THE CONSTITUENTS OF THE EXTRACTIVES FROM PONDEROSA PINE BARK (PINUS PONDEROSA, LAWS.)

by

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THE CONSTITUENTS OF THE EXTRACTIVES FROM PONDEROSA PINE BARK (PINUS PONDEROSA, LAWS.)

INTRODUCTION

The purpose of this investigation was to determine by chemical analysis the constituents of the extractives of ponderosa pine bark (Pinus ponderosa, Laws.). At present it is estimated that approximately three billion board feet of ponderosa pine lumber are produced annually (28, p.1). Currently the sawmills use the bark for hog fuel or burn it in a waste burner as a method of quick disposal. An examination of the literature revealed that there were no previous reports on the chemical analysis of the bark extractives from this particular pine tree.

With the source of sawlog timber slowly being depleted there is a move for greater utilization of the wood and bark, which is continually wasted in converting trees into usable products. In many cases, even though there is a potential market for the products made from forest waste, the cost of manufacturing and marketing these products is greater than their selling price. This opens the path for products that do not require extensive manufacturing or products that bring premium prices because of special properties.

Clean logs and a source of wood-free bark result from the installation of barkers at pulp mills and sawmills. These barkers, whether mechanical or hydraulic, facilitate the utilization of the logs in the mills. At the sawmills the bark free slab wood can be disposed of for pulp or hard board manufacture. The bark free log saws economically and does not dull the saws as fast as the logs with their bark intact.

At the present time the bark of Douglas fir trees (Pseudotsuga taxifolia, Britt.) is the only bark of commercial timber that is utilized on a large scale in the Northwest. One of the largest uses of this bark at present is based on its separation into cork, fiber, and an amorphous powder. These products can be used in connection with various industries, such as plastics, oil, and insecticides. The extractives of Douglas fir bark are also being exploited currently. The tannin is being used to produce a light-brown, well-plumped leather at a tannery in Dallas, Oregon. One plant in Springfield, Oregon, is extracting from the bark a wax, which is a light-colored, hard material comparable to other high grade vegetable waxes.

The example being set by the Douglas fir bark is hoped to be a pattern for future utilization of bark

waste of other species in the Northwest. With large amounts of ponderosa pine in the western states being cut every year, an analysis of the bark extractives will give an answer to the possible chemical utilization of the bark of this species.

COLLECTION AND PREPARATION OF BARK SAMPLES

The ponderosa pine bark used in this work was taken from sawlogs on the land of the Brooks-Scanlon Company, Bend, Oregon, about five miles east of Black Butte, Oregon, in June, 1948. All of the trees were felled about one month previous to the time of collection. The stand of trees was about one-third of the way up a slope covered with thim topsoil over rock. Most of the trees in this stand were about 250 years old.

Samples were taken separately from the bottom (2-foot height), middle (32-foot height), and top (62-foot height) sections of five trees in each of the following three age groups: 150 to 200 years; 200 to 250 years; 250-300 years. Bark was removed from a total of fifteen trees to give forty-five samples, from which the nine composites were prepared for analysis. This gave a bottom, middle, and top composite sample for each age group as shown in Table 1. The bark of the ponderosa pine is relatively thin; thus, the bark from the top of the trees had a high proportion of inner bark, whereas the bark samples from the bottom section had little inner bark. The data recorded at the time of sampling are summarized in Table 1.

Age group	Tree	Bottom Section (2-foot height)		Middle Section (32-foot height)		Top Section (62-foot height)	
		Dia. outside bark	Dia. inside bark	Dia. outside bark	Dia. inside bark	Dia. outside bark	Dia. inside bark
Years		In.	In.	In.	In.	In.	In.
150 to 200	1	27.5	23.5	20	18	14	13
	2	25	23	16	15	11	10.5
	3	24	22.5	15.5	15	9	8.5
	4	27	24	19.5	17	13	11.75
	5	24	22.5	18	17	12.5	12.0
200 to 250	6	32	29	22.5	20.75	16	15.5
	7	29	27	20	19	13.5	13
	8	26	25	20.5	19.25	14	13.5
	9	26	23	16	15	13	12
	10	41	36.5	25	23.5	13	12
250 to 300	11	42	39	27.25	24.75	15.5	13.5
	12	37	34	28	26.5	19.25	17.75
	13	33	30.75	22.5	21.5	14	13
	14	34.5	32.0	22	21	14	13
	15	43	41	32.25	31	23	22

TABLE 1

COLLECTION DATA ON PONDEROSA PINE BARK SAMPLES

The bark of this species is scaly, nonfibrous, and has a characteristic yellow color. An anatomical examination indicated the presence of thin-walled parenchyma cells, cork cells, and sieve tubes. Several views of the bark are shown in Plates 1, 2, and 3. The yellow material formed a thin film on the outer surfaces of each scale and was found to be more pronounced in the butt logs of the oldest trees. The color is found throughout the bark.

After collection the samples were passed through a Greundler-Peerless hammer-mill shredder and air-dried to a moisture content of about 10%. Subsequently, they were ground for analysis in a Wiley mill to pass a twenty-mesh sieve. The hammer-mill reduced the moist bark to amorphous particles and a fine powder; no well-defined structural elements were distinguishable in the shredded bark. The ground material was placed in brown, stoppered bottles for analysis.

Distribution of extractives in samples. The general plan of analysis was to extract the bark with diethyl ether, acetone, and water successively and to examine the extractives of each solvent. In addition, the standard tannin analysis of the American Leather Chemists Association for woody materials was performed. It was thought that such a plan would reveal the constituents

