NEW POLYOXYPHENOLS FROM WESTERN HEMLOCK SAPWOOD[1]

G. M. Barton

Department of Fisheries and Forestry, Canadian Forestry Service, Forest Products Laboratory, Vancouver 8, British Columbia

ABSTRACT

Mild treatment of western hemlock (*Tsuga heterophylla*) sapwood sawdust with methanol resulted in extractives largely uncontaminated with colored polymeric material. Further separation into chloroform solubles followed by gel column chromatography and preparative layer chromatography resolved the extractives into cyclitols and sugars (0.66%); unknown phenolics (0.13%); chloroform soluble nonphenolics (0.12%); phenolic glycosides (0.12%); previously reported polyoxyphenolics such as α-conidendrin (0.01%), hydroxymatairesinol (0.10%), matairesinol (0.02%), and catechin (0.05%); and two new compounds, liovil (0.01%) and a coumaran (0.05%). Liovil previously was found only in black spruce (*Picea mariana*), and the coumaran is a new lignin dimer with a unique β, γ-linkage. Its structure has been determined as 2-(α-hydroxy-vanillyl)-5-(ω-hydroxypropyl)-7-methoxy coumaran by a combination of nuclear magnetic, infrared, and mass spectroscopy as well as chemical analysis and degradation.

The discovery of liovil in western hemlock reinforces the similarity in extractives between hemlock and spruce and should be of interest to taxonomists to reexamine the genetic relationship between the two genera. The new coumaran, on the other hand, will be of particular interest to lignin chemists who have postulated phenylcoumaran linkages in lignin.

The better utilization of western hemlock [*Tsuga heterophylla* (Raf.) Sarg] for lumber and pulp depends on a thorough understanding of its chemical extractives. Of these extractives the polyoxyphenols are the most important for brown stain on sapwood lumber and low brightness of groundwood pulp. Recent papers (Barton and Gardner 1966; Barton 1968) have explored this aspect in detail. In addition, however, polyoxyphenols are often useful in providing clues in the wider interests of chemotaxonomy and lignin biosynthesis, to which a valuable introduction has been made by Goldschmid and Hergert (1961). This paper reports the discovery of two more extractives, a unique lignin dimer I with an unusual β, γ-linkage and the lignan liovil (II) found previously only in black spruce [*Picea mariana* (Mill.) B.S.P.] (Freudenberg and Knof 1957).

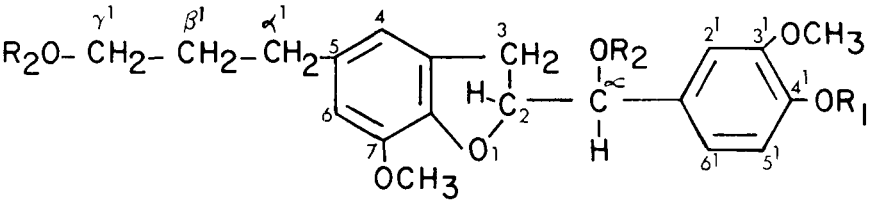
EXPERIMENTAL

Starting material

A 40-inch-long bolt of western hemlock (age 42 yr) 11 inches in cross section was obtained from the Point Grey Campus of the University of British Columbia, Vancouver, B.C. The bark and a 3-inch-diameter core of heartwood were discarded, and the remainder was reduced to wood meal by grinding in an intermediate Wiley mill to pass a 20-mesh screen.

A total of 19 kg of sapwood (moisture-free basis) was extracted in five batches with a total of 164 liters of methanol. In a typical extraction, 4 kg of sapwood sawdust (moisture content 41%) was steeped in 27 liters of methanol for 24 hr at room temperature. The methanol extract was filtered and concentrated to 2 liters under water-tap vacuum at 50 C. Further concentration would result in precipitation of water insolubles, owing to the lowering of the methanol-to-water ratio.

[1] The author is indebted to many scientists for key reference compounds and helpful advice. The following men are gratefully acknowledged: Drs. E. Adler, K. Freudenberg, J. A. F. Gardner, L. O. Hall, J. M. Harkin, J. P. Kutney, J. F. Manville, H. Nimz, I. A. Pearl, J. M. Pepper, E. Piers, K. V. Sarkanen, R. Segal, A. F. A. Wallis. Technical assistance by A. C. Harris and C. R. Daniels is gratefully acknowledged.



