

ELEMENTARY COMPOSITION OF "LIGNIN" MATERIALS

Per Cent by Weight

	Carbon	Hydrogen
Douglas fir wood Klason lignin	64.9	4.9
Redwood bark "acid lignin"	57.1	5.0
Douglas fir bark fiber phenolic acid	54.12	5.33
Braun's spruce native lignin	63.89	6.07
Douglas fir bark fiber dioxane-HCl lignin	63.88	6.04

II. DIOXANE LIGNIN OF THE DOUGLAS FIR BARK FIBERS

Isolation. Wedekind and Engel found that dioxane in the presence of 0.1 to 0.7 per cent hydrochloric acid removed lignin from wood. They succeeded in obtaining a German patent for this procedure (45). Nikitin and Orlowa (34), in reviewing Wedekind's work,

recognized the fact that this reactive and soluble type of dioxane-lignin should more closely resemble the lignin as it appears in wood than the lignin obtained by more drastic reagents or by alcohols which may react with the lignin. Because of the foregoing reasons, it was decided to attempt to extract lignin from the bark fibers by this method and characterize the product obtained.

The extractive-free bark fibers were extracted in Alundum crucibles with a 0.4 per cent hydrochloric acid-dioxane solution by means of Soxhlet type extractors. After nine hours the dioxane was no longer removing lignin, as evidenced by lack of color in the solvent returning to the receiving flask. The dioxane-lignin solution was distilled to remove most of the dioxane, and the concentrated solution was poured into a liter of distilled water. A small amount of ammonium sulfate was added, and soon a voluminous light-brown precipitate formed. This was allowed to settle overnight. The aqueous layer was decanted from the precipitated lignin, which was then washed with distilled water in centrifuge tubes, dissolved in purified dioxane and dried over anhydrous sodium sulfate. This dried dioxane-lignin solution was filtered and the lignin precipitated in a large volume of ether. It was filtered on a sintered glass crucible, washed with ether and hexane, then dried in a vacuum oven at 60° C. for five hours. The yield of purified lignin was 7.7 per cent based on oven dry fiber or 17 per cent of the total Klason lignin content of the fiber. A Klason lignin determination on the dioxane extracted fiber residue revealed that 10.8 per cent of the total lignin content had

actually been removed by the dioxane. The loss in purification may be attributed to the observed tendency of the dioxane lignin to form colloidal solutions especially during the washing process.

Properties of dioxane lignin. The dried powder was found to be soluble in dilute alkali solutions producing a dark red coloration. It was dissolved by dioxane, methyl and ethyl alcohols, acetone, pyridine, and ethyl acetate. Phloroglucinol-hydrochloric acid reagent gave a negative color reaction.

Methylation of dioxane lignin. The methods employed for methylation studies have been discussed in the foregoing section dealing with methylation of the high molecular weight phenolic acid from the bast fibers. These methods were applied to the dioxane lignin. A phenylhydrazone of the dioxane lignin was also prepared by the procedure which Brauns employed in native lignin studies (5). Results obtained in the methylation procedures are shown in Table 15.

TABLE 15

METHOXYL DETERMINATIONS ON BARK FIBER DIOXANE-HCl LIGNIN

	Per Cent by Weight
A - Residual lignin insoluble in dioxane-HCl	9.5
B - Dioxane-HCl lignin	14.3
C - (B) methylated with diazomethane	21.6
D - (B) methylated with dimethyl sulfate	27.6
E - (B) methylated with dimethyl sulfate and diazomethane	29.3
F - Methoxyl content of phenylhydrazone	11.4

TABLE 16

METHOXYL GROUPS ADDED TO THE DIOXANE-HCl LIGNIN

	Per Cent by Weight
G - Due to phenolic and carboxylic groups, (C-B)	7.3
H - Due to carboxylic hydroxyl groups, (E-D)	1.7
I - Due to phenolic groups, (G-H)	5.6
J - Due to alcoholic groups, (D-B-I)	7.7

Table 17 shows the calculated functional groups present in dioxane lignin before methylation. As a means of comparison, the values available for Braun's spruce native lignin (5), redwood bark phenolic acid (30), and Douglas fir bark fiber phenolic acid are included.

TABLE 17

FUNCTIONAL GROUPS IN VARIOUS "LIGNIN" PREPARATIONS

	Per Cent by Weight			
	Methoxyl	Alcoholic	Phenolic	Carboxylic Acid
Spruce native lignin	14.8	5.1	3.8	---
Redwood bark acid	2.7	2.1	7.8	6.4
Fir bark phenolic acid	4.3	4.2	8.3	4.9
Fir bark dioxane lignin	14.3	4.4	3.2	2.5

The dioxane lignin from the fir bark fibers shows methoxyl content similar to what is found in conventional lignin preparations from wood. This 14.3 per cent methoxyl content is considerably higher than the 4.3 per cent methoxyl of the phenolic acid extracted from the fibers with one per cent sodium hydroxide. The dioxane lignin was then treated with one per cent sodium hydroxide in the same manner as employed in the extraction of the phenolic acid to ascertain if the alkali had split off methoxyl due to ester linkages. A methoxyl determination on the dioxane lignin so treated was found to be 13.2 per cent. This indicated that the dioxane lignin did not contain an appreciable amount of saponifiable methoxyl groups.

The dioxane lignin was then subjected to a Klason lignin determination with 72 per cent sulfuric acid; 87.6 per cent appeared as Klason lignin, methoxyl content 13.1 per cent. Kudzin in a study of native lignins from various woods has shown that they yield approximately 90 per cent Klason lignin when treated with 72 per cent

sulfuric acid (25).

Extraction studies. The data in the foregoing sections indicated that the sodium hydroxide fraction and the dioxane fraction differed considerably in many respects, particularly in methoxyl content and solubility. It was interesting to note that the "Klason lignin" from dioxane lignin of the fibers was able to be redissolved in warm dioxane-HCl solution. The "Klason lignin" from the sodium hydroxide soluble phenolic acid was not soluble in warm dioxane-HCl solution.

The extractive-free bark fibers were treated with one per cent NaOH, dioxane containing 0.4 per cent HCl, and 72 per cent sulfuric acid to determine the "lignin" yields obtained with each reagent when it was used in various sequences. This procedure revealed the behavior of the "lignin" fraction removed by each and gave an indication of the homogeneity of the fraction. Methods of extraction were those described in the foregoing sections. All "lignin" yields reported are based upon the Klason lignin found in the extracted residue.

An extraction was also made using dioxane without the 0.4 per cent hydrochloric acid catalyst. After twelve hours of extraction, only 1.7 per cent of an oily, amorphous material was removed from the fibers. A dark red color appeared in the dioxane solution immediately upon continued extraction with the addition of the 0.4 per cent hydrochloric acid to the dioxane. This indicated that the acid was necessary for the lignin extraction.

