HEARTWOOD EXTRACTIVES OF A WESTERN LARCH TREE (*LARIX OCCIDENTALIS* NUTT.)[1]

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ABSTRACT

Heartwood meal of a western larch (*Larix occidentalis* Nutt.) was successively extracted with solvents of increasing polarity. The total extractable material collected was 15%. Compounds in each solvent fraction were separated by column chromatography followed by preparative-layer chromatography. Some isolates were characterized by comparison with standards, IR, UV, and NMR spectra, and various chromatographic parameters.

Compounds previously unreported found in western larch heartwood were: four resin acids (0.2% total yield), sandaracopimaric, isopimaric, abietic and dehydroabietic, together with larixol and larixyl acetate from the petroleum ether extract; pinocembrin (5,7-dihydroxyflavanone) from the benzene extract (0.003% yield); isolariciresinol from the ethanol extract; and free L-arabinose and secoisolariciresinol from the water extract.

Additional keywords: Chromatography, resin acids, lignans, flavonoids, L-arabinose, larixol, larixyl acetate.

INTRODUCTION

Compounds of various chemical classes are known to occur in western larch (*Larix occidentalis* Nutt.) wood extractives. For example, the flavonoids, dihydroquercetin (3, 5, 7, 3′, 4′-pentahydroxy flavanone), dihydrokaempferol (3, 5, 7, 4′-tetrahydroxy flavanone), and quercetin (3, 5, 7, 3′, 4′-pentahydroxy flavone) have been described and studied [Barton and Gardner 1958; Gardner and Barton 1960], and western larch arabinogalactan [Ekman and Douglas 1962] has been intensively investigated.

The purpose of the present investigation was to isolate and characterize heartwood extractives from western larch. There is an extensive literature on studies of other *Larix* species. For example, the above flavonoids plus kaempferol were found in *L. leptolepis* by Demachi et al. [1968]; Norin et al. [1965] have determined the structure of the diterpenes larixol and larixyl acetate found in the oleoresin of *L. europa* and *L. sibirica*; Leptova et al. [1971] found the lignans conidendrin, pinoresinol, lariciresinol, isolariciresinol, secoisolariciresinol, and divanillyltetrahydrofuran in the heartwood of *L. dahurica* and *L. sibirica*.

In North America, Nair and von Rudloff [1959] obtained dihydroquercetin (0.3%)[3] and dihydrokaempferol (0.05%), as well as traces of quercetin and eicosanyl ferulate from the acetone extract of tamarack (*L. laricina*). Saponification of neutrals gave phthalic acid (1.9%) and long-chain fatty acids (31.7%), sitosterol (19.5%), eicosanol

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[3] Yields quoted by Nair and von Rudloff were calculated on acetone solubles basis; they have been recalculated here and are quoted on an O.D. wood basis.

(2.2%) and 2-nonanol (3.1%). No resin acids were identified. The same workers isolated dihydroquercetin and dihydrokaempferol in 1.25% and 0.82% yields, respectively, from the acetone extract of subalpine larch (*L. lyalli*) heartwood [Nair and von Rudloff 1960]. Small amounts of conidendrin were also isolated. Other compounds obtained from the extracts were β-sitosterol, 2-nonanol together with two unidentified alcohols, and phthalic, palmitic, a C_{16} and a C_{20}, stearic, oleic, linoleic, linolenic acids were also isolated.

The present work was undertaken to establish the existence or absence of the above compounds in the extractives of western larch heartwood.

EXPERIMENTAL

Wood was obtained from the trunk of an 89-year-old tree grown near Armstrong, B.C., felled in 1972 and shipped to the Western Forest Products Laboratory. Cross sections were cut and the heartwood was separated from the sapwood. The heartwood was split into pieces, air-dried, and ground in a Wiley mill to pass a 5-mm sieve. The wood meal was air-dried and its moisture content determined on four samples.

Extraction procedure

Air-dried wood meal (926 g O.D. basis) was weighed into a large cloth thimble placed in a 12 *l* soxhlet and was successively extracted for 48 h each with 8 *l* of the following distilled solvents in the order listed. Percent yields (in parentheses) are calculated on an O.D.-wood basis.

i. petroleum ether (65°–80°) (0.7);
ii. benzene (0.2);
iii. benzene-ethanol 1:1 (1.3);
iv. ethanol (0.6);
v. distilled water (11.9);
vi. ethanol (0.3).

