HYDROXYSTILBENES FROM THE BARK OF Pinus sibirica

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Continuing an investigation of the extractive compounds of the bark of Pinus sibirica R. Mayr (Siberian pine) [1, 2], we have isolated two phenolic compounds with the compositions $C_{15}H_{14}O_3$ (A) and $C_{14}H_{12}O_3$ (B) (according to mass spectrometry and elementary analysis). Compounds A and B are unstable to oxidation, and therefore their subsequent isolation from the extracts was performed under acetylating conditions.

Both compounds decolorize bromine water and possess a strong blue fluorescence on UV irradiation. The IR spectra of these compounds show the bands of the deformation vibrations of the CH groups of para-disubstituted (835, 840 cm^{-1}) and 1,3,5-trisubstituted (675, 810, 870 cm^{-1}) benzene rings (1590, 1613 cm^{-1}). The compounds contain hydroxy groups (3243, 3300, 3435 cm^{-1}), and compound A also has a methoxy group (2851, 2951 cm^{-1}).

Qualitative reactions and the nature of the IR and UV spectra give grounds for assuming that these compounds have related structures and belong to the class of hydroxystilbenes.

The PMR spectrum of compound A has signals corresponding to the positions of the resonance lines in the spectra of the stilbenes [3]. A broad singlet ($\approx$2.5 Hz) in the weak field (δ 8.38 ppm) and a singlet in the strong field (δ 3.69 ppm) show the presence of hydroxy and methoxy groups in substance A. The results of a comparison of the integral intensities of the signals in the spectra of compound A and its acetate showed that this compound contains one methoxy and two hydroxy groups. It was established by the double-resonance method (collapse) that the triplet in the PMR spectrum of compound A is due to the H_4 proton interacting with the H_2 and H_6 protons (J = 2.3 Hz), the signal of which is a doublet (δ 6.56 ppm). The value of J confirms the meta arrangement of the three protons in this aromatic ring. On irradiation with a second weak field (Overhauser effect) it was found that the two doublets with δ 6.79 ppm and 7.31 ppm (J = 8.5 Hz) are due to the $H_{2'}$ and $H_{6'}$ and the $H_{3'}$ and $H_{5'}$ protons. The olefinic protons, forming an AB system, are responsible for the appearance of two doublets (δ 7.05 and 6.81 ppm, J = 16.5 Hz) with the distortion of the resonance lines in the doublets that is characteristic for such a situation. The value of the spin–spin coupling constants shows the trans arrangement of the protons on the double bond.

However, it is difficult on the basis of the spectra to establish the position of the methoxy group in compound A unambiguously. The location of the substituents was judged from the oxidation reaction, leading to the formation of p-hydroxybenzoic acid and 3-hydroxy-5-methoxybenzoic acid.

According to the results obtained, compound A is 3,4'-dihydroxy-5-methoxystilbene, which has not been described in the literature previously and which we have called pinostilbene.

The PMR spectrum of the acetate of compound B, unlike the acetate of pinostilbene, lacks the signal of the protons of a methoxy group, and the integral intensity of the signal of the acetoxy groups (δ 2.16 ppm) corresponds to nine protons. Consequently, compound B has three hydroxy groups. The signals in the region of the aromatic protons correspond to the signals in the spectrum of pinostilbene acetate, although their position has changed somewhat. Thus, the H_4 signal has shifted downfield by 0.25 ppm, and the signals of the H_2 and H_6 protons have also shifted downfield, being superposed on the signals of the olefinic protons, as is shown by double resonance.

The oxidation of the acetate of compound B forms p-hydroxybenzoic and 3,5-dihydroxybenzoic acids. We have identified compound B as 3,4',5-trihydroxystilbene (resveratrol).

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The stilbene compounds isolated have the trans structure, which is confirmed by the frequency of the deformation vibrations of the CH groups on the double bond in the IR spectrum (970 cm^{-1}), the position of the absorption maxima in the UV spectrum (307, 327 nm) [4], and the PMR spectra [5].

EXPERIMENTAL

The UV spectra were taken on a Unicam SP-8000 recording spectrophotometer, the IR spectra (KBr) on a UR-10 spectrophotometer, and the PMR spectra on a Bs 487B spectrometer (with acetone and chloroform as the solvents and HMDS as the internal standard; δ scale). The molecular weight was determined by mass spectrometry on an MS902 instrument.

Chromatography was performed with Leningrad paper of type C (medium). Thin-layer chromatography (TLC) was performed on polyamide powder [6]. The following solvent systems were used for paper chromatography (PC): 1) 2% acetic acid; 2) acetic acid–hydrochloric acid–water (30 : 3 : 10); and the following for TLC: 3) chloroform–ethanol (80 : 20), 4) chloroform–methanol (70 : 30); and 5) methanol–acetic acid (9 : 1).

The elementary and functional analyses of all the compounds corresponded to the calculated figures.

