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The Flavonoids of Lodgepole Pine Bark

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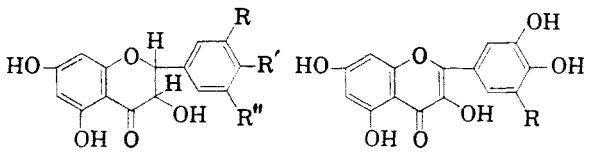
The principal flavonoid present in lodgepole pine bark is myricetin 3,3′,4′,5′,5,7-hexahydroxyflavone. A small amount of quercetin, dihydromyricetin, dihydroquercetin, aromadendrin, and pinobanksin are also present.

The infrared curve of myricetin is presented and compared with that of myricetin hydrochloride. A new structure is proposed for the latter which is consistent with both spectral and chemical evidence.

Recent investigations have shown that the bark of certain conifers contains considerable quantities of flavonoids.[1-3] Although extensive studies of the flavonoids from pine wood have been made,[4] relatively little is known of pine bark flavonoids. Dihydroquercetin (I) has recently been found in the bark[5] of Jeffrey pine (*Pinus jeffreyi*), tuscan pine (*P. pinaster*), and ponderosa pine (*P. ponderosa*). The latter species also contains a yellow flavonoid coloring matter of disputed structure.[1,6-9]



I R = R′ = OH, R″ = H
II R = R′ = R″ = OH
III R′ = OH, R = R″ = H
IV R = R′ = R″ = H

V R = OH
VI R = H

In this laboratory, an examination of the bark of a large number of pine species has indicated that lodgepole pine (*Pinus contorta* Dougl.) bark was relatively rich in flavonoids. Lodgepole pine is a tree indigenous to the Pacific Northwest and most of the Rocky Mountain region. Some authorities distinguish two varieties of this pine, one of which is limited to the coastal region (*P. contorta* var. Contorta), and the other to the interior (*P. contorta* var. *latifolia Engelm.*). The extractive content of lodgepole pine bark from the coastal variety was compared with bark from trees obtained inland (see Table I.) No apparent differences in the chemical nature of the extractives were observed; however, the coastal variety contained much larger amounts of flavonoids.

TABLE I

YIELD AND NATURE OF LODGEPOLE PINE BARK EXTRACTIVES

Nature	Solvent	Yield,[a] % I[b]	II[c]	III[d]
Yellow wax, resin acids	Petroleum ether	3.41	6.94	6.23
Brown wax, oxidized resin acids	Benzene	2.29	2.87	2.14
Flavonoids, phenolic wax	Ethyl ether	2.86	0.90	1.01
Tannin and carbohydrate	Hot water	12.95	16.42	19.01
Phlobaphene	Ethanol	2.30	2.02	1.82
	Sum	23.81	28.15	30.21

[a] Yield based on oven-dry weight of unextracted bark. [b] Bark obtained from Grays Harbor, Washington. [c] Bark from Mt. Hood National Forest, Oregon. [d] Bark from Ochocho National Forest, Oregon.

A large-scale ether extraction was made of coastal lodgepole pine bark which had been pre-extracted with benzene to remove wax, fats, and resin acids. The dried ether extract then was digested with warm acetone and filtered. The yellow crystalline residue from this treatment was chromatographically homogeneous. It was found to be myricetin (V), a hexahydroxy flavone, by direct comparison with a synthetic derivative. The myricetin was obtained in about 2.0% yield of the bark and accounted for 90% of the total flavonoid fraction. Small amounts of a second flavone, quercetin (VI), were obtained from the mother liquor.

A flavanone fraction was obtained by hot water extraction of unextracted bark. Paper chromatography[10,11,12] of the crude extract indicated the presence of traces of myricetin and quercetin (these compounds are slightly water soluble) and four flavanones. The Rf's in several different solvent systems and the color reactions indicated the flavanones to be dihydroquercetin (I), dihydromyricetin (ampelopsin) (II), aromadendrin (III) and pinobanksin (IV). Recrystallization of the crude ex-

(1) Kurth and Hubbard, *Ind. Eng. Chem.*, **43**, 896 (1951).
(2) Hergert and Kurth, *Tappi*, **35**, 59 (1952).
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(8) Gupta, Kurth and Seshadri, *J. Sci. and Ind. Research (India)*, **13B**, 886 (1954).
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(12) Lindstedt, *Acta Chem. Scand.*, **4**, 448 (1950).