PLATE 1

A VERTICAL VIEW

OF THE OUTSIDE OF PONDEROSA PINE BARK

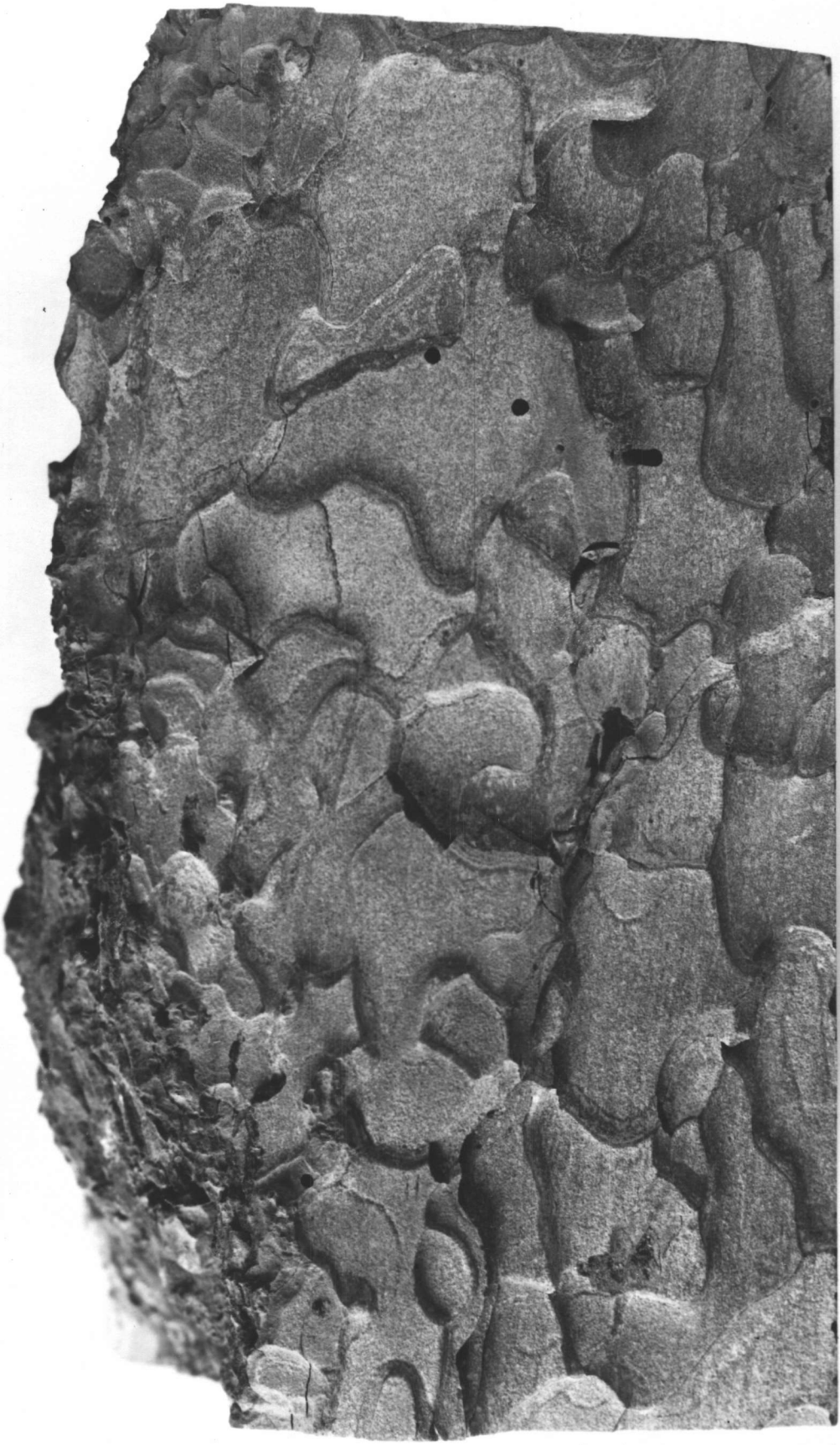

PLATE 2

AN OBLIQUE VIEW

OF A BEVELED PIECE OF PONDEROSA PINE BARK

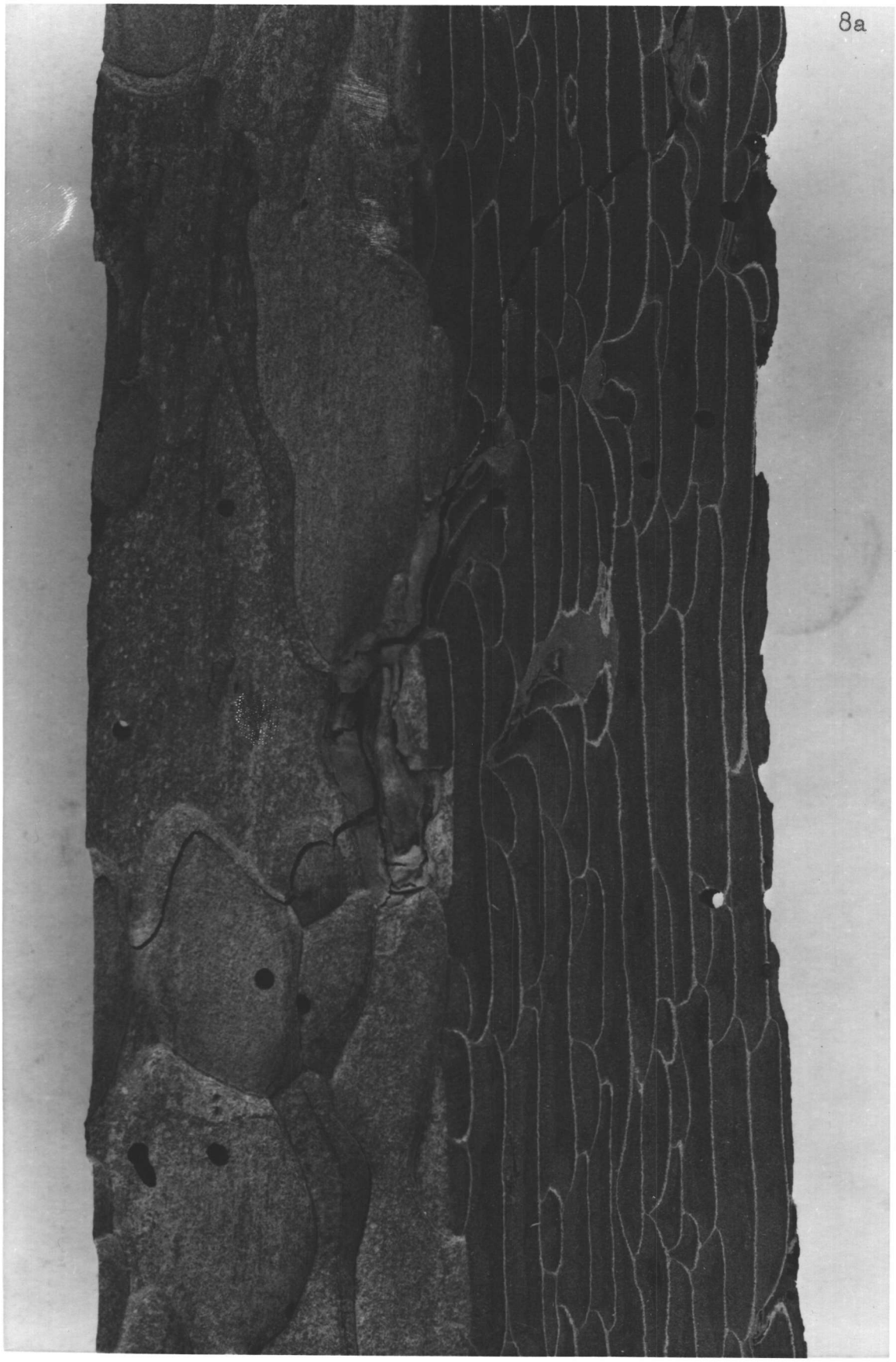

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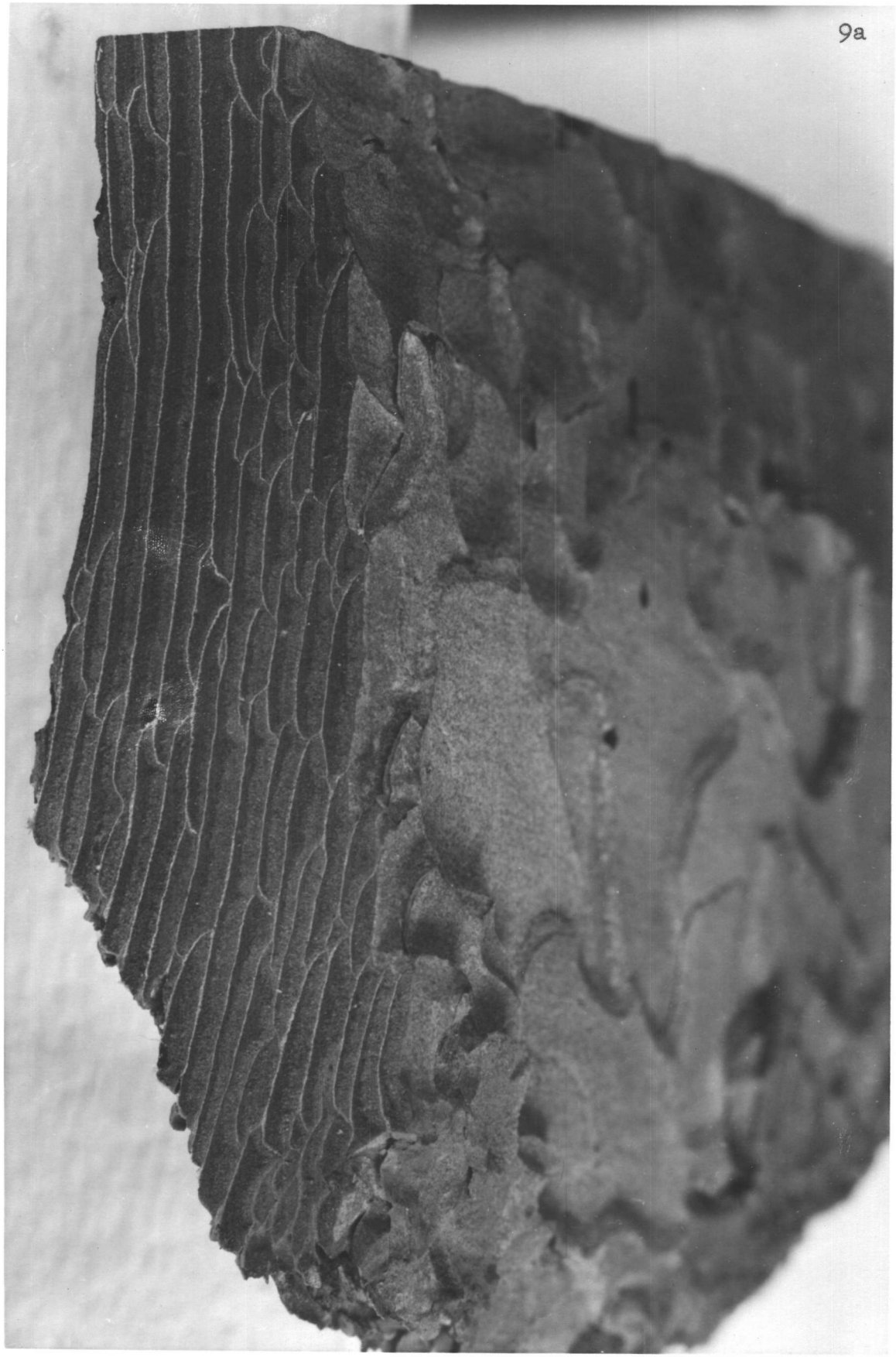
PLATE 3

AN OBLIQUE VIEW

OF A CROSS-SECTION OF PONDEROSA PINE BARK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of the extractives of ponderosa pine bark.

The nine bark samples previously mentioned were extracted in a Pyrex glass Soxhlet extractor with diethyl ether for eight hours. After removal of the ether, the dried extract from the composite samples was found to vary between 5.13 and 8.13%, based on the oven-dry weight of bark. The residual ether on the bark was allowed to evaporate in the hood, and then the samples were extracted in a similar manner with acetone.

This acetone extract, after drying, comprised 4.67 to 8.84% of the oven-dry bark. Upon removal of the acetone in the hood, the bark was next extracted with hot water. The hot water solubles, which were 11.05 to 13.20% of the oven-dry bark, were found by difference. As can be seen in Table 2, the total extractive content of ponderosa pine bark varied between 23.21 and 28.69% of the weight of oven-dry bark.

Age group	Sample	Moisture	Ethyl ether solubles	Acetone solubles	Water solubles	Total of three extractives
Years		Percent	Percent	Percent	Percent	Percent
150 to 200	Bottom	9.90	6.06	6.10	11.05	23.21
	Middle	9.76	5.47	5.47	12.94	23.73
	Top	9.25	6.54	6.54	13.15	25.84
200 to 250	Bottom	9.78	6.44	8.24	12.30	26.98
	Middle	9.32	5.13	4.67	12.42	23.22
	Top	9.68	6.17	5.58	12.20	23.95
250 to 300	Bottom	10.25	8.13	8.84	11.72	28.69
	Middle	9.64	6.11	5.78	11.89	23.78
	Top	9.05	7.33	6.59	13.20	27.12

TABLE 2

EXTRACTIVE CONTENT OF PONDEROSA PINE BARK

(Percent of oven-dry weight of the unextracted bark)

EXPERIMENTAL PROCEDURE

CONSTITUENTS OF THE HEXANE EXTRACT

In order to have a better separation of the ether-soluble material of the ponderosa pine bark, the bark was extracted successively with Skelly B solvent, B.P. 60-70° C.; benzene; and ether. Three kilograms of bark ground to pass a twenty-mesh sieve were extracted in five hundred gram batches in a Pyrex glass Soxhlet type extractor for eight hours with Skelly B solvent. The bark used for this work was a random sample taken from trees 24-36 inches in diameter located in the vicinity of Sisters, Oregon. The yield of hexane soluble extract, based on the oven-dry weight of bark, was 2.62%. The solvent was removed, and the extract recovered. This wax-like material had a melting point of 56-58° C. The hexane soluble extract was then dissolved in ether and extracted with 5% potassium carbonate to remove the free acids present. This left the neutral material present in the extract in the ether layer. The potassium salts of the acids were freed by adding mineral acid, and the aqueous solution was extracted with ether to remove the free acids.

Free acids. Upon removal of the ether at reduced

pressure the free acids were found to comprise 42.1% of the hexane soluble extract. The solid acids, insoluble in cold acetone, were recrystallized from hot acetone, and were found to be saturated aliphatic acids with a melting point of 71-72° C. and a yield of 21.2%. These acids had a neutral equivalent of 351.5, which indicated that they were equivalent to a mixture of C_{22} (behenic) and C_{24} (lignoceric) acids. No attempt was made to separate this natural mixture of saturated fatty acids.

After filtration of the saturated fatty acids from the cold acetone solution, the solvent was removed from the filtrate in order to examine the residue. Since it was somewhat viscous and sticky at room temperature, two types of acids were indicated, resin acids and unsaturated fatty acids. The resin acids were removed by the preferential esterification method of Wolff and Scholze (29, p.369). These resin acids gave a positive Liebermann-Storch test (15, p.412). The acids had a melting point of 82-83° C., a neutral equivalent of 346.5, and were obtained in a yield of 19.5%. The specific rotation in ethanol was found to be $[\alpha]_D^{25^\circ} = +39.5^\circ$. While the resin acids present in ponderosa pine bark did not have a neutral equivalent as low as abietic acid, a common resin acid,

these resin acids were optically active, gave a positive Liebermann-Storch test, and were not esterified. These properties indicated the presence of resin acids.

The methyl esters of the acids remaining after the removal of the resin acids were saponified with sodium hydroxide. The solution was acidified and the acids removed by extraction with diethyl ether. This acidic fraction was a dark-brown viscous material which absorbed bromine and decolorized aqueous potassium permanganate. The unsaturated acids, found in a yield of 1.4%, had a neutral equivalent of 194 and an iodine number of 93.4, as determined by the Hanus method (19, p.494). In view of the small yield no attempt was made to purify the material by vacuum distillation. The acids were oxidized by cold alkaline potassium permanganate by the Lapworth and Mottram method (17, p.1629). The recovered hydroxy acid had a melting point of 129-130° C. and a neutral equivalent of 314, which demonstrated the presence of dihydroxystearic acid. Thus the unsaturated acid fraction present in ponderosa pine bark contained oleic acid with small amounts of more highly unsaturated acids.

Neutral fraction. The neutrals of the hexane solubles comprised 57.9% of the extract. After removal of the ether, the neutral fraction was steam distilled to recover any volatile oil present.