The following table presents the yields and methoxyl contents

TABLE 18

LIGNIN YIELDS UNDER VARIOUS EXTRACTION SEQUENCES

Per Cent Oven Dry Extractive-free Fibers

Extraction Sequence	Lignin Yield	% Methoxyl in Lignin
A		
1. 1% NaOH	22.0 (diff.)	4.3
2. 72% H_2SO_4	22.8	12.5
B		
1. Dioxane-HCl	10.8 (diff.)	14.3
2. 72% H_2SO_4	24.0	9.5
C		
1. 1% NaOH	22.0	4.3
2. Dioxane-HCl	11.8 (diff.)	13.5
3. 72% H_2SO_4	11.0	11.1
D		
1. Dioxane-HCl	10.8	14.3
2. 1% NaOH	5.2 (diff.)	8.3
3. 72% H_2SO_4	28.8	6.6

of lignin extractions conducted in various sequences. Calculations were based on the 44.8 per cent Klason lignin content of extractive-free fibers.

The data in Table 18 show that the amount of dioxane-HCl lignin removed did not vary greatly (10.8 to 11.8 per cent) regardless of the order of extraction. The sodium hydroxide soluble phenolic acid, however, appeared to become insolublized by dioxane-HCl, and therefore appeared in the Klason lignin of the fiber residue. This was substantiated by the fact that isolated sodium hydroxide phenolic acid "lignin" before and after treatment with 72 per cent sulfuric acid was found to be practically insoluble in hot dioxane-HCl. The dioxane-HCl lignin remained soluble under these conditions. These data therefore indicated that when the extractive-free fibers were extracted with one per cent sodium hydroxide or dioxane-HCl or with sequence "C" of Table 18, reasonably homogeneous fractions of "lignin" materials were obtained.

III. INFRARED ABSORPTION SPECTRA OF THE "LIGNIN" FRACTIONS

The infrared absorption spectrum of an organic substance is probably its most important and informative physical property. This is especially true in the case of a material such as lignin, where most other physical properties are not significant. Infrared studies of lignins have not been extensive, and interpretations of the spectra have not been developed to the point where a conclusive answer to the lignin problem can be given; however, infrared data have served to

substantiate certain chemical data and thus aid in bringing the problem closer to solution.

A Perkin-Elmer single beam infrared spectrometer was used in this investigation. A Nujol blank was run prior to each sample, which had been prepared as a Nujol mull. It was found after considerable experimentation that the best performance was obtained by using a 25 per cent concentration of the sample in Nujol. The thoroughly dried sample and Nujol were first well ground in a mortar and then mulled on a glass plate with a glass muller.

The phlobaphene used for the infrared data was prepared by pouring the alcohol extractives from the Douglas fir bark bast fibers into water in which it is insoluble. It was filtered off; washed with hot water, then dissolved in acetone and dried over anhydrous sodium sulfate. The dried acetone solution of phlobaphene was then precipitated into ether; filtered, washed with ether and then hexane to remove occluded ether. The prepared phlobaphene was dried in a vacuum oven at 60° C. for five hours. A portion of this purified phlobaphene was treated with one per cent sodium hydroxide in the same manner as that employed in the isolation of the phenolic acid from the fibers.

These prepared phlobaphene materials were then compared with the sodium hydroxide soluble phenolic acid to ascertain suspected similarities. Methoxyl content of the phlobaphene was found to be 2.77 per cent; the sodium hydroxide treated phlobaphene contained 2.31 per cent methoxyl.

Regions of the curves where Nujol and atmospheric carbon dioxide

show strong absorption were smoothed out. For Nujol these regions were in the vicinity of 2945 centimeters^{-1}, 1460 centimeters^{-1} and 1380 centimeters^{-1}. For carbon dioxide the region was in the vicinity of 2320 centimeters^{-1}.

Figure 7 shows the similarities and differences between the phenolic acid, dioxane-HCl lignin and the 72 per cent sulfuric acid lignin from the Douglas fir bark bast fibers. It is at once apparent that the curves possess an overall similarity to each other. This basic pattern is also evident when these curves are compared to the infrared curves of various lignins studied by Kudzin (25) and Jones (23). The broad band at 3350 centimeters^{-1}, which was found in all of the various "lignin" spectra, is attributed to the presence of hydrogen bonded hydroxyl groups. (Nitrogen compounds are an exception.) (38, p.6).

These bonded structures do not permit distinguishing between the various types of hydroxyl groups other than carboxylic. Absorption in the region of 1725 centimeters^{-1} is attributed to the presence of ester or carboxylic acid groups. Herein lies the greatest difference observable between the Douglas fir bark fiber lignin fractions and the spruce native lignin spectra of Jones (24). Spruce native lignin displayed only a slight absorption plateau in this 1725 centimeters^{-1} region, whereas all of the Douglas fir bark "lignin" preparations exhibit definite absorption in this region. This substantiates the carboxylic acid evidence obtained by methylation studies. Figure 11 shows the effect of ester formation by means of diazomethane upon

the phenolic acid of the bark fibers. The increased absorption in the carboxylic acid and ester region (1725 centimeters^{-1}) is at once apparent.

Another difference between the spectra of the Douglas fir bark fiber "lignins" and those of Jones (23) is the apparent lack of carbonyl absorption in the region of 1663 centimeters^{-1} exhibited by the bark fiber "lignins". The dioxane-HCl lignin of the fibers is the only fraction which resembles the native lignin in respect to the presence of carbonyl absorption. This is shown in Figure 9 and further verifies the chemical evidence of similarities between native spruce lignin and the dioxane-HCl lignin fraction from Douglas fir bark bast fibers.

Absorption in the region of 1600 and 1500 centimeters^{-1} is attributed to be a function of the aromatic nucleus (38, p.16). This type of absorption is strongly in evidence in all of the spectra. The significance of the absorption in the region of 1260 centimeters^{-1} to 1075 centimeters^{-1} shown by all the lignins has not been verified but is probably due to aromatic and aliphatic carbon to oxygen bonds (24). The spruce native lignin and dioxane lignin show great similarity in this region as shown by Figure 9.

Figure 8 shows spectra made of the fractions removed from the Douglas fir bast fibers in the sequence: one per cent sodium hydroxide, dioxane-HCl and 72 per cent sulfuric acid. The spectra of these fractions were almost identical to those taken from the fibers in other extraction sequences (Table 18), as is evident by comparing

Figure 7 with Figure 8. This similarity of spectra substantiates the evidence of homogeneity in the NaOH soluble phenolic acid, dioxane-HCl lignin and Klason lignin fractions.

Figure 10 is a comparison of the phenolic acid from the Douglas fir bark bast fibers and phlobaphene from the same source. A spectrum is also shown for some of this phlobaphene which had been treated with one per cent sodium hydroxide in the same manner that was used in isolating the bark fiber phenolic acid. These spectra are almost identical, particularly in the case of the NaOH treated phlobaphene and the phenolic acid. The presence of carboxylic acid or ester groups at 1725 centimeters^{-1} and the absence of carbonyl groups at 1663 centimeters^{-1} is in evidence. These similarities definitely establish a relationship between the phlobaphene and the fiber phenolic acid.

