LIGNAN COMPOUNDS FROM Picea obovata

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The isolation of four lignan compounds from an acetone extract of the wood of Picea sibirica (Siberian spruce) has been reported previously [1-3].

The present paper reports the identification of another four substances and gives a discussion of the NMR spectra of the lignans isolated from spruce.

We isolated two fractions from the petroleum ether-soluble fraction (A) of an acetone extract by percolation through polyamide in the chloroform–methanol (95:5) system. The first fraction, A-1, contained components giving a red coloration on azo coupling with diazotized sulfanilic acid (DSA), and the second, A-2, gave a yellow coloration.

On this basis, it may be assumed that fraction A-1 contains substances in which an aliphatic hydroxyl in the para position to a phenolic hydroxyl is either esterified or is absent, while in the substances of fraction A-2 it is in the free state [4].

The preparative separation of the components of fraction A-1 presents considerable difficulty. Chromatography on polyamide using an aqueous methanol (80:20) eluent system enables some individual compounds to be obtained, but in the working up of the aqueous methanolic eluates the labile compounds present do not withstand the temperature conditions. Consequently, silica gel impregnated with sodium bisulfite was more useful in our work. The compounds were eluted with a low-boiling mixture of chloroform and acetone (95:5).

In addition to conidendrin and oxomatairesinol [1, 2], two crystalline compounds were isolated which we have called B_1 and B_2.

Substances B_1 and B_2, when recrystallized from ethanol, had mp 72-74°C and 120-121°C, respectively. From an analysis of the products of nitrobenzene oxidation and IR spectra (1225, 1200, 1035 cm^{-1}), guaiacyl structures are proposed for both compounds. But the IR spectrum of B_1 shows the characteristic vibration of the carbonyl group of a lactone ring (1756 cm^{-1}), and in B_2 they are absent.

The empirical formula $C_{20}H_{22}O_6$ was established for B_1 on the basis of a peak at m/e 358 in the mass spectrum and from the results of elementary analysis. Substance B_1 was identified as matairesinol and B_2 as pinoresinol. A study of the NMR spectrum of substance B_2 showed its complete similarity to the spectrum of (+)-pinoresinol [5].

In addition to the isolation of matairesinol and pinoresinol, 3,4-divanillyltetrahydrofuran was identified in one of the fractions by TLC on polyamide and silica gel in the water–methanol (40:60) and chloroform–ethyl acetate (40:60) systems, respectively.

The NMR spectra of the lignans isolated from the wood were recorded for the purposes of a structural and analytical investigation. The spectra of a number of lignans (hydroxymatairesinol, liovil, and 3,4-divanillyltetrahydrofuran) were obtained first.

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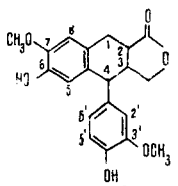
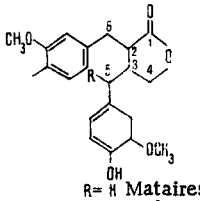
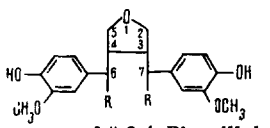
The NMR spectrum of hydroxymatairesinol acetate (Fig. 1) showed seven groups of lines. In the strongest field was a signal from two resonance lines (δ 2.00 and 2.04 ppm) with an intensity ratio of 1:2, which shifted relative to one another with a change in the working frequency of the spectrometer. This signal must be ascribed to the methyl protons of an aliphatic acetate group.



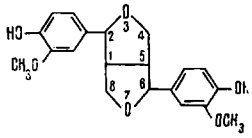
α-Conidendrin



R= H Matairesinol
R=OH Hydroxymatairesinol
R= O Oxomatairesinol



R=H 3,4-Divanillyltetrahydrofuran
R=OH Liovil



Pinoresinol

A similar signal, also consisting of two resonance lines (δ 3.68 and 3.70 ppm) with an intensity ratio of ~ 1:2 was found in a weaker field. This signal is due to the protons of two methoxy groups.

The assignment of the weak-field multiplet in the 6.38-7.05 ppm region and of the singlet in the strong field (δ 2.24 ppm) causes no doubt: the multiplet is due to six aromatic protons and the singlet to the protons of two aromatic acetate groups. The H_2-6, H-3, and H-2 protons give an unresolved multiplet in the 2.48-3.08 ppm region. A signal in the weak field consisting of two doublets (δ 5.58 and 5.74 ppm) is characteristic. The distance between the centers of the doublets varies with a change in the frequency of the spectrometer while the distances between the lines in them remain unchanged. When the sample under investigation was irradiated with an additional radiofrequency field having a frequency corresponding to the center of the multiplet in the 2.48-3.08 ppm region (doublet-resonance method), the doublets changed into a singlet. Consequently, we ascribe these two doublets (intensity ratio 1:2) to the signal of the H-5 proton.

