THE CHEMICAL NATURE OF THE EXTRACTIVES FROM WHITE FIR BARK; *ABIES CONCOLOR* (ENGELM.) GORD.

by

HERBERT LAWRENCE HERGERT

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APPROVED:

Redacted for Privacy

Professor of Chemistry

In Charge of Major

Redacted for Privacy

Chairman of the Department of Chemistry

Redacted for Privacy

Chairman of School Graduate Committee

Redacted for Privacy

Dean of Graduate School

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INTRODUCTION

White fir, Abies concolor (Engelm.) Gord., is one of the important softwood species found growing in nine western states (47). Its principal use is in the production of paper pulp (22); smaller amounts are used for general building construction, crating, and as a raw material for hardboard. Although the western pine region contains over 66 billion board feet of white fir stumpage, the present annual cut is only about 500 million board feet (47). This is due, in part, to its inferior strength properties and to increased difficulty in drying the lumber, in comparison to Douglas-fir, for example. As useful species, such as Douglas-fir and ponderosa pine, decrease in availability white fir undoubtedly will become increasingly important in lumber production.

At present, white fir bark is either disposed of in refuse burners or, more rarely, used as fuel. A study of the chemical constituents of the bark and their possible industrial utilization would have a twofold objective: the utilization of a waste material and the establishment of an additional source of income

to the sawmill or the pulp mill operator. A survey of the literature showed that the only available information about this bark was a report on the oleoresin in the bark (2) and a report on the nature and amount of volatile oils present (44).

OBJECTIVES OF THE INVESTIGATION

The primary objective of this investigation was to study the chemical constituents of white fir bark extractives. Particular attention was devoted to the extractives which appeared to be of potential commercial importance, i.e. wax, flavanoids and tannin. An attempt was made to make use of some of the more recent experimental techniques. For this reason special emphasis was placed on the use of infrared spectroscopy since this technique holds promise of solving heretofore unsolved problems in wood chemistry. In addition to determining the spectra of some of the bark constituents, a series of carbonyl-containing compounds was analyzed to provide fundamental information on characteristic frequencies of this group in various types of compounds. It is believed that this information will be very useful in subsequent spectroscopic studies of wood and bark lignin, tannin, and phlobaphenes.

EXPERIMENTAL

Raw Material

An anatomical examination of white fir bark shows it to consist of several distinct components (see Figure 1). The inner bark is light brown to reddish brown in color. It varies from ¼ to 1½ inches in thickness and is composed of sieve cells, phloem parenchyma, and bast fibers. The outer bark varies from ½ to 4 inches in thickness and consists of salmon-colored, corky layers interspersed with areas of dark red phloem tissue.

In order to determine the distribution of the extractives, a series of bark samples was taken separately from the bottom, middle, and top of trees of different ages. The collection data are shown in Table I. The samples were obtained from a mixed stand approximately 12 miles northeast of Fort Klamath, Oregon, in July, 1950. The trees were felled two days prior to sampling. Bark samples were also obtained from the Lakeview Lumber Company at Lakeview, Oregon, and the Ivory Pine Lumber Company at Dinuba, California.

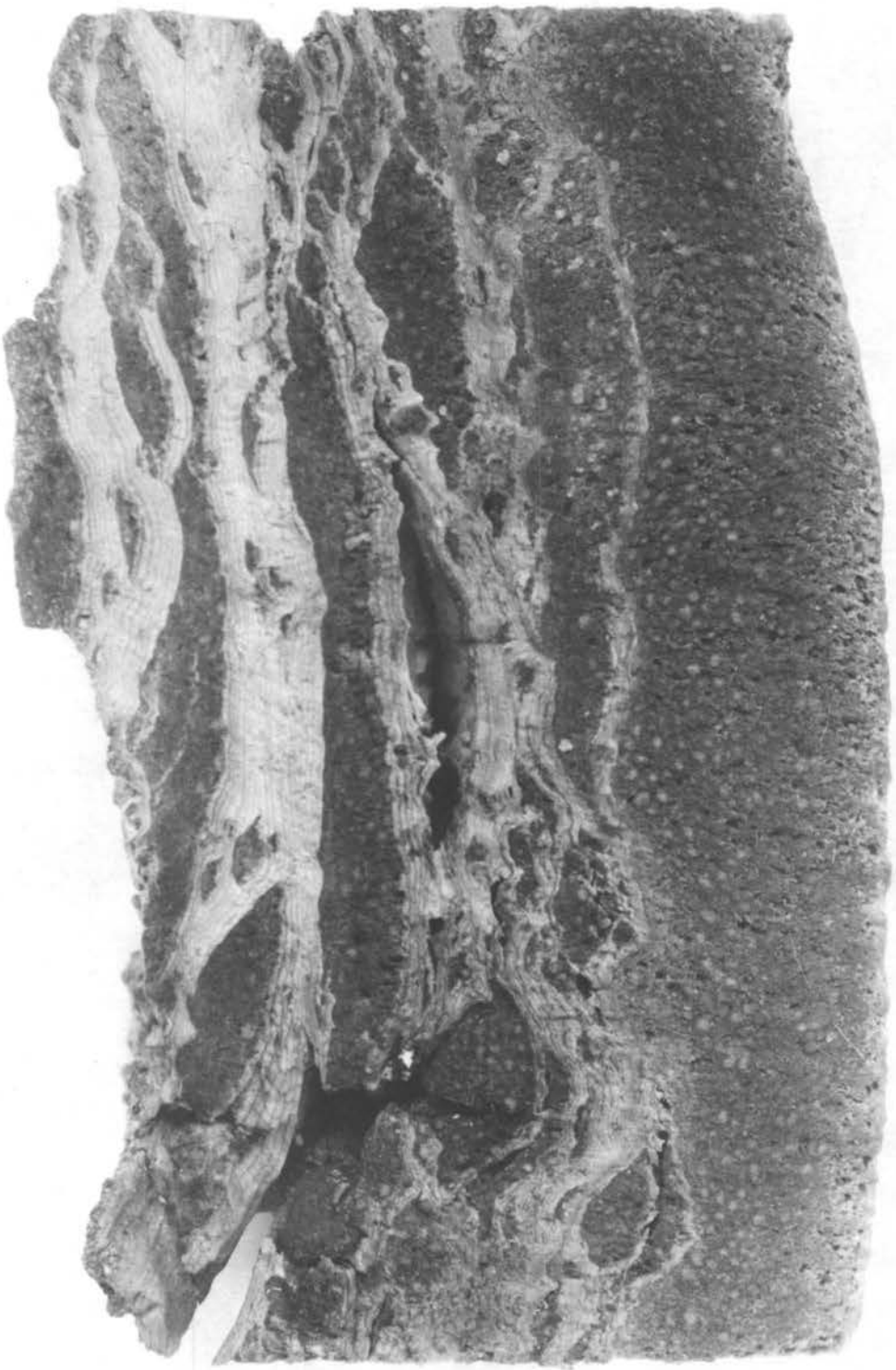


Figure 1. Cross section of white fir bark from the butt of a 150-year-old tree

Table I. Collection Data on White Fir Bark Samples

Age group no.	Average age, yr.	Tree age, yr.	Bottom section, dia. of bark, in. Outside	Inside	Middle section, dia. of bark, in. Outside	Inside	Top section, dia. of bark, in. Outside	Inside
			(2.0 to 3.5 ft. high)		(20 to 30 ft. high)		(40 to 60 ft. high)	
I	116	123	30	27	22.5	21	16	15
		105	29	26	22.0	20	16	15
		120	27.5	24	20.5	19	15	14
			(2.0 to 3.5 ft. high)		(40 to 50 ft. high)		(80 to 90 ft. high)	
II	152	167	40.0	37	23.5	22	16	15
		146	38.0	35	22.5	21	15	14
		143	34.5	31.5	19	18	16	15
			(2.C to 3.5 ft. high)		(50 to 55 ft. high)		(100 to 110 ft. high)	
III	209	226	43	39	31.5	29	20	19
		194	37.5	34	28	26	18	17

Preparation of the Samples

Within 24 hours of the bark collection, it was reduced in a Mitts and Merril hog to pass a 5-mesh screen. The hogged bark was air-dried to a moisture content of approximately 10% and then further reduced in size in a Rietz disintegrator to pass a 20-mesh screen. Equal parts of the bark from each section of each tree were mixed, giving nine composite samples representing the bottom, middle, and top sections of trees from three different age groups. After thorough air-drying, the samples were stored in glass jars.

A method for the separation of the cork and the bark phloem was developed in order to determine the extractive content of these components. Pure analytical samples of cork and phloem could be obtained by splitting off the inner bark by hand and then purifying the cork by a previously described, alternate grinding and screening process (20). This procedure is slow and would be impractical from an industrial standpoint.

On a large scale, it was found that reasonably good separation could be achieved by first air-drying the bark to about 15% moisture content and then passing it through a hog equipped with a ½-inch screen. This material was screened through a 20- and a 100- mesh sieve. The fraction retained on the 20-mesh sieve

represented about 40% of the bark by weight and contained 90% cork. The fraction retained on the 100-mesh screen represented about 55% of the bark by weight and consisted of bark phloem admixed with about 20% cork. The fraction passing through the 100-mesh sieve was composed of broken phloem, cork, etc., and represented about 5 to 10% of the bark.

Distribution of the Extractives

The bark samples were successively extracted with hexane, benzene, diethyl ether, hot water, and ethanol. The nature of the material removed by each of these solvents is shown in Table II.

Table II. Nature of White Fir Bark Extractives

Solvent	Extractives
Hexane	Light brown wax
Benzene	Dark reddish-brown wax
Ethyl ether	d-Catechin and l-epicatechin
Hot water	Tannin and carbohydrates
Ethanol	Phlobaphene

Thirty-five grams of each of the nine samples, with known moisture content, were placed in cloth bags and extracted successively with hexane, benzene, and ether in borosilicate glass, Soxhlet extractors. Each extractive was then dried to constant weight in a vacuum oven at 55°C. The hot-water solubility was determined by extracting the solvent-extracted residues in Erlenmeyer flasks with four successive 500-ml. portions of hot water over a period of 4 hr. A 250-ml. aliquot of the aqueous extract was evaporated to dryness and weighed. The hot-water extracted bark was then air-dried and subsequently extracted with ethanol in a Soxhlet extractor. All determinations were made in duplicate.

The distribution of the extractives in the nine composite samples is shown in Table III. Although the total extractive content does not markedly vary between the samples, there are significant differences among the individual extractives. In young trees, the hot-water solubility is considerably higher in samples obtained from the tops. The sum of the hexane, benzene, and ether extractives increases with increasing age of the tree. This extractive distribution pattern is similar to that in Douglas-fir bark (33).

The distribution of the extractives among the various bark fractions is shown in Table IV. These fractions

Table IV. Percentage Extractives from White Fir
Values Based on Oven-Dry Weight of Material

Solvent	Wood	Inner bark	Outer bark phloem	Cork
Hexane	0.20	0.53	1.23	3.46
Benzene	0.03	0.19	0.52	1.93
Ethyl ether	0.12	0.07	1.28	8.98
Hot water	2.11	12.74	10.47	7.69
Ethanol	3.12	0.98	1.90	3.89
Sum of five extractives	5.58	14.51	15.40	25.95

were obtained from the bottom bark of trees in age group III. Comparison is made with extractives from a sample of white fir wood obtained from the bottom of trees of approximately the same age. The bark cork fraction was found to contain the greatest amounts of hexane, benzene,

Table III. Percentage Extractives from White Fir Bark
Values Based on Oven-Dry Weight of Material

Age group	Position on tree	Solvent					Sum of five extractives
		Hexane	Benzene	Ether	Hot water	Ethanol	
I	Top	2.14	0.78	0.41	14.45	0.67	18.45
	Middle	2.44	0.92	1.02	13.54	1.95	19.87
	Bottom	2.91	1.02	1.63	9.85	2.84	18.25
II	Top	2.10	1.03	0.93	15.67	0.98	20.71
	Middle	1.30	0.70	0.59	12.83	1.65	17.07
	Bottom	2.44	0.69	1.94	15.14	2.70	22.91
III	Top	2.46	0.89	2.79	11.43	2.31	19.88
	Middle	2.61	1.00	3.42	10.98	2.09	20.10
	Bottom	2.72	1.10	3.80	10.61	3.31	21.54

and ether-soluble components. The yield of the extractives from cork obtained from the barks of trees of different ages is shown in Table V. The percentage

Table V. Percentage Extractive from White Fir Cork Values Based on Oven-Dry Weight of Material

Solvent	Average age of trees 152	209	241a
Hexane	4.03	3.46	4.50
Benzene	2.03	1.93	2.07
Ether	5.93	8.98	16.62
Hot water	7.64	7.69	6.86
Ethanol	3.13	3.89	7.21
Sum of five extractives	22.76	25.95	37.26

[a]Bark from Ivory Pine Lumber Co.

of hexane, benzene, and hot water-soluble extractives shows small variation. The percentage of ether-extractive and the alcohol-extractive, however, markedly increases with age of the tree. Similar behavior was noted in the case of Douglas-fir cork (20).