The spectra of Klason lignin preparations from the bark fibers show diminution of absorption by functional groups. This was also found to be true in the case of spruce Klason lignin (23). Behavior of this nature may be attributed to the fact that 72 per cent sulfuric acid may have destroyed some of these groups or that the physical nature of lignin isolated by this method does not permit suitable grinding and mulling to obtain a fine enough particle size to reveal the spectra. It was found in this investigation that thorough grinding of the samples in Nujol was highly essential.

Ultraviolet absorption spectra, Figures 12 and 13. A Beckman quartz spectrophotometer was used to obtain ultraviolet spectra of the sodium hydroxide soluble phenolic acid as well as the dioxane-HCl lignin and its methylated derivatives. All of the spectra exhibit maximums in the range of 280 millimicrons and minimums in the range of 260 millimicrons. Differences are not apparent between the dioxane-HCl lignin and its methylated derivatives. Nord and Schubert (35) studied ultraviolet spectra of native lignin and lignin isolated from wood decayed by various fungi. All of these spectra show the same structure and maximums as the curves in Figures 12 and 13.

In the light of work on the ultraviolet spectra of various lignin preparations, the results of the ultraviolet spectra examinations are not very conclusive. Glading (17) holds that the characteristic absorption maximum of many lignin preparations and related compounds at 280 millimicrons can be attributed to the presence of pyran or furan ring structures as proposed by Freudenberg (15). Aulin-Erdtman also supports this view (2). Jones (23) contends that the characteristic bands are specifically attributable to the oxygen-substituted nucleus in lignin.