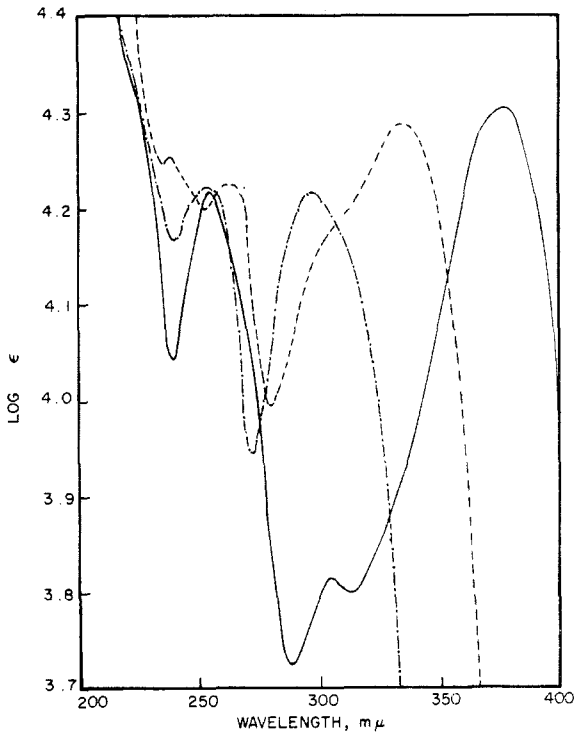


FIG. 1.—ULTRAVIOLET SPECTRA OF MYRICETIN (———), HEXAMETHYL MYRICETIN (– – – –), AND MYRICETIN HEXAACETATE (–·–·–·–).

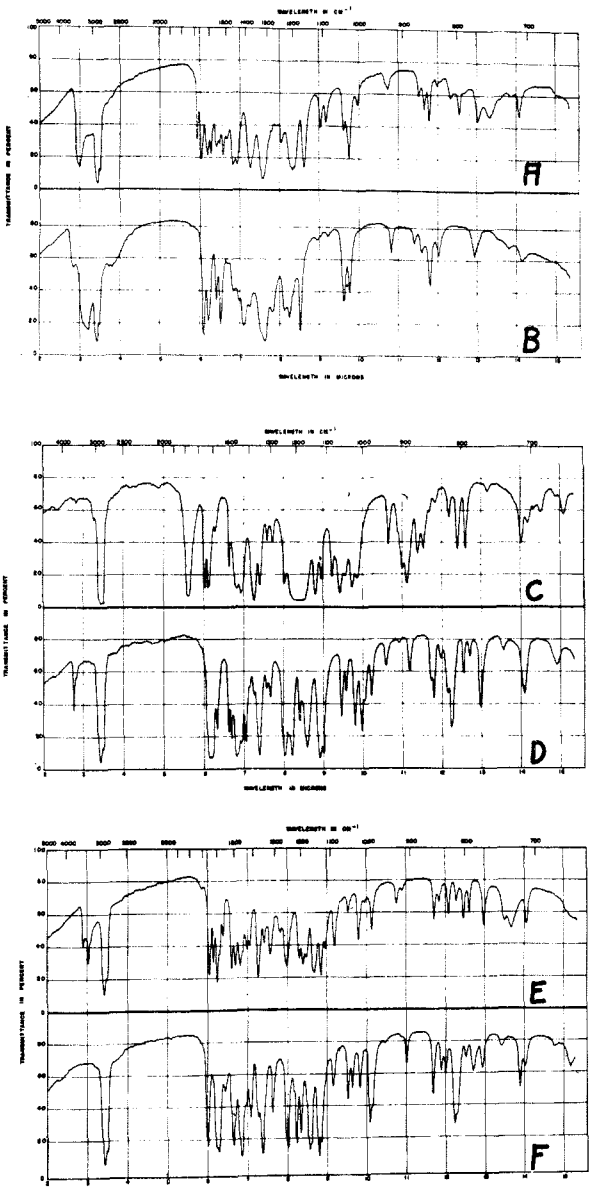


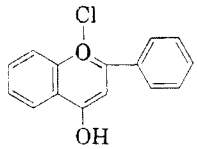
FIG. 2.—INFRARED SPECTRA OF (A) MYRICETIN (dotted line shows acetone of crystallization) (B) Myricetin hydrochloride, (C) Hexaacetylmyricetin, (D) Hexamethylmyricetin, (E) Trimethylmyricetin, and (F) Pentamethylmyricetin.