Each solvent was evaporated from the wood meal before the next extraction. After extraction, solvent was removed from the extract in a tared flask at reduced pressure on a rotary evaporator.

Chromatography

Column chromatography (CC) was the separation method for the above extracts. Chromatographic media employed were primarily silica-gel, Sephadex A25, and Sephadex LH20. Separation on a silica-gel column was always preceded by a silica-gel TLC (see later) of the material. Two solvent systems were employed to elute the column. Stepwise increase in eluting solvent polarity from petroleum ether (65°–110°) was obtained by addition of varying amounts of ethyl acetate. Similarly ethanol was added stepwise to benzene. Fractions were collected in 25-ml increments. A 45 cm × 3.7 cm column was used first to separate the components. Subsequently, a smaller 28 cm × 1.6 cm column was used for purification, eluting with a less polar solvent mixture.

The method of Zinkel and Rowe [1964] was used (CC with Sephadex A25) for separation of acids (fatty and resin) from neutrals.

CC with Sephadex LH20 was used in a search for flavonoid glycosides [Repas et al. 1969]. The eluting solvent was 100% ethanol.

Thin-layer chromatography (TLC) was used to compare compounds (R_f values) in column eluates from extracts with standards. Quantum Industries Analytical silica-gel GF with gypsum binder and phosphor Q4F plates stored in a 70 C oven to prevent deactivation were used [0.25-mm-thick layer for TLC, and thicker plates (2.5 mm) for separation and purification].

The developing solvents used for silica-gel TLC were: methylene chloride (CH_2Cl_2) and benzene-ethanol, 9:1 (BE). All solvent ratios are given by volume.

Carbonyl compounds were detected by spraying the plates with 2,4 dinitrophenylhydrazine reagent in ethanol (2,4-DNPH); upon heating on a hot plate other compounds were detected.

The identification of 3-hydroxyflavanones, as distinct from other flavonoids, was made on silica-gel TLC plate by the method of Barton [1968]. Other nonspecific detecting

reagents were iodine vapor and mixed nitric-sulphuric acids.

Cellulose TLC plates were used with the developing solvent n-butanol, acetic acid, and water, 60:15:25 (BAW). Detection of carbohydrates on cellulose plates was with *p*-anisidine hydrochloride spray, followed by heating in an oven (110 C) for 15 min.

Silver nitrate-impregnated alumina TLC was used for the fatty and resin acid methyl esters. Glass plates (20 cm × 20 cm) were coated (0.25 mm) with a slurry of alumina (aluminum oxide G with binder, Research Specialities Co.) and silver nitrate solution. They were dried at 110 C for 30 min before use. The developing solvent was diethyl ether-petroleum ether (40–60°) (1:3). Concentrated sulphuric acid and diethyl ether, 1:4, was used as spray reagent. The plate was heated in an oven (110 C) for 15 min and then charred (200 C) for 1 h [Zinkel and Rowe 1963].

Reverse-phase TLC (RPC) was used for the most lipophilic neutrals. A cellulose plate was washed with 10% Nujol in petroleum ether. The petroleum ether was evaporated from the plate and after spotting, the developing solvent was methanol saturated with Nujol, and 2,4-DNPH detecting reagent.

Paper chromatography (PC) was used for flavonoids and lignans. R_f values, colors under visible and ultraviolet light and ease of color formation with reagents such as DSA make such compounds readily identifiable.

Descending one-dimensional (1-D) and two-dimensional (2-D) PC were run on Whatman No. 1 paper. The developing solvent systems were BAW above; and the top layer of a mixture consisting of n-butanol, concentrated ammonia and water, 20:3:10 (BNW); and 2% acetic acid in water (AW). Detecting reagents were DSA and Barton's reagent (0.5% aq. ferric chloride and 0.5% aq. potassium ferricyanide).

Gas-liquid chromatography (GLC) with a 10% EGSS-X on 100–120 mesh Gas Chrom Q stainless-steel column (5 ft × ⅛ in) gave good separations of fatty acids and resin acids (Me esters), and also of neutrals obtained in the petroleum ether extract. An Aerograph 204 was used with injection temperature 250°, detector (F.I.D.) temperature 275°, and the column oven was programmed from 150° to 250° at 5° per min.

Chemical techniques

O-methyl esters and O-methyl ethers were obtained for acids and phenols, respectively, by treating the compounds to be methylated with diazomethane in ether-methanol.