FIGURE 7

DOUGLAS FIR BARK FIBER LIGNINS
INFRARED SPECTRA

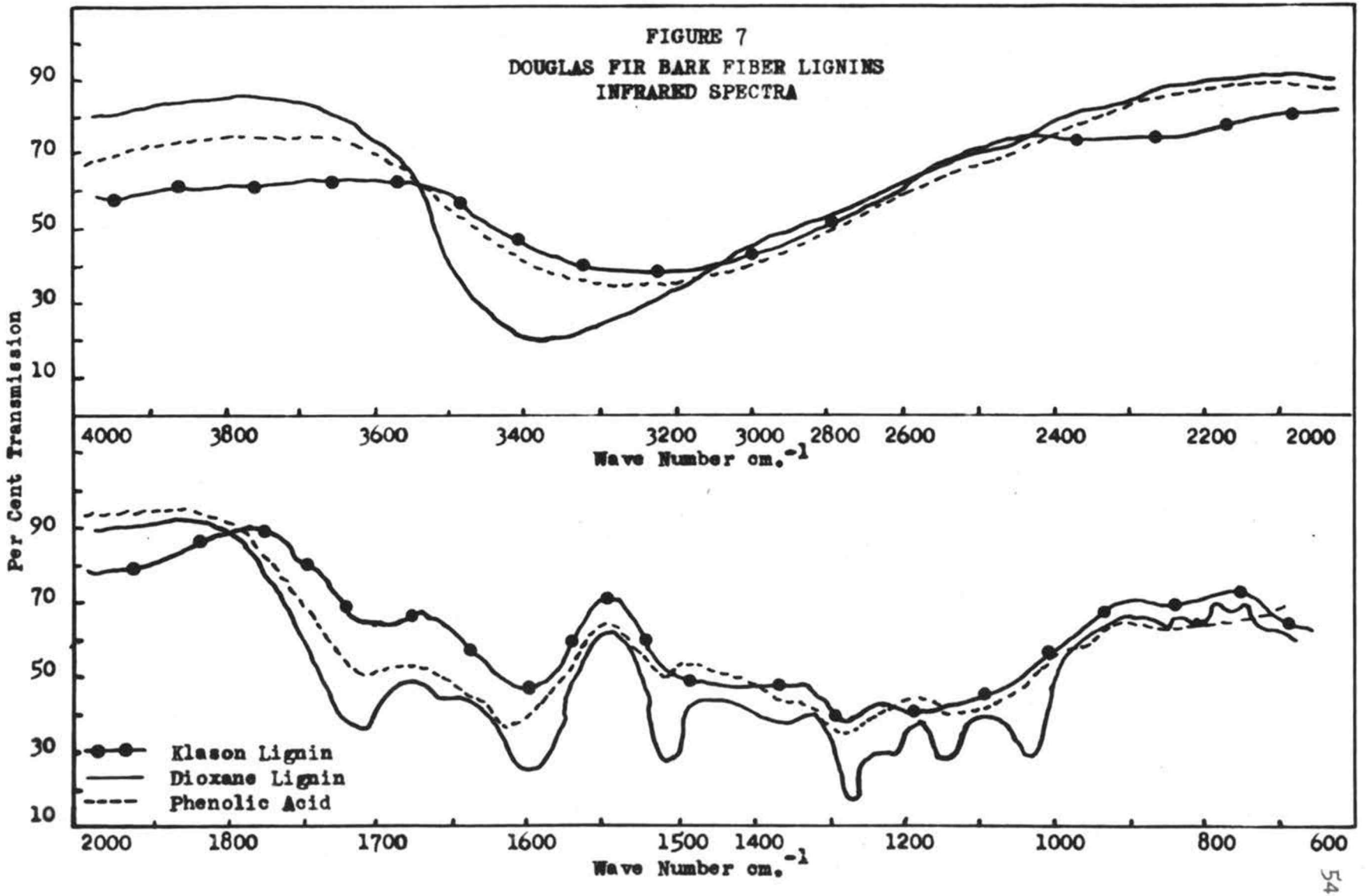


FIGURE 8
STEPWISE REMOVAL OF LIGNIN FRACTIONS
INFRARED SPECTRA

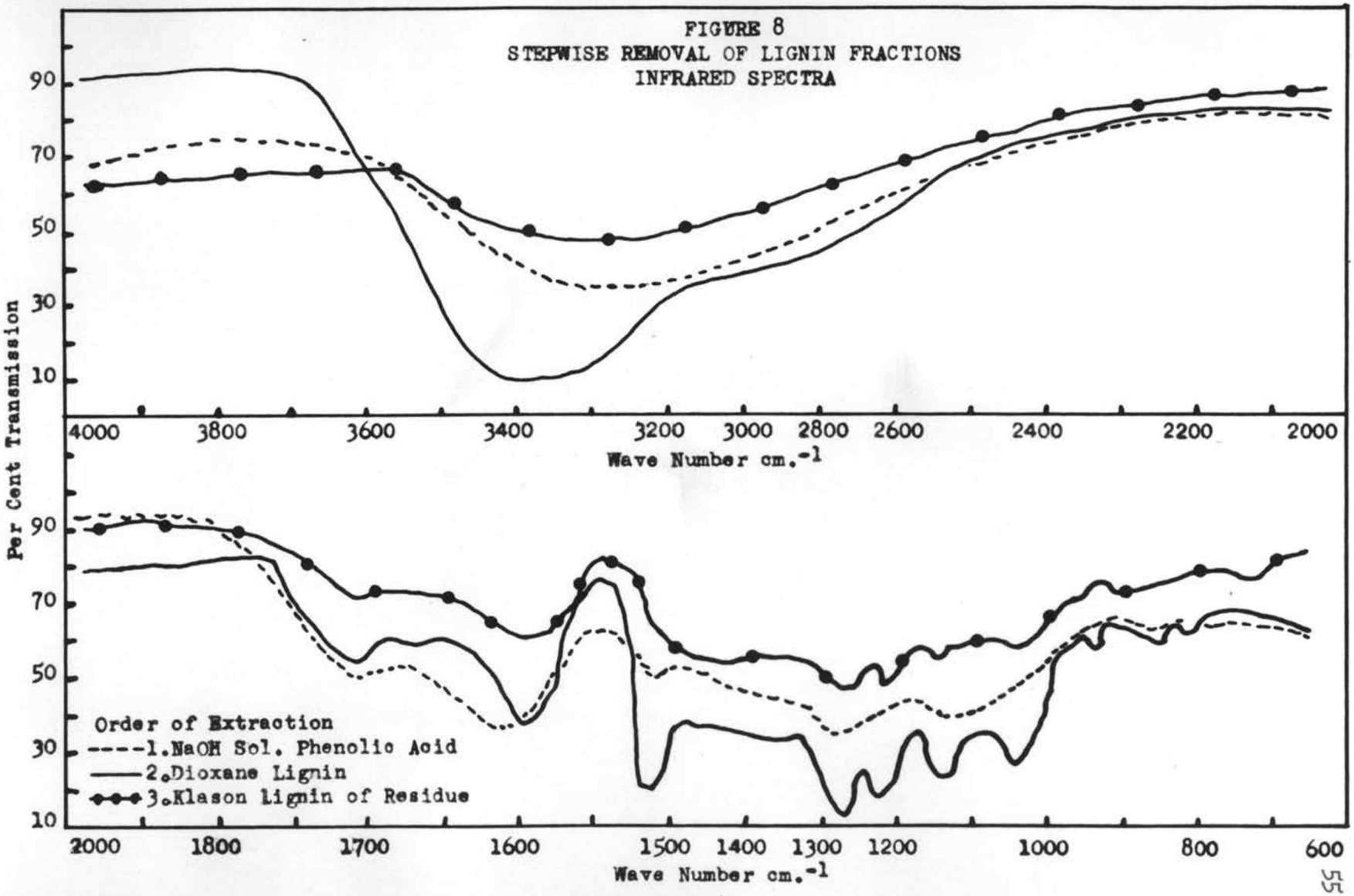
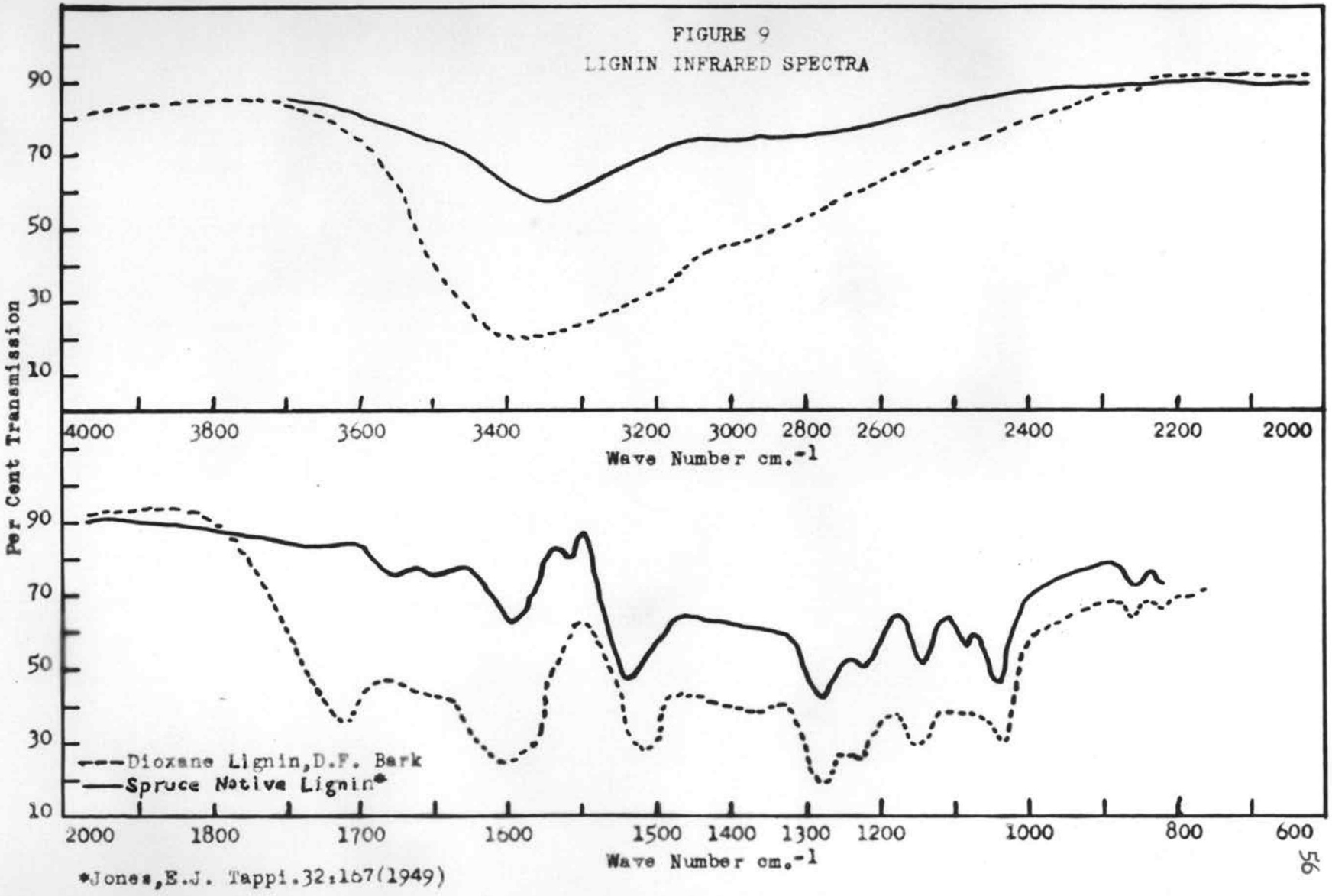


FIGURE 9

LIGNIN INFRARED SPECTRA



*Jones,E.J. Tappi.32:167(1949)