Constituents of the Extractives

Hexane Extract

This extract is a moderately hard wax, ranging in color from light brown to a cream. It was obtained by direct extraction of the whole bark or bark fractions with Skellysolve B, a petroleum fraction with a boiling point of 60 to 70°C. The extract was evaporated to dryness and then steam distilled to remove volatile oils. The volatile oils were not further examined, since they have been previously characterized (44) and the yields were relatively small (usually less than 0.2% by weight of the bark). The yields and properties of this wax are shown in Table VI.

Table VI. Properties of Hexane-Soluble Wax from White Fir Bark

Bark fraction.....	Whole bark	Cork	Cork-free bark
Color.....	Yellow-brown	Cream	Brown
M.p., °C.[a].....	52-53	56-57	47-48
Yield (O.D.basis),%..	2-3	3-6	0.5-1.5
Unsaponifiables, %...	37.8	39	30.6
Acid number.....	61	53	74
Saponification number	113	107	136

[a] Fisher-Johns melting point block.

The chemical constituents from a wax sample representative of the whole bark were separated and isolated by the procedure shown in Figure 2. Fifty grams of wax

were dissolved in 150 ml. of hot acetone, and the solution was cooled to 35°C. A white precipitate of uncombined, fatty alcohols, I, was rapidly filtered off with suction, and the filtrate evaporated to dryness. This residue was dissolved in ether and extracted with 5% potassium carbonate in order to remove free acids, II. The neutral fraction, which remained in the ether layer, was evaporated to dryness and then saponified for 2 hours with 300 ml. of 2 N alcoholic potassium hydroxide. The alcohol was removed by boiling, replaced with water, and the resultant aqueous solution extracted with ether to remove the unsaponifiables, III. The aqueous soap solution was acidified with hydrochloric acid and extracted with ether to remove the combined acids. No constituents were found in the residual aqueous layer. The yields of the various constituents are shown in Table VII.

Uncombined alcohol, I. The white, crystalline precipitate, I, was purified by several recrystallizations from hot acetone. The resulting, white, crystalline solid melted at 68 to 69°C., gave no test for unsaturation and a negative Liebermann-Burchard test for sterols. An acetate derivative, melting point 51 to 52°C., was prepared with pyridine and acetic anhydride. These properties suggested

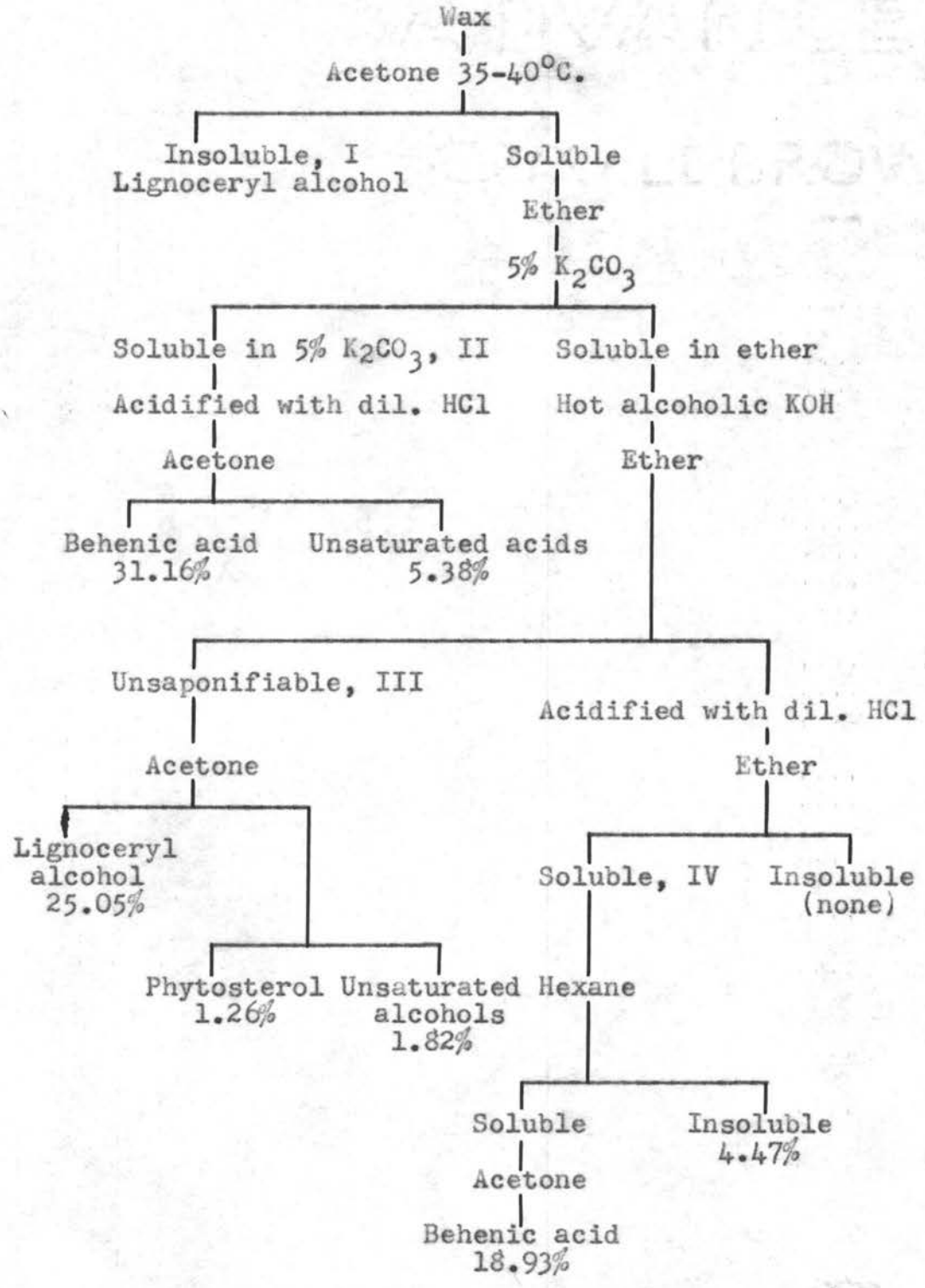


Figure 2. Separation of components of hexane-soluble wax
Percentages based on oven-dry weight of wax.

Table VII. Percentage Constituents of Hexane-Soluble Wax from White Fir Bark
Values Based on Oven-Dry Weight of Wax

	Whole bark	Cork	Cork-free bark
Lignoceryl alcohol	34.7	36.7	27.5
Sterol and unsaturated alcohols	3.1	1.8	5.1
Free acids	36.5	32.3	42.5
Behenic acid	31.2	30.0	30.3
Unsaturated acids	5.3	2.3	12.2
Combined acids	23.5	26.8	20.6
Behenic acid	19.0	24.4	12.4
Hexane-insoluble acids	4.5	2.4	8.2
Loss (by difference)	2.2	2.4	4.3

that the material was a saturated, aliphatic alcohol, a conclusion that was verified by oxidizing the alcohol to the corresponding acid by a potash fusion (42). Extraction of the alkaline fusion mixture with ether yielded only traces of ether-soluble material, indicating the absence of hydrocarbons in this fraction. The fusion mixture was acidified with hydrochloric acid, and the liberated acids recrystallized from acetone. They melted at 71 to 72°C. and had a neutral equivalent of 364. These properties correspond to those of a mixture of lignoceric acid and near homologs, indicating the original alcohol to be lignoceryl alcohol. A mixed melting point with the lignoceryl alcohol from Douglas-fir bark hexane wax (30) was undepressed.

Free acids, II. The potassium soaps of the free acids were acidified with hydrochloric acid. The

liberated free acids were filtered off, dried, and then recrystallized three times from acetone. A white, microcrystalline product was obtained with a melting point 70 to 71°C., and neutral equivalent of 346. A negative test for unsaturation was obtained with bromine water, and also with potassium permanganate solution. These properties suggest that the product was a mixture of behenic acid and near homologs.

The filtrates from the acetone recrystallizations were combined and evaporated to dryness. The residue comprised a small amount of a sticky, semiliquid mixture of acids that gave positive tests for unsaturated acids, but a negative Liebermann-Storch test for resin acids. Since oxidation with cold alkaline permanganate (34) yielded no crystallizable products, this fraction was not further characterized.

Unsaponifiable, III. The neutral fraction remaining after the separation of free alcohols and acids was a cream-colored wax with a melting point of 54°C. It was saponified for 3 hours with 2 N alcoholic potassium hydroxide. The alcohol was replaced with water, and the aqueous soap solution was extracted with ether in a separatory funnel in order to remove the unsaponifiable fraction. The ether extract was washed with 5% sodium carbonate solution and water. After drying over sodium sulphate, the solution was evaporated and

yielded a cream-colored residue that was separated into three components by fractional crystallization from hot acetone.

The first, and largest, fraction, obtained by solution of the residue in a large volume of hot acetone and recrystallization at room temperature, was a white, crystalline, saturated alcohol with a melting point of 68.5 to 69.5°C. (cor.). A mixed melting point with the free wax alcohol was undepressed. Oxidative fusion with potash yielded a crystalline acid with a melting point of 71 to 72°C. and a neutral equivalent of 365. These properties indicated that the alcohol belonged to the lignoceryl series and that it was identical with the free wax alcohol.

When the filtrates from the preceding crystallization were concentrated to a small volume and cooled in an icebox, long, needle-shaped crystals were formed, that had a melting point of 134 to 135°C. and gave a positive Liebermann-Burchard test for sterols. A mixed melting point with Douglas-fir wood phytosterol (19) was undepressed. The filtrate from the sterol separation was evaporated to dryness and yielded a yellow-orange sticky residue that gave a positive test for unsaturation. It was not further characterized.

Combined acids, IV. The aqueous soap solution remaining after the separation of the unsaponifiables

was acidified with hydrochloric acid and extracted with ether. The ether extract of liberated acids was washed with water and then dried over sodium sulphate. The solvent was removed by evaporation, and the acids were crystallized from hot hexane and then from acetone. A white, crystalline acid was obtained, which had a melting point of 70.5 to 71.5°C., a neutral equivalent of 344, and gave no test for unsaturation. A mixed melting point with the crystalline free acid was undepressed, indicating the two acids to be identical, and to consist essentially of behenic acid. The filtrates from the preceding crystallizations, after being evaporated to dryness, yielded a dark-colored, sticky residue, that had a melting point of about 40°C., and was not crystallizable from any of several solvents. This fraction was not further characterized.

Benzene Extract

This fraction, a hard, reddish-brown wax that softened at 70 to 72°C., was obtained by extracting the hexane-extracted bark residue with benzene. It was very soluble in benzene, dioxane, and ethyl acetate; moderately so in acetone, methanol, and ethanol; and sparingly soluble in ether. It could not be crystallized nor separated into simpler components by the use of any

of these solvents. The wax was insoluble in either cold sodium bicarbonate, sodium carbonate, or sodium hydroxide solutions, indicating the absence of free acids or acidic groupings.

Preliminary investigation showed the wax to be an ester of aliphatic acids, aliphatic hydroxy acids, and a phenolic acid, similar to the hexane insoluble wax in Douglas-fir bark (20). The wax was separated into simpler components by saponification, following the scheme shown in Figure 3. Forty grams of wax were saponified for 3 hours in 300 ml. of 40% ethanol containing 25 grams of potassium hydroxide. Saponification was carried out in this medium because the phenolic acid formed a potassium salt that was insoluble in alcohol but soluble in water. The dilute alcohol solution, therefore, permitted saponification to take place in a homogeneous system.

After saponification, the mixture was boiled to remove ethanol, and the aqueous solution extracted with ether to remove the unsaponifiable fraction. The aqueous soap solution was acidified with hydrochloric acid. The resultant precipitate was filtered off and washed with water to remove mineral acid. After thorough air-drying, the precipitate was placed in a paper thimble and successively extracted with hexane, benzene, and absolute ether in a Soxhlet extractor.