I $R_1 = R_2 = H$

Ia $R_1 = CH_3, R_2 = H$

Ib $R_1 = CH_3, R_2 = CH_3CO$

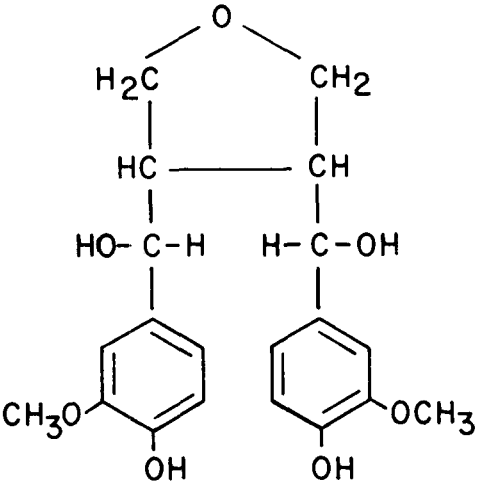


FIG. 1. New polyoxyphenols from western hemlock sapwood.

The concentrated extract from the preliminary processing treatment was extracted with boiling chloroform. In a typical extraction, a 2-liter quantity of the concentrate was stirred vigorously with 500 ml of chloroform under reflux for 4 hr. The solution was cooled, and the chloroform solubles were removed in a separatory funnel. This process was repeated until all of the concentrated extract had been chloroform extracted. Chloroform removed 46 g of solids (0.24% of the moisture-free wood). The remainder methanol-water extract contained 200 g of solids (1.06% of the moisture-free wood).

Composition of the chloroform and methanol-water solubles

Based on thin-layer chromatographic (TLC) separations in two solvent systems chloroform-methanol 7:1 and 7:3 and detection with diazotized sulfanilic acid (DSA), as well as charring with sulfuric-nitric acid (1:1), the composition of these two main fractions was estimated to be as in Table 1.

Separation of polyoxyphenols

Two general methods were used to separate the components described in Table 1

TABLE 1. *Estimated composition of chloroform and methanol-water solubles in percentage*

Chloroform solubles	%	Methanol-water solubles	%
Nonphenolics	46	Cyclitols and sugars	62
α-conidendrin	6	Hydroxymatairesinol	6
Hydroxymatairesinol	14	Coumaran I	4
Matairesinol	10	Phenolic glycosides	11
Liovil (II)	3	Catechin	5
Coumaran I	6	Unknown phenolics	12
Unknown phenolics	15		100
	100		

from the mixture, namely preparative layer silica gel chromatography and Sephadex gel column chromatography.

(a) *Preparative layer chromatography*

Glass plates 20 × 40 cm were spread with a silica gel G slurry in the usual manner to a thickness of 0.75 mm. The plates were allowed to dry at room temperature until they could be removed from the spreader, at which time drying was completed by infrared heating. After cooling, the plates were streaked with concentrated chloroform or methanol-water solubles by means of the hypodermic needle technique (Barton 1967). In a typical operation, 10 g of chloroform solubles dissolved in 25 ml of chloroform were applied to six large plates. The plates were developed in chloroform-methanol (6:1) and the bands located by using ultraviolet light and spraying a portion of the plate with DSA. The bands were removed with a spatula and eluted with hot methanol-chloroform (1:1). Because of the small differences in R_f values, overlapping of components was unavoidable, and it was often necessary to rechromatograph using preparative TLC techniques.

(b) *Sephadex gel column chromatography*

Separations of hemlock polyoxyphenols were made on a column (2.5 × 45 cm) packed with either LH-20 Sephadex for the chloroform solubles or G-25 Sephadex for the methanol-water solubles. In a typical run, 4.0 g of chloroform solubles were added to the LH-20 gel, which had been packed in ethanol and the ethanol displaced with chloroform. Chloroform was used as the eluant. Unknown high R_f [chloroform-methanol (7:1)] compounds as well as colored impurities were eluted first, followed by α-conidendrin, hydroxymatairesinol, liovil (II), and an unknown phenolic. The coumaran I and further unknown compounds were eluted only after 5 to 6 liters of eluant had been collected. In the case of the methanol-water solubles, 0.6 g were added to a G-25 medium gel that had been swelled and packed in 0.1 molar aqueous acetic acid. Elution with 0.1 molar aqueous acetic acid resulted in the cyclitols and sugars being removed in the first fraction followed by phenolic glycosides, unknown phenolics, hydroxymatairesinol, coumaran I, and catechin.