Volatile oil. The volatile oil of ponderosa pine bark, separated by steam distillation from the neutral fraction, was a clear liquid with a characteristic odor. Since it was present only in traces, i.e. 0.2% of the hexane soluble wax, no extensive analysis was conducted. The liquid had a boiling range of 195-208° C. and a refractive index, $n_D^{25°} = 1.4780$ with a dispersion of 39.4 Z scale divisions. The refractive index was determined with an Abbe type refractometer. The density of the material was 0.844 grams/cc at 25° C.

Ponderosa pine wood is known to contain a volatile oil of the following composition: 51% l-beta-pinene, 21% l-alpha-pinene, 12% dipentene, 8% borneol, and 1.5% bornyl acetate (1, p.959). This volatile oil had a density of approximately 0.85 grams/cc and a refractive index, $n_D = 1.4740$. Since the volatile oil from the ponderosa pine bark had a comparable density and refractive index to that of the wood it was indicated that the oils were similar. Because of its access to the air the oil from the bark would be expected to contain a smaller percentage of the more volatile constituents.

Combined acids. After it had been steam distilled, twelve grams of the neutral fraction of the hexane solubles were saponified with 100 milliliters 0.8 normal alcoholic potassium hydroxide. The melting point of

the neutral wax was 59-60° C. and the saponification number was 84.0. Following the saponification the alcohol was removed by reduced pressure and water added. The mixture of potassium salts and unsaponifiables was then extracted with ether. The ether removed the unsaponifiable fraction whereas the combined acids remained in the water layer as the potassium salts. These acids were freed by adding mineral acid, and then they were recovered by extraction with ether. After removal of the ether and drying to constant weight, the combined acids were found to comprise 29.8% of the total hexane soluble extract. The acids were recrystallized from acetone to a constant melting point of 69-70° C. and a neutral equivalent of 330, which indicated that they were equivalent to a mixture of C_{20} (arachidic) and C_{22} (behenic) acids.

Unsaponifiables. The material which remained in the ether was the unsaponifiable fraction that comprised 27.9% of the hexane solubles. Upon removal of the ether at reduced pressure the material was recrystallized from ethanol. This material yielded a trace, i.e. 0.7% of the hexane soluble wax, of a sterol that gave a positive Liebermann-Burchard test (12, p.261). This phytosterol had a melting point of 137-138° C., and

the acetate derivative melted at 124-125° C.

The sterol crystals gave a precipitate with digitonin in ethanol. The melting point of the phytosterol and its acetate were similar to those of the sterol found in Douglas fir wood (10, p.409). Mixed melting points of the two phytosterols showed no depression in melting point.

After removal of the ethanol the residue of the unsaponifiable portion, which comprised 27.2% of the hexane soluble extract, was recrystallized from acetone. The white precipitate that was filtered off had a melting point of 65-67° C. This white neutral material formed an acetate with a melting point of 51-52° C. when refluxed with acetic anhydride in the presence of fused sodium acetate. In addition a phenyl urethane, melting point 81-82° C., was prepared. The nature of the separation of the unsaponifiables and the derivatives formed indicated that this fraction was composed of saturated fatty alcohols. These alcohols were oxidized to the corresponding acids by means of fusion with potassium hydroxide (13, p.1737). The acids formed had a melting point of 66-67° C. and a neutral equivalent of 344, hence were equivalent to a mixture of C_{22} (behenyl) and C_{24} (lignoceryl) alcohols. The alkaline fusion mixture was extracted with ether

before acidification. The complete oxidation of the alcohols and the absence of hydrocarbons in the hexane soluble extract was indicated by the presence of a very small amount of residual material after removal of the ether.

Table 3 shows the percentages of the constituents in the hexane soluble extract. The method of separation described above is illustrated by means of a flow diagram in Figure 1.

CONSTITUENTS OF THE BENZENE EXTRACT

After the ponderosa pine bark had been extracted with Skelly B solvent, it was further extracted with benzene in a Pyrex glass Soxhlet type extractor, and a brown wax-like substance was removed. A yield of 0.92%, based on the oven-dry weight of bark, was obtained. The benzene was removed from the extract by reduced pressure. This wax had a melting point of 58-60° C.

Fatty acids. Ten grams of this benzene soluble material was saponified with 100 milliliters of 0.8 normal alcoholic potassium hydroxide. After removal of the alcohol by reduced pressure, water was added, and the unsaponifiables were extracted from the mixture with diethyl ether. The aqueous layer contained the potassium salts of the acids present. Upon the addition of mineral

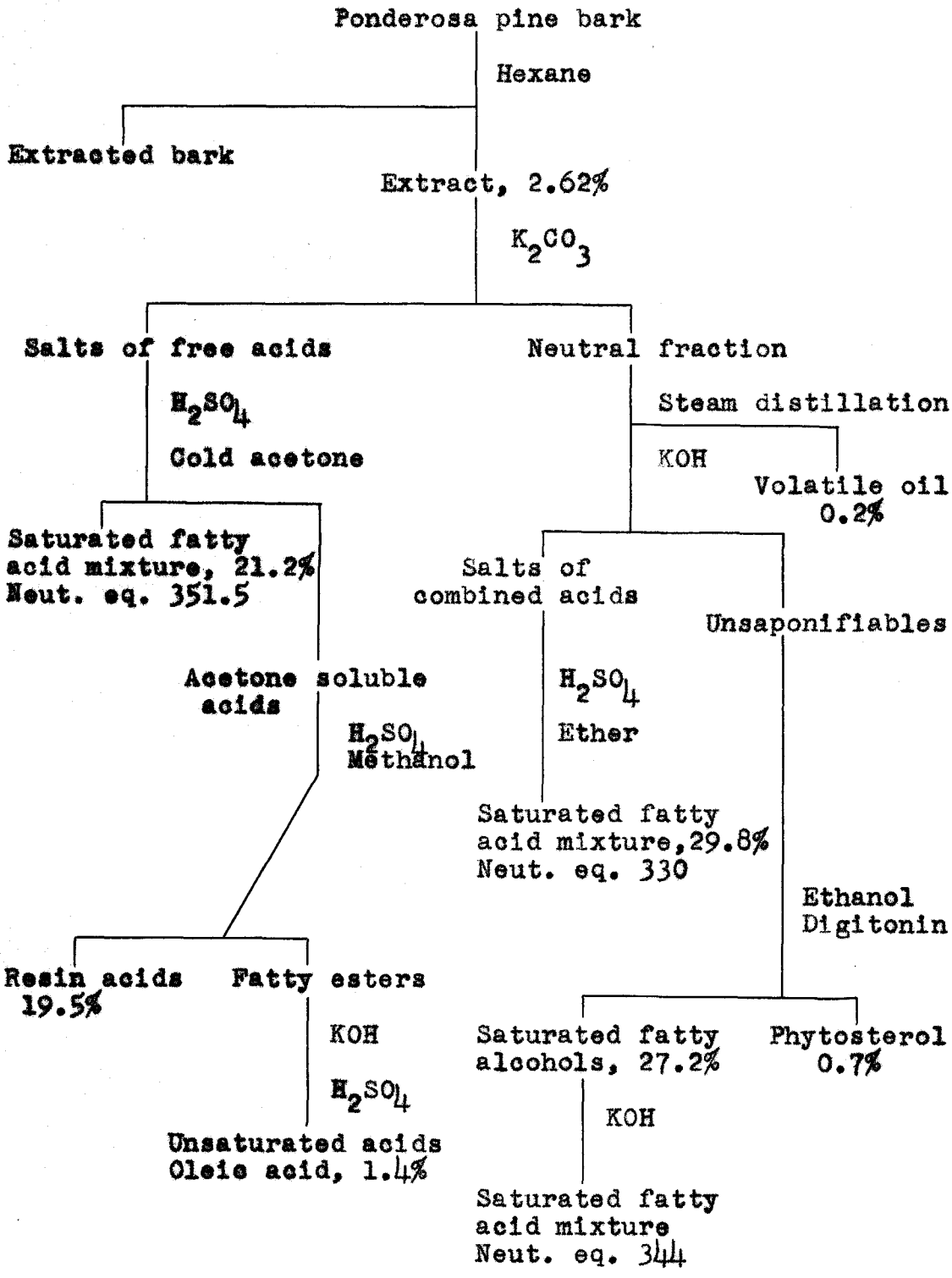
TABLE 3

CONSTITUENTS OF THE HEXANE SOLUBLE EXTRACT

Free Acids		42.1%
Saturated Fatty Acids	21.2	
Unsaturated Acids (Oleic Acid present)	1.4	
Resin Acids	19.5	
Combined Acids		29.8%
Saturated Fatty Acids	29.8	
Unsaponifiables		28.1%
Fatty Alcohols	27.2	
Phytosterols	0.7	
Volatile Oil	0.2	

FIGURE 1

SEPARATION OF HEXANE SOLUBLE EXTRACT



acid, the wax acids were freed and removed from the aqueous layer by ether. After removal of the ether, the acids were redissolved in hexane and recrystallized from this solvent. Following this these acids were recrystallized from acetone.

These acids, which comprised 34% of the benzene extract, were found to melt at 68-70° C. and to have a neutral equivalent of 358, which indicated that they were equivalent to a mixture of C_{22} and C_{24} acids, that is behenic and lignoceric acids. The Wolff and Scholze preferential esterification method revealed an absence of resin acids. The lack of absorption of iodine from the Hanus iodine solution showed that there were no unsaturated acids present in the benzene soluble fatty acids.

Phlobaphene material. When the potassium salts of the fatty acids of the benzene soluble wax were acidified after separation from the unsaponifiables, there settled out a brown amorphous material which was insoluble in acid solution. This material was filtered off, washed with water, dissolved in acetone, and then precipitated in water. The material, when dry, was a dark-brown amorphous powder, which amounted to 39% of the benzene soluble wax. It was soluble in ethanol, acetone, and potassium

carbonate and insoluble in water, diethyl ether, and benzene. The material was obtained only upon saponification of the benzene soluble wax. This phlobaphene-like material gave a dark-brown coloration when aqueous ferric chloride was added to an alcoholic solution of the material.

Unsaponifiables. After the ether was removed from the unsaponifiable material, it was separated into ethanol soluble and ethanol insoluble fractions. The ethanol soluble material, which was 23.5% of the benzene soluble wax, was found to have a melting point of 65-67°C. The soluble material was acetylated with acetic anhydride and fused sodium acetate. The acetate derivative recovered had a melting point of 55-56° C. These properties and the derivative formed indicated that the ethanol soluble portion of the benzene wax unsaponifiables was composed of saturated fatty alcohols. These fatty alcohols were oxidized to the corresponding acids with molten potassium hydroxide. The recrystallized solid fatty acids had a melting point of 66-67° C. and a neutral equivalent of 343. Therefore the ethanol soluble fraction appeared to be equivalent to a mixture of C_{22} and C_{24} fatty alcohols.

The ethanol insoluble portion of the unsaponifiables, which was 3.5% of the extract, was not fully characterized.