Isolation of the Individual Compounds. An ethereal extract of the bark of Siberian pine, after the elimination of acids [2] by chromatography on polyamide sorbent in the methanol–water system, gave a fraction enriched in the hydroxystilbenes. Chromatography on the sorbent in the methanol–chloroform system yielded the individual hydroxystilbenes, which did not crystallize from the usual solvents. After repeated purification of ethereal solutions of these substances on Al_2O_3, pale pink plates of pinostilbene (0.3 g) and of resveratrol (0.11 g) were obtained from chloroform–methanol.

Pinostilbene, $C_{15}H_{14}O_3$, mp 117-118°C (chloroform–methanol). UV spectrum: λ_{max} (ethanol) 218, 240, 307, 322, 338 nm (log ε 4.44, shoulder, 4.55, 4.43, shoulder). IR spectrum, cm^{-1}: 838 (para-substituted benzene ring), 678, 808 (1,3,5-substituted benzene ring), 965 (nonplanar deformation vibrations of a trans-CH = CH group), 1593, 1608 (C_6H_5), 1050, 1147, 1193 (C–O), 2851, 2951 (OCH_3), 3243, 3435 (OH); mol. wt. 242.

Acetylation. A fraction of the ethereal extract of the bark of Siberian pine containing pinostilbene according to TLC (9.6 g) was acetylated with acetic anhydride (5 ml) in the presence of pyridine (4 ml). The reaction mixture was washed with water and with ethanol and was chromatographed on Al_2O_3 (activity grade 6, eluent diethyl ether) and on silica gel (eluent benzene–ethyl acetate).

Pinostilbene acetate (5.1 g), mp 91-92°C (ethanol), UV spectrum: λ_{max} (ethanol) 228, 300, 311, 327 nm (log ε: shoulder, 4.58, 4.57, shoulder). IR spectrum, cm^{-1}: 980 (nonplanar vibrations of a trans-CH = CH group) 1510, 1600, 1625 (C_6H_5), 1760 (acetate C=O), 1020, 1060, 1140, 1170, 1200 (C–O).

Oxidation of Pinostilbene Acetate. Over 30 h, $KMnO_4$ (0.06 g) was added to a solution of the acetate (0.02 g) in acetone (2 ml). The reaction mixture was filtered, the solvent was distilled off, and the residue was heated with 0.1 N NaOH (5 ml) for 2 h. The mixture was acidified with 0.1 N HCl and extracted with ether. p-Hydroxybenzoic and 3-hydroxy-5-methoxybenzoic acids were identified in the ethereal extract by the PC method in systems 1 and 2 and by TLC in systems 3, 4, and 5.

Resveratrol, $C_{14}H_{12}O_3$, mp 246°C (decomp. chloroform–methanol). Literature data: mp 263°C (6% CH_3COOH) [7], 256-257°C (aqueous methanol) [8], 216°C (water) [9]. UV spectrum: λ_{max} (ethanol) 218, 240, 307, 322, 338 nm (log ε 4.42, shoulder, 4.50, 4.48, shoulder). IR spectrum, cm^{-1}: 840 (para-substituted benzene ring), 670, 710 1,3,5-substituted benzene ring), 967 (nonplanar deformation vibrations of a trans-CH = CH group), 1585, 1613 (C_6H_5), 1013, 1155, 1215 (C–O), 3300 (OH), mol. wt. 228.

Acetylation. A fraction of the ethereal extract of the bark of Siberian pine containing resveratrol according to TLC was acetylated and treated in a similar manner to that described above.

Resveratrol acetate (0.16 g), mp 111-112°C (ethanol). Literature data: mp 109-110°C (ethanol) [6]; 111-112°C (ethanol) [7]. UV spectrum: λ_{max} (ethanol) 228, 300, 311, 327 nm (log ε: shoulder, 4.62, 4.60 shoulder). IR spectrum, cm^{-1}: 975 (nonplanar vibrations of a trans-CH = CH group), 1503, 1581, 1613 (C_6H_5) 1773 (acetate C=O), 1024, 1114, 1210 cm^{-1} (C–O).

Oxidation of Resveratrol Acetate. Over 30 h, $KMnO_4$ (0.06 g) was added to a solution of the acetate (0.02 g) in acetone (2 ml) until decoloration ceased. The reaction mixture was treated as in the oxidation of pinostilbene acetate. The ethereal extract was shown to contain p-hydroxybenzoic and 3,5-dihydroxybenzoic acids.

An authentic sample of resveratrol was given to us by Dr. V. Benešova (Czechoslovakian SSR).

SUMMARY

From the bark of Pinus sibirica R. Mayr have been isolated 3,4',5-trihydroxystilbene (resveratrol), not previously known for the genus Pinus, and a new stilbene – pinostilbene – for which the structure 3,4'-dihydroxy-5-methoxystilbene has been established.

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