Identification of coumaran I

(a) *Chemical and spectroscopic properties*

Milligram quantities of the coumaran, obtained from preparative TLC [R_f 0.2 in chloroform methanol (7:1)] and gel-column chromatography, failed to crystallize, although TLC indicated purity. A positive test for phenolics resulted with the ferric-ferricyanide reagent (Barton, Evans, and Gardner 1952) and a bright orange color, indicative of an α-hydroxy vanillyl derivative (Goldschmid and Hergert 1961), was obtained with DSA. A positive Gierer test (Gierer 1954) further supported this concept.

Spectra: λ max 283 (log $\epsilon = 3.78$) λ min 257 mμ (methanol) (Beckman DK-2); νKBr 740(w), 800(br.m), 850(m), 1030 (s), 1120(m), 1150(w), 1200(w), 1230 (w), 1270(s), 1340(br.m), 1430(m), 1450

(s), 1490(w), 1500(s), 1600(s), 2850 (w), 2870(w), 2925(m), 34(br.s), cm^{-1} (Perkin-Elmer 521)[2].

The integrated n.m.r. spectrum (deuterioacetone) indicated 24 protons and included signals showing the presence of five aromatic protons (τ 3.02–3.50); one proton, a doublet, centered at 4.58; six protons corresponding to two methoxyl groups at 6.28; two protons, a triplet, centered at 6.5; two protons, a triplet, centered at 7.5; two protons, a multiplet, centered at 8.26; four protons hidden under the methoxyl region (6–6.4); and two protons accounted for by a rise in the base line (6.6–7.3). (Varian HA-100). The molecular weight by mass spectroscopy was 360 (Nuclide 12-90-C); the most important peaks together with their relative m/e intensities were 360(3), 346(14), 328(50), 316(M-44, 61), 313 (22), 269(16), 153(17), 151(22), 149(23), 137 (100). No optical activity was noted during an optical rotary dispersion examination (JASCO Model ORD/UV5 spectropolarimeter).

(b) *Degradation studies*

Methylated coumaran Ia (10 mg), made by treatment of I with diazomethane, was refluxed in benzene (2 ml) for one hour with 2, 3-dichloro- 5, 6-dicyano- 1, 4-benzoquinone (DDQ)(6.6 mg). Solvent was removed under vacuum, and a 1-ml mixture of acetic acid-hydrogen peroxide (3 ml glacial acetic acid, 0.3 ml of 30% hydrogen peroxide) was added. After heating under reflux at 60 C for 4½ hr, water (3 ml) was added and the mixture evaporated to dryness under vacuum. The residue was dissolved in 2 N potassium hydroxide (1 ml) and heated on a water bath (100 C) for two hr. After cooling, the extract was acidified with 6 N sulfuric acid, extracted three times with ethyl acetate and the ethyl acetate extract concentrated on a TLC plate by multiple spotting. The TLC plate was developed in chloroform-acetone-acetic acid (2:7:1) and detected with 3% aqueous ferric chloride. Formation of a blue spot at R_f 0.7 corresponded to one from an authentic sample of styraxinolic acid (Segal, Milo-Goldzweig, Sokoloff, and Zaitschek 1967).

(c) *Preparation of derivative Ib*

A small quantity of coumaran I (50 mg) was methylated with an excess of diazomethane in methanol. Acetylation overnight at room temperature with a mixture of anhydrous pyridine and acetic anhydride (1:1) produced acetate Ib, which did not crystallize. The product was purified by TLC on silica gel using cyclohexane-ethyl acetate (3:2) as a developing solvent (R_f 0.23). Elemental analysis (Mr. Peter Borda, Chemistry Department, University of British Columbia) gave C, 65.6%; H, 6.59%; calculated for structure Ib, $C_{25}O_8H_{30}C$, 65.5%; H, 6.55%.

Spectra: νKBr 720(w), 850(w), 1040 (s), 1150(m), 1240(br.s), 1360(m), 1380(w), 1420(w), 1450(w), 1470(m), 1500(w), 1520(s), 1600(br.m), 1730(s), 2950(w) cm^{-1} (Baird Atomic KM-1).