The material, when recrystallized from acetone had a melting point of 108° C. It did not give a positive Liebermann-Burchard test for sterols (12, p.261). The white crystals were acetylated with acetic anhydride and pyridine. The acetate derivative melted at 58° C. All of the normal saturated monohydric aliphatic alcohols melt below 100° C. (27, p.11). Thus this ethanol insoluble portion of the benzene wax unsaponifiables must be either a branched chain alcohol or an alcohol with a ring structure.

The percentages of the constituents present in the benzene soluble extract are shown in Table 4, and the method of separation is illustrated by Figure 2.

TABLE 4

Constituents of the benzene soluble extract

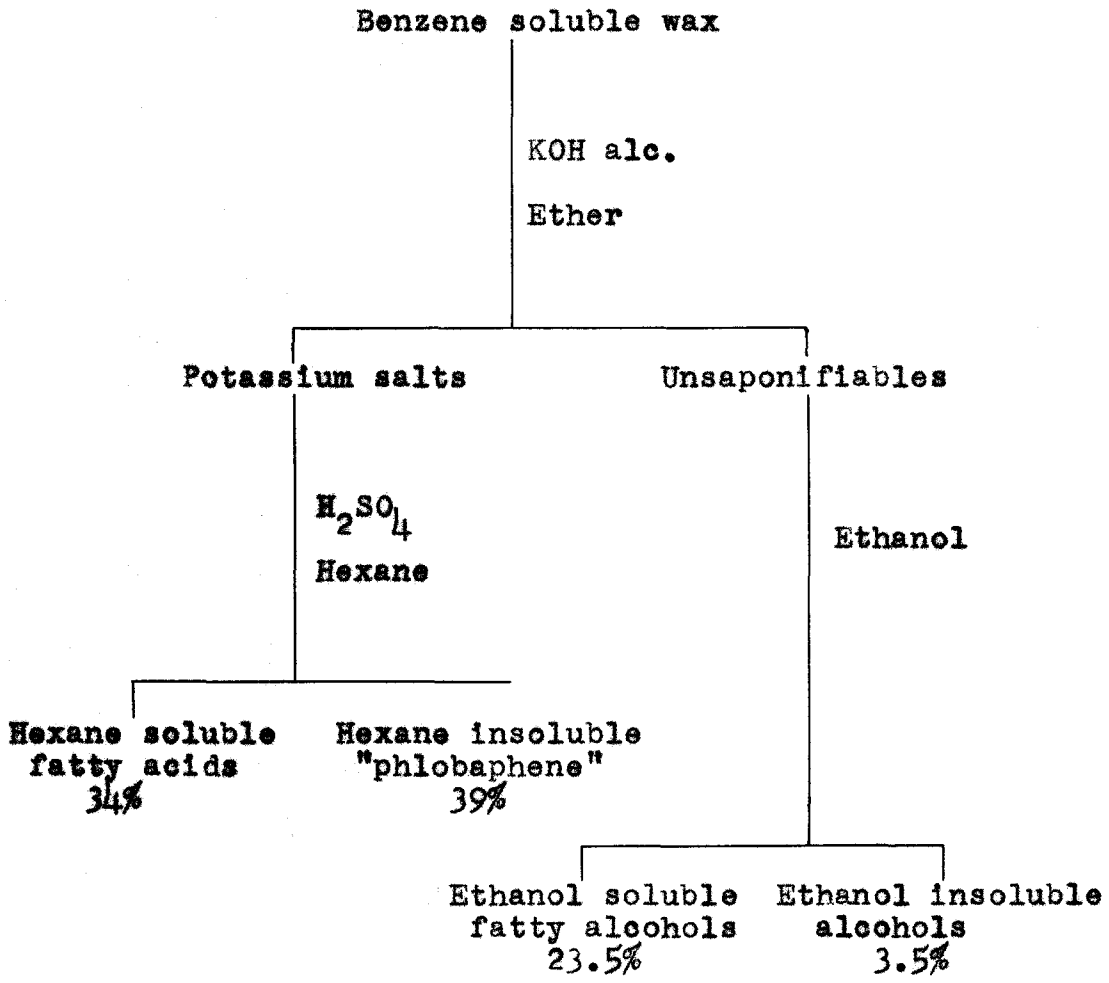
Hexane soluble fatty acids.....	34.0%
Ethanol soluble fatty alcohols.....	23.5%
Ethanol insoluble alcohols.....	3.5%
"Phlobaphene".....	39.0%

CONSTITUENT OF THE ETHER EXTRACT

After having been successively extracted with hexane and benzene, the ponderosa pine bark was next extracted in a Pyrex glass Soxhlet type extractor with diethyl ether. Upon removal of the ether at reduced

FIGURE 2

SEPARATION OF THE BENZENE SOLUBLE WAX



pressure, a bright yellow material remained that was soluble in ether, acetone, and alcohol; slightly soluble in water, and insoluble in benzene, hexane, and ethyl acetate. The yield of this ether soluble extract was 0.83%, based on the oven-dry weight of bark. The material was recrystallized from either acetone, ether, or alcohol, and in each case gave a constant melting point of 305-307° C. with decomposition.

Table 5 shows the color reactions given by the purified yellow material. These color reactions indicated the presence of a polyhydroxyflavone.

TABLE 5

Color reactions of
the yellow coloring matter

Reagent	Color developed
Sodium hydroxide, 0.1N	dark green
Alcoholic ferric chloride	dark green
Potassium carbonate, 5%	dark green
Concentrated sulfuric acid	bright orange
Dilute ammonium hydroxide	dark green
Lead acetate	orange
Reduction with Mg, HCl, and H_2O	pink
Wilson's boric acid reaction for flavone	positive

The molecular weight of the flavone by the ebullioscopic method in ethanol was 305 and 314.

Analysis of the flavone: calculated for

$C_{15}H_{10}O_7$: C, 59.6; H, 3.31; mol. wt. 302

Found: C, 59.4, 59.3; H, 3.80, 3.85; mol. wt.305

Suitable derivatives for such a material are the acetate and methoxy compounds. The acetate was prepared by refluxing two grams of the yellow flavone with excess acetic anhydride in the presence of fused sodium acetate. The excess acetic anhydride was destroyed with water and a white acetylated product separated. This derivative was filtered off and recrystallized from ethanol to a constant melting point of 237-239° C. It gave no coloration with alcoholic ferric chloride, which indicated that all of the phenolic hydroxyl groups had been acetylated. Using Clark's acetyl procedure (6, p.487) the acetate was found to contain 40.7% acetyl. With an indicated molecular weight of 305 the acetylated material must be a penta-acetate derivative. The molecular weight determination of the penta-acetate by the Rast camphor method (21, p.217) gave an average molecular weight of 511. The theoretical molecular weight for the penta-acetate is 512.

Analysis of the penta-acetoxyflavone: calculated for $C_{15}H_5O_7(CH_3CO)_5$: C, 58.6; H, 3.91; CH_3CO, 42.0; mol. wt. 512

Found: C, 58.3, 58.3; H, 4.21, 4.28; CH_3CO, 40.7; mol. wt. 505, 517

A methoxyl determination by Zeisel's method (30,p.989) indicated the complete absence of methoxyl groups on the compound. A methoxyl derivative was made, using diazomethane as the methylating agent. The diazomethane was prepared by the decomposition of 41.2 grams of nitrosomethyl urea with 50% aqueous potassium hydroxide. Five grams of the flavone were dissolved in dry ether with a trace of methanol, which enhances the methylation under these conditions (24, p.747). The ethereal solution of the diazomethane was added over a period of three days. After each addition of ethereal diazomethane the material was placed in a stoppered flask and stored in the icebox for twenty-four hours. A white material separated from the cold ethereal solution and was filtered off and found to contain 38.5% methoxyl groups. The melting point of the methylated compound was 198-199° C. Remethylation under similar conditions did not increase the methoxyl content. By the Rast camphor method the methylated material was found to have a molecular weight of 370 and 374. In alcoholic ferric chloride the

methylated compound did not give a coloration, which indicated the complete methylation of the phenolic hydroxyl group. The derivative was also insoluble in 5% aqueous sodium hydroxide.

Analysis of the pentamethoxy flavone: calculated for $C_{15}H_5O_2(OCH_3)_5$: C, 64.5; H, 5.38; OCH_3, 41.6; mol. wt. 372

Found: C, 63.9, 64.3; H, 5.81, 5.88; OCH_3, 38.5; mol. wt. 370, 374

An examination of the literature indicated that no compound with these properties had been reported.

Degradation of the pentahydroxyflavone. By means of degradation reactions it was possible to clarify the structure of this flavone. In general it was found expedient to conduct the experiments with the methylated material as it was stable in acid and base solution and the fragments of the molecule were more easily isolated after the reaction. Five grams of the pentamethoxy-flavone were oxidized with hot alkaline permanganate. After the manganese dioxide had been filtered off the solution was made acidic with mineral acid. Under these conditions an acid separated. This acid was removed by extraction with ether, and the ether distilled off at reduced pressure. The material was recrystallized from

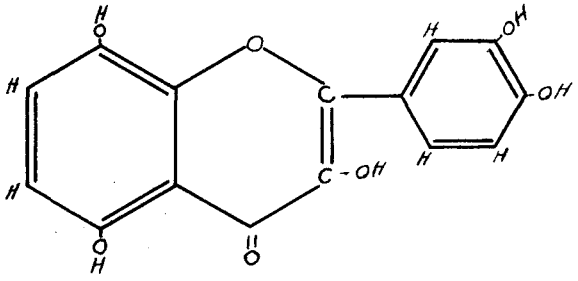
dilute ethanol to a constant melting point of 179-180° C. and a neutral equivalent of 181. A mixed melting point determination of this material with veratric acid, melting point 180-181° C., showed no depression in melting point. From these data it was known that the flavone was a 3',4'-polyhydroxyflavone.

Next, five grams of the flavone were fused with potassium hydroxide. Upon completion of the fusion, water was added to the black fusion mixture. This alkaline solution was then methylated with dimethyl sulfate. Over a period of four hours dimethyl sulfate was added in sufficient quantity to neutralize the base, and the mixture was stirred for an additional four hours. Then the methoxy derivatives of the phenols were removed with ether from the acid solution. First, the diethyl ether was removed and then the aromatic ethers were distilled at reduced pressure. Under these conditions it was possible to separate the distillate into two fractions. The first fraction had a boiling point of 203-206° C. at atmospheric pressure, whereas the second fraction boiled at 250-253° C. From the previous oxidation the lower-boiling ether was thought to be veratrole, B.P. 207° C. The dibrome-derivative was prepared by Underwood's method (26, p.4090). The 4,5-dibromoveratrole was recrystallized from alcohol to a constant melting point

of 91-92° C. (literature value, 92-93° C.).

The higher-boiling ether was thought to be a trimethoxy ether. A methoxyl determination showed that there was 55.0% OCH_3 present, whereas trimethoxybenzene contains 55.4% OCH_3. Since all three of the possible trimethoxybenzenes form suitable bromine derivatives, the compound was brominated in acetic acid. The bromo-derivative obtained was recrystallized from ethanol to a constant melting point of 54-55° C. (literature value 54-55° C.). These constants are those of 5-bromo-1,2,4-trimethoxybenzene, which was derived from 1,2,4-tri hydroxybenzene (4,p.375), boiling point 250-255° C.