The doublets are due to spin-spin coupling between the H-5 and H-3 protons ($J_{5,3}$ = 5.0 Hz). Finally, the quartet of signals belonging to the lines with centers at δ 3.98 ppm must be ascribed to the two H_2-4 protons. These protons are chemically nonequivalent and they form an AB system. From the spectra taken at three frequencies with allowance for the possible value of the spin-spin coupling constant between them, it can be seen that the appearance of the quartet depends on the splitting of the internal resonance lines of the AB system on spin-spin coupling of the H-2 and H-3 protons ($J_{2,3}$ = 6.0 Hz).

The outer, weaker, lines are apparently lost in the noise. The nature of the signals of the protons of the aliphatic acetate and the methoxy groups and the H-5 proton show the presence of two conformers.

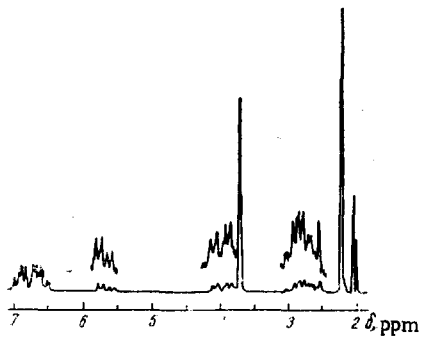


Fig. 1. NMR spectrum of hydroxymatairesinol.

The NMR spectra of liovil with pyridine and of hydroxymatairesinol are similar. The splitting of the signal of the methoxy group into three singlets (δ 3.48, 3.53, and 3.58 ppm) also shows the presence of a mixture of conformers. A detailed description of the NMR spectrum of liovil, which contains four asymmetric centers, is difficult at the present stage because of its complexity.

In the NMR spectrum of matairesinol, a singlet with δ 3.73 ppm is due to the protons of two methoxy groups, and weak-field signals (δ 6.28-6.84 ppm) to the protons of aromatic rings. It follows from a comparison of integral intensities that a broad singlet (δ 2.74 ppm) and a poorly resolved doublet (δ 2.83 ppm) are due to the H-2, H-3, H_2-5, and H_2-6 protons. The two protons of the $-O-CH_2$ group give a multiplet with a center at δ 4.00 ppm, and the two hydroxy protons a broad singlet at 5.56 ppm.

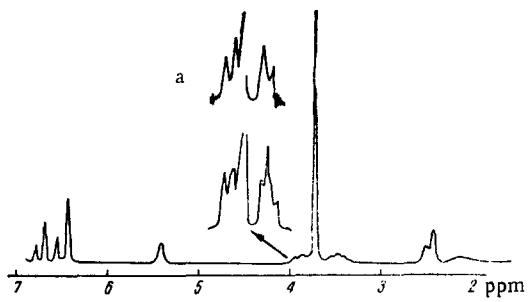


Fig. 2. NMR spectrum of 3,4-divanillyltetrahydrofuran; signals of the H_2-5 and H_2-2 protons with suppression of the signals of the H-3 and H-4 protons (a).

In the NMR spectrum of pinoresinol acetate, a doublet with δ 4.74 ppm is due to the H-2 and H-6 protons ($J_{2,1} = J_{6,5} = 4.5$ Hz), a quartet with δ 4.21 ppm to equatorial protons at C-4 and C-8 ($J_{gem} = 9.2$ Hz; $J^{cis}_{4,5} = J^{cis}_{8,1} = 7.2$ Hz), and a quartet with δ 3.80 ppm to the axial protons on the same carbon atoms ($J_{gem} = 9.2$ Hz; $J^{trans}_{4,5} = J^{trans}_{8,1} = 3.8$ Hz. Such values of the spin-spin coupling constants of these protons are characteristic for the diequatorial isomer of pinoresinol [5]. The protons at C-1 and C-5 are represented by a multiplet in the δ 3.15-2.83 ppm region. A singlet with δ 2.21 ppm corresponds to the six protons of two acetate groups.