tract from hot water gave crystals of dihydromyricetin (II) admixed with small amounts of dihydroquercetin (I). The other two flavanones could not be isolated due to the small amount present in the bark. The identification of the two compounds III and IV is, therefore, based on chromatographic evidence and must be regarded as tentative. The flavonoid fraction from the lodgepole pine bark obtained east of the Cascade range (inland variety) was found to be identical with that obtained from the coast. As far as may be ascertained, this is the first report of the authentic isolation of myricetin from a conifer. Lodgepole pine bark appears to be the only presently known domestic source of the compound.

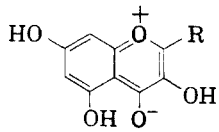
Comparison of the ultraviolet absorption spectra of myricetin (V) and its derivatives (see Figure 1) with the corresponding quercetin derivatives[13] shows much similarity. This is due to the same chromophore in both molecules,[14] *i.e.*, both compounds have the same number of hydroxyl groups conjugated with the carbonyl group. Examination of the infrared spectra indicates that the carbonyl bands also occur at approximately the same position in the two compounds and their derivatives (see Figure 2). The position of the carbonyl band in flavones has been shown to be related to substitution in the 5 and 3 positions.[15,16] The phenyl ring stretching vibrations in the region 1610–1500 cm.$^{-1}$ differ considerably from those of quercetin, and thus permit spectral differentiation of the two compounds.

There has been considerable controversy as to the structure of the salts formed by the flavones and mineral acids. The common view had been that they are represented by structure VII.[13] Comparison of the infrared spectrum of myricetin hydro-

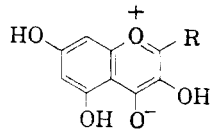
(13) Briggs and Locker, *J. Chem. Soc.*, 3136 (1951).
(14) Aronoff, *J. Org. Chem.*, 5, 561 (1940).
(15) Hergert and Kurth, *J. Am. Chem. Soc.*, 75, 1622 (1953).
(16) Shaw and Simpson, *J. Chem. Soc.*, 655 (1955).



VII



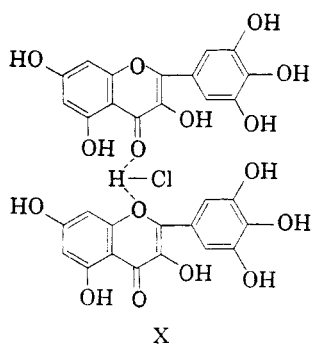
VIII



IX

chloride with that of myricetin indicates that this structure is incorrect. The salt spectrum shows a band at 1639 cm.$^{-1}$ attributable to the carbonyl group. The carbonyl band is intensified, which is generally due to an ionic structural contribution, and shifted from 1656 cm.$^{-1}$, the position in myricetin. This indicates the increased importance of resonance structures VIII and IX.

New bands appear at ca. 2550 cm^{-1} which are similar to absorption bands due to OH stretching vibrations in carboxylic acid dimers.[17] A somewhat similar NH stretching band occurs in the mineral acid salts of organic nitrogen bases.[18] This suggests that in myricetin hydrochloride the hydrogen chloride molecule lies between the oxygen atom (which is basic, due to the contribution of VIII and IX) of the pyrone nucleus and an adjacent carbonyl group of a neighboring flavone molecule in the crystalline lattice (see X).



X

Infrared spectra of corresponding quercetin salts showed the same intensification and shifting of the carbonyl band and the OH stretching bands at 2500–2575 cm^{-1}. Further evidence for a benzenoid structure of the pyrone ring in the flavonols is that the 3-hydroxyl group shows many of the properties of a typical phenol, i.e., color with ferric chloride,[13] salt formation with inorganic bases, etc. The infrared carbonyl band of the acetate of the 3-hydroxyl group is at the same position as the carbonyl band of acetates of phenolic hydroxyl groups.

(17) Flett, *J. Chem. Soc.*, 962 (1951).
(18) Witkop, *J. Am. Chem. Soc.*, **76**, 5597 (1954).