From the foregoing data two structures are possible, i.e. the 3,3',4',5,6-pentahydroxyflavone and the 3,3',4',5,8-pentahydroxyflavone. Since the 3,3'4',5,6-pentahydroxyflavone is known (22, p.130), and its melting point is 318-320° C. whereas the pentamethoxy and penta-acetate melt at 196° C. and 121-122° C., respectively, the yellow coloring matter in ponderosa pine bark must be the 3,3',4',5,8-pentahydroxyflavone.



Absorption spectra. The ultraviolet absorption spectra of the 3,3',4'5,8-pentahydroxyflavone is shown in Figure 3. The ultraviolet curves were determined by means of a Beckman model DU quartz photoelectric spectrophotometer. The solvent used was ethanol, which was purified by Leighton's sulfuric acid method (18, p.3017). The absorption curve of the flavone, which is very similar to the curve of quercetin, has a maximum absorption at 255 millimicrons. Such a situation is quite possible in view of the fact that certain hydroxyl groups in appropriate positions on a flavone molecule do not cause a shift in the absorption peaks of the curve (3, p.565).

The infrared spectra of the 3,3',4'5,8-pentahydroxyflavone, dihydroquercetin, and quercetin are shown in Figures 4, 5, and 6. These were determined by means of a Baird Associates twin-beam infrared spectrophotometer. All of the materials were mulled in Nujol paste for analysis.

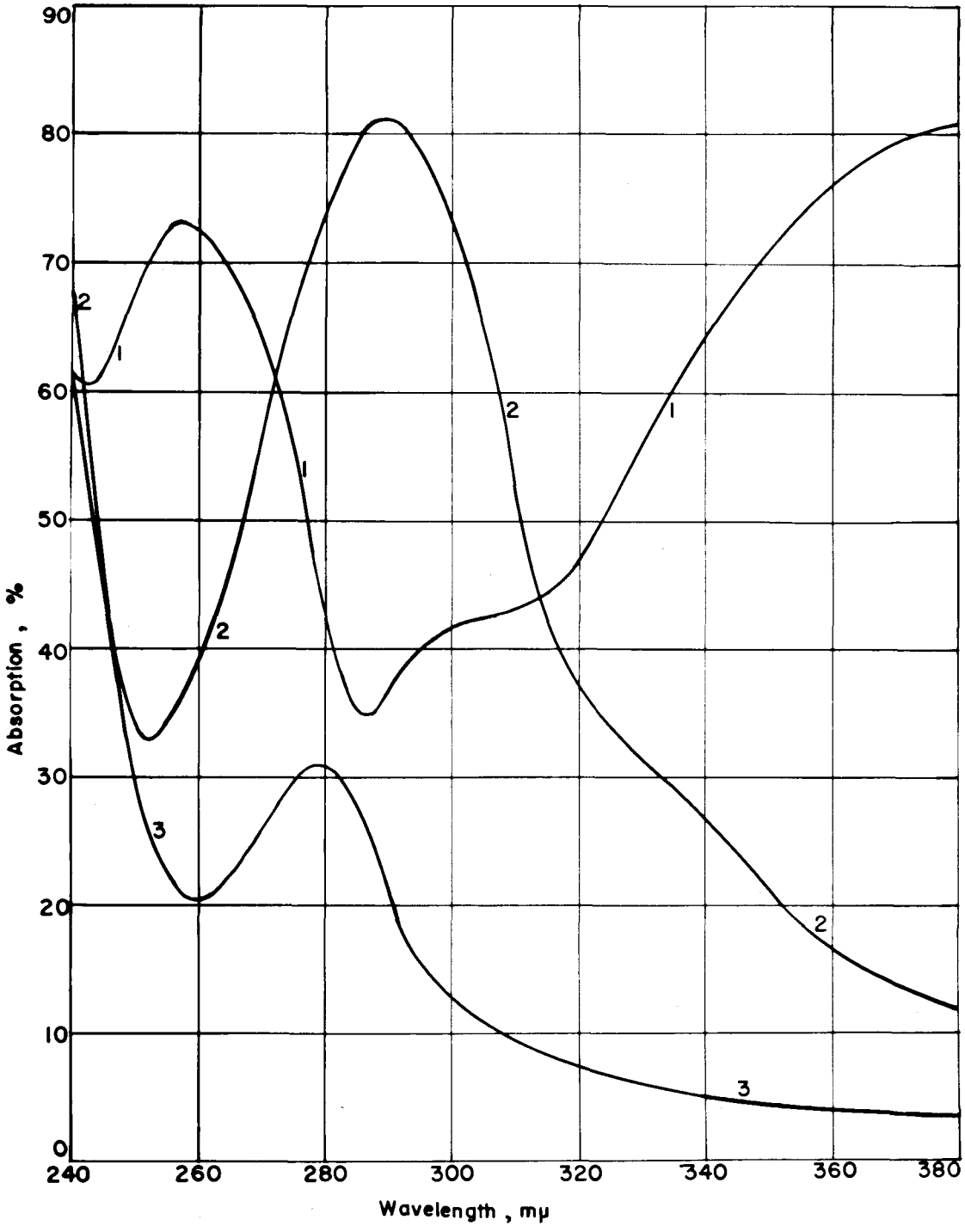
In view of the fact that no two organic molecules have completely identical infrared absorption spectra (23, p.1), these curves demonstrate the dissimilarity of the three compounds. The quercetin is 3,3',4',5,7-pentahydroxyflavone, whereas the dihydroquercetin is the 3,3',4',5,7-pentahydroxyflavanone. It will be noted that the main absorption band of the hydroxyl groups