In the spectrum of α-conidendrin, the protons of the methoxy groups are more highly nonequivalent ($\Delta\alpha^{OCH_3} = \delta_1^{OCH_3} = 0.07$ ppm) than in the spectrum of its optical isomer obtained by thermal inversion (β-conidendrin, $\Delta\beta^{OCH_3} = 0.03$ ppm). In the spectrum of β-conidendrin, the signals of the methylene protons of the lactone ring are represented by quartets (δ 3.97 and 4.26 ppm) due to the coupling of these protons with one another ($J_{gem} = 9.8$ Hz) and with the H-3 proton (J = 6.5 and 2.7 Hz). The signal of the latter, together with the signals of the H-4 and H_2-1 protons, forms an unresolved multiplet, which prevented us from using the value of $J_{2,3}$ for a conformational analysis of α- and β-conidendrins. This is due to the fact that the distances between the resonance lines in the signal of the H-2 proton (δ 3.65 ppm), some of which are apparently masked by the signals of the protons of the methoxy groups (two singlets with δ 3.72 and 3.75 ppm), are not the true values of the $J_{2,3}$ and $J_{2,1}$ constants. In the spectrum of α-conidendrin, the difference in the values of the chemical shifts of the signals of the methylene protons of the lactone ring decreases and the H-2 signal shifts downfield. These protons are responsible for a multiplet in the 3.58-4.43 ppm region upon which the signals of the protons of the two CH_3O groups (δ 3.67 and 3.74 ppm) are superposed. Two singlets in the strong field ($\delta^{\alpha} = 2.15$ and 2.23 ppm, $\delta^{\beta} = 2.17$ and 2.26 ppm) and also signals in the weak field in the 6.44-7.04 ppm region (α isomer) and the 6.34-6.96 ppm region (β isomer) are due to the protons of acetyl groups and of aromatic rings, respectively.

3,4-Divanillyltetrahydrofuran has a simpler structure of the aliphatic chain. In the NMR spectrum of this compound (Fig. 2), the signals in the strong field (multiplet with δ 2.13 and doublet with δ 2.48 ppm) we assigned to the H-3, H-4, H_2-6, and H_2-7 protons. The multiplet has approximately half the intensity of the doublet and relates to the H-3 and H-4 protons. The doublet nature of the signal of the benzyl protons can be explained by their spin-spin coupling with the H-3 and H-4 protons (J = 6.0 Hz) if it is assumed that they are chemically equivalent, as, for example, in the spectrum of pluviatolide [6].

The H_2-2 and the H_2-5 protons give two multiplets in the 3.25-4.03 ppm region upon one of which the singlet of the protons of the methoxy groups (δ 3.71 ppm) is superposed. When the signal in the strong field (δ 2.13 ppm) is suppressed, these multiplets change into the doublets characteristic for an AB system. Consequently, we assumed that the shape of the signal of the H_2-2 and H_2-5 protons in the absence of irradiation is determined by their spin-spin coupling with one another and also with the H-3 and H-4 protons; $J^{gem}_3 = J^{gem}_2 = 8.0$ Hz, and the coupling constant of the H-3 and H-4 protons with one of the two neighboring protons of the $-OCH_2$ group is ~ 7 Hz; we could not determine the second constant because of the signal of the methoxy protons.

In matairesinol, in contrast to 3,4-divanillyltetrahydrofuran, the α-protons (with respect to the benzene ring) are chemically nonequivalent. The signal of one of these CH_2 groups is superposed on the signals of the H-2 and H-3 protons and forms a broad singlet (see above). The signal of the other CH_2 group is a doublet, which we have ascribed to the H_2-6 protons. We made this assignment on the basis of a comparison of the values of the integral intensities of the H_2-6 H_2-7, H-3, and H-4 signals and also the H_2-5, H_2-6, H-2, and H-3 signals in the spectra of 3,4-divanillyltetrahydrofuran and matairesinol, respectively.

EXPERIMENTAL

The melting points were determined on a Kofler block. The IR spectra were taken on a UR-10 spectrophotometer in KBr, the UV spectra on a Unicam 8000, and the NMR spectra on HA-100, A-60 (Novosibirsk Institute of Organic Chemistry of the Siberian Branch of the Academy of Sciences of the USSR) and

BS 487B instruments. The molecular weights were determined mass-spectroscopically on an MI-1305 instrument (energy of the ionizing electrons 50 eV).

The internal standard for the NMR spectra was HMDS, and the chemical shifts are given on the δ scale. Deuterated chloroform was used as the solvent. The conidendrins, the hydroxymatairesinol, and the pinoresinol were obtained in the form of the acetates. To record the NMR spectrum, 3,4-divanillyltetrahydrofuran isolated from Larix sibirica and kindly donated by K. I. Lapteva was used.