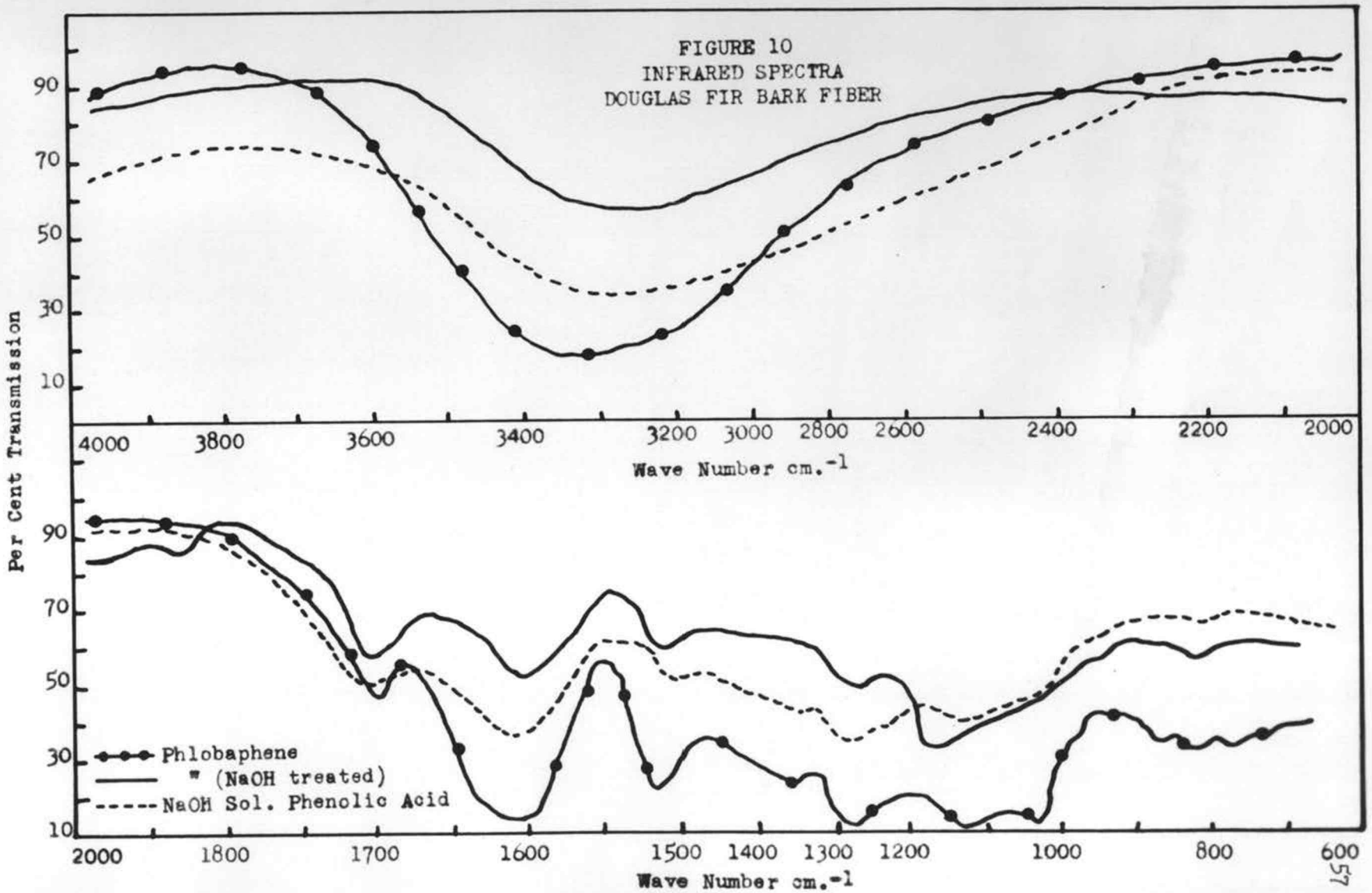


FIGURE 10
INFRARED SPECTRA
DOUGLAS FIR BARK FIBER
Per Cent Transmission
90
70
50
30
10
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
Wave Number cm.-1
90
70
50
30
10
2000
1800
1700
1600
1500
1400
1300
1200
1000
800
600
Wave Number cm.-1
Phlobaphene
" (NaOH treated)
NaOH Sol. Phenolic Acid