Fatty acids were separated from resin acids by precipitation of their amine salts. Mixtures containing both were treated with 10% cyclohexylamine in ethanol. The resin acid amine salts precipitated, while the fatty acid amine salts remained in solution. The precipitate was collected, washed with ethanol, partitioned between chloroform and 1 *N* hydrochloric acid, and the hydrochloric acid layer was discarded. The chloroform was evaporated. The residues are methylated (above) to obtain the resin acid methyl esters. The solution containing the fatty acid amine salts was similarly partitioned between chloroform and 1 *N* hydrochloric acid, and the chloroform layers were then concentrated and methylated (above) to obtain the fatty acid methyl esters.

Saponification of esters (in the neutrals) converted the esters to fatty acids and alcohols. The fatty acids were identified after methylation by using GLC. The saponification reagent was ethanolic sodium ethoxide. This reagent and the material to be saponified were heated under reflux for 1-½ h. The solution was allowed to cool and 1 *N* hydrochloric acid was added to pH 3. The fatty acids were extracted with petroleum ether (65°–80°) and the solvent evaporated to dryness. The collected fatty acids were methylated as above.

Spectroscopy

Nuclear magnetic resonance (NMR) spectra were obtained on a Varian HA-100, 100 MHz NMR spectrometer. The samples were dissolved in either chloroform-d, acetone-d_6, or deuterium oxide, depending on com-

TABLE 1. *Heartwood extractives of various* Larix *species*

	L. europa	*L. siberica*	*L. dahurica*	*L. laricina*	*L. lyalli*	*L. leptolepis*	*L. occidentalis* (1)	*L. occidentalis* (2)
dihydroquercetin				X	X	X	X	X
dihydrokaempferol				X	X	X	X	X
quercetin				X		X	X	X
larixol	X	X						X
larixyl acetate	X	X						X
conidendrin		X	X		X			X
pinoresinol		X	X					
lariciresinol		X	X					
isolariciresinol		X	X					X
secoisolariciresinol		X	X					X
L-arabinose								X
divanillyltetrahydrofuran		X	X					
arabinogalactan							X	X
eicosanyl ferulate				X				X
pinocembrin								X
sistosterol				X	X			X
sistosterol palmitate								X
2-nonanol				X	X			
resin acids								X
long-chain fatty acids				X	X			

(1) - from other studies
(2) - from this study

pound solubility. Tetramethylsilane was added as an internal standard and lock signal at $\delta = 0$. Sample dilution was approximately 15 mg/300 μl of solvent.

Infrared spectroscopy (IR) was performed using a Perkin-Elmer 521 Infrared Spectrophotometer to obtain spectra of samples, either in potassium bromide pellets or as smears on sodium chloride plates.

Ultraviolet and visible spectra were obtained on a Beckman DK-2 spectrophotometer with samples dissolved in ethanol.

RESULTS AND DISCUSSION

The total amount of extract obtained from the heartwood of western larch by successive extraction with five solvents of increasing polarity and finally with ethanol was 15%. Individual compounds were identified, provided the standard itself was available as a reference, and the amount present in the wood was in some cases quantitatively determined. The presence of some compounds could only be inferred from literature descriptions of compounds found in other *Larix* species, and some compounds expected to be present were not found. The compounds present in the extractives removed by each of the solvents are discussed below in order of increasing solvent polarity. Table 1 collectively summarizes the literature data and compounds found in this research.

Petroleum ether (65°–110°) solubles (0.7%)

Separation of the neutrals from the acids (fatty and resin) was effected by CC of a portion of the petroleum ether extract on Sephadex A25 [Repas et al. 1969]. Total yield of fatty acids was 0.1%; resin acids yield was 0.017%. The compounds found are discussed in the order neutrals, fatty acids, and resin acids as follows.

A portion of the neutrals was fractionated by CC on silica gel using gradient elution of petroleum ether with increasing concentrations of diethyl ether. Fractions were examined by TLC and like fractions com-

bined. Individual compounds were obtained by preparative TLC. Sitosterol, sitosteryl palmitate, eicosanyl ferulate, larixol and larixyl acetate (these are their first reported occurrence in western larch), and fats were indicated and identified by other chromatographic techniques, e.g., reversed-phase TLC. Larixol and larixyl acetate were not found in the oleoresin from *L. occidentalis* (Mills 1973), but several resin acids and other neutral diterpenes, e.g. thunbergol, epimanool, were present. The fats were saponified and the fatty acids (after methylation) were palmitic, stearic, linolenic, and arachidic.