The integrated n.m.r. spectrum (carbon tetrachloride) indicated 30 protons and included signals showing the presence of five aromatic protons (τ 3.28–3.54); one proton, a doublet, centered at 4.74; two protons, a multiplet, centered at 5.80; two protons, a triplet, centered at 6.10; three protons, a singlet, at 6.25; six protons, a singlet, at 6.32; one proton, a multiplet, centered at 6.50; two protons, a triplet, centered at 7.50; six protons, a singlet, at 8.10 and two protons, a multiplet, centered at 8.2. (Varian HA-100).

Spin-spin decoupling-irradiated multiplet at 6.50 τ, doublet at 4.74 collapsed to a singlet and multiplet at 5.80 changed shape; irradiated doublet at 4.74, multiplet at 6.50 changed shape; irradiated multiplet at 8.2, triplets at 7.5 and 6.10 collapsed to singlets. Protons on the following carbon atoms can be assigned as: C-α, doublet 4.74; C-3, multiplet, 5.80; C-γ', triplet, 6.10; C-4′, singlet, 6.25; C-3′ & C-7, singlet, 6.32; C-2,

[2] (w) = weak, (m) = medium, (s) = strong and (br) = broad.

multiplet, 6.50; C-α', triplet, 7.50; C-β', multiplet, 8.2.

Identification of liovil II
Chemical and spectroscopic properties

A bright orange spot (DSA) that ran in front of the coumaran I in chloroform-methanol (7:1), R_f 0.54 formed crystals, melting at 173–174 C from a chloroform solution. These crystals gave no depression in melting point when mixed with liovil (II) (m.p. 173.5–174.5°) (Freudenberg and Knof 1957) obtained from black spruce. Crystals from both spruce and hemlock gave identical quantitative infrared spectra. Elemental analysis (Clark Microanalytical, Urbana, Illinois) gave C, 63.8%; H, 6.87%; OCH_3, 15.68%.

Calculated for liovil, $C_{20}O_7H_{24}$ - C, 63.8%; H, 6.42%; OCH_3, 16.49%.

Spectra: νKBr 470(w), 550(w), 610(w), 630(br.m), 720(m), 760(m), 775(m), 795(w), 820(m), 840(m), 845(m), 880 (w), 900(w), 920(s), 925(m), 929(w), 1000(m), 1035(s), 1060(w), 1080(m), 1110(w), 1120(m), 1150(m), 1170(w), 1230(m), 1260(s), 1310(w), 1330(w), 1370(br.m), 1420(s), 1460(br.s), 1510 (s), 1600(s), 2840(w), 2890(w), 2910 (w), 2950(br.m), 2990(w), 3330 (br.s), cm^{-1} (Perkin-Elmer 521).

The integrated n.m.r. spectrum (deuterated dimethyl sulfoxide) indicated 24 protons and included signals showing the presence of two protons, a multiplet, centered at τ 1.4; six aromatic protons, a multiplet, centered at 3.22; two protons, broad singlets at 3.8, 4.2, 4.6 and 4.8; two protons, multiplets at 5.05 and 5.5; six methoxyl protons, a singlet, at 6.25; four protons, a doublet centered at 6.54 (Varian HA-100).

The molecular weight by mass spectroscopy was 376 (Hitachi, Morgan-Schaffer Corp., Montreal, Quebec); the most important peaks together with their relative m/e intensities were 376(9), 358(M-18, 4), 340 (M-36, 3), 206(14), 153(51), 138(42) and 137(100). Calculation of (P + 1)/P ratio agreed with the empirical formula $C_{20}O_7H_{24}$.

TABLE 2. *Estimated yield of major methanol-soluble sapwood extractives on moisture-free wood basis in percentage*

Cyclitols and sugars	0.66
Unknown phenolics	0.13
Chloroform soluble nonphenolics	0.12
Phenolic glycosides	0.12
Hydroxymatairesinol	0.10
Catechin	0.05
Coumaran I	0.05
Matairesinol	0.02
α-conidendrin	0.01
Liovil (II)	0.01
	1.27

DISCUSSION OF RESULTS

Room temperature methanol extraction of hemlock sapwood gave only 1.27% (Table 2) of total extractives compared with an average of 2.4% for an alcohol-benzene soxhlet extraction. The former system was chosen, however, not for high yields but to minimize structural changes due to excessive heating as well as to limit the extraction of higher molecular weight colored impurities. The relatively low amount (0.13%) of these unknown phenolics (Table 2) would indicate the success of this procedure.