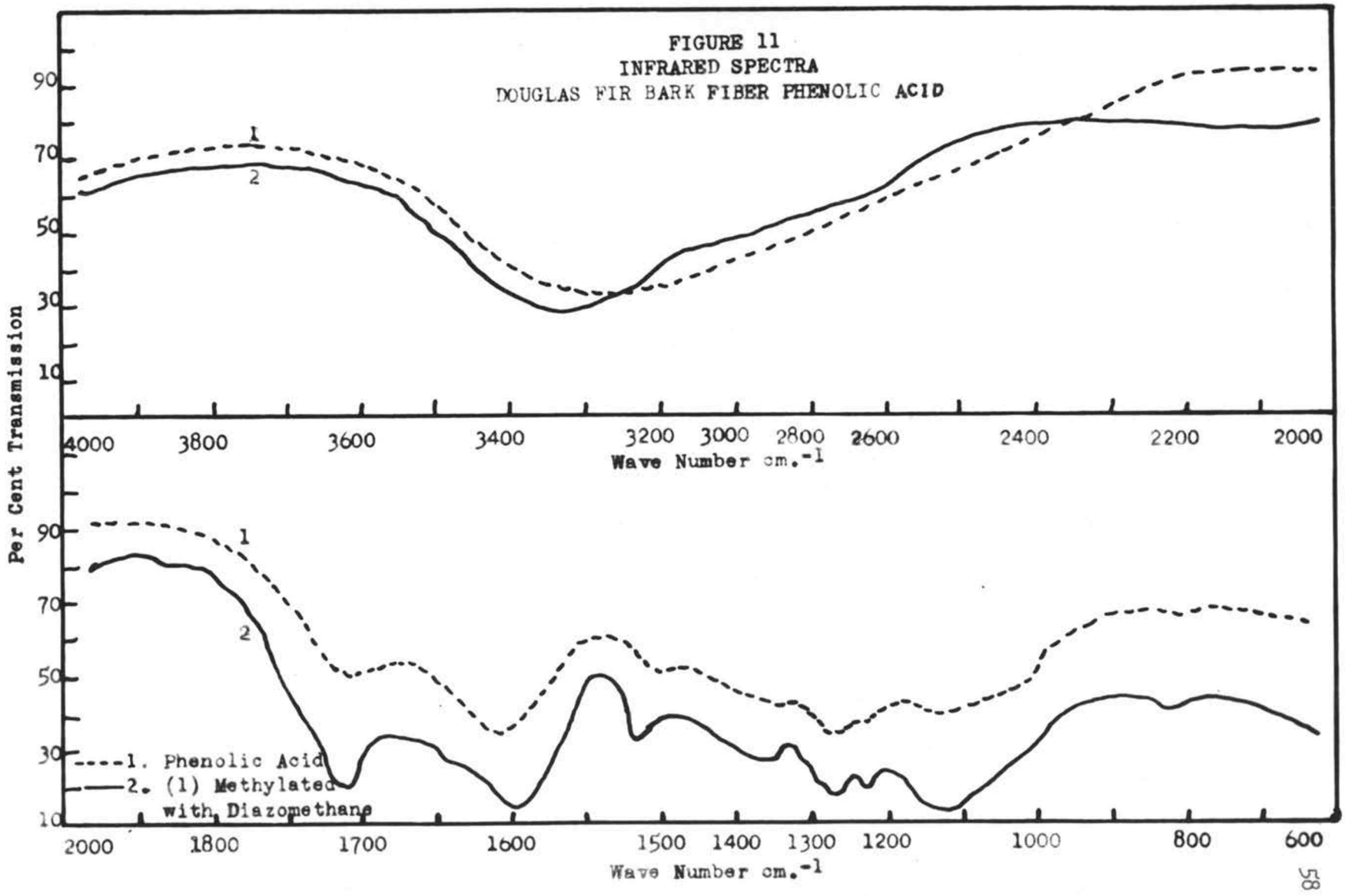
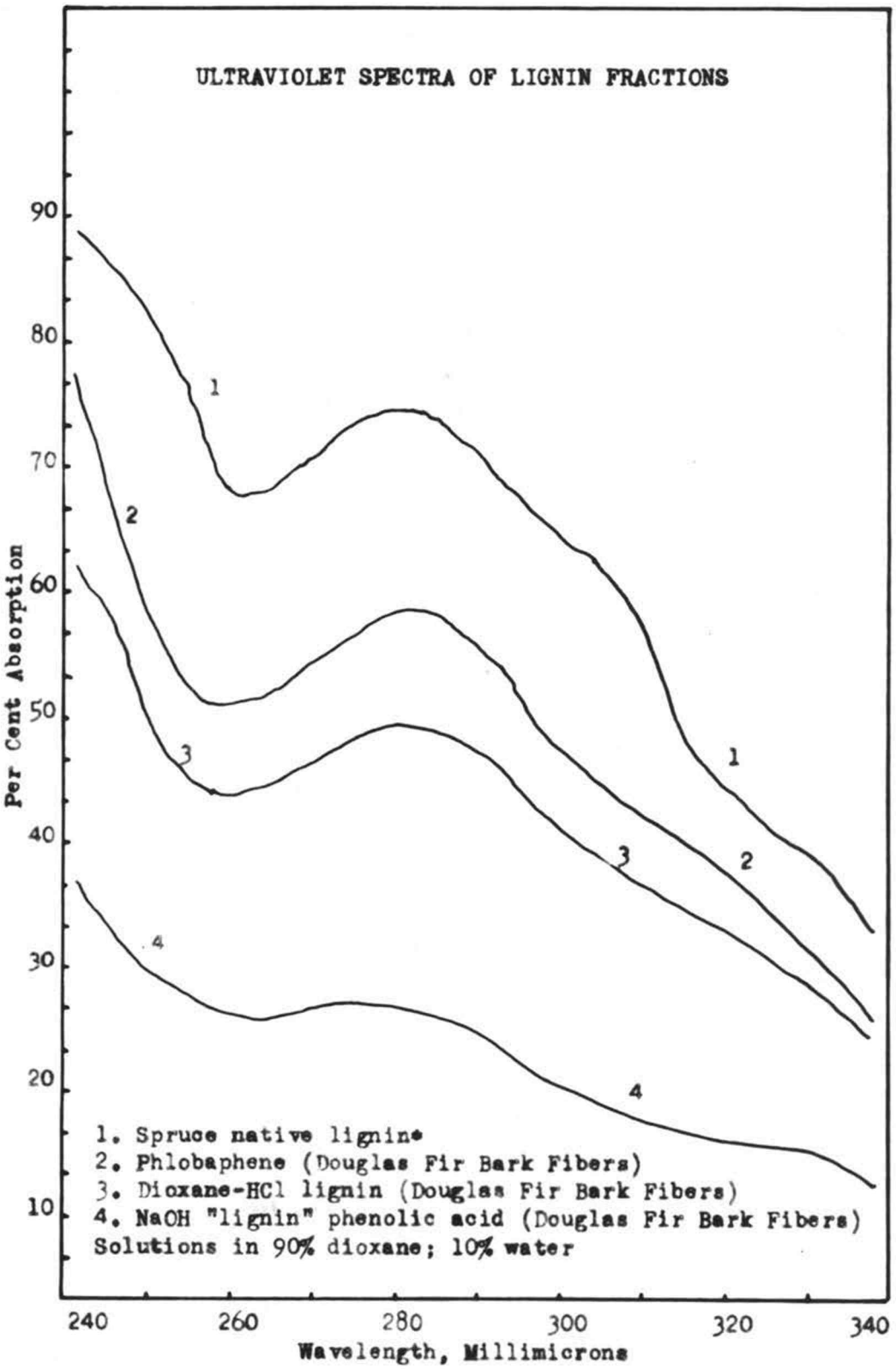


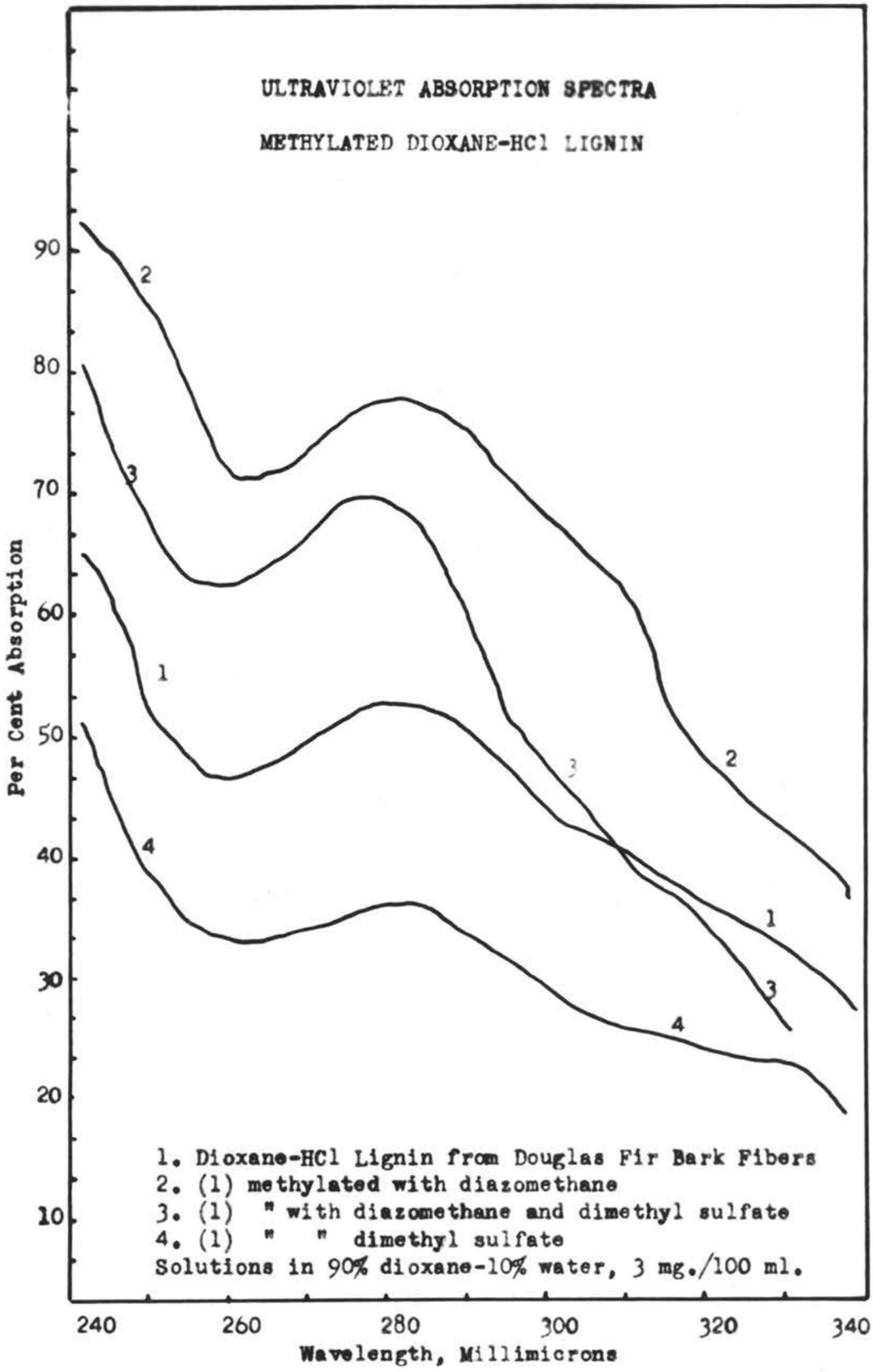
FIGURE 11
INFRARED SPECTRA
DOUGLAS FIR BARK FIBER PHENOLIC ACID
Per Cent Transmission
90
70
50
30
10
1
2
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
Wave Number cm.-1
90
70
50
30
10
1
2
----1. Phenolic Acid
——2. (1) Methylated with Diazomethane
2000
1800
1700
1600
1500
1400
1300
1200
1000
800
600
Wave Number cm.-1

