Summary

3-Acetocoumarin reacts with certain ketones in the presence of amides, forming derivatives of 5-keto-4-methyl[1]benzopyrano[3,4-c]pyridine. The condensation involves a Michael reaction, in which 3-acetocoumarin functions as an acceptor and a ketone as an addendum. It is remarkable that cyanoacetamide, like other amides, acts only as a source of nitrogen and not as an addendum.

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[CONTRIBUTION FROM THE OREGON FOREST PRODUCTS LABORATORY, AND THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

The Composition of the Wax in Douglas-Fir Bark

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Douglas-fir [*Pseudotsuga taxifolia* (Poir.) Britt.] bark was reported previously to contain from 3.6 to 6.0% of a light-colored wax, which was extracted with hexane.[1] At that time an examination of the literature revealed that little information was available concerning the chemical composition of this wax. Warth[2] reported the presence of a wax in the corky layers of the Douglas-fir bark with m. p. 63°. Although no experimental data were presented, he mentioned that free melissic acid, an unsaturated alcohol and an ester of melissic acid were possible constituents of this wax. Evidence is now presented that the chief constituents of this wax are lignoceric acid, lignoceryl alcohol, ferulic acid (4-hydroxy-3-methoxycinnamic acid), and their corresponding esters. Oleic acid and phytosterol are minor constituents. The acid constituents of the wax are present in the free and combined state. Treatment of a hexane solution of the wax with 5% potassium carbonate solution extracted both the fatty acid and the aromatic acid. The largest part of both acids was obtained only after saponification. The composition of the bark wax is different from that obtained from the lignin residue produced in the hydrolysis of Douglas-fir sawmill residues.[3]

The properties of the hexane soluble waxes produced in this Laboratory from different lots of bark were

Melting point, °C.	60 to 63
Acid number	30 to 70
Saponification number	130 to 164
Unsaponifiable, %	18 to 30
Hanus iodine number	18 to 22

The color was yellow to light brown. They were non-tacky and brittle when cold. When broken they presented a granular non-crystalline fracture. The specific gravity of the solid waxes was greater than one and hence they commonly separated as a granular powder on cooling a hot water suspension.

On saponification of the waxes with alcoholic potassium hydroxide solution followed by extraction of the unsaponifiable matter with ethyl ether or hexane there was obtained a mixture of lignoceryl alcohol and phytosterol crystals. Acidification of the alkaline soap solution and extraction with hexane gave white crystals of lignoceric acid on evaporation of the solvent. Suspended in the aqueous solution following the removal of the fatty acid fraction, was a light brown resin. This was completely soluble in ethyl ether and partially soluble in benzene. It was crystallized from both these solvents. The identity of ferulic acid was established by methoxyl group determination, preparation of the acetyl derivative, methylation, and oxidation of the fully methylated acid to veratric acid. The presence of this aromatic acid does not appear to have been established previously in a natural wax. It has long been of interest in lignin chemistry. The basic nucleus of the lignin molecule, $(CH_3O)(OH)C_6H_3CH{=}CH{-}C$, from conifers is patterned after ferulic acid. Lignin conceivably could be derived from ferulic acid in nature.

Other extractives in addition to the above wax have been isolated from Douglas-fir bark in this Laboratory. These were a reddish-brown wax insoluble in hexane, but soluble in benzene, dihydroquercetin, tannin and sugars. The dihydroquercetin has been obtained in yields of 5 to 8% in relatively pure form by extracting wax-free bark with diethyl ether. Similarly, it has been obtained in yields as high as 19% from the cork in the bark. The hexane insoluble wax appeared to possess a more complicated composition than the hexane soluble wax. It had a melting point of 60 to 63°, and there has been tentatively isolated from this material on saponification a fatty acid mixture in about 25% yield, a dark-colored phlobaphene in 24% yield, a dark colored ether soluble acid fraction in 26% yield, unsaponifiable matter in 5% yield and glycerol.

Experimental

Extraction of the Wax from the Bark.—In the course of the experimental work, several lots of bark were extracted with hexane in a stainless steel extractor which had a capacity of about 50 pounds of bark ground to pass a screen containing $^3/_8$ inch perforations. Stainless steel was used for the reason that iron and copper caused discoloration of

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[1] Kurth, Kiefer and Hubbard, *The Timberman*, **49**, no. 8, 130-131 (1948).

[2] Warth, "The Chemistry and Technology of Waxes," Reinhold Publishing Corp., New York 18, N. Y., 1947, p. 148.

[3] Kurth, *The Chemurgic Digest*, **6**, 366 (1947).

the wax, particularly in the presence of water. In this extractor, the ground bark was charged into a vertical vessel 12 inches in diameter and 5 feet high. Hot solvent was pumped upward through the bark and into an evaporator where the solvent was removed from the wax by steam distillation. At the end of the extraction period the molten wax was drained from the apparatus, cooled, and dried. The limit of extraction was reached in four hours. Bark was extracted with a moisture content ranging from 10 to 30% with little observed difference in extraction efficiency.

Saponification.—A mixture of 20 g. of wax, 20 g. of potassium hydroxide and 300 ml. of 70% ethanol was refluxed for three hours. Following this, 100 ml. of water was added, the alcohol removed in a hot-water-bath, and the residue extracted with hexane in a separatory funnel to remove unsaponifiable compounds.