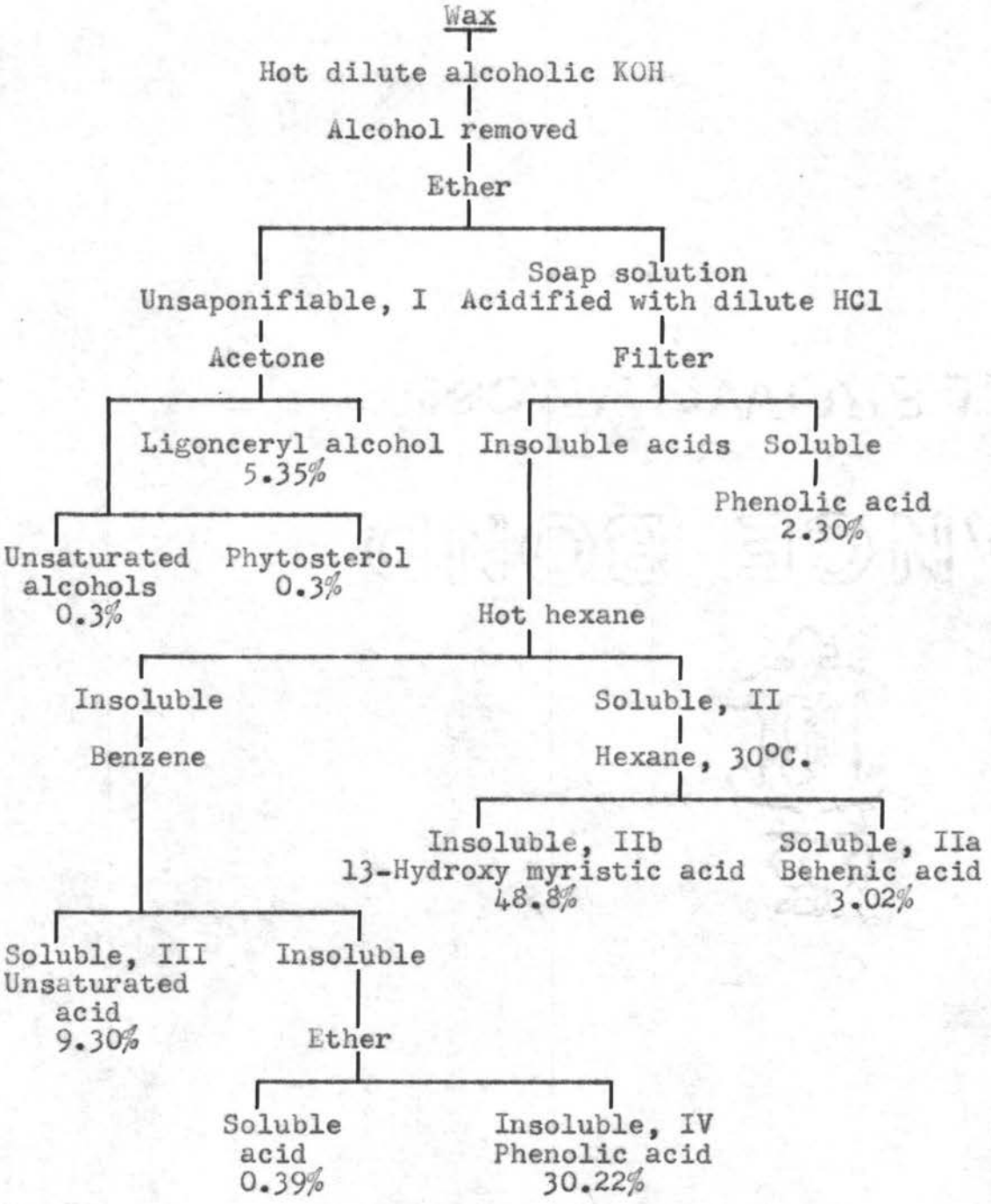


Figure 3. Separation of components of hexane-insoluble wax
Percentages based on oven-dry weight of wax.

The mother liquor and the washings from the acid precipitation were combined, neutralized, and evaporated to dryness *in vacuo*. A small amount of ether-insoluble phenolic acid was recovered in this way. Neither glycerol nor any other organic compounds were detected in this residue.

Unsaponifiables, I. The unsaponifiable fraction was recrystallized several times from acetone. A white crystalline alcohol, melting point 68 to 69°C., was obtained which, upon oxidative fusion with KOH, yielded an aliphatic acid with a melting point of 69 to 71°C. and a neutral equivalent of 368, indicating that the original alcohol was lignoceryl alcohol. A mixed melting point with the lignoceryl alcohol from the hexane wax was undepressed. Evaporation of the filtrates from the recrystallizations yielded small amounts of a phytosterol, melting point 134 to 135°C., and unsaturated materials that were similar to those isolated from the hexane-soluble wax.

Hexane-soluble acids, II. This fraction consisted of a saturated aliphatic acid and a hydroxy aliphatic acid. Their separation was based on the relative insolubility of the hydroxy acid in hexane at 25°C. and the relative insolubility of the saturated acid in acetone at 25°C. The mixture (20 grams) was dissolved in 750 ml. of hot hexane, and the solution cooled to

room temperature. The precipitated hydroxy acid, IIb, was filtered off and washed with hexane. The mother liquor and washings were combined and evaporated to dryness. The residue was dissolved in hot acetone, a white precipitate was obtained upon cooling. After several recrystallizations from acetone, the acid, IIa, melted at 70 to 71°C. and had a neutral equivalent of 346. The melting point was undepressed upon admixture with the behenic acid from the hexane-soluble wax.

The hydroxy acid, IIb, was purified by resaponification in alcoholic potassium hydroxide and subsequent acidification. After recrystallization from hexane, it melted at 81 to 82°C. (cor.) and had a neutral equivalent of 246.0. Contact with heat or mineral acids caused etholide or lactone formation with a consequent lowering of the melting point and an increase in the neutral equivalent value.

The hydroxy acid was methylated with dimethyl sulphate and potassium hydroxide in 50% alcoholic solution. A large excess of dimethyl sulphate was used and the solution kept basic. The methylated acid was refluxed in alcoholic potassium hydroxide to decompose any methyl ester present, acidified, and recrystallized from hexane. It melted at 67 to 68°C., had a neutral equivalent of 259.5, and contained 11.9% methoxyl. These properties corresponded to a mono-

methoxy myristic acid, $C_{15}H_{30}O_3$, with a calculated neutral equivalent of 258.3 and a methoxyl content of 12.0%, indicating that the original material was a mono-hydroxy myristic acid.

Fusion of the hydroxy acid with potassium hydroxide at 325°C. for 30 min. produced a small yield of a di-carboxylic acid, that could be separated from unchanged hydroxy acid by its insolubility in hexane. It had a melting point of 111°C., a neutral equivalent of 119, and a molecular weight of 240 by the Rast method. These properties indicated that the hydroxyl group was attached to the 13 carbon atom and that the original acid was 13-hydroxy myristic acid. A literature search showed this to be the first reported isolation of this acid from natural or synthetic sources. Previously prepared mono-hydroxy myristic acids are the 2-, 3-, 11-, and 14-hydroxy acids (46), all of which have been obtained synthetically.

Benzene-soluble acids, III. The benzene-soluble (hexane-insoluble) fraction was purified by resaponification and acidification. A mixture of light-brown sticky acids, melting point 35 to 45°C., was obtained that could not be crystallized from a variety of solvents. The ether-soluble acid fraction also was a dark sticky mixture. Neither fraction was further characterized.

Ether-insoluble acid, IV. This fraction was purified by dissolving in dioxane and precipitating in water. It was then redissolved in a minimum amount of dioxane and precipitated in absolute ether. The precipitate, a brown powder, was soluble in ethanol, methanol, dioxane, aqueous sodium hydroxide or sodium carbonate, sparingly soluble in acetone, only slightly soluble in sodium bicarbonate, and insoluble in ether, benzene, or hexane. An alcoholic solution gave a greenish-brown color with 1% ferric chloride solution.

The infrared spectra of this fraction and its methylated derivative (prepared by treatment with diazomethane) are shown in Figure 4. A model 12-c Perkin-Elmer spectrometer, fitted with a Brown Recorder and adapted to automatic double beam operation by the method of Savitsky and Halford (20), was used to obtain the spectra. The samples were mulled in Nujol or perfluorokerosene and run against a salt plate as a blank. Table VIII shows the tentative absorption band assignments and a comparison with the tannin, phobaphene, and methylated phlobaphene fractions from white fir bark.

Examination of the spectra indicates the presence of a carbonyl group which from its position (1703 cm.-1) could only be an aliphatic aldehyde, ketone, or carboxyl group. The shift to higher frequency upon methylation

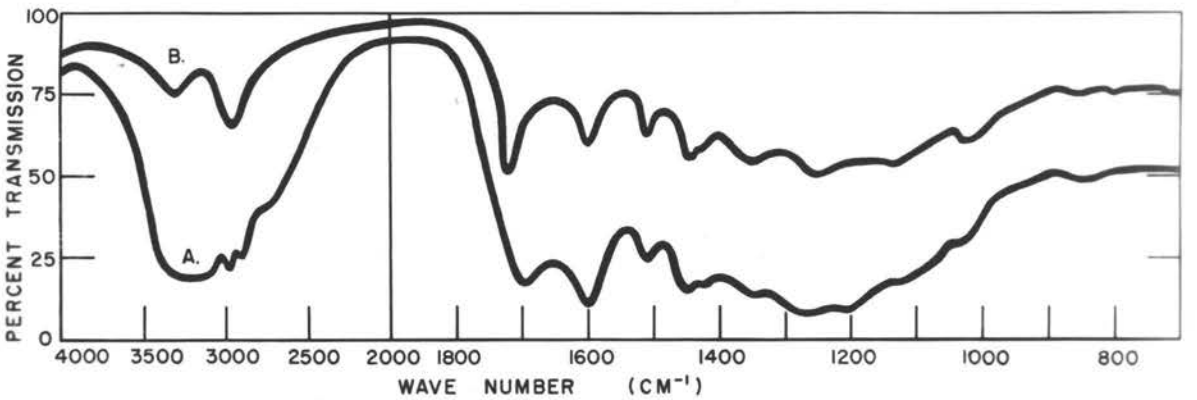


Figure 4. Infrared spectra of A--ether insoluble acid IV, B--IV methylated with diazomethane

Table VIII. Infrared Absorption Bands of White Fir Tannin, Phlobaphene, and Wax Phenolic Acid

Assignment	Wave number ($cm.^{-1}$) Tannin	Phlobaphene	Methylated phlobaphene	Phenolic acid	Methylated phenolic acid
Hydroxyl	3300	3320	3490	3230	3390
CH_2 or CH_3	2925	2925	2950	2950, 2890	2950
Carbonyl	....	1701	1718	1696	1720
Phenyl rings	1603	1608	1599	1602	1598
	1514	1513	1513	1512	1512
--C--H--	1451	1447	1458	1450	1455
	1352	1350	1422	1420	1355
	....	....	1355	1350	....
Unassigned	1283	1275	1263	1270	1260
	1195	1200	1199	1200	....
	1102	....	1122	1125	1144
	980	1036	1025	1035	1027
	860	860	861	860	860
	808	792	800	....	790
	724	....	748	....	....
	....	....	720	....	....

indicates that this band is due to a carboxyl group, since methyl esters show a shift of 10 to 30 cm.$^{-1}$ from the unsubstituted carbonyl position. A close similarity to the spectra of phlobaphene (Figure 8) was observed. This band was much more intense than the corresponding band in the phlobaphene, however, and a higher percentage of carboxyl in the phenolic acid is indicated.

Ether Extract

One-kg. samples of the various bark fractions were extracted with benzene in a large, borosilicate glass, Soxhlet-type extractor. The benzene-extracted bark was then extracted with diethyl ether (U.S.P. grade containing 2.5 percent ethanol as an impurity) for 24 hours. The ether extract was evaporated to dryness and weighed. The yield of crude materials thus obtained are reported in Table IX.

The ether-soluble extractive was found to consist of three fractions: a water-insoluble red colored material, a water-soluble white crystalline substance, and a water-soluble red colored substance. These materials were separated by suspending the vacuum-dried ether extract in a 5 fold excess of hot water (85°C.). The insoluble material was filtered off, dried, and weighed. The filtrate was then placed in icebox for several days until crystallization occurred.

Table IX. Yield of Crude, Ether-soluble Extractive from White Fir Bark

Source	Age of tree[a]	Yield,[b] %
White fir wood	145	0.12
Whole bark	209	3.80
Inner bark	209	0.07
Outer bark[c]	209	1.28
Cork	209	8.98
Cork	152	5.93
Cork	241	16.62

[a] Average age of three tree samples. Samples obtained 3' from base of tree. [b] Percentage of oven-dry weight of material. [c] Excluding cork.

The crude crystals were filtered off by suction, dried and weighed. The filtrate was evaporated to dryness and weighed. The yields of these fractions obtained from a sample of cork (from trees of an average age of 209 years) and whole bark (a sample composed of nine equal portions by weight of bark from the top, middle, and bottom of trees of three different age groups) are shown in Table X.