FIGURE 12



*Erdtmann, G. A. T.A.P.P.I. 32;161. 1949.

FIGURE 13


ULTRAVIOLET ABSORPTION SPECTRA
METHYLATED DIOXANE-HCl LIGNIN
Per Cent Absorption
90
80
70
60
50
40
30
20
10
240
260
280
300
320
340
Wavelength, Millimicrons
1. Dioxane-HCl Lignin from Douglas Fir Bark Fibers
2. (1) methylated with diazomethane
3. (1) " with diazomethane and dimethyl sulfate
4. (1) " " dimethyl sulfate
Solutions in 90% dioxane-10% water, 3 mg./100 ml.

DISCUSSION

The investigation to determine the quantity of fiber in Douglas fir bark revealed that it composes 36 to 47 per cent of the air dried bark as received in the mills. These values compare quite well with the data from the Weyerhaeuser Company, where production of fiber from Douglas fir bark amounts to approximately 38 per cent of the bark. Redwood bark wastes have been estimated to contain more than 25 per cent fiber (30).

Structural examinations of the fibers in bark sections by means of a microscope reveal the presence of a ring type or layer structure (Figure 4). A center cavity or lumen appears to contain living protoplast. Pits terminating in the lumen are also in evidence. The fiber is surrounded by a wall or matrix of reddish-brown lignified material which by microscopic examination appears to be removed by dilute aqueous alkali solutions. It seemed reasonably apparent after completion of this investigation that this lignified matrix is responsible for the relatively high lignin content of the fibers. The presence of this lignified wall also appears to be at least partly responsible for the difficulty experienced in isolating holocellulose from the fibers. It was found that after the fiber had been treated with dilute alkali, conventional holocellulose procedures could be applied.

Figure 1 shows how the fibers first become prominent in the relatively inactive outer bark. As the tree grows, the outer bark becomes compressed and the softer portions become distorted. The

fibers, however, because of their high density, retain essentially the same shape as shown in the inner bark (Figures 2 and 3).

Extractive contents of the fibers were found to be relatively low at 13.5 per cent as compared to the bark cork fraction extractives content of 41.3 per cent found by Hergert (20). The fiber extractives show more similarity in content and composition to Douglas fir wood than to the bark cork fraction (Table 3). It is apparent that it would not be feasible to utilize the fiber fraction as a source of wax and dihydroquercetin.

Holocellulose determinations on the Douglas fir bark fibers revealed that the T.A.P.P.I. method T 9 m-45 employing monoethanolamine was not applicable due to filtration difficulties because of the one per cent NaOH soluble phenolic acid material surrounding the fibers. The chlorite method (50) was found to be more successful, but when attempts were made to reduce the lignin content of the holocellulose to less than 5.4 per cent, degradation of the cellulose occurred. It was found that after the bark fibers had been extracted with a dilute alkali solution, both the monoethanolamine and the chlorite holocellulose method could be successfully applied to yield a holocellulose fraction containing from 0.1 to 0.3 per cent lignin. This fraction could not by definition be correctly called "holocellulose" since 9.3 per cent carbohydrate material was removed by the one per cent sodium hydroxide extraction along with the 22.0 per cent "lignin". The carbohydrate material removed by the alkali was found to consist of 1.89 per cent pentosans and 2.62 uronic acid anhydride.

The remaining 5.49 per cent consisted of hexosan material characterized in the total carbohydrate analysis.

The "holocellulose" isolated after the alkali extraction was found to contain 77.8 per cent alpha cellulose, 1.6 per cent beta cellulose and 20.6 per cent gamma cellulose. According to Kraemer (54, p.180), alpha cellulose from wood pulps has a molecular weight of 90,000 to 150,000; beta cellulose, 3000 to 15,000; and gamma cellulose, less than 3000.

The composition of the alpha cellulose was found to be: ash, 0.24 per cent; lignin, 0.27 per cent; mannose, 1.04 per cent; xylose, 0.51 per cent; galactose, none; glucose, 97.94 per cent. An analysis of the total carbohydrate of the extractive-free fiber was found to be: galactose, 3.54 per cent; mannose, 6.31 per cent; xylose, 11.03 per cent by fermentation or 9.90 per cent by pentosan determination; arabinose, none; and glucose, 39.85 per cent, based on the weight of the extractive-free fiber. It was interesting to note that bark fibers from redwood were found to contain no galactose or arabinose (30).

Three fractions of "lignin" materials from the Douglas fir bark fibers were isolated and studied. Each fraction was found to display its own individual homogeneity upon analysis and extraction. The fraction of "lignin" removed by one per cent sodium hydroxide represented 49 per cent of what appeared as Klason lignin on the whole extractive-free fiber. This easily removed "lignin" material was found by infrared and chemical analysis to resemble a high molecular

weight phenolic acid rather than what is generally designated lignin.

A functional group analysis by methylation studies revealed that this phenolic acid contained: methoxyl, 4.3 per cent; carboxy group, 4.9-5.3 per cent; phenolic hydroxyl, 8.3 per cent; and alcoholic hydroxyl, 4.2 per cent. On this basis, one building unit of molecular weight, 850-918, would contain one carboxyl, one methoxyl, two alcoholic hydroxyl and four phenolic groups.