FIGURE 3

Ultraviolet absorption curves

Concentrations

1. Yellow coloring matter	0.00125 grams /ml.
2. Tannin	0.0025 grams /ml.
3. Phlobaphene	0.00125 grams /ml.



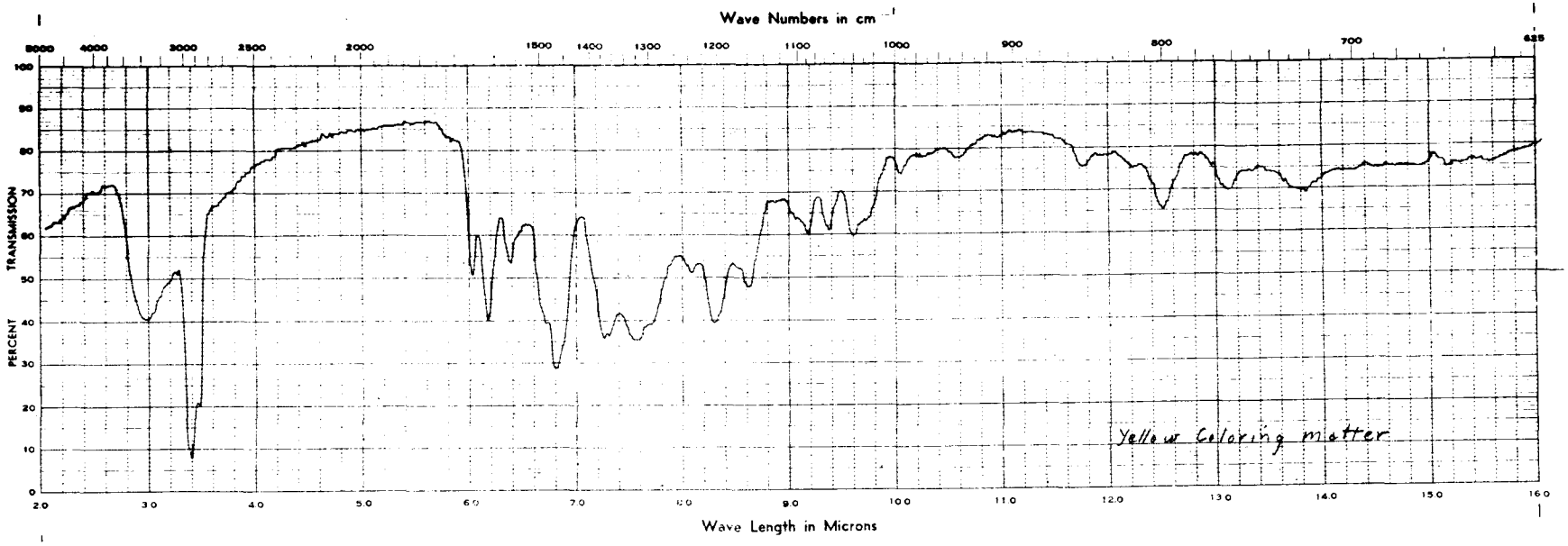


FIGURE 4

INFRARED ABSORPTION CURVE OF 3,3',4',5,8-PENTAHYDROXYFLAVONE

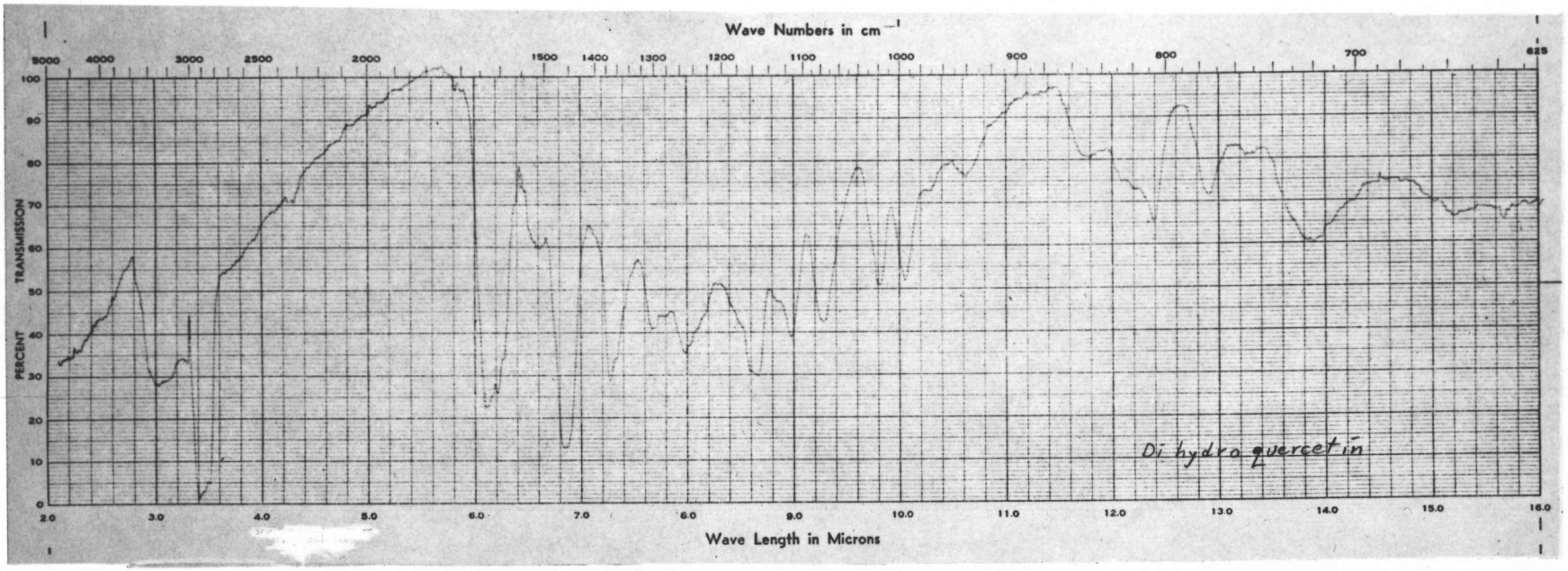


FIGURE 5

INFRARED ABSORPTION CURVE OF 3,3',4',5,7-PENTAHYDROXYFLAVANONE

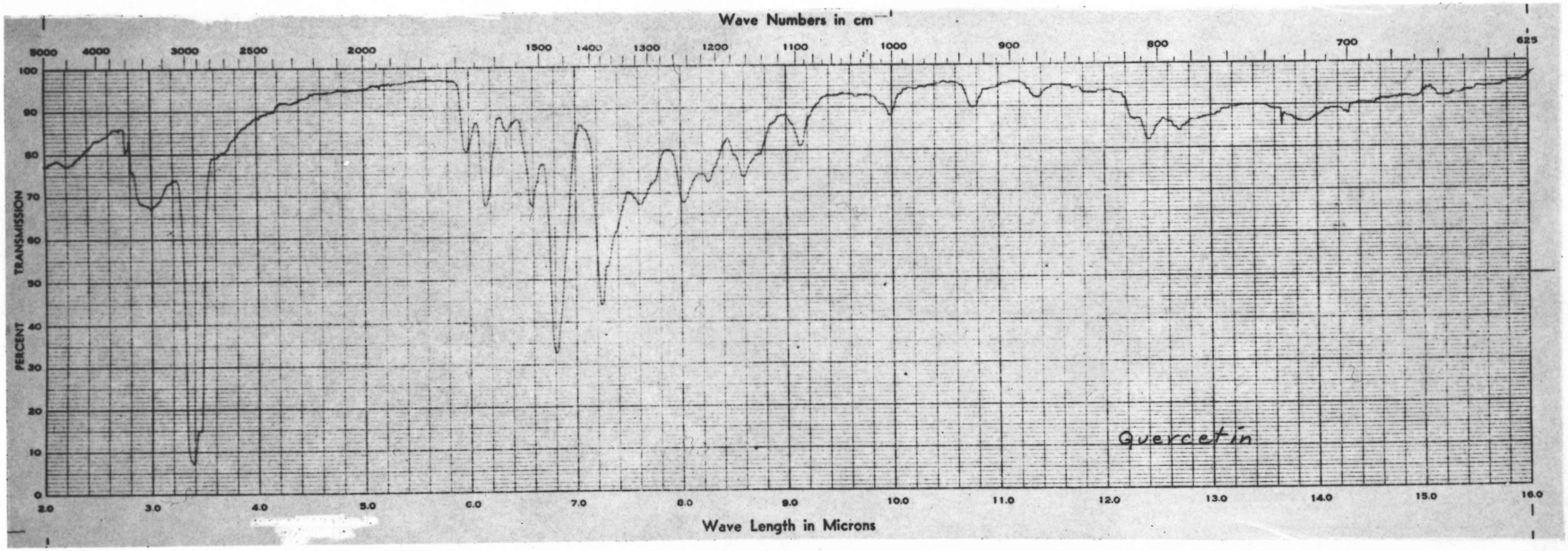


FIGURE 6

INFRARED ABSORPTION CURVE OF 3,3',4',5,7-PENTAHYDROXYFLAVONE

occurred at 3.0-3.1 microns. If greater dispersion of this particular absorption band had been possible, a more detailed analysis of the hydroxyl groups could have been made. The most obvious difference in the infrared spectra of quercetin and the 3,3',4',5,8-pentahydroxyflavone can be noted in the 9-10 micron region.

ETHANOL SOLUBLE CONSTITUENTS

Tannin and phlobaphene. A 500 gram sample of bark previously extracted with diethyl ether was extracted in a Pyrex glass Soxhlet type extractor with 95% ethanol. The yield of ethanol solubles was 8.30%. When the volume of the solution had been decreased to 250 milliliters at reduced pressure, the dark-brown alcoholic solution of tannin and phlobaphene was poured into 1175 millliliters of water. Under these conditions the phlobaphene was insoluble and could be filtered off, washed free of tannin, and dried in a vacuum oven at 55° C. and 3" of mercury. The phlobaphene comprised 37.8% of the ethanol soluble extract. The percentage of tannin in the solution was determined to be 62.2% by taking an aliquot and analyzing it for tannin in accordance with the standard methods of the American Leather Chemists Association (2, p.7).

Two methods were used to purify the tannin from

ponderosa pine bark. In the first method a concentrated aqueous solution of the tannin, i.e. 20% solids, was extracted with ethyl acetate in a liquid-liquid extractor. After an eight-hour extraction the ethyl acetate was removed at reduced pressure and the material dried in a vacuum oven at 55° C. and 3" of mercury. Of the tannin available in solution, only 20.8% was extracted with the ethyl acetate. The product was a brown powder characterized as follows:

1. It was soluble in water, ethanol, acetone, and ethyl acetate.
2. It was precipitated in aqueous solution by gelatine, lead acetate, and bromine.
3. It was colored a brown-black by the addition of a drop of ferric chloride solution.
4. It tanned a sheepskin skiver a light brown color in aqueous solution.

The second method of isolating the pure tannin was based upon the fact that the tannin was precipitated from an aqueous solution by sodium chloride. The salt was added to the tannin solution which had been previously extracted with ethyl acetate, and the tannin precipitated. The sodium chloride precipitated tannin was filtered off and dried in a vacuum oven at 55° C. and 3" of mercury. The tannin was then extracted with acetone in the Pyrex glass Soxhlet apparatus. The acetone soluble portion of the sodium chloride precipitated tannin, which had the

same properties as those listed for the ethyl acetate soluble tannin, was 4.5% of the ethanol extract. The percentage of residual tannin was found by difference.

Table 6 shows the percentages of the constituents found in the ethanol extract and Figure 7 demonstrates the method of separation of the extract.

TABLE 6

Constituents of the ethanol extract

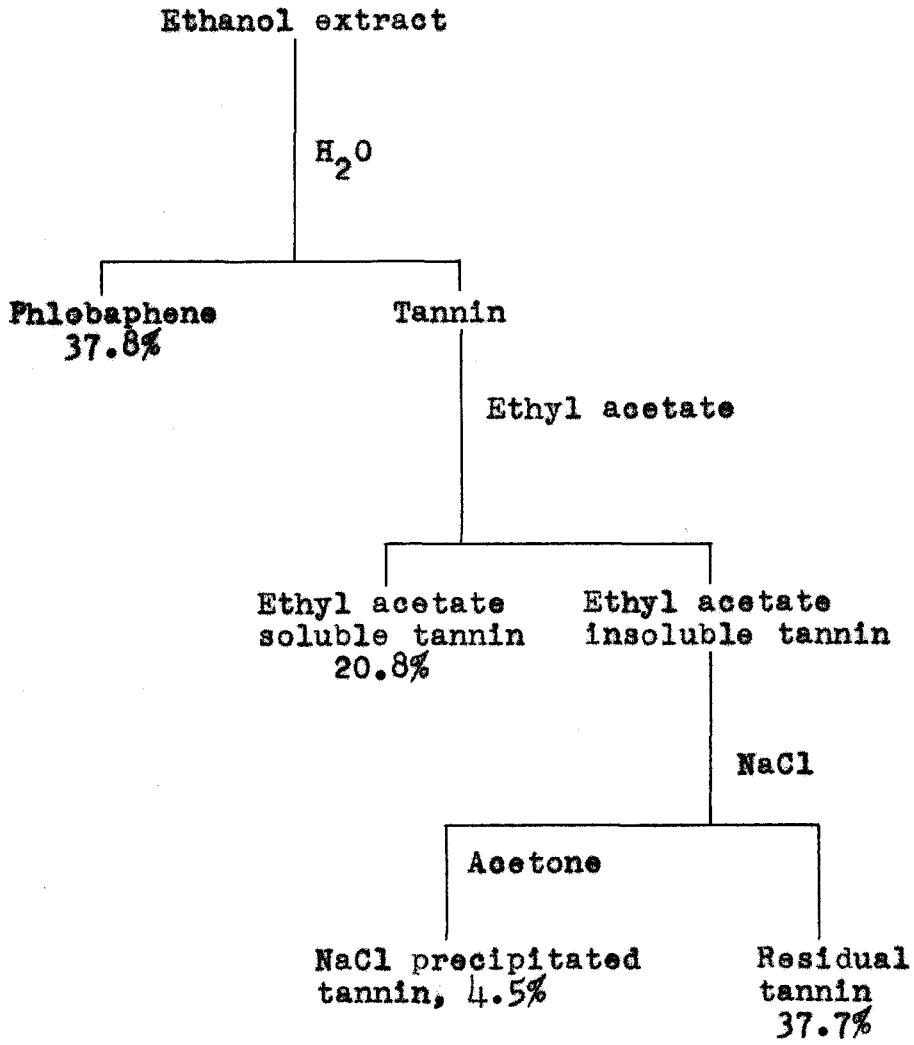
(8.30% yield on the basis of oven-dry bark)

Phlobaphene.....	37.8%
Ethyl acetate soluble tannin.....	20.8%
NaCl precipitated tannin.....	4.5%
Residual tannin (by difference)..	37.7%

Methylation of tannin. Since the hydroxyl groups present on the tannin molecule are believed to be active in the tanning of leather, it was desirable to know the percentage of aliphatic and phenolic hydroxyl groups present. Two three-gram samples of the ethyl acetate soluble tannin were methylated to constant methoxyl. The first sample was dissolved in 1.0% aqueous sodium hydroxide and methylated by adding dimethyl sulfate while stirring. The solution was kept basic at all times by adding additional base. When twenty-five milliliters of dimethyl sulfate had been added, the solution was

FIGURE 7

SEPARATION OF THE ETHANOL SOLUBLE EXTRACT



allowed to become acidic and the methylated product filtered off and remethylated under the same conditions to constant methoxyl. Finally the methylated tannin was dissolved in acetone and precipitated by adding the solution to a large volume of water. The light brown material was filtered off and dried in a vacuum oven at 55° C. and 3" of mercury.

The second sample of tannin was dissolved in dry dioxane containing a trace of dry methanol, which facilitated the reaction, and methylated with diazomethane. The diazomethane was produced by the decomposition of nitrosomethyl urea with potassium hydroxide. After each addition of diazomethane, the solution was allowed to stand over night in the icebox. Five such methylations produced a light brown product of constant methoxyl. Upon removal of the diethyl ether and dioxane at reduced pressure the material was dissolved in acetone and precipitated by the addition of water. The diazomethane methylated tannin was filtered off and dried in a vacuum oven at 55° C. and 3" of mercury.

The dimethyl sulfate was used to methylate all of the hydroxyl groups present, while the diazomethane reacted only with the acidic hydroxyl groups. In this way it was possible to determine by difference the percentage of

aliphatic hydroxyl groups present in this tannin molecule. Since it was necessary to know the amount of methoxyl present in the tannin molecule before methylation, the tannin was purified in such a manner that no methoxyl or ethoxyl group was added. For this, 0.5 grams of the ethyl acetate soluble tannin was dissolved in hot water and boiled. The solution was then placed in a vacuum desiccator and dried over sulfuric acid.