Table X. Percentage Composition of the Ether Extract from White Fir Bark
Values based on oven-dried weight of material

	Whole bark	Cork
Water-soluble coloring matter	38.93	12.01
Water-insoluble coloring matter	12.39	11.41
Crude crystalline material	48.68	76.58

The water-soluble coloring matter gave a green coloration when treated with one percent ferric chloride solution. It was adsorbed on hide powder and when boiled with mineral acid, a flocculent red precipitate was formed. This suggested that the material was a phlobatannin. The water-insoluble coloring matter when dissolved in alcohol also gave a green-color with ferric chloride solution. Both of these materials were practically insoluble in ether and when anhydrous, alcohol-free ether was used in the extraction, these constituents were not removed from the bark. The crystalline material was also much less soluble in alcohol-free ether, from 5 to 6 times longer extraction times being required to obtain amounts equivalent to those obtained with U. S. P. ether.

d-Catechin. The crude crystalline material was recrystallized several times from hot water and then decolorized with a little charcoal. The white crystals thus obtained were recrystallized from a dilute aqueous solution (1 gram in 40 cc. water). After filtration, they were air-dried for 24 hours and then dried for 3 days over P_2O_5 at 105° in vacuo. The crystals sintered at 150°C. and melted at 176-177° (corrected) C. The specific rotation in 1:1 acetone-water was + 16.6°. An absolute alcohol solution did not show any rotation.

The crystalline matter was very soluble in hot water, ethanol, acetone, ethyl acetate, or methanol. It was insoluble in benzene and all hydrocarbon or chlorinated solvents less polar than ether.

The white crystalline compound gave a negative Wilson boro-citric acid test for flavones. No color was developed upon treatment with zinc or magnesium in alcoholic hydrochloric acid indicating that the compound was not a flavanone. An intense greenish coloration was obtained with 1% ferric chloride solution and an insoluble white precipitate with basic lead acetate indicating the presence of a catechol grouping. A reddish-purple color was imparted to a pine wood splint in 12% hydrochloric acid. This indicated a phloroglucinol nucleus. When the compound was treated with strong mineral acid, it decomposed forming a red amorphous precipitate. These tests indicated that the compound was a polyhydroxy flavan.

The melting point of the crystalline compound was undepressed when mixed with an authentic sample of d-catechin, $C_{15}H_{14}O_6$, from Uncaria gambir (furnished by the S. B. Penick Company and further purified by repeated recrystallization from water). The infrared spectrum of the crystalline matter was compared with that of d-catechin and found to be identical (see Figure 5). The spectra

were obtained as mineral oil (Nujol) mulls. Two salt plates containing a layer of Nujol (equivalent to the amount in the sample) serving as a blank. The spectra in Figures 5 and 6, therefore, represent only bands due to the sample.

Acetylation of the compound with pyridine-acetic anhydride (38) for 24 hours at room temperature gave colorless prisms from acetone-alcohol, m.p. 132-133°C., $(\alpha)_D^{25}$ + 39.1° (chloroform, C.4.32). Freudenberg (9) reported a m.p. of 131 to 132°C. and $(\alpha)_{5780}$ + 40.6° for d-catechin. The infrared spectrum is shown in Figure 6.

Analysis of the acetate derivative:

Calculated for $C_{15}H_9O_6(CH_3CO)_5$: CH_3CO 43.0.

Found: CH_3CO 42.7.

When the compound was dissolved in boiling water to form a saturated solution the crystals obtained upon cooling melted at 219°C. after drying in vacuo for 5 days over P_2O_5 at 105°C. The specific rotation was + 16.4° in 1:1 acetone-water. This higher melting form did not sinter at 145 to 155°C., in contrast to the low-melting form. Acetylation of this high melting form with acetic anhydride-pyridine gave white crystals, m.p. 132 to 133°C. This melting point was undepressed by admixture with the pentaacetate of the lower-melting form.

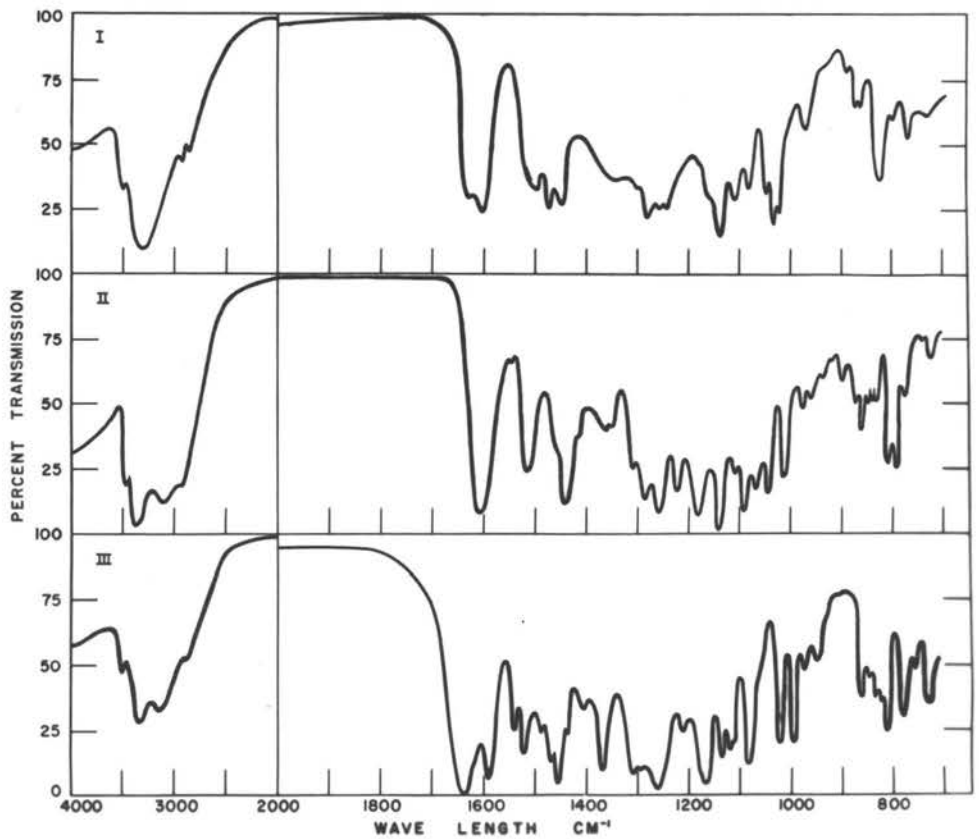


Figure 5. Infrared Spectra (solid mulls) of (I) d-Catechin; (II) l-Epicatechin; (III) Dihydroquercetin

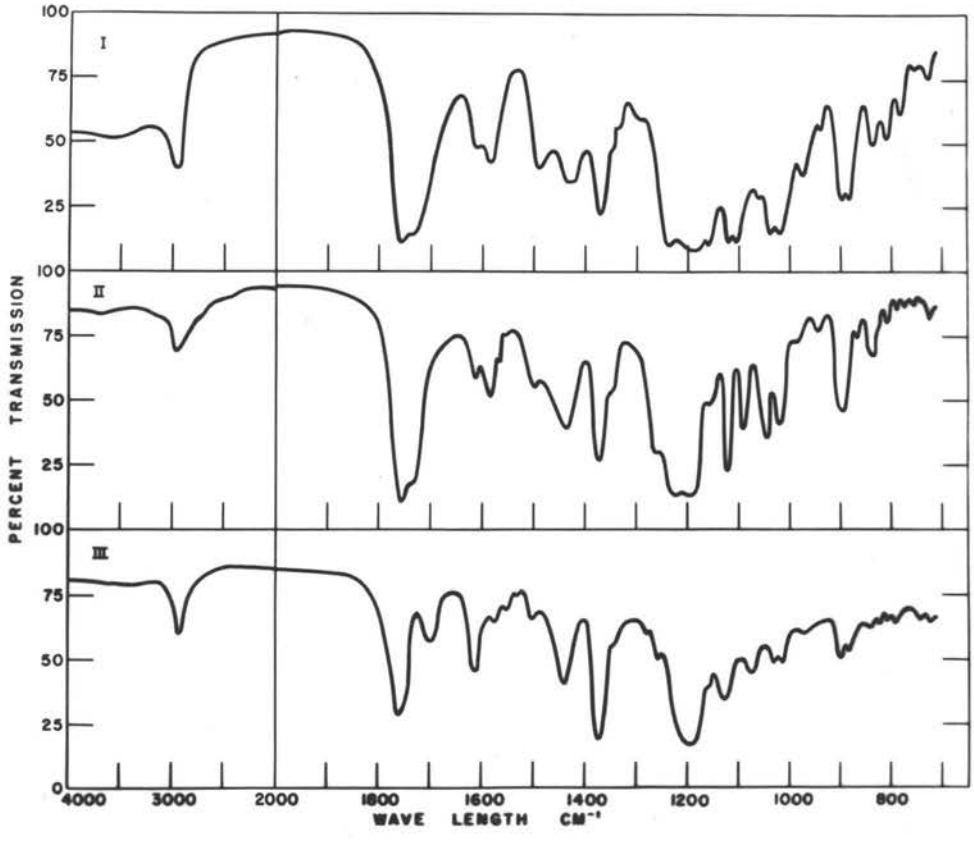


Figure 6. Infrared Spectra (solid mulls) of (I) d-Catechin Pentaacetate; (II) l-Epicatechin Pentaacetate; (III) d-Dihydroquercetin pentaacetate

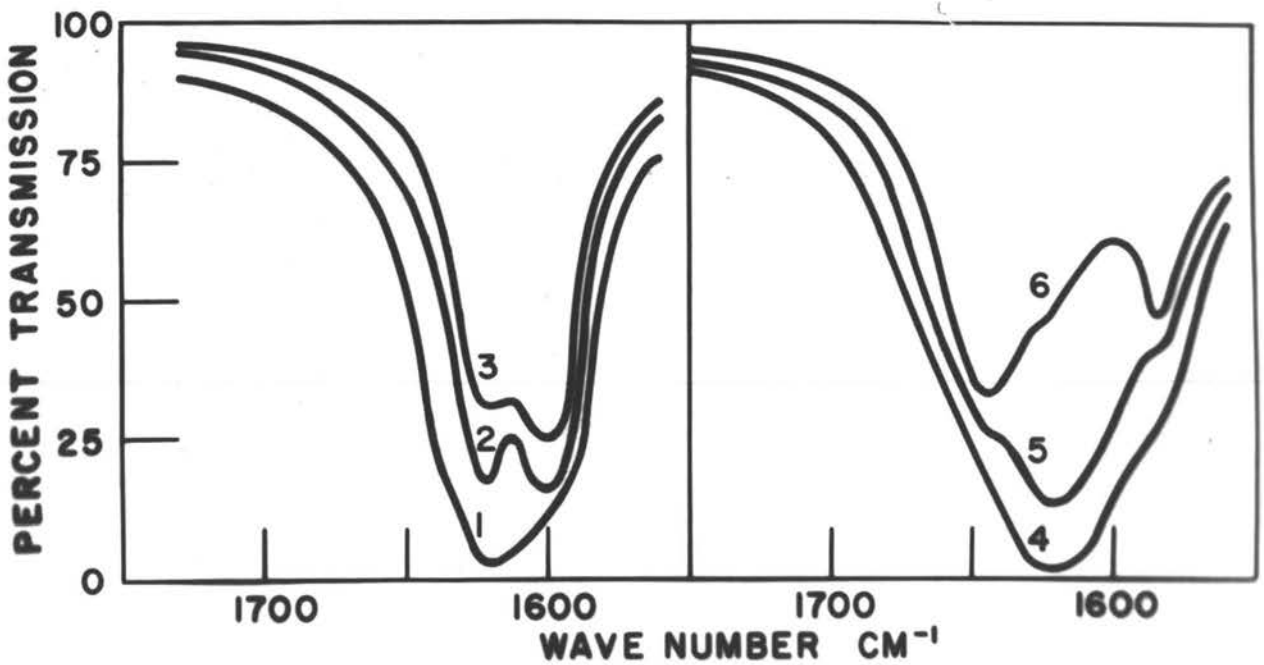


Figure 7. Infrared Spectra of (1) d-Catechin, air-dried; (2) d-Catechin, vacuum dried over P_2O_5 at 56°C; (3) d-catechin vacuum-dried over P_2O_5 at 110°C. for seven days; (4) d-dihydroquercetin, air-dried; (5) d-dihydroquercetin, vacuum-dried over P_2O_5 at 56°C.; (6) d-dihydroquercetin, vacuum-dried over P_2O_5 at 110°C. All samples are solids mulled in Nujol.