The free acids (after methylation) were examined by GLC and this fraction contained palmitic, palmitoleic, linoleic, linolenic, and arachidic acids. Dimethyl phthalate was not observed; phthalic acid esters probably do not occur in *Larix* spp. Their discovery in *L. laricina* by Nair and von Rudloff [1959] may have been an error, as the esters came not from the wood but from plasticizers in their solvents.

The resin acids were of interest because they have not been reported previously in this wood. Nair and von Rudloff [1959] had reported minor amounts of uncharacterized C_{20} acids in *L. laricina*. The resin acids found in this study (0.017%) were methylated and examined by GLC. Those present were sandaracopimaric (minor), isopimaric (major), abietic (minor), and dehydroabietic (minor) acids. The identity of each particular fatty and resin acid (methylated) was confirmed by argentative TLC [Zinkel and Rowe 1963].

Benzene solubles (0.2%)

The benzene soluble fraction was fractionated by CC on silica gel using gradient elution of benzene with increasing concentrations of ethanol. Like fractions were combined and rechromatographed on a smaller column using the same solvent system. Individual components were collected and dried, and three were crystallized. These were dihydrokaempferol, pinocembrin (the first time reported from this wood, 0.003%), and α-conidendrin. Their 2-D PC and TLC behavior, and their NMR and IR spectra were identical to standards. Two minor phenolic components were isolated after the above compounds were eluted from the column. Color tests in various TLC and PC systems suggested that the compounds were both lignans, but amounts were insufficient for any structural identification.

Benzene-ethanol (1:1) solubles (1.3%)

A portion of this extract was placed on a large column and purified by CC as described above for the benzene solubles. Three compounds isolated were dihydrokaempferol, dihydroquercetin (the major component), and quercetin.

Ethanol solubles (0.6%)

A 2-D PC of this extract (BAW and AW solvents) showed that it was almost all dihydroquercetin. The extract was therefore crystallized from ethanol. After removing the crystalline dihydroquercetin on a filter, the mother liquors were fractionated by CC with the benzene-to-ethanol gradient elution solvent system. Minor components of this extract were combined, and two were further purified by preparative TLC. Their spectra and TLC R_f values were close to the published data on 3,4-divanillyltetrahydrofuran and isolariciresinol [Leptova et al. 1971; Anderegg and Rowe 1974], but only the latter was available as a standard and its identity was proved by TLC and PC comparison with the isolated isolariciresinol. The mother liquors were also examined for flavonoid glycosides [Repas et al. 1969], but none were found. This confirmed the work of Hergert and Goldschmid [1958], who found dihydroquercetin-3-glucoside in the sapwood but not the heartwood of *L. occidentalis*.

Water solubles (11.9%)

This extract was dried and washed twice with ethanol. The ethanol insoluble gum (11.10%) was the well-known arabinogalactan [Ekman and Douglas 1962]. The mother liquors were examined by TLC and PC;

the phenolics isolated by CC contained the lignan, secoisolariciresinol, as shown by TLC and PC comparison with a reference standard.

The mother liquors were fractionated by CC on Sephadex LH20 with ethanol as eluant [Repas et al. 1969]. Flavonoid glycosides again were absent, but one fraction was obtained from this column and crystallized. PC, IR spectrum, and comparison with standards showed that it was L-arabinose, found for the first time in this wood. Its presence was not unexpected, since it could come from decomposition of the arabinogalactan or be its precursor.

Final ethanol extraction (0.3%)

After the water extraction, the wood meal was dried and then reextracted with ethanol to determine the yield of ethanol-solubles available after removal of the arabinogalactan, which may have acted as a membrane substance preventing complete removal of the extractives in the first place. PC and CC on Sephadex LH20 showed this extract contained a series of five highly polar phenolics (not examined further), more L-arabinose, and some Braun's native lignin.

CONCLUSIONS

This work confirmed that the two major extractives of western larch are arabinogalactan (11.1%) and dihydroquercetin (about 0.5%). The remaining compounds in the extractives are the same as those described in the literature for European and Russian larches summarized in Table 1. No conclusions may be drawn about the absence of several compounds searched for in this work, because only one tree was investigated here. Further research might show variation of extractives in western larch within its growth range.

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