The methoxyl content was determined in accordance with TAPPI standard, T 2 M-43, which is based on Zeisel's method (25, T 2). The results of these analyses are shown in Table 7.

TABLE 7

Analysis of the tannin

	Methoxyl,%	Increase in methoxyl,%
Tannin	2.54	
Diazomethane methylated tannin	24.15	21.61
Dimethyl sulfate methylated tannin	36.80	34.26

Hydroxyl groups in the tannin

Total hydroxyl groups	22.2%
Phenolic hydroxyl groups	13.2%
Aliphatic hydroxyl groups	9.0%

Oxidation of the methylated tannin. A twenty-gram sample of ponderosa pine tannin was dissolved in a 1% solution of sodium hydroxide. To this solution was added dropwise 40 milliliters of dimethyl sulfate and an equivalent amount of 30% sodium hydroxide over a period of two hours. The solution was basic until the end of the reaction, at which time it became acidic and the methylated product separated. This product was filtered off and remethylated in the presence of acetone.

Upon removal of the acetone, the methylated product was recovered, dissolved in acetone, and precipitated in water. The precipitated product was recovered. This methylated tannin was then oxidized by hot alkaline potassium permanganate. The manganese dioxide was removed and mineral acid added to the filtrate. The precipitated acid was filtered off and recrystallized from water and ethanol to a constant melting point of 179-180° C. The neutral equivalent of the acid was 181. These constants and the fact that no depression was noted when a mixed melting point was made with veratric acid demonstrated the presence of this dimethoxy benzoic acid in the oxidation products. Since the veratric acid was derived from a catechol nucleus, this nucleus must have been present in the original tannin, which would, by this fact, be called a catechol-type tannin.

Ultraviolet absorption spectra of the tannin and phlobaphene. The absorption spectra of the tannin and phlobaphene from ponderosa pine bark are shown in Figure 3. The solvent used was ethanol. The absorption maxima of the tannin and phlobaphene occur at 280 and 290 millimicrons, respectively. These curves are quite similar to those of the tannin and phlobaphene from Douglas fir bark (14, p.611). This might be expected in view of the fact that both are catechol type tannins. The absorption spectra of the methylated materials, as shown in Figure 8, have a maximum absorption at 280 millimicrons.

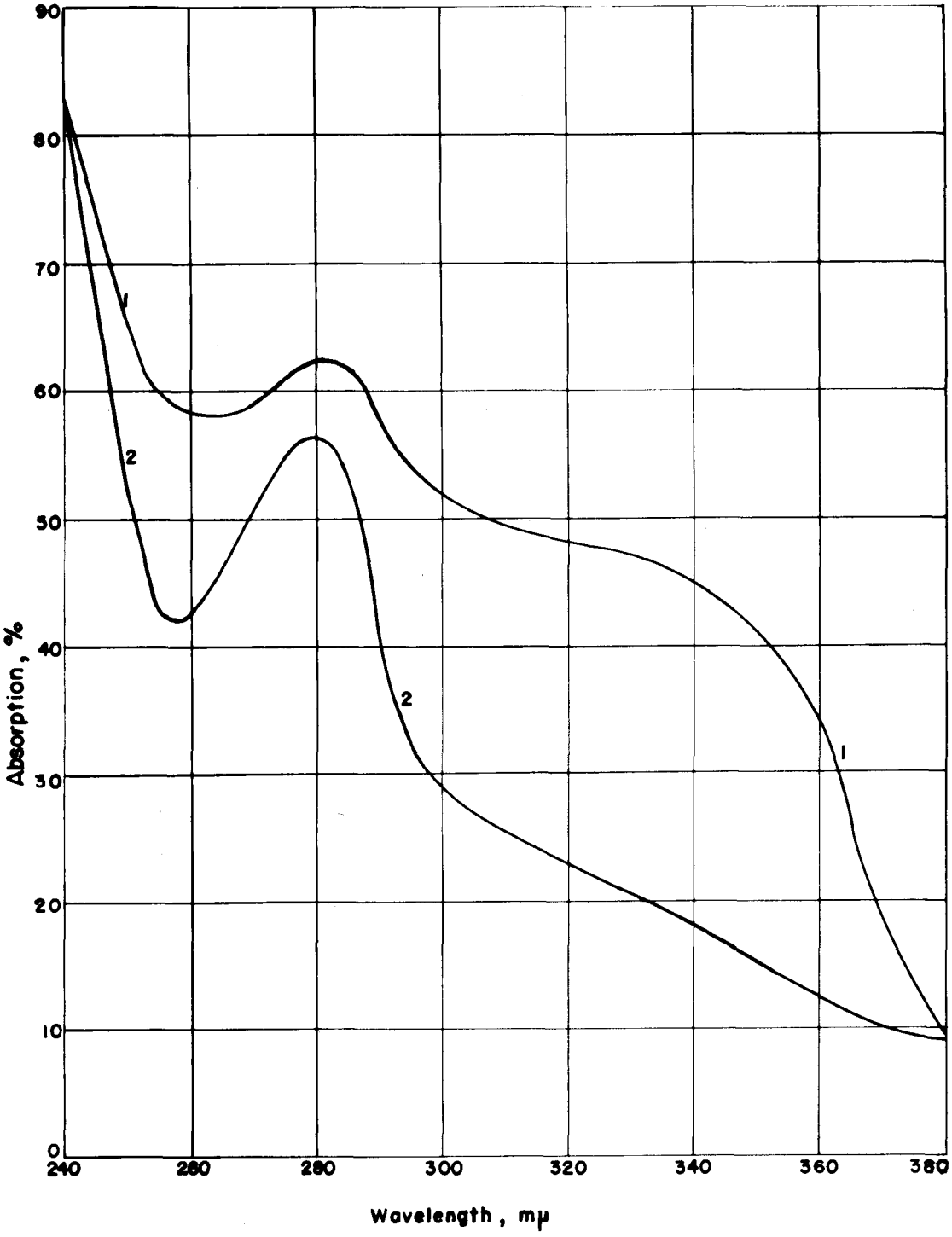
Aqueous extraction of the tannin. The tannin from ponderosa pine bark can also be extracted with hot water. In this method the extractives will include along with the tannin all of the water soluble constituents. The phlobaphene was not soluble in the hot water. The nine composite samples listed in Table 1 were extracted with water and analysed, with the exception of the sugars, in accordance with the standard methods of the American Leather Chemists Association (2, p.7). The sugars were determined before and after hydrolysis by Somogyi's modification of the Shaffer and Hartmann method (5, p.196). The data of the analyses are given in Table 8.

FIGURE 8

Concentrations

1. Dimethyl sulfate methylated tannin — 0.0025 grams /100ml.

2. Diazomethane methylated tannin — 0.0025 grams / 100ml.



Age group	Sample	Total solids	Soluble solids	Insol-ubles	Tannin	Non-tannin	pH	Acid, as acetic	Reducing sugar	Total sugar after hydrolysis
Years		Percent	Percent	Percent	Percent	Percent		Percent	Percent	Percent
150 to 200	Bottom	16.2	13.9	2.3	7.7	6.2	3.4	0.016	2.61	2.88
	Middle	14.1	12.9	1.2	5.7	7.2	3.6	.012	3.02	3.11
	Top	16.9	14.9	2.0	6.5	8.4	3.7	.011	3.14	4.55
200 to 250	Bottom	20.2	17.8	2.4	10.4	7.4	3.5	.027	2.86	4.33
	Middle	16.3	14.5	1.8	5.6	8.9	3.8	.024	2.56	3.90
	Top	16.1	13.8	2.3	5.7	8.1	4.0	.020	3.18	4.32
250 to 300	Bottom	23.1	19.5	3.6	11.4	8.1	4.0	.020	5.60	6.16
	Middle	18.0	16.4	1.6	7.1	9.3	4.1	.018	2.22	2.77
	Top	17.9	16.1	1.8	7.0	9.1	4.1	.018	1.52	2.18

TABLE 8

ANALYSIS OF TANNIN EXTRACTS FROM PONDEROSA PINE BARK

(Percentages based on oven-dry weight of bark)

It will be noted that in each age group the greatest percentage of tannin occurred in the butt-log samples, and that the greatest amount of tannin was found in the oldest age group. This is the reverse of the tannin distribution found in the bark of Douglas fir trees (Pseudotsuga taxifolia, Britt.) (14, p.605). The tannin comprised approximately one-half of the soluble solid material leached from the bark. The tannin content of the bark ranged from 5.6 to 11.4%. The tannin extract was used to tan sheepskin skivers a light brown color, which indicated that the tannin possessed good leather-making qualities.

Ponderosa pine bark tan liquor contains a high percentage of reducing sugar compared to the bark of other species (7, p.91). The reducing sugar was found to be from 1.52 to 5.60% of the weight of oven-dry bark. After hydrolysis with hydrochloric acid, the total sugar was found to be between 2.18 and 6.16%. A certain amount of sugar in tan liquors is necessary for the proper curing of hides in commercial tanning processes. The pH of the liquor, as measured by a Beckman glass electrode pH meter, Model H., varied from 3.4 to 4.1. It has been shown that hides more readily absorb the tannin at low pH values (20, p.571).

Since most vegetable tanning materials are shipped in the dry state, it was desirable to know the changes that take place when ponderosa pine bark tan liquor was dried.

TABLE 9

Effect of evaporation on tannin solutions

	Before evaporation	After evaporation no sulfite	After evaporation Na_2SO_3
Soluble solids	1.25%	1.10%	1.30%
Insolubles	0.16%	0.31%	0.11%
Total solids	1.41%	1.41%	1.41%

These changes were found by evaporating one liter of tan liquor with 1.41% total solids, based on the weight of the solution. When this liquor was dried at 55-60° C. with reduced pressure there was a negligible loss of tannin. On the other hand when it was evaporated over a steam bath to dryness and then redissolved to make a liquor with 1.41% total solids, the insoluble portion almost doubled while the soluble solids decreased to 1.10%. When 0.7 grams per liter of sodium sulfite was added to the liquor before evaporation, it was noted that there was a decrease in the insolubles with a corresponding increase in the soluble solids and tannin in the liquor. The values in Table 9 have been corrected for

the Na_2SO_3 added. Thus, it can be seen that the tan liquor from ponderosa pine bark possesses possibilities as a commercial tanning agent.

WATER SOLUBLE CONSTITUENTS

Extraction and separation of the water solubles. Ponderosa pine bark that had been previously extracted with diethyl ether and ethanol was next extracted with water. This was accomplished by extracting the bark in 50 grams batches in the conventional laboratory tannin extraction apparatus. The extract from 500 grams of bark was combined and filtered before evaporation at reduced pressure. The water soluble material was reduced to a thick syrup, which was purified by precipitation in a large volume of ethanol. Under these conditions the carbohydrate material was insoluble, whereas the tannin and other colored materials not previously removed remained in the ethanol. The precipitated material was centrifuged and then washed with alcohol. The final product was a light tan material.