The x-ray diffraction patterns of the high and low-melting forms of d-catechin were compared. Powder diffraction patterns were obtained by using a North American-Phillips x-ray spectrometer with a Geiger-tube detector. Cu radiation with a Ni filter was used as a source. The samples were ground to pass through a 300-mesh screen. They were then pressed into a depression in an aluminum plate to form a layer about 1/8" thick, 1/2" wide, and 3/4" long. The interplanar spacings and intensities of catechin are shown in Table XI. The intensities are expressed as a percentage of the intensity of the strongest line of each pattern.

Many discrepancies appear in the literature concerning the melting point of d-catechin. Zwenger (48) reported the m.p. of gambir catechin (d-catechin) to be 217°C., while Gautier (15) reported the isolation of three gambir catechins melting at 163°C., 176 to 177°C., and 204 to 205°C. Perkin (39) indicated that gambir catechin sintered at 145 to 150°C. and then melted at 175 to 177°C. Clauser (5) stated that catechin normally melted at 96°C., but if dried over sulfuric acid it then melted at 176°C.; further drying at 100°C. raised the m.p. to 210°C. Perkin (38) later stated that he was unable to obtain a m.p. higher than 177°C. This was confirmed by Freudenberg (10), whose work suggests (37) that Clauser's

Table XI. Interplanar Spacings and Intensities of Catechin

l-epicatechin pentaacetate	l-epicatechin	d-catechin (low melting)	d-catechin (high melting)
16.1 (100)	9.60 (3)	11.47 (29)	11.47 (27)
12.6 (28)	6.28 (13)	10.77 (12)	9.90 (4)
10.8 (10)	6.02 (20)	9.60 (41)	7.89 (13)
10.0 (11)	5.50 (29)	8.11 (15)	7.49 (8)
9.50 (18)	4.90 (100)	7.37 (15)	5.79 (100)
8.50 (17)	4.57 (25)	6.32 (59)	5.01 (5)
7.49 (7)	4.23 (7)	5.79 (88)	4.60 (19)
6.60 (13)	3.86 (27)	5.37 (100)	4.44 (28)
6.28 (19)	3.74 (32)	5.01 (29)	4.11 (64)
5.71 (13)	3.56 (70)	4.53 (59)	3.97 (60)
5.43 (18)	3.35 (3)	4.31 (37)	3.80 (45)
5.18 (15)	3.25 (7)	4.11 (71)	3.66 (39)
5.04 (10)	3.14 (38)	4.02 (49)	3.60 (35)
4.90 (15)	3.07 (38)	3.83 (39)	3.46 (44)
4.74 (12)	2.98 (4)	3.70 (34)	3.34 (19)
4.53 (15)	2.74 (4)	3.57 (34)	3.23 (11)
4.31 (21)	2.68 (9)	3.42 (71)	3.08 (13)
4.21 (27)	2.62 (2)	3.23 (22)	2.96 (8)
3.98 (16)	2.57 (3)	3.09 (24)	2.70 (8)
3.81 (13)	2.43 (3)	2.95 (7)	2.48 (7)
3.62 (33)	2.34 (3)	2.78 (5)	2.43 (7)
3.40 (12)	2.21 (5)	2.69 (5)	2.34 (9)
	2.14 (3)	2.50 (12)	2.10 (3)
	2.07 (6)	2.42 (5)	2.07 (8)
	2.01 (2)	2.38 (7)	2.00 (4)
	1.93 (6)	2.30 (5)	1.81 (4)
	1.86 (1)	2.27 (5)	1.76 (3)
	1.79 (2)	2.22 (12)	
	1.71 (3)	2.10 (5)	
		2.07 (5)	
		2.00 (5)	

high m.p. was due to racemization during drying at high temperature. Recently, Keller and Berger (24) reported a dimorphic form of d-catechin, m.p. 210 to 213°C., obtained by extracting unwashed gum gambir.

The source of these discrepancies lies in the occurrence of two crystalline modifications of d-catechin. The high-melting modification is obtained by crystallizing from an aqueous solution saturated at the boiling point and vacuum-drying over P_2O_5 at 110°C. This form melts sharply at 219°C. The low-melting form is obtained by crystallization from a large volume of water; it sinters at 145 to 155°C. and melts at 176 to 177°C., in agreement with previous investigators. The infrared spectra of both forms are identical, but the x-ray diffraction patterns differ. Both forms show the same optical rotation and give identical pentaacetate derivatives, indicating that racemization is not responsible for the high melting point. The form reported by Keller and Berger (24) is apparently a mixture and not a result of using unwashed gambir as they have suggested, but rather a function of the concentration of the solution from which crystallization takes place.

A further difficulty is involved in the removal of the water of crystallization, since it apparently cannot all be removed without degrading the molecule. Even

d-catechin which has been vacuum dried at 115° over P_2O_5 for two weeks still contains water, as evidenced by the persistence of the 1627 to 1635 cm.$^{-1}$ band in the infrared spectrum (see Figure 7).

l-Epicatechin. The mother liquor and filtrates from the recrystallization of I were combined and slowly evaporated at room temperature by placing the solution in a large porcelain bowl and blowing air across the surface. Pink crystals were formed after two or three days. When the solution had been evaporated to about 50 ml., the mixture was filtered. The crude crystalline material was washed with warm (30 to 35°C.) water to remove any residual d-catechin, and then recrystallized from hot water. The yield was 9% of the original crude crystalline material. The purified white crystalline compound melted at 245°C. (cor.) and had a specific rotation of -58.9° in 1:1 acetone-water. The color reactions were identical with those obtained with d-catechin. This suggested that the compound might be l-epicatechin, $C_{15}H_{14}O_6$, which has a reported m.p. of 237 to 239°C. and an optical rotation of -58° (11).

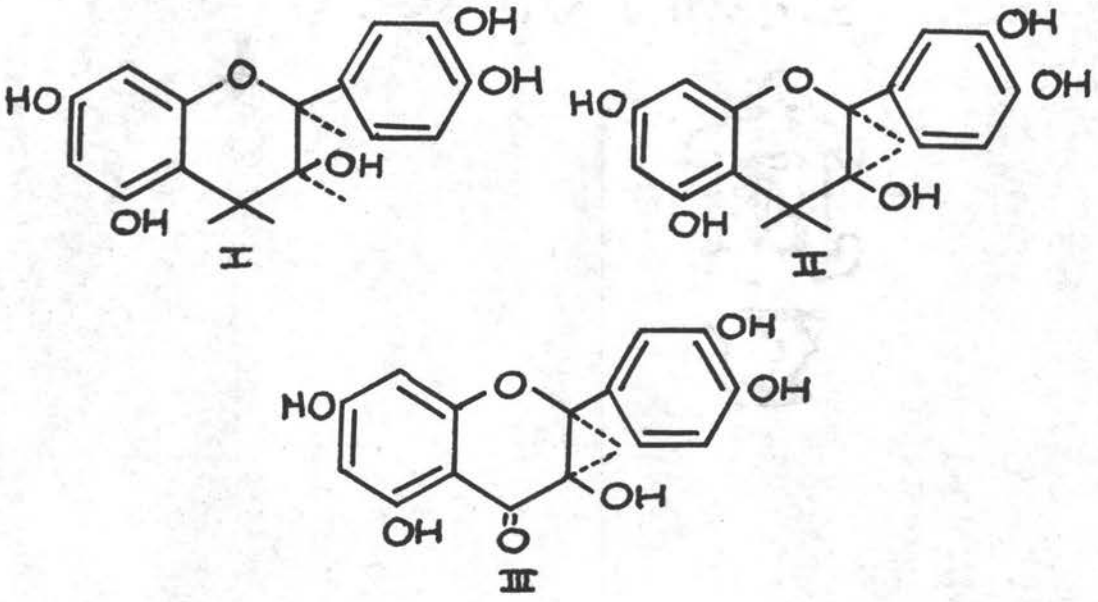
Acetylation with pyridine-acetic anhydride gave the acetate derivative, m.p. 152 to 153°C., with a specific rotation of -15.6° in chloroform. The penta-acetate derivative of l-epicatechin is reported to

melt at 151 to 152°C. and to have a specific rotation of -14.3 to 15.0° in $C_2H_2Cl_4$ (11).

An 15 mg. sample of l-epicatechin, which had been prepared by Dr. Charles Horton, was procured from Dr. Simon H. Wender of the University of Oklahoma. The infrared spectra and x-ray diffraction patterns of l-epicatechin were found to be identical to the crystalline material thus confirming its identity. The interplanar spacings of l-epicatechin pentaacetate have previously been reported by Bradfield and Penny (3) and are in good agreement with those reported here.

Structure of the catechins. Considerable discrepancy exists in the literature concerning the structure of the catechins (9; 37, pp.32-50) and the relation of d-catechin to l-epicatechin. Nierenstein and his coworkers believed that the two compounds are structural isomers (37) while Freudenberg has stated that they are optical isomers (9), epicatechin having a cis configuration II and catechin a trans configuration I. This latter view is now generally accepted (21, p.159).

It was thought that comparison of the infrared spectra of these compounds and that of d-3,3',4',5,7-penta hydroxy flavanone, dihydroquercetin, III, which differs from the catechins by having a carbonyl group in the 4 position, might reveal similarities or



differences which would support either the Nierenstein or Freudenberg work. Douglas fir bark cork (20) was used as a raw material for the extraction of d-dihydroquercetin. The purified dihydroquercetin melted at 240 to 242°C. and had a specific rotation of +48° in 1:1 acetone-water. Acetylation with pyridine-acetic anhydride gave an acetate derivative, m.p. 128 to 129°C. from methanol. The spectra are shown in Figures 5 and 6 and the probable band assignments in Table XII.

Examination of Figure 5 indicates that the infrared spectrum of l-epicatechin closely resembles that of d-dihydroquercetin, especially in the appearance of the two sets of strong bands at 1000-1050 cm.$^{-1}$, and 775-825 cm.$^{-1}$, and the series of four weak bands at 825-860 cm.$^{-1}$. However, the spectra of the two catechins do not closely resemble each other. This

Table XII. Tentative Infrared Frequency Assignments

Assignment	Absorption Maximum, $cm.^{-1}$		
	d-catechin	l-epicatechin	d-dihydroquercetin
Hydroxyl stretching	3475, 3318	3486, 3409, 3162	3510, 3355, 3150
C--H stretching	2938, 2869	2935	2875
Conjugated carbonyl			1642
Phenyl ring	1601, 1500	1603, 1516	1593, 1541, 1524
C--H bending	1451, 1347	1442, 1352	1458, 1369

Assignment	d-catechin pentaacetate	l-epicatechin pentaacetate	d-dihydroquercetin pentaacetate
Aromatic ester carbonyl	1756	1758	1764
Aliphatic ester carbonyl	1737	1738	
Conjugated carbonyl			1703
Phenyl ring	1612, 1584, 1487	1613, 1583, 1570	1613, 1573, 1553
		1505	1505
C--H bending	1434, 1347	1437, 1350	1438, 1346
Acetyl CH_3	1371	1373	1372
Acetyl C--O	1195-1185	1207-1190	1200-1184

dissimilarity is not nearly so marked in the case of the pentaacetate derivatives, in which a closer resemblance between the catechin derivatives may be noted. Even in this case, l-epicatechin pentaacetate shows a close relationship to dihydroquercetin pentaacetate. The wide divergence between the infrared spectra of cis- and trans-isomers is well known and would seem to be a reasonable explanation for the differences observed here. In spite of the usefulness of the spectra for identification purposes, they could not be used to further clarify structural relationships of catechin and epicatechin. This is due to the lack of fundamental information concerning the significance of bands in the 600-1200 $cm.^{-1}$ region of the spectrum.

The close spectral relationship between dihydroquercetin and epicatechin suggested that the former should also have a cis configuration and that reduction of dihydroquercetin should therefore yield epicatechin rather than catechin. Attempts to reduce dihydroquercetin by a Clemmensen reduction were unsuccessful, inasmuch as both I and II are very unstable in an acid medium. However, a modified Clemmensen reduction (35) of the pentamethyl derivative of dihydroquercetin was successful.

d-Dihydroquercetin (30.0g.) was dissolved in 300 cc.

of acetone. Over a period of two hours, 100 ml. of methyl sulfate and 60 grams of KOH (in water to make 100 cc. of solution) were added in small quantities, with stirring. The temperature was not allowed to exceed 10°C. during the reaction. An additional 60 grams of KOH and 100 ml. of dimethyl sulfate were added over another two-hour period. Then 100 ml. of water was added to the mixture and the mixture was placed in an icebox. After standing 24 hours, the crystalline precipitate was filtered off, washed with cold 80% ethanol, and dried. Two recrystallizations from 95% ethanol gave colorless prisms, m.p. 134 to 135°C. The yield was 26.5 grams (72%). The basic mother-liquor was acidified, and the precipitate was filtered off and remethylated as above. An additional 4.4 g. (12%) was obtained. The compound was insoluble in base.