Chromatography was performed with type KSK silica gel (150-200 and 200-250 mesh) impregnated with 2% of sodium bisulfite, on polyamide powder [7], and on type "M" ["slow"] paper.

The phenolic compounds were revealed on the plates with a solution of diazotized sulfanilic acid (DSA) and with a 1% solution of vanillin in conc. sulfuric acid.

Extraction and Separation of the Extract. Spruce sawdust with a size of 20-35 mesh (48.2 kg), dried in the air, was charged into a glass vessel (20 liters) and was covered with acetone. After steeping for 7 days, the extract was decanted off. The solvent was distilled off in a circulation evaporator. Two changes of solvent were used to extract the bulk of the extractive substances. This gave 835 g of a brownish resinous mass which was then extracted exhaustively with petroleum ether (40-70°C). Fraction A (410 g), insoluble in petroleum ether, was chromatographed on a polyamide column in the chloroform–methanol (98:2) system. This gave 280 g of fraction A-1.

Isolation of the Lignan Compounds. A suspension in chloroform of 15 g of fraction A-1 and 20 g of sorbent was charged into a column filled with 300 g of impregnated silica gel. Elution was performed with a mixture of chloroform and acetone (95:5). Fractions enriched with one or two components were eluted successively from the column: 1) a substance of phenolic-terpenoid nature 0.49 g; 2) a mixture of substances giving a lemon-yellow coloration with DSA, 0.3 g; 3) vanillin, 0.35 g; 4) α-conidendrin 0.7 g; 5) 3,4-divanillyltetrahydrofuran; 6) pinoresinol, 0.1 g; 7) matairesinol, 0.5 g; and 8) oxomatairesinol.

Matairesinol. The fraction containing the matairesinol was thrice chromatographed on a column of silica gel in the ethyl acetate–chloroform (70:30) system. After recrystallization from aqueous methanol white crystals were obtained with mp 72-74°C, $[\alpha]_D^{20}$ –45.2° (c 0.12; methanol); λ_{max}: 283, 231 nm (log ε 3.73, 4.08). IR spectrum, cm^{-1}: 3430 (broad, assoc. OH), 2850 (OCH_3), 1760 (C=O of a lactone ring), 1613, 1520, 1425 (C_6H_5), 1270, 1240, 1035 (C–O–C).

Found, %: C 66.98; H 6.53. Mol. wt. 358 (mass-spectrometrically). $C_{20}H_{22}O_6$. Calculated, %: C 67.01; H 6.13.

Pinoresinol. The fraction enriched in pinoresinol was purified by chromatography on columns in a similar manner to matairesinol; mp 120-121°C (ethanol). IR spectrum, cm^{-1}: 3450 (broad, assoc. OH); 2897, 2875 (OCH_3); 1612, 1520, 1425, (C_6H_5); 1265, 1235, 1030 (C–O–C).

The acetylation of 0.08 g of pinoresinol in 1 ml of dry pyridine with the subsequent addition of 0.5 ml of acetic anhydride gave the acetate in the form of an amorphous powder which, after washing with water, was repeatedly recrystallized from ethanol. Yield 0.04 g, mp 163-164°C. The acetate was used to identify the pinoresinol by the NMR-spectroscopic method.

3,4-Divanillyltetrahydrofuran. The compound was shown to be identical chromatographically with the 3,4-divanillyltetrahydrofuran characterized earlier [8].

Nitrobenzene Oxidation. A 1-ml bomb was charged with 0.02 g of the substance under investigation together with 0.016 ml of nitrobenzene and 0.12 ml of 2 N NaOH solution and was hermetically sealed. Then it was heated at 180°C for 1 h. Paper chromatography of the products in the petroleum ether–n-butyl ether–water (6:1:1) system showed the presence of vanillin, which was revealed with a 0.4% solution of 2,4-dinitrophenylhydrazine in 2 N HCl.

In addition, the vanillin was separated preparatively by the TLC method on polyamide in the ethanol–water (60:40) system and was identified by UV spectroscopy.

SUMMARY

1. In addition to the four lignan compounds studied previously, we have isolated and identified matairesinol and pinoresinol and have also shown the presence of 3,4-divanillyltetrahydrofuran by thin-layer chromatography.

2. The NMR spectra of the lignans of Siberian spruce have been discussed.

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