Subsequent treatment with boiling chloroform resulted in a further removal of highly colored impurities as well as separating the cyclitols, sugars, and phenolic glycosides from the main lignan group. The presence of hydroxy-matairesinol and courmaran I in both chloroform and methanol-water solubles (Table 1) illustrates the difficulty in purifying hemlock polyoxyphenols on the basis of solubility differences alone and the need for chromatographic techniques.

The application of preparative layer and gel column chromatography in particular to the separation of the two main fractions of chloroform and methanol-water soluble extractives has been extremely useful. The latter technique using Sephadex LH-20 gel with chloroform resulted in the primary elution of high molecular weight colored impurities as would be expected from gel permeation theory. The majority of the components, however, were eluted not on

the basis of molecular weight but according to the number of hydroxyl groups present. Thus α-conidendrin, hydroxymatairesinol, and liovil II were collected in that order. Coumaran I did not follow this pattern no doubt because of its different ring structure.

The detection of coumaran I on silica gel thin-layer plates was enhanced by its reaction with DSA. The initial orange color development with this reagent, diagnostic for α-hydroxy guaiacyl compounds, changes to a characteristic red after several hours. This color change has been useful in its purification from closely associated α-hydroxy guaiacyl compounds in hemlock extractives that remain yellow-orange after detection with DSA.

The proposed structure for I was strongly suggested by n.m.r. studies of I and its chromatographically pure derivative, Ib. In particular, spin-spin decoupling experiments were useful in assigning the dihydrobenzosuran protons as well as those of the 3-hydroxypropyl side chain. Mass spectrometry confirmed this side chain structure by virtue of the very strong M-44 peak attributable to the removal of a C_2H_4O fragment from the side chain (Segal, Milo-Goldzweig, Sokoloff, and Zaitschek 1967). Color reactions of I with DSA and quinone monochloroimide (Gierer 1954) reagents established the presence of the α-hydroxy guaiacyl pendant ring. Degradation studies paralleled those of Segal (Segal, Milo-Goldzweig, Sokoloff, and Zaitschek 1967) once the dihydrobenzofuran had been aromatized with DDQ.

The phenylcoumaran linkage in lignin proposed by Freudenberg and investigated by Adler (Adler, Delin, and Lundquist 1959) dealt only with the α, β-type as exemplified in the model compound dihydrodiconiferyl alcohol. The proposed structure I for the hemlock coumaran suggests that lignin also could have some linkages of the β, γ-type. The preferred linkage, however, would be α, β, based on the sealed tube experiments of Nimz (1966), in which α-(4-hydroxy-3-methoxyphenyl) glycerol β-dihydroconiferyl ether rearranged in the presence of water at 100 C to give dihydrodehydrodiconiferyl alcohol. Attempts to obtain evidence of the hemlock coumaran by repeating his experiments failed to produce a detectable amount of I by TLC.

Evidence that the hemlock coumaran also occurs as a glycoside was obtained by hydrolyzing the phenolic glycoside fraction (Table 1) with 2% aqueous oxalic acid (100 C for 1 hr). Since the glycoside gave a positive phenolic test (orange with DSA) indicating a free phenolic group and since coumaran I has only one phenolic hydroxyl group, the sugar moiety must, therefore, be attached to one of the alcoholic hydroxyl groups. Work on this aspect is continuing.

In view of the potential interest of this coumaran as a model substance to lignin chemists, numerous attempts to synthesize it were tried. Two main routes were examined. The first was a Claisen-type condensation of styraxinaldehyde and chloroacetoveratrone, cyclization to the benzofuran and reduction to give the fully methylated hemlock coumaran I. Alternatively, experiments were designed to condense α-(4-hydroxy-3-methoxyphenyl) glycerol β-dihydroconiferyl ether to give the coumaran directly. Neither route was successful, but interesting intermediates were synthesized. (Results will be published later.)

Confirmation by means of quantitative infrared spectra that the crystals (m.p. 174–175 C) obtained from hemlock and spruce were the same compound, liovil (II), adds to the list of lignans already shown to be common to both genera. These include α-conidendrin, hydroxymatairesinol, oxomatairesinol, matairesinol and pinoresinol (Goldschmid and Hergert 1961; Freudenberg and Knof 1957; Barton and Gardner 1962). Thus far, lariciresinol has been found in spruce (Freudenberg and Knof 1957) but not in hemlock. Also, no trace of the hemolck coumaran I was detected in the one sample of black spruce examined in our laboratory. These common extractives should, however, be of considerable interest to forest taxonomists since it is unusual for tree species so vaguely related

genetically to have so many common extractives.