Analysis calculated for $C_{15}H_7O_2(CH_3O)_5$: CH_3O, 41.44.

Found: CH_3O, 41.32.

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Pentamethyl dihydroquercetin (5g.) was dissolved in 75 cc. of toluene and added to 10 grams of amalgamated zinc (17), 20 cc. of water, and 10 cc. of concentrated hydrochloric acid. The mixture was slowly brought to reflux temperature over a period of 8 hours with constant stirring and then refluxed for an additional 40 hours. During this time, an additional 10 cc. of concentrated

hydrochloric acid was added in 4 portions. The two layers that formed were then separated. The aqueous portion was extracted with four 25-cc. portions of ether. The ether and toluene solutions were combined, washed with aqueous Na_2CO_3 solution, and dried over Na_2SO_4. The solution was evaporated to dryness in vacuo and the residues extracted with CCl_4. This solution was evaporated nearly to dryness and ethanol was added. After one week, crystals of pentamethyl d, l-epicatechin were obtained. These were recrystallized from 95% ethanol, m.p. 110 to 113°C. (reported (9) m.p. 113 to 114°C.); $(\alpha)_D^{25}$ +0.3° (chloroform, c, 0.75). The yield was 0.7 g. (14%).

Analysis calculated for $C_{15}H_9O(OCH_3)_5$: CH_3O, 43.06.
Found: CH_3O, 42.81.

The isolation of pentamethyl epicatechin by reduction of pentamethyl dihydroquercetin confirmed the similarity in configuration.

Alcohol Extract

Phlobaphene. Ether-extracted bark was extracted with 95% ethanol for 8 hours in a large, Soxhlet-type extractor. When the alcohol extract was concentrated to a syrup and poured into water, a voluminous red precipitate was obtained that was filtered off and

washed with water to remove any adsorbed tannin. The precipitate was twice reprecipitated in water and then purified further by being dissolved in a minimum amount of dioxane and reprecipitated in absolute ether. A salmon-colored, amorphous powder, decomposing at about 275°C., was obtained; it gave a bright green coloration with alcoholic ferric chloride solution. The infrared spectra of this material and of a derivative prepared by treatment with ethereal diazomethane are shown in Figure 8.

Tannin. The aqueous filtrate from the phlobaphene precipitation was concentrated, in vacuo, to a solution with a total solids content of about 5%. This was poured into a saturated salt solution to precipitate the tannin, which was then filtered off by suction and dried in a desiccator. An analysis of the precipitated tannin by the hide powder method showed a purity of 93%. Other procedures such as lead precipitation, or adsorption on hide powder and subsequent recovery with alcohol gave a less pure tannin.

The infrared spectrum of the tannin is shown in Figure 8, and the absorption band assignments in Table VIII. Comparison of the curve with that of phlobaphene shows marked dissimilarities. The greatest difference is in the presence of the carbonyl band (presumably

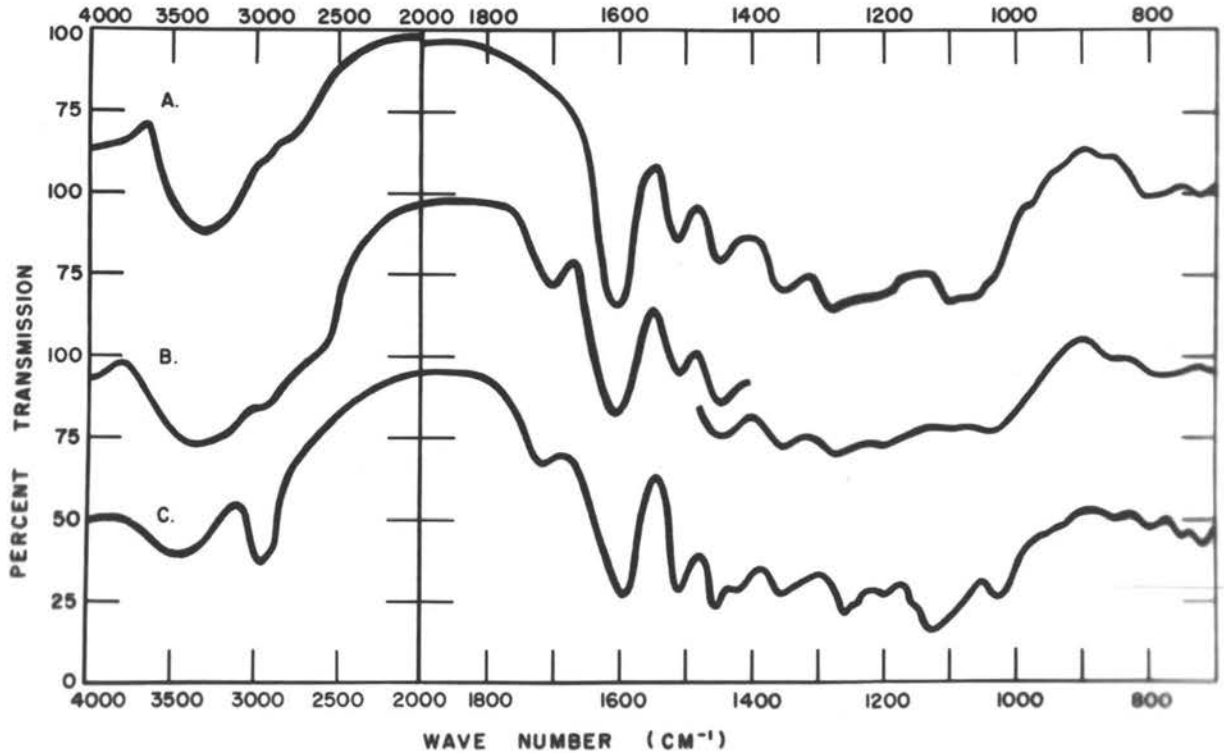


Figure 8. Infrared spectra of A--white fir tannin, B--white fir phlobaphene, C--phlobaphene methylated with diazomethane

due to a carboxyl group, since methylation shifts it to a position out of the range of aldehydes and ketones, but normal to esters) and a pronounced band attributable to CH_2 or CH_3 groups in the phlobaphene. Both of these bands are absent from the tannin spectrum and, furthermore, do not appear there after the tannin has been "condensed" by the action of acid or by long boiling. This indicates that the tannin molecule does not give rise to the phlobaphene in the bark by the simple process of condensation. On the contrary, the marked similarity of the phlobaphene to the wax phenolic acid and to the bark "lignin" or phenolic acid, both in spectra and properties, suggests that the phlobaphene bears the same relationship to the bark "lignin" that Brauns' native lignin (4) bears to wood lignin.

Aqueous extraction of the tannin. The tannin in white fir bark may also be extracted with hot water. Tannin analyses on air-dry, white fir bark were carried out by the American Leather Chemists Association method (1). In order to ascertain the optimum tannin extraction time, a series of hot-water extractions were made in which the length of extraction time was varied. The middle section bark sample of age group II was selected for these extractions. As can be seen from the results in Table XIII, a 6 hour extraction period gave optimum

tannin yields. This period, therefore, was used for the determination of tannin distribution by age group and location on the tree; The results of these analyses are shown in Table XIV. The bottom bark from the oldest trees had the highest tannin content, whereas the top bark from young trees had the highest.

Table XIII. Effect of Time on Tannin Extraction of White Fir Bark
Percentages Based on Oven-Dry Weight of Bark

	Time of extractions, hr.				
	1¼	2	3	6	9
Total solids, %	11.21	12.53	12.87	14.33	14.57
Insoluble solids, %	0.06	0.34	0.59	0.64	2.67
Soluble solids, %	11.15	12.19	12.28	13.69	11.90
Tannin, %	6.26	6.73	6.92	7.40	5.57
Nontannin, %	4.89	5.46	5.36	6.29	6.33
Tannin/sol. solids	0.562	0.552	0.562	0.541	0.468

Since the distribution of tannin among the various bark fractions might prove of interest, it is shown in Table XV. The fractions were obtained by separation from the bottom bark of age group III. The analyses indicate that the cork fraction has the highest tannin content and also gives the extract with the highest degree of purity (ratio of tannin to soluble solids).

The stand of trees from which these samples were obtained was quite dense and contained some trees of small diameter. These trees were as old as those of larger diameter, but apparently had been suppressed by

Table XIV. Analysis of Tannin Extracts from White Fir Bark
Percentages Based on Oven-Dry Weight of Bark

Age group	Sample	Total Solids, %	Soluble solids, %	Insoluble solids, %	Tannin, %	Nontannin, %	Tannin/sol. solid
I	Top	17.42	14.76	2.66	6.96	7.80	0.47
	Middle	12.18	11.44	0.69	6.18	5.26	0.54
	Bottom	12.53	10.94	1.59	6.26	4.68	0.57
II	Top	17.85	16.50	1.35	8.82	7.68	0.53
	Middle	14.33	13.69	0.64	7.40	6.29	0.54
	Bottom	17.12	15.37	1.75	10.01	5.36	0.65
III	Top	13.04	12.14	0.90	6.31	5.83	0.52
	Middle	12.30	11.45	1.85	6.30	5.15	0.55
	Bottom	17.58	16.29	1.29	10.17	6.12	0.62

Table XV. Tannin Distribution in White Fir Bark Fractions
Percentages Based on Oven-Dry Weight of Bark

Sample	Inner bark	Outer bark phleom (cork-free)	Cork
Total solids, %	14.31	12.06	18.43
Soluble solids, %	12.88	10.00	17.43
Insoluble solids, %	1.43	2.06	1.00
Tannin, %	7.19	5.48	10.70
Nontannin, %	4.69	4.52	6.73
Tannin/sol. solids	0.55	0.54	0.61

the density of the stand. A comparison of the tannin content of the bark of such trees with that of larger diameter trees is shown in Table XVI. Although the tannin content of the bark from small trees is somewhat lower, it is still sufficiently high for commercial extraction.

Table XVI. Effect of Tree Diameter Upon Tannin Distribution in White Fir Bark
Percentages Based on Oven-Dry Weight of Bark

Average age, yr.	143	152
Average diameter, in.	9	35
Average thickness of bark, in.	5/8	1½
Distance of sample from tree base, ft.	2-6	2-3½
Total solids, %	15.26	17.12
Soluble solids, %	14.54	15.37
Insoluble solids, %	0.72	1.75
Tannin, %	7.53	10.01
Nontannin, %	6.91	5.36
Tannin/sol. solids ratio	0.518	0.651

An examination of the nontannins indicated the presence of catechin. The boiling of catechin solutions, however, especially in the presence of tannins, appeared to cause some change in the catechin nucleus that made it partially absorbable on hide powder. This was evidenced by a study of the tannin extract from both the unextracted and ether-extracted white fir barks. The top and bottom barks from a tree with an approximate age of 210 yr. were analyzed; the analyses are given in Table XVII.

Table XVII. Tannin Analysis of White Fir Bark Before and After Ether Extraction
Percentages Based on Oven-Dry Weight of Unextracted Bark

Sample	Unextracted top bark	Ether-extracted top bark	Unextracted bottom bark	Ether-extracted bottom bark
Ether-soluble, %	1.97		4.75	
Total solids, %	15.46	12.86	17.92	11.79
Soluble solids, %	14.91	12.54	17.25	10.74
Insoluble solids, %	0.55	0.32	0.67	1.05
Tannin, %	8.70	8.68	10.49	7.85
Nontannin, %	6.21	3.86	6.76	3.89
Tannin/sol. solids	0.584	0.692	0.608	0.689

The data in Table XVII indicate that the purity of the tannin extract was markedly increased when ether-extracted (catechin-free) bark was used. This result suggested a procedure which gave a tannin extract with increased purity, and at the same time, a good

yield of catechin. White fir bark, preferably the cork fraction, was extracted with hot water. The hot-water extract was evaporated to about 5% total solids content, and a small amount of sodium bisulphite was added to prevent the tannin from acting as a colloidal dispersant of the catechin. After standing 12 hours, catechin had precipitated from the dark red solution and was filtered off. One recrystallization from hot water yielded pure d-catechin. The filtrate contained tannin with a purity ranging from 60% for the whole bark to 75% for the cork fraction.

In general, the tannin from white fir bark is light reddish-brown in color and appears to be of good quality. A well-plumped, pliable, light reddish-brown leather resulted when a sheepskin skiver was tanned with an aqueous solution of the tannin. The tannin belongs to the phlobatannin classification, since it gave an emerald-green color with ferric chloride solution, and precipitated phlobaphene "reds" when boiled with mineral acids. It appeared much more stable under heat than Douglas-fir tannin; most of the tannin was still water soluble after the dried extract had been heated for 6 hours at 105°C.