Unsaponifiables.—The hexane solution containing the unsaponifiables was washed with water, dried over anhydrous sodium sulfate, and then evaporated to dryness; the yield was 3.9 g. (19.5%).

Lignoceryl Alcohol.—The unsaponifiable matter was recrystallized from acetone. A white crystalline solid was obtained that melted at 68 to 69°, gave a phenylurethan derivative melting at 76 to 77°, and gave no test for unsaturation. An acetyl number determination indicated an alcohol with the molecular weight of 360. Oxidation of the alcohol by fusion with soda-lime[4] gave the corresponding acid, m. p. 70 to 71°. An ether extraction of the alkaline fusion mixture prior to acidification yielded only traces of residue. This indicated a practically complete conversion of the alcohol to the corresponding acid and also indicated the absence of hydrocarbons in the unsaponifiable fraction. Tetracosanol-1 (lignoceryl), $C_{24}H_{49}OH$: Calcd.: mol. wt., 354.4. Found: mol. wt., 360. Tetracosanoic acid (lignoceric) $C_{24}H_{48}O_2$: Calcd: neut. equiv., 368.4. Found: neut. equiv., 370.

Phytosterol.—The filtrate from the lignoceryl alcohol crystallization gave a positive Liebermann–Burchard test for sterols and a precipitate with digitonin. After removal of the acetone, the residue was recrystallized from dilute alcohol as white needles, m. p. 134 to 135°. A mixed melting point test with the phytosterol isolated from Douglas-fir wood[5] did not lower the melting point. This phytosterol has been shown previously to be a mixture of α- and β-sitosterols. The yield of crystals was 0.3% of the wax.

Lignoceric Acid.—The alkaline solution from the separation of the unsaponifiable matter was acidified with sulfuric acid, and extracted with hexane in a separatory funnel. The hexane solution was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness; the yield was 12.1 g. (60.5%). The fatty acid residue was recrystallized from acetone and then from hexane; a white product was obtained, m. p. 73 to 74°, neut. equiv. 360.1; *p*-bromophenacyl ester, m. p. 86 to 88°. Synthetic lignoceric acid has a neut. equiv. of 368.4 and its *p*-bromophenacyl ester melts at 90 to 91°. It has been recognized for a long time that natural lignoceric acid is a mixture of near homologs that cannot be separated by crystallization methods or fractional distillation of the esters.

The small yellow-solid residue in the filtrate from the lignoceric acid crystallization was oxidized with cold potassium permanganate.[6] This gave a small amount of dihydroxystearic acid, m. p. 129–131°, which indicated oleic acid was a minor constituent. Although several samples of the fatty acid fraction have been examined from the bark, wood,[5] and the lignin residue from the saccharification of Douglas-fir sawdust[3] in this laboratory, melissic acid, $C_{31}H_{62}O_2$, thus far has not been actually found in any of them.

Ferulic Acid.—A brown resin remained suspended in the aqueous liquor after the extraction of the lignoceric acid. This was soluble in ethyl ether and incompletely soluble in benzene. The aqueous liquor was shaken out repeatedly in a separatory funnel with ethyl ether. After removal of the solvent and drying in the oven at 105° the brown residue weighed 4.4 g. (22%). Attempts at redissolving the dry residue with small amounts of ethyl ether or benzene left a crust of crystals, m. p. 168 to 169°. Recrystallization from hot benzene gave fine crystals, m. p. 169–170°; whereas recrystallization from dilute ethanol gave yellow prisms, m. p. 169 to 170°. The crystals were very soluble in alcohol, and soluble in warm sodium bicarbonate solution. Titration with standard base indicated an acid with a neut. equiv. around 190; an exact end-point was not obtained with phenolphthalein indicator. Tests with ferric chloride gave a pink to red coloration which indicated a para substituted phenol. Methylation with dimethyl sulfate in sodium hydroxide solution gave white crystals insoluble in acid solution. Oxidation of the methylated product with potassium permanganate gave veratric acid, m. p. 180°. The acetyl derivative prepared with acetic anhydride and pyridine melted at 196–197°. Ferulic acid, 4-hydroxy-3-methoxycinnamic acid, melts at 168–169° (170°) and the acetyl derivative melts at 196–197°. Calcd. for $C_{10}H_{10}O_4$: OCH_3, 15.9. Found: OCH_3, 15.9.

In subsequent work on the isolation of ferulic acid from the aqueous layer remaining after the fatty acid separation, it was found that benzene extraction afforded the most convenient means of separation and purification.

Water Layer.—The aqueous layer from the ferulic acid separation on neutralization and evaporation to dryness gave no indication of the presence of other wax constituents.

Summary

The wax extracted with hexane from Douglas-fir bark was examined for its properties and chemical constituents. Based on the saponification products, this wax contains approximately 20% lignoceryl alcohol, 60% lignoceric acid, and 20% ferulic acid (4-hydroxy-3-methoxycinnamic acid). Phytosterol is a minor constituent and oleic acid may be present. The acid constituents are present in both the free and combined states.

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[4] Ripper and Heiduschka, *Ber.*, **56**, 1736 (1923).

[5] Graham and Kurth, *Ind. Eng. Chem.*, **41**, 409–414 (1949).

[6] Lapworth and Mottram, *J. Chem. Soc.*, **127**, 1629 (1925).