EXPERIMENTAL[19]

Lodgepole pine bark extractives. Lodgepole pine bark was peeled from 12 logs cut in September, 1954, in the vicinity of Grays Harbor, Washington (Sample I). The trees had an average age of 80 years and were 12 to 16 inches in diameter. The bark was dark brown in color and ranged in thickness from $^3/_4$ to 2 inches. Anatomical investigation indicated the presence of sieve cells, phloem parenchyma, and a well developed periderm. Fiber or schlerenchyma cells were absent. Bark was also obtained from trees (average age, 90 years) growing 35 miles southeast of Mt. Hood, Oregon, in the Mt. Hood National Forest (Sample II) and from trees (average age, 70 years) at a site about 30 miles east of Prineville, Oregon in the Ochocho National Forest (Sample III). The bark from the two latter places was only $^3/_8$ to $^5/_4$ inch in thickness. The bark samples were ground in a hammermill to pass a 20 mesh sieve. The air-dried samples were successively extracted with petroleum ether (b.p. 30–60°), benzene, ethyl ether, hot water, and ethanol. The amounts and qualitative nature of the extractives were determined by a procedure previously described[20] and are shown in Table I.

Chromatography. The crude ether extract obtained after benzene extraction was chromatographed on Whatman No. 1 paper by the descending technique with the solvent systems described by Gage, Douglass, and Wender.[10] Best results were obtained with acetic acid-water (60:40). Yellow spots were obtained at Rf 0.31 (myricetin) and 0.40 (quercetin), colorless, non-fluorescent spots at 0.65 (dihydromyricetin), 0.72 (dihydroquercetin), 0.81 (aromadendrin) and 0.87 (pinobanksin), and a colorless, fluorescent (ultraviolet light) spot at 0.70 (unidentified, stilbene derivative?). Chromatography with the organic and aqueous phases of benzene-ligroin-methanol-water[12] confirmed the presence of pinobanksin. Authentic compounds were used for comparison.

Isolation of flavones. Bark (Sample I) was extracted with ethyl ether in a large, borosilicate glass, Soxhlet-type extractor. The ether extract was evaporated to dryness, placed in a paper thimble and extracted with benzene in a Soxhlet extractor. The benzene-insoluble residue (containing the flavonoids) was triturated with warm acetone and filtered. The insoluble yellow crystalline residue consisted of nearly pure myricetin. The filtrate was evaporated to dryness and retriturated to yield additional small amounts of myricetin. The acetone-soluble fraction was diluted with water and placed in the icebox. A crude, yellow precipitate of quercetin was obtained. The yields of the crude flavones were 2.0 and 0.1 per cent, respectively.

Myricetin. The crude myricetin was recrystallized several times from acetone. Yellow crystals, decomp. pt. 350°, containing acetone of crystallization, thus were obtained. Drying at 55° *in vacuo* over P_2O_5 failed to remove the acetone, as evidenced by the persistence of the 1710 cm.$^{-1}$ band in the infrared spectrum (see Figure 2). Paper chromatography indicated the flavone to be completely homogeneous.

Anal. Calc'd for $C_{15}H_{10}O_8 \cdot C_3H_6O$: C, 57.44; H, 4.29. Found: C, 57.60; H, 4.87.

The compound gave a positive Wilson borocitric acid test[21] for flavones. Treatment of an aqueous solution with bases or basic salts gave an intense deep blue to purple

(19) All melting points are corrected; microanalyses by Weiler and Strauss, Oxford, England; infrared spectra were obtained as Nujol mulls on a Perkin-Elmer Model 21 double-beam spectrophotometer; ultraviolet spectra were determined in ethanol on a Cary Model 11 recording spectrophotometer.
(20) Hergert and Kurth, *Tappi*, **36**, 137 (1953).
(21) Wilson, *J. Am. Chem. Soc.*, **61**, 2303 (1939).

coloration characteristic of flavones containing a pyrogallol nucleus.

The *acetate derivative* was prepared with acetic anhydride and pyridine. Long white fibrous crystals, m.p. 215–216° (reported[22] 214–216°), were obtained from ethanol.

Anal. Calc'd for $C_{27}H_{22}O_{14}$: C, 56.84; H, 3.89. Found: C, 56.27; H, 3.99.