Hot Water Extract

Bark which had been previously extracted with diethyl ether and ethanol was extracted with hot water. The bark was treated for one hour with a five-fold excess of boiling water in a stainless steel beaker. This was repeated twice more. The combined filtrates were concentrated *in vacuo* to about thirty percent total solids content and then poured into ethanol. The flocculent precipitate was filtered off and dried in a desiccator. A yield of 0.4% was obtained from cork and one of 2.3% from the inner bark of age group III. The crude carbohydrate material was purified by treating an aqueous solution with hide powder to remove occluded tannin and then reprecipitating the carbohydrate in ethanol. A light tan powder was obtained which gave 83% reducing sugar after acid hydrolysis. A conventional sugar determination on the acid-hydrolyzed hot water extracts of cork and inner bark yielded 0.9 and 3.3% reducing sugar (calculated as glucose), respectively. The individual sugars were not characterized.

Infrared Studies of Flavanones, Flavones, Chalcones and Acetophenones

Infrared spectroscopy has proved to be an especially useful tool in the field of lignin chemistry since it provides a rapid method of evaluating lignins isolated by different methods. Application of the infrared method toward the structural elucidation of lignin and other natural materials such as tannin and phlobaphene has been hindered, however, by the lack of sufficient data concerning certain functional group frequencies in solid organic compounds. This information is necessary, since lignin may be prepared only as a mull or a film because of its insolubility in the solvents ordinarily used in infrared analysis.

Examination of the infrared spectra of lignin and lignin derivatives indicates the presence of two bands, 1666-1668 $cm.^{-1}$ and 1705-1710 $cm.^{-1}$, (6, 12, 26, 27, 28) which presumably originate from conjugated aldehyde and ketone carbonyl groups, respectively. Other materials related to lignin, such as tannin and phlobaphene, also show bands assignable to carbonyl groups. It seemed to be advantageous, therefore, to study a series of known solid compounds that may be related to lignin and contain hydroxyl, methoxyl and carbonyl groups.

Spectra. The instrument used in this work was a

model 12C Perkin-Elmer spectrometer and Brown recorder adapted to automatic, double-beam operation by the method of Savitsky and Halford (43). Lithium fluoride optics were used in the 2500-4000 $cm.^{-1}$ region and sodium chloride in the 1550-1800 $cm.^{-1}$ region. Solid samples were mulled in mineral oil (Nujol) or perfluorokerosene and run against a salt plate as a blank.

Compounds. Benzalacetophenone (chalcone) and the hydroxy acetophenones were purchased from the Eastman Kodak Company. Purification was effected by recrystallization or fractional distillation, whichever was appropriate. Derivatives were prepared by known methods.

Flavanone was prepared by the method of Kostanecki and Szabranski (25). The method of Kurth (29) was used to prepare 3',4'-dihydroxyflavanone and its corresponding chalcone. Douglas fir bark cork (20) was used as a source of d-3,3',4',5,7-pentahydroxyflavanone. The pentaacetate derivative, m.p. 128 to 129°C. from methanol, was prepared by the acetic anhydride-pyridine method. The 3',4',7-trimethoxy and pentamethoxy derivatives of this flavanone were prepared by diazomethane and dimethyl sulfate methylation, respectively. The pentahydroxyflavanone was converted to the corresponding flavone (quercetin) by treatment with aqueous sodium bisulfite (31). Pentamethoxy-and 3,3',4',7-tetramethoxy-

quercetin were prepared by the method of Gomm and Nierenstein (17). The method of Pew (40) was used to prepare 3',4',5,7-tetrahydroxyflavanone. The 3',4',7-trimethoxy derivative was obtained by diazomethane methylation. Ponderosa pine bark was used as a source of 3,3',4',5,8-pentahydroxyflavone (32).

The carbonyl and hydroxyl frequencies of this series of compounds are presented in Table XVIII.

Table XVIII

Compound	Frequency, cm.$^{-1}$ Carbonyl	Hydroxyl
Flavanone	1680	....
3',4'-Dihydroxy-	1665	3395, 3105[a]
3',4'-Diacetoxy-	1762, 1680	....
3',4',5,7-Tetrahydroxy-	1620	3260[a]
3',4',5,7-Tetraacetoxy-	1763, 1680	....
5-Hydroxy-3',4',7-trimethoxy-	1610[b]	....
3,3',4',5,7-Pentahydroxy-	1642	3510, 3355[a]
3,3',4',5,7-Pentaacetoxy-	1764, 1703	....
3,3',4',5,7-Pentamethoxy	1649	....
3,5-Dihydroxy-3',4',7-trimethoxy	1606[b]	3380
Acetophenone	1687	....
2-Hydroxy-[c]	1635	....
2-Acetoxy-	1762, 1678	....
2-Benzoxy-	1736, 1684	....
2-Methoxy-[c]	1649	....
4-Hydroxy-	1638	3100[a]
4-Acetoxy-	1763, 1685	....
4-Methoxy-[c]	1657	....
4-Methoxy-2-hydroxy-	1615[b]	....
2,4-Dihydroxy-	1620	3260, 3150[a]
2,4-Dimethoxy-	1643	....
2,4-Diacetoxy-	1764, 1688	....
Chalcone (benzalacetophenone)	1659	....
2',3,4-Trihydroxy-	1621	3280[a]
2',3,4-Triacetoxy-	1762, 1661	....
2',3,3',4,4'-Pentahydroxy-	1619[b]	3250[a]
2',3,3',4,4'-Pentabenzoxy-	1744, 1656	....
Flavone		
3,3',4',5,7-Pentaacetoxy-	1763, 1640	....
3,3',4',5,7-Pentahydroxy-	1654	3290[a]
3,3',4',5,7-Pentamethoxy-	1627	....
3',4',5,7-Tetramethoxy-5-hydroxy	1657	....
3',4',5,7-Tetrahydroxyflavone-3-rutinoside	1655	3270[a]
3,3',4',5,8-Pentahydroxy-	1655	3340
3,3',4',5,8-Pentaacetoxy-	1764, 1645	....

[a] Broad band, not sharply defined. [b] Exact position in doubt because of interference by phenyl band at 1605-1590 cm.$^{-1}$. [c] Liquid.

DISCUSSION OF THE CONSTITUENTS OF THE BARK EXTRACTIVES

The total extractive content of white fir bark varied from 17 to 23 percent depending upon the age and position on the tree from which the bark was obtained. Slightly higher yields of hexane and benzene soluble extractives were obtained from the butt of the tree but the total amount obtained varied only slightly from top to bottom. The ether-soluble extractive markedly increased from top to bottom and with increasing age. The hot water-soluble extractive was obtained in the highest yields from the top of young trees and the bottom of old trees. This extractive distribution is very similar to that in Douglas-fir (33) and ponderosa pine bark (32). The highest yield of benzene and ether soluble constituents were obtained from the cork fraction, while the lowest was obtained from the inner bark. Douglas fir-cork was previously shown to contain larger quantities of these constituents than whole bark (20).

The hexane soluble extractive was a light brown to cream colored wax with a melting point of 52 to 53°C. Analyses indicated it to consist of free lignoceryl alcohol, free behenic acid, an ester of lignoceryl alcohol and behenic acid, and phytosterol, and small

quantities of unsaturated alcohols and acids which were not characterized. The yields of the wax obtained from whole bark were rather low. Thus commercial extraction does not appear to be feasible from an economic standpoint at the present time. The relatively higher yields obtained from the cork fraction might warrant separation of the cork for wax extraction.

The hexane insoluble, benzene-soluble extractive was a hard, reddish-brown wax with a softening point of 70 to 72°C. Saponification of the wax indicated it to consist of 5% lignoceryl alcohol, 3% behenic acid, 49% of an acid characterized as 13-hydroxy myristic acid, 9% of a benzene-soluble unsaturated acid, and 30% of an ether-insoluble phenolic acid. The infrared spectra of the phenolic acid showed the presence of a carboxyl group, hydroxyl groups and phenyl rings. Inasmuch as the hexane-insoluble component was not soluble in cold sodium bicarbonate of hydroxide solution, the absence of free acids or acidic groupings was indicated. Apparently the acids are esterified to each other through an etholide type linkage and to the phenolic acid through its phenol groups. Further work is yet necessary to verify this assumption, particularly on the phenolic acid. A comparison of the wax phenolic acid with the phenolic acids obtained from extractive-free bark would be of considerable interest.

The ether soluble extractive was found to consist primarily of d-catechin along with small amounts of l-epicatechin. These compounds are stated to be optical isomers of 3,3',4',5,7-pentahydroxy flavan. The relationship of l-epicatechin to d-dihydroquercetin, 3,3',4',5,7-pentahydroxy flavanone, was established through the reduction of the pentamethyl derivative of the latter compound by a modified Clemmensen reduction to racemic pentamethyl epicatechin. The relationship between catechin and epicatechin was not further clarified, however, on the basis of infrared spectra which were markedly different. The differences may be ascribed to cis-trans isomers, but there was no direct proof of this. Two polymorphic forms of d-catechin were noted. The x-ray diffraction patterns were different but the infrared spectra were identical. The concentration of the solution from which crystallization took place was found to determine the form obtained.

Tannin analyses on whole white fir bark indicated yields varying from 6 to 10 percent. The yields were high enough to justify commercial recovery. The tannin appeared to be of good quality and had good heat resistances. A crude carbohydrate fraction was obtained by pouring a concentrated hot-water extract (from alcohol-extracted bark) into ethanol. The carbohydrate

material gave 83% reducing sugar after acid hydrolysis.

Phlobaphene was obtained by extracting ether and hot-water extracted bark with alcohol or fractionating an alcohol extract of ether-extracted bark. The infrared spectra was very similar to the benzene soluble wax phenolic acid but differed from the spectra of the tannin which lacked bands attributable to a carbonyl group and CH_2 of CH_3 groups.

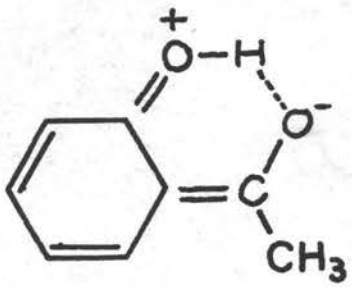
These analyses indicated that white fir bark contained sufficient quantities of extractives to warrant commercial extraction provided suitable markets are available. The presence of considerable amounts of catechin in the cork fraction warrants further investigation as to the best means of recovery of the catechin and possible usage.

DISCUSSION OF INFRARED STUDIES

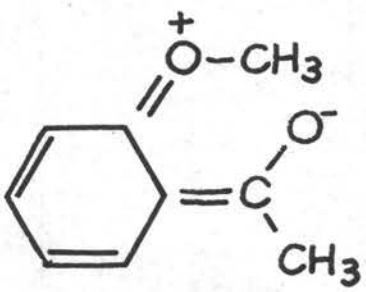
It is a well-known observation in infrared spectroscopy that conjugation of ethylenic double bonds, or a carbonyl group and double bonds causes a shift from the normal position to a longer wave length. Thus the unconjugated carbonyl group in acetone shows a band at 1718 cm.$^{-1}$ while conjugation with one phenyl group, as in acetophenone, lowers the frequency to 1687 cm.$^{-1}$ and conjugation with two phenyl groups, as in benzophenone, lowers the frequency to 1655 cm.$^{-1}$. Whether this shift is due to mesomerism or to an actual lengthening of the bond is not known. Examination of Table XVIII indicates that whenever there is an increased opportunity for the contribution of the ionic resonance structure, A, to the carbonyl group, B, a shift to lower frequencies is observed. Generally, the shifted band also has a greater intensity, which would be expected from participation of A.