Analysis of the carbohydrate material. The pentosan determination (19, p.412) demonstrated the presence of 22.8% pentosans in the water solubles. When one gram of the carbohydrate material was oxidized with nitric acid in accordance with the Bureau of Standards method (5, p.528),

the yield of mucic acid showed that the material contained 25.3% galactan. The filtrate was then neutralized with potassium carbonate. When this mixture was acidified with acetic acid, potassium acid saccharate was found. The white crystals were recrystallized from water. Microscopic identification of the salt was made as outlined by Hassid (11, p.685). The saccharic acid accounted for 32.4% of the fraction as glucosan. The uronic anhydride was determined by Dickson's method (8,p.775) and was found to be 2.1% of the carbohydrate material. An aqueous solution of the carbohydrate fraction was hydrolyzed with 2% sulfuric acid to test for mannose. After neutralization of the hydrolysate and filtration, the solution was concentrated under reduced pressure. Phenylhydrazine was added to the cold solution, which had been acidified with acetic acid. The phenylhydrazone of mannose was formed while the solution was in the icebox. The recrystallized derivative had a melting point of 196° C.

The analysis of the carbohydrate material is shown in Table 10.

TABLE 10

Constituents of the carbohydrate material

Pentosans	22.8%
Galactan	25.3%
Glucosan	32.4%
Uronic anhydride	2.1%
Mannan	9.2%
Undetermined	8.2%

DISCUSSION

This investigation has revealed that the total extractives of ponderosa pine bark varied from 23.21 to 28.69% of the oven-dry weight of bark. The yield of total extractives appears within the range of that of the bark of other species (16, p.35). The bark samples used in this analysis were gathered with respect to the age of the tree and position of the bark on the log. A greater percentage of extractives occurred in the top and bottom sections than in the middle section.

Solvent extraction demonstrated that the diethyl ether solubles comprised 5.47 to 8.13% of the weight of oven-dry bark, whereas the acetone solubles were 4.67 to 8.84% of the bark. The water solubles, which were found by difference, amounted to 11.05 to 13.20%.

In this species the hexane soluble extract was found to be a waxy material with a melting point of 56-58° C. The yield of wax was 2.62% based on the weight of oven-dry bark. This wax, which was light in color, was a hard, non-tacky material that appears to be suitable for industrial or household purposes. This hexane extract contained 42.1% free acids, which

included 21.2% saturated fatty acids with a neutral equivalent of 331.5, 19.5% resin acids, and 1.4% unsaturated acids. The combined acids, neutral equivalent 330, comprised 29.8% of this extract; whereas the unsaponifiables were found to be composed of 27.2% fatty alcohols, 0.7% phytosterol, and 0.2% volatile oil. When the fatty alcohols were oxidized, a fatty acid mixture, neutral equivalent 344, was obtained.

The benzene soluble wax, found to be 0.92% of the bark, was darker in color than the hexane soluble wax, but the dark color would not limit its use as an industrial wax. The benzene soluble wax composition was: saturated fatty acids, 34.0%, with a neutral equivalent of 358; ethanol soluble fatty alcohols, 23.5%; ethanol insoluble alcohols, 3.5%; and a phlobaphene-like material, 39.0%. When the ethanol soluble fatty alcohols were oxidized, a saturated fatty acid mixture, neutral equivalent 343, was obtained. It is thought that this benzene soluble extract contains a phenolic-fatty acid complex. Upon saponification, the benzene soluble wax yielded a phlobaphene-like material along with fatty acids and unsaponifiables. The phlobaphene material was insoluble in benzene and water, although soluble in ethanol and aqueous base.

The characteristic yellow coloring matter of ponderosa pine trees was isolated and found to be a new polyhydroxyflavone not previously described in the chemical literature. Its structure was determined and found to correspond to 3,3',4',5,8-pentahydroxyflavone. The yield of the flavone, based on the oven-dry weight of bark, was found to be 0.83%. An absorption maximum was shown at 255 millimicrons in the ultraviolet absorption curve of the 3,3',4',5,8-pentahydroxyflavone. The crystalline flavone is somewhat similar to the vitamin P compounds. Even though this new compound is not a glycoside, as is rutin, it may find some use in pharmaceutics. It is interesting to note that this crystalline pigment was found in conjunction with a catechol-type phlobatannin. Freudenberg believed that the phlobatannins were polymerization products of such polyhydroxyflavonols (9, p.156).

The ethanol solubles in this investigation were found to be 37.8% phlobaphene and 62.2% tannin. This tannin, which was characterized as a catechol-type phlobatannin, was shown to contain 2.54% methoxyl groups and 22.2 hydroxyl groups. The ultraviolet absorption spectra showed a maximum at 280 and 290 millimicrons for the tannin and phlobaphene, respectively.

The tannin from ponderosa pine bark can also be extracted with hot water. The samples were analyzed in accordance with the methods of the American Leather Chemists Association. The tannin content was found to vary from 5.6 to 11.4%. The greatest amount of tannin occurred in the butt log sample of the oldest trees, whereas the top log sample of the bark yielded the least amount of tannin. The extracts contained a high percentage of reducing sugar before and after acid hydrolysis. It is believed that tannin can be extracted at a profit from woody materials if the tannin content is 5% or more. If this is true, the overall average tannin content of 7.5% found in ponderosa pine bark would be slightly above the minimum. The tannin was used to tan sheepskin skivers, and the skivers had a light brown color, which would be suitable for most leathers.

The water soluble constituents, which were extracted with hot water, were found to contain 22.8% pentosans. Oxidation of the carbohydrate fraction to mucic acid demonstrated the presence of 25.3% galactan. Potassium acid saccharate which was identified microscopically accounted for 32.4% of the carbohydrate material as glucosan. Mannan was found in the amount of 9.2% and was

characterized by the formation of a phenylhydrazone. The yield of uronic anhydride was 2.1%.

Since ponderosa pine bark is a potential source of both wax and tannin, extraction of the bark should be planned to remove both of these valuable products. One such plan would be to extract the bark first with benzene to remove the waxes, and then to extract it with water to remove the tannin. The residual bark could then be burned to produce steam for the sawmill and the extraction plant. Thus the lumber industry would be one step closer to the complete utilization of the ponderosa pine logs brought to the mill.

SUMMARY

Nine samples of ponderosa pine bark taken from the bottom, middle and top sections of logs of three age groups were analyzed for their extractive content. Successive extraction of the bark with ether, acetone, and water was performed. Further analysis showed that the hexane soluble extract contained both saturated and unsaturated fatty acids, resin acids, volatile oil, phytosterol, and saturated fatty alcohols. Also saturated fatty acids, saturated fatty alcohols, ethanol insoluble alcohols, and a phlobaphene-like material were found in the benzene soluble extract. The ether extract, after previous extraction with hexane and benzene, contained a crystalline coloring material, which was characterized as 3,3',4',5,8-pentahydroxyflavone. Phlobaphene and a catechol-type phlobatannin were found by extraction with ethanol. The water soluble constituents were found to contain: pentosans, mannan, glucosan, galactan, and uronic anhydride.

LITERATURE CITED

1. Adams, Maxwell. Composition of wood turpentine. The Journal of industrial and engineering chemistry 7:957-960. 1915.

2. American leather chemists association. Methods of sampling and analysis. Proposed methods, 1946. Section A. Cincinnati, Ohio, The Association, 1946. 19p.

3. Aronoff, S. Some structural interpretation of flavone spectra. Journal of organic chemistry 5:561-571. 1940.

4. Baker, Wilson and C. Evans. Derivatives of 1,2,3,4-tetrahydroxybenzene. Part 4. Attempted synthesis. Journal of the chemical society 1938:372-375. 1938.

5. Bates, Frederick J. Polarimetry and saccharimetry and the sugars. Washington, D.C., U.S. Govt. printing office, 1942. 810p. (U.S. Dept. of commerce. National bureau of standards. Circular C440)

6. Clarke, E.P. Semi-microdetermination of acetyl. Industrial and engineering chemistry. Analytical edition 8:487-488. 1936.

7. Clarke, I.D. and R.W. Frey. Determination of sugars in tanning materials. Journal of American leather chemists association 23:91-109. 1928.

8. Dickson, Allan D. A Method for the determination of uronic acids. The Journal of the American chemical society 52:775-778. 1930.

9. Freudenberg, K. and P. Maitland. Chemistry of quebracho tannin. Journal of the international society of leather trades chemists 18:156-159. 1934.

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

11. Hassid, W.Z. and R.M. McCready. Identification of sugars by microscopic appearance of crystalline osazones. Industrial and engineering chemistry. Analytical edition 14:683-686. 1942.

12. Hawk, Philip B., Bernard L. Oser, and William H. Summerson. Practical physiological chemistry. 12th ed. Philadelphia, Blakiston, 1947. 1323p.

13. Heiduschka, A. and J. Ripper. Uber Heptadecylsaure. Berichte der deutschen chemischen Gesellschaft 56:1736-1739. 1923.

14. Hubbard, James K. and Ervin F. Kurth. Douglas fir bark tannin. The Journal of the American leather chemists association 44:604-614. 1949.

15. Jamieson, George S. Vegetable fats and oils. 2d ed. New York, Reinhold, 1943. 508p.

16. Kurth, Ervin F. The Chemical composition of barks. Chemical reviews 40:33-49. 1947.

17. Lapworth, Arthur and Edward N. Mottram. Oxidation products of oleic acid. Journal of the chemical society 127:1639-1633. 1925.

18. Leighton, Philip A., R.W. Crary, and L.T. Schipp. The Ultraviolet light absorption of ethyl alcohol purified by different methods. Journal of the American chemical society 53:3017-3019. 1931.

19. Lepper, Henry A.(ed.). Official and tentative methods of analysis of the association of official agricultural chemists. 6th ed. Washington, The Association, 1945. 932p.

20. McLaughlin, George D and Edwin R. Theis. The Chemistry of leather manufacture. New York, Reinhold, 1945. 800p.

21. Niederl, Joseph B. Micromethods of quantitative organic analysis. 2d ed. New York, Wiley, 1948. 347p.

22. Ramachandra Row and T.R. Seshadri. Synthesis of 5,6-dihydroxyflavonols. II. 3,3',4',5,6-pentahydroxyflavone. Proceedings of the Indian academy of science 21A:130-133. 1945.

23. Randall, H.M. Infrared determination of organic structures. New York, Van Nostrand, 1949. 239p.

24. Schonberg, A. and A. Mustafa. Action of diazomethane on hydroxy-compounds and of diazomethane derivatives on phenanthraquinone. Journal of the chemical society 1946: 746-748. 1946.

25. Technical association of the pulp and paper industry. Standards and suggested methods. New York, The Association, September, 1946.

26. Underwood, H.W. Preparation of solid derivatives for the identification of ethers. Journal of the American chemical society. 52:4087-4092. 1930.

27. Warth, Albin H. The Chemistry and technology of waxes. New York, Reinhold, 1947. 519p.

28. Western pine association. Ponderosa pine, the pick o' the pines. Portland, Ore., Western pine association, 1949. 72p.

29. Wolff, H. and E. Scholze. Determination of colophony in boiled linseed and other oils and soaps. Chemiker Zeitung 38:369-370. 1914.

30. Zeisel, Simon. Uber ein Verfahren zum quantitativen Nachweise von Methoxyl. Monatshefte fur Chemie und verwandte Theile anderen Wissenschaften. 6:989-996. 1885.