Hexamethoxymyricetin. Myricetin (2.0 g.), anhydrous K_2CO_3 (30 g.), and 100 cc. of acetone were mixed in a flask. The mixture was maintained at reflux and 10 cc. of dimethyl sulfate was added over a period of 3 hours. The methylated derivative was isolated from the filtered mixture and recrystallized several times from 80% ethanol. Yield 1.75 g. of colorless prisms, m.p. 155–156° (reported[23] 155–156°) containing water of crystallization.

Anal. Calc'd for $C_{21}H_{22}O_8 \cdot H_2O$: C, 60.00; H, 5.75; CH_3O, 44.29. Found: C, 60.69; H, 5.33; CH_3O, 44.2.

Synthetic hexamethoxymyricetin was prepared by a similar methylation of 3′,4′,5′-trimethylmyricetin[24] obtained by condensation of ω-benzoyloxyphloracetophenone, sodium O-trimethyl gallate, and 3,4,5-trimethoxybenzoic anhydride. The synthetic derivative melted at 155–156° and gave an undepressed mixture m.p. with hexamethoxymyricetin from the bark. The infrared spectra of the naturally-occurring and synthetic derivatives were identical.

Pentamethylmyricetin was obtained by methylation with smaller quantities of dimethyl sulfate for a shorter period of time. The crude product was recrystallized from 95% ethanol yielding pale yellow needles, m.p. 139–140° (lit.[25] 139–140°).

Anal. Calc'd for $C_{20}H_{20}O_8$: C, 61.85; H, 5.19. Found: C, 61.16; H, 5.18.

Myricetin hydrochloride, bright orange crystals, was prepared according to Perkin.[26]

Quercetin. The crude quercetin fraction was recrystallized several times from aqueous acetone. Yellow crystals, m.p. 310–314°, were obtained which still contained traces of myricetin. The mixture then was acetylated and fractionally crystallized from acetone-ethanol (1:1) to yield white needles, m.p. 193–194°, which were identical with an authentic sample of quercetin pentaacetate by direct comparison (mixture m.p., spectra, and color reactions).

Isolation of flavanones. Unextracted lodgepole pine bark (Sample I, 2 kg.) was extracted with four 5-liter portions of boiling water over a period of four hours. The aqueous filtrates were combined and evaporated to about 20% total solids in a natural circulating, glass vacuum evaporator. This solution then was extracted with one liter of ether in a separatory-funnel. The ether extract was evaporated to a small volume, filtered to remove a small amount of myricetin, and evaporated to dryness. The residue was taken up in 20 cc. of warm (60°) water, decolorized with charcoal and filtered. To the filtrate was added 0.5 g. of K_2HPO_4 in order to precipitate potassium salts of 3-hydroxy flavanones. After several days, the precipitate was filtered off, suspended in 10 cc. of water, acidified with dilute hydrochloric acid, and set aside to crystallize. Yield 0.15% (based on original oven dried bark) of colorless needles, m.p. 236–238°.

Dihydromyricetin and dihydroquercetin. Paper chromatograms of the crystalline 3-hydroxy flavanone fraction indicated it to be a mixture of dihydromyricetin and dihydroquercetin (in a ratio of 3 to 1). The mixture gave an intense violet-cerise coloration (positive color test for 3-hydroxy flavanones) when treated with powdered zinc and alcoholic hydrochloric acid.[27] The two compounds could not be separated by fractional crystallization of the original mixture or of the acetate derivatives. The structure of the two compounds was verified by conversion to the corresponding flavonols with hot 20% sodium bisulfite solution.[28] This gave a mixture of yellow flavones, m.p. 308–320°, which was separated into myricetin, m.p. 345° (acetate derivative, m.p. 212°) and quercetin, m.p. 310–312° (acetate derivative, m.p. 193°) by fractional crystallization from acetone.

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(23) Seshadri and Venkateswarlu, *Proc. Indian Acad. Sci.*, **23A**, 296 (1946).

(24) Kalff and Robinson, *J. Chem. Soc.*, 181 (1925).

(25) Rao and Seshadri, *Proc. Indian Acad. Sci.*, **25A**, 444 (1947).

(26) Perkin and Hummel, *J. Chem. Soc.*, **69**, 1287 (1896).

(27) Pew, *J. Am. Chem. Soc.*, **70**, 3031 (1948).

(28) Kurth, *Ind. Eng. Chem.*, **45**, 2096 (1953).