Acetophenones. When a hydroxyl group is introduced ortho to the keto group in acetophenone, the carbonyl frequency is shifted from 1687 to 1635 cm.$^{-1}$. This effect has been previously noted by Gordy (18) who attributed it to hydrogen bonding between the hydroxyl

group and the keto group. Rasmussen and co-workers (41) reinterpreted this shift as being due to a conjugate-chelate system. They suggested that such a system is necessary for the occurrence of extreme shifts. Careful examination of the 3500-2500 cm.$^{-1}$ region of the spectrum of o-hydroxyacetophenone indicates the absence of any band attributable to a hydroxyl group. This behavior was previously noted in the o-hydroxyanthraquinones (8) and attributed to intramolecular hydrogen bonding. Furthermore, acetylation of o-hydroxyacetophenone shifts the band back to 1678 cm.$^{-1}$ (the 1762-1764 cm.$^{-1}$ band in all the acetoxy derivatives is caused by the acetoxy carbonyl group). These three facts seem to indicate further that conjugated chelation is responsible for the shift to shorter wave length. However, o-methoxyacetophenone shows a band at 1649 cm.$^{-1}$; clearly neither hydrogen bonding nor chelation is possible in this case. It seems likely, therefore, that the lowering is due to the participation of resonance forms, such as I in the



I

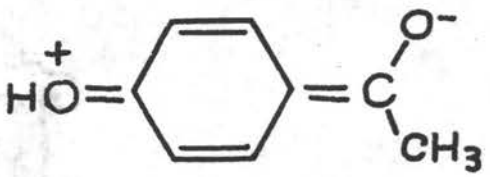


II

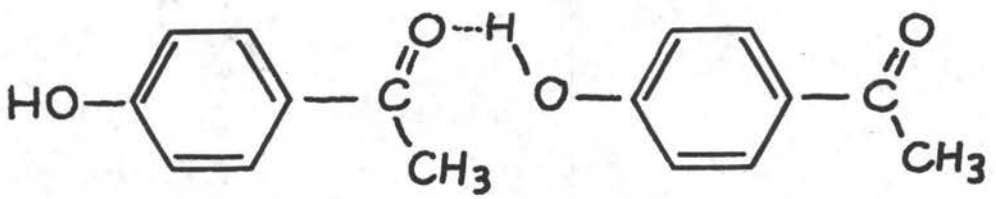
case of the hydroxyl derivative and II in the case of the methoxyl derivative. The increased stabilization in I, due to the chelate ring plus the presumedly greater ability of the hydroxyl group to donate electrons to the ring, is responsible for the lower carbonyl frequency in I than II. The ability of the acetoxy group to donate electrons is very meager and thus accounts for the acetoxy derivatives not markedly differing from unsubstituted acetophenone.

The hydroxyl band in p-hydroxyacetophenone occurs at 3250 $cm.^{-1}$, which indicates strong hydrogen bonding. Resonance structure III is likely responsible for the carbonyl shift to 1635 $cm.^{-1}$; structure III may be stabilized by hydrogen bonding as in IIIa.

The 2,4-derivatives of acetophenone require the same considerations as previously noted. The dimethoxyl

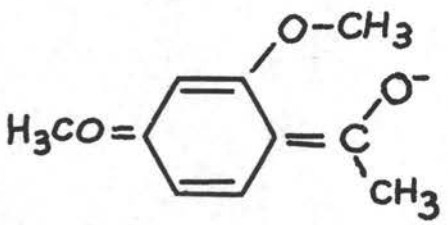


III

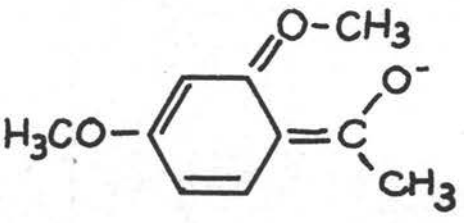


III a

derivative shows a shift lower than either the para or ortho compound. Increased electron supply to the ring present in both forms IV and IVa is very likely responsible. It is interesting to note that the datum of Soloway and Friess (45) indicates that m-methoxylaceto-



IV

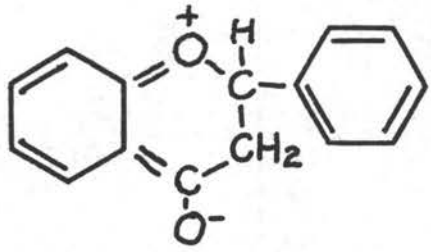


IVa

phenone shows a carbonyl band at 1681 cm.$^{-1}$. The meta position is not conjugated with the carbonyl group, which indicates that even though the methoxyl group is an electron donor, it must be in a conjugated position to affect the carbonyl frequency.

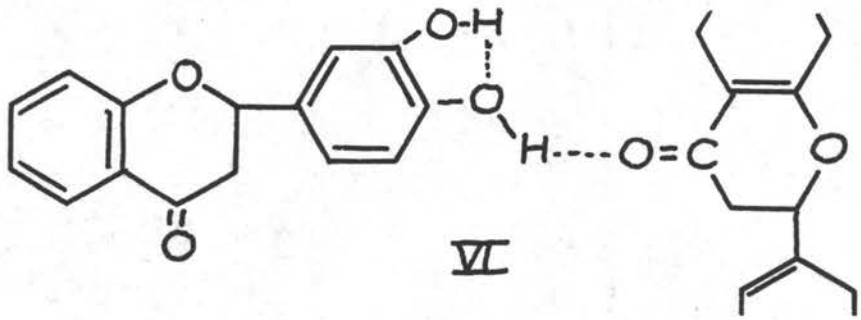
Flavanones. Unsubstituted flavanone shows a band at 1680 cm.$^{-1}$. This is only a slightly longer wave length than that of acetophenone, indicating that the oxygen in the pyran ring does not have the same influence as the o-methoxyl group in acetophenone. Consequently, resonance structure V is of small importance. Introduction of hydroxyl groups in the 3'- and 4'-positions causes the carbonyl frequency to shift to 1665 cm.$^{-1}$. Acetylation of these groups causes a shift back to 1680 cm.$^{-1}$, a frequency identical with that of unsubstituted

flavanone. Since these hydroxyl groups are not conjugated



V

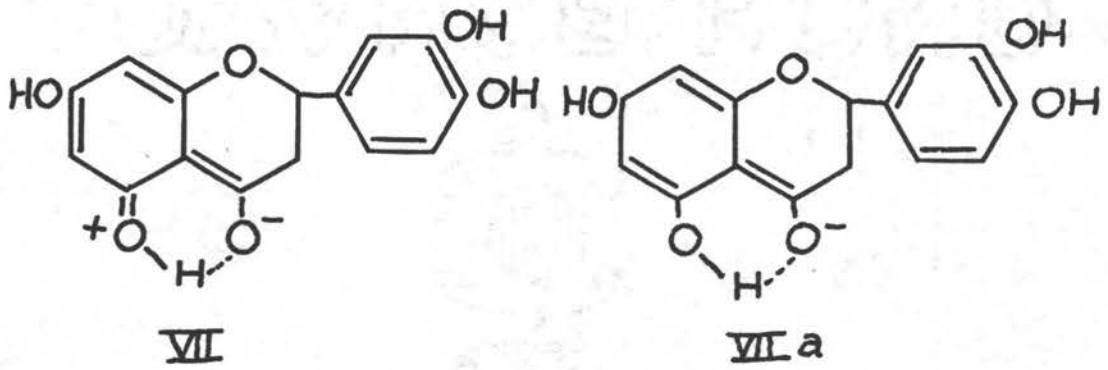
with the carbonyl group, their electron-donating character is not responsible for the shift to a lower wave number. This shift must be attributed to intermolecular hydrogen bonding of the type VI. Further evidence of this is found by examining the hydroxyl bands at 3395 and 3105 $cm.^{-1}$. The former indicates a moderately strong hydrogen bond. The latter is shifted further than



VI

normal for such phenols as catechol and is very likely due to hydrogen bonding to the keto group.

Introduction of hydroxyl groups into the 5- and 7-positions shifts the carbonyl frequency to 1620$cm.^{-1}$, which is identical with that of 2,4-dihydroxyacetophenone. It is concluded that VII and VIIa are important resonance structures. Hydrogen bonding between the



VII VII a

5-hydroxyl group and the keto group has previously been suggested to explain the resistance of the 5-hydroxyl group to methylation by diazomethane (17) and the stabilization of 5-hydroxyflavanones toward ring opening (36). Methylation of 3',4',5,7-tetrahydroxyflavanone with diazomethane yields the 3',4',7-trimethoxy-5-hydroxy derivative, which has a carbonyl frequency of 1610 cm.$^{-1}$ and shows no band attributable to a hydroxyl group. Although this shift is very likely enhanced by the methoxyl group in the conjugated 7-position, the electron-donating property of the 5-hydroxyl group enhanced by chelation, as in VII, must be chiefly responsible for this shift.

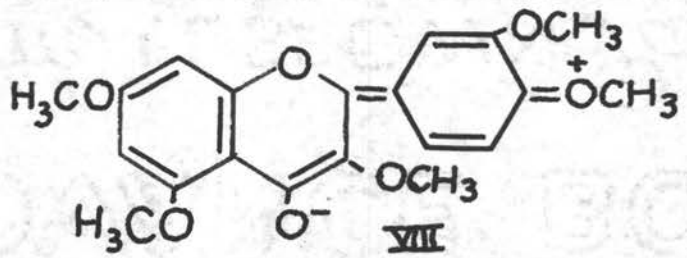
Acetylation of 3,3',4',5,7-pentahydroxyflavanone shifts the carbonyl band from 1642 to 1703 cm.$^{-1}$. Since this acetoxy derivative has a higher carbonyl frequency than unsubstituted flavanone and 3',4',5,7-tetraacetoxyflavanone, it appears likely that the 3-substituent is responsible for this effect. Further

work is necessary to verify this assumption.

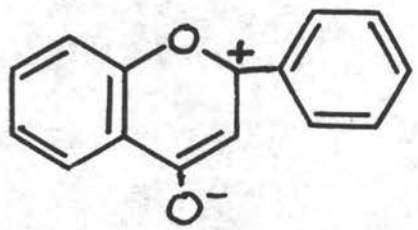
Chalcones. Unsubstituted chalcone (benzalacetophenone) shows a carbonyl band at 1659 cm.$^{-1}$ which is due to conjugation with a phenyl group and an aliphatic double bond. Introduction of a hydroxyl group in the conjugated-chelated 2'-position lowers the carbonyl frequency to about 1620 cm.$^{-1}$. Acetylation causes a return to the original unsubstituted position. The same considerations governing the carbonyl frequencies in acetophenone and flavanone derivatives apparently are operative in the chalcones, with the exception that enhancement due to the conjugated 2- and 4-positions is increased.

Flavones. The flavone derivatives examined did not show marked lowering of carbonyl frequency when a hydroxyl group was present in the 5-position. Acetylation of the hydroxyl groups decreased, rather than increased, the carbonyl frequency. That the 5-hydroxyl group is involved in chelation is apparent, since the OH band is absent in 5-hydroxy-3,3',4',7-tetramethoxyflavone. Introduction of a methoxyl group in the 5-position causes a shift to 1627 cm.$^{-1}$ for 3,3',4',5,7-pentamethoxyflavone. This value is 22 cm.$^{-1}$ lower than that of the corresponding flavanone derivative. The lowering is due, at least partially, to increased

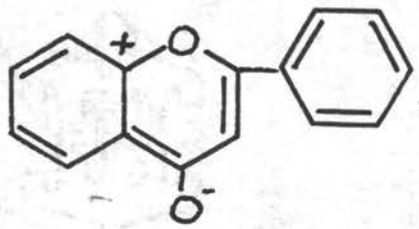
conjugation, as in VIII, which is not possible in the flavanones. The chemical behavior of flavones (the formation of salts, difficulty of carbonyl derivative



VIII



IX



IX a

formation, etc.) and the infrared spectra indicate that flavones are not closely analogous to flavanones, chalcones or acetophenones. This may be due to the importance of resonance structures, such as IX and IXa, in which conjugation between the carbonyl group and the 5- and 7-position is not favored.

Comparison of these results with studies of the polarographic reduction of the carbonyl group in flavones, flavanones and chalcones by Geissman and his coworkers (7, 16) indicates that the same factors that are operative in altering the infrared frequency of the carbonyl group are responsible for the ease of reduction of this group.

SUMMARY

The extractive content of white fir bark ranged from 17 to 22% of the dry weight of the bark. The hexane extractive, a light-brown wax obtained in about a 2.5% yield, was composed of free lignoceryl alcohol and behenic acid, and of combined lignoceryl alcohol, phytosterol, unsaturated alcohols, combined behenic acid, and unsaturated acids. Benzene extraction of the hexane-extracted bark residue produced a dark brown wax, in about a 1% yield, that was composed of lignoceryl alcohol, phytosterol, behenic acid, 13-hydroxy myristic acid, hexane-insoluble unsaturated acids, and a phenolic acid. By extracting this bark residue with ethyl ether, d-catechin and l-epicatechin were obtained in about a 2% yield. A phlobatannin was obtained in about a 7% yield by hot water extraction. Alcohol extraction of the solvent and water extracted bark residue yielded about 2% phlobaphene. The cork fraction, which comprised roughly 40% of the bark and was easily separable by screening hogged bark, contained 6% wax, 6 to 16% catechin, 5% tannin, and 3 to 7% phlobaphene.

The hydroxyl and ketone carbonyl infrared frequencies of a series of flavanones and related compounds have been measured. An o-hydroxyl group chelated to a

carbonyl group effects a shift of 60 cm.$^{-1}$ to a longer wave length. Shifts to shorter or longer wave lengths were observed when electron-withdrawing or supplying constituents were introduced into ring positions conjugated with the carbonyl group. The effects of intramolecular and intermolecular hydrogen bonding upon the carbonyl frequency are shown and discussed.

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