Based upon a molecular weight of 850, functional group data, and the elementary composition (54.12 per cent carbon, 5.33 per cent hydrogen), the following empirical formula was proposed for the Douglas fir bark fiber phenolic acid:

$$\begin{array}{lll} HO & & OH \\ HO & & OH \\ HO & C_{36}H_{35}O_{16} & OCH_3 \\ HO & & COOH \end{array}$$

Infrared studies confirmed the presence of the carboxyl group in this phenolic acid and served to show the similarity of the acid to the phlobaphenes isolated from the Douglas fir bark bast fibers. An ultraviolet absorption spectrum of the sodium hydroxide soluble phenolic acid showed the maximum absorption peak in the 280 millimicron region. This behavior has been found to be common to many lignin, tannin and phlobaphene preparations. Various theories of its significance have appeared in the literature. Jones (23) contends that absorption in this region is due to oxygen substituted benzene nuclei. Aulin-Erdtmann (2) and Glading (17) support the view that this absorption at 280 millimicrons can be attributed to

the presence of the pyran or furan ring as proposed by Freudenberg (15) in his proposed lignin structures.

Oxidation of the phenolic acid from the bast fibers yielded 1.63 per cent vanillin. This is a low yield compared to the 17 to 24 per cent yields obtained from materials which are generally termed lignin. Evidently, the phenolic acid does not resemble lignin in respect to the presence of a vanillin nucleus. This is in accord with the infrared spectrum which shows little indication of the presence of carbonyl groups. It is also in agreement with its low methoxyl content of 4.3 per cent. Softwood lignins are generally considered to contain about 14.8 per cent methoxyl. The low methoxyl content of the bark fiber phenolic acid is comparable to the methoxyl content of the phlobaphene which was found to contain 2.77 per cent methoxyl. This relationship to phlobaphene was further displayed when the infrared spectra of the two materials were found to be almost identical. Both spectra displayed strong carboxyl or ester absorption and little or no carbonyl absorption. It is interesting to note that a low methoxyl lignin fraction was found by Wacek and Schon in pine bark (44). Also redwood bark fiber contains a high molecular weight phenolic acid of low methoxyl content (30).

The lignin extracted from Douglas fir bark fibers by means of dioxane-HCl solution was obtained in 10.8 per cent yield. An 11.8 per cent yield based on the weight of oven dry, extractive-free fibers was obtained when the fibers had been previously extracted with one per cent sodium hydroxide solution. These yields are equivalent to

about 24 per cent of the total "Klason lignin" in the extractive-free fiber. Dioxane lignin was found to possess more similarity to soft wood native lignin than to the phenolic acid of the fibers. By methylation studies its functional groups were found to be: methoxyl, 14.3 per cent; alcoholic hydroxyl, 4.4 per cent; phenolic hydroxyl, 3.2 per cent; carboxyl group, 2.5 per cent. This content of functional groups is comparable to that of spruce native lignin (5) with the exception of the carboxylic acid groups which are presumably not present in native lignin. In this regard it is interesting to note that Jones in his infrared study of spruce wood native lignins (23) and Kudzin in his infrared study of hardwood lignins (25) mentioned the presence of absorption bands and plateaus in the ester and carboxylic acid band region of 1710 to 1725 centimeters^{-1}. However, no explanation for this observation was given by either investigator. This evidence for the presence of ester or carboxyl groups has not been accounted for in any of the proposed formulae for native lignin, of which Braun's structure is an example (5).

The preparation of a phenylhydrazone derivative and infrared absorption at 1663 centimeters^{-1} show the presence of a carbonyl group in the dioxane-HCl lignin. This dioxane lignin was the only fraction of the bark fiber lignins which exhibited decisively the presence of carbonyl groups.

The 72 per cent sulfuric acid or Klason lignin amounted to 44.8 per cent of the extractive-free bark fibers and represented

the total lignin content as it is conventionally determined. This yield is considerably higher than the Klason lignin content of Douglas fir wood which is reported as 30.1 per cent (19). Residual lignin in the fibers as determined by the Klason method varied according to the prior treatment of the fibers. Treatment of the fibers first with dioxane-HCl solution, then with one per cent sodium hydroxide solution (sequence D, Table 18), yielded 10.8 per cent dioxane lignin, 5.2 per cent sodium hydroxide soluble phenolic acid and 28.8 per cent residual Klason lignin. When the sequence was changed to treatment first with sodium hydroxide followed by dioxane-HCl (sequence C, Table 18), the yields were 22.0 per cent phenolic acid, 11.8 per cent dioxane-lignin and 11.0 per cent residual or Klason lignin. These data indicated that, by employing sequence C, the prior dioxane-HCl treatment converted much of the phenolic acid into a form no longer soluble in one per cent sodium hydroxide; it therefore appeared in the Klason lignin. This is substantiated by the fact that Klason lignin from sequence D (Table 18) contained a correspondingly lower methoxyl content (6.6 per cent) than Klason lignin from sequence C (11.1 per cent methoxyl). All of the Klason lignin preparations from the bast fibers displayed infrared spectra which were almost identical to each other and to the phenolic acid spectra. The infrared spectrum of spruce Klason lignin (23) showed the same absorption characteristics but with less intensity, thereby displaying a somewhat flatter curve.

It seems evident from an observation of infrared spectra determined on various types of lignin preparations (23, 25) and phlobaphene that an overall similar pattern exists for these materials even though they have been isolated by different means.

SUMMARY

An investigation was made to determine the approximate portion of bast fibers in Douglas fir bark. Thin bark sections were prepared and examined under the microscope to ascertain the general appearance and position of the fibers in the bark layer. Successive extraction of the fibers with appropriate solvents revealed that the extractive contents were relatively low. Conventional holocellulose procedures were found to be satisfactory after the fibers had been subjected to a dilute alkali extraction. Alpha, beta and gamma cellulose determinations were made on the holocellulose fraction. Sugars found in the hydrolyzed holocellulose fraction were galactose, mannose, xylose and glucose; no arabinose was found. The alpha cellulose was essentially glucose anhydride (glucosan), accompanied by small amounts of mannan, xylan; galactan was not found in the alpha cellulose. The "lignin" portion of the bast fibers was separated into three distinct fractions: one per cent sodium hydroxide soluble, dioxane-HCl soluble, and the residual 72 per cent sulfuric acid lignin, which was insoluble in either of these solvents. The sodium hydroxide fraction resembled a high molecular weight phenolic acid as shown by methylation, oxidation, infrared spectra and ultraviolet spectra studies. These same procedures revealed that the dioxane-HCl lignin strongly resembled spruce native lignin with the exception that a small amount of carboxyl groups was present. The residual 72 per cent sulfuric acid lignin

spectra showed it to be similar to the other two preparations but possessing decreased functional group contents. Therefore, the "lignin" in the bast fibers is not a homogeneous material and further work on its chemistry should be performed on purified homogeneous fractions.

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