REFERENCES

ADLER, E., S. DELIN, AND K. LUNDQUIST. 1959. Phenylcoumaran elements in spruce lignin. Acta Chem. Scand., **13**: 2149–2150.

BARTON, G. M. 1967. Application of solutions to preparative chromatoplates using basic thin-layer equipment. Chem. and Ind., **25**: 1079.

———. 1968. Significance of western hemlock phenolic extractives in pulping and lumber. Forest Prod. J., **18**(5): 76–80.

———, AND J. A. F. GARDNER. 1962. The occurrence of matairesinol in mountain hemlock, western hemlock and balsam. J. Org. Chem., **27**: 322–323.

———, AND ———. 1966. Brown stain formation and the phenolic extractives of western hemlock. Dept. of Forest. Publ. No. 1147. p 20.

———, R. S. EVANS, AND J. A. F. GARDNER. 1952. Paper chromatography of phenolic substances. Nature, **170**: 249–251.

FREUDENBERG, K., AND L. KNOF. 1957. Die Lignane des Fichtenholzes. Berichte, **90**: 2857–2869.

GOLDSCHMID, O., AND H. L. HERGERT. 1961. Examination of western hemlock for lignin precursors. TAPPI, **44**: 858–870.

GIERER, J. 1954. Die Reaktion von Chinonmonochlorimid mit Lignin. Acta Chem. Scand., **8**(8): 1319–1331.

NIMZ, H. 1966. A new type of rearrangement in the lignin field. Angew. Chem. Internat. Edit., **5**(9): 843.

SEGAL, R., I. MILO-GOLDZWEIG, S. SOKOLOFF, AND D. V. ZAITSCHEK. 1967. A new benzofuren from the seeds of styrax officinalis L. J. Chem. Soc., **C**: 2402–2404.

KOŠÍK, M., M. DANDÁROVÁ, and R. DOMANSKÝ. 1969. Pyrolysis of beech wood at low temperatures. IX. Chemical variations in beech wood during pyrolysis. *Holzfroschung und Holzverwertung* 21(2): 40–43 (G. ge). Residue of beech-wood pyrolysis was studied. During pyrolysis the residue became richer in nonhydrolyzed substances, and aliphatic double bonds increased steadily. No aromatic portion increase was proved. Treatment of wood with 1N H_2SO_4 caused decomposition reactions to occur at considerably lower temperatures and increased the carbon content of the residue. (A)

KOŠÍK, M., V. REISER, and R. DOMANSKÝ. 1969. Pyrolysis of beech wood at low temperatures. VIII. Conditions of furfural formation during pyrolysis. *Holzforschung und Holzverwertung* 21(2): 38–40 (G.ge). Conditions of furfural formation during pyrolysis of beech treated with sulfuric acid were studied. Furfural yields were increased by water extraction and molecular milling of wood, and yield was best at 250–275 C. Hydrogen was not a suitable filtering gas. Oxygen in the filtering gas was not as harmful as oxygen present during pyrolysis without filtering layer. (A)

ARNDT, U., and H. WILLEITNER. 1969. On the resistance behaviour of wood in natural weathering. *Holz als Roh- und Werkstoff* 27(5): 179–188 (G.e). After weathering treatment, heartwood samples of *Thuja plicata, Sequoia sempervirens, Chlorophora excelsa* and *Tectona grandis* were exposed to the attack of subterranean termites and fungi to study the effect of weathering on the resistance of wood. Results reveal that not all wood species of a high initial resistance possess a truly long-lasting durability. (J.Y.)

MATSUKURA, M., and A. SAKAKIBARA. 1969. On the heterogeneous distribution of chemical structure in lignin. III. *J. Jap. Wood Res. Soc.* 15(1): 35–39 (J.ej). Milled wood lignin, lignin-carbohydrate complex, and wood residue of *Picea jezoensis* were subjected to ethanolysis and oxidation with $KMnO_4$ and nitrobenzen alkali. The products were subjected to gas chromatograph analysis. There are significant differences in quantities of oxidation products among milled lignin, lignin-carbohydrates, and wood residues. It was concluded that there exist heterogeneity of lignin molecules and its distribution. (R.L.)