HYDROXYSTILBENES OF THE INNER BARK OF *Pinus sibirica*

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Continuing an investigation of the extractive phenolic compounds of the inner bark (phloem) of the Siberian pine (*Pinus sibirica* R. Mayr), in addition to the pinostilbene (Ia) and resveratrol (IIa) isolated previously [1], we have found two new glycosidated stilbenes, which we have called pinostilbenoside (I) and resveratroloside (II). Both compounds were present in the ether-soluble fraction of the extracts that were isolated by preparative chromatography on polyamide and silica gel.

The structures of the new stilbenes have been established by a detailed analysis of their PMR spectra (Figs. 1 and 2). The parameters of the PMR spectra of compounds (I) and (II) and of the corresponding aglycones (Ia) and (IIa) are given in Table 1.

A comparison of the integral intensities of the signals of the aglycone and carbohydrate moieties in the PMR spectra of compounds (I) and (II) has shown that both compounds are monosides. The trans configuration of the olefinic protons is shown both by the spin–spin coupling constants (SSCCs) of these protons (16.3 Hz) and by the results of a comparison of their chemical shifts (CSs) with the values found previously for a number of trans-stilbenes [1-3]. Meta disubstitution in ring A and para substitution in ring B is obvious from the

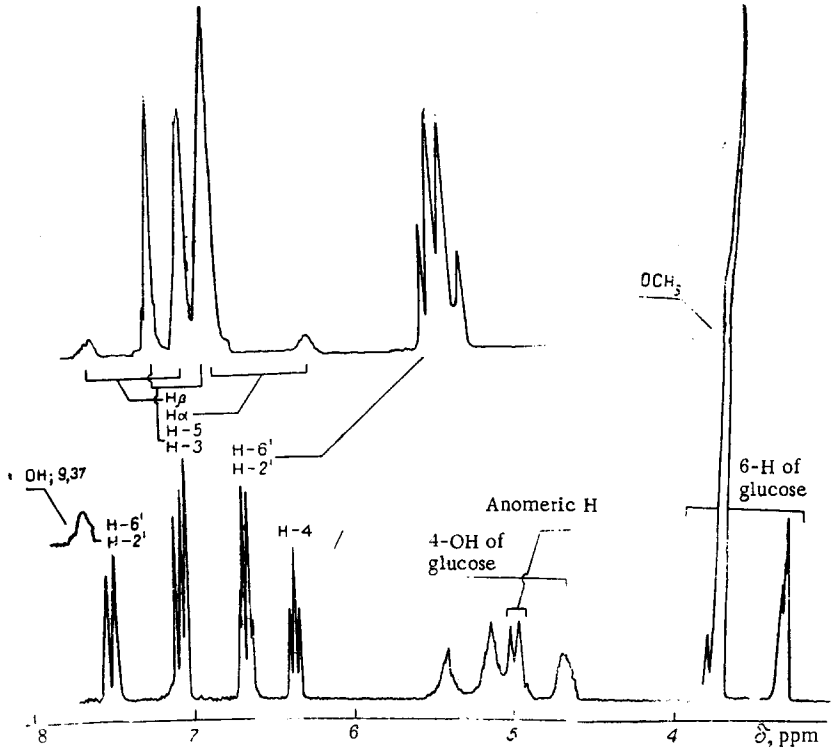

Fig. 1. PMR spectrum of pinostilbenoside [solvent $(CD_3)_2CO$].

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SSCC values ($J_{2,4} = J_{4,6} = 2.1$ Hz; $J_{2,6} = 1.7$ Hz; $J_{2',3'} = J_{5',6'} = 8.6$ Hz), and from the CSs of the aromatic protons, which agree well (± 0.05 ppm) with those calculated on the basis of the additivity of the contributions of the substituents in an aromatic nucleus to the screening of the unsubstituted protons. The values of the contributions were taken from the literature [4, 5], and the CSs of the aromatic protons of unsubstituted trans-stilbene, which were used as the basis for the calculation, were taken from Brugel's compilation [6]. The results of this comparison show that the carbohydrate substituent in each of compounds (I) and (II) is present in the para position of the ring B. This is confirmed unambiguously by the complete equivalence of the H-2 and H-4 protons and the hydroxyl protons (see Fig. 1) in compounds (II), which is possible only with the above-mentioned position of the carbohydrate substituent. An analysis of the nature of the changes in the CSs of the protons on passing from compounds (II) to (I), the presence in the spectrum of compound (I) of the signal of a methoxy group, and the relative decrease (twofold) of the integral intensity of the signal of the hydroxy group shows that in compound (I) the carbohydrate residue is present in the para position of ring B. The meta position of the methoxy group A of compound (I) is shown additionally by the appearance of an Overhauser effect on the H-2 and H-6 protons on irradiation with a second radiofrequency field at the frequency of the signal of the methoxy group. This position of the substituents in compounds (I) and (II) is confirmed by the results of a comparison of the PMR spectra with the spectra of the corresponding aglycones ((Ia) and (IIa), see Table 1).

The structures of compounds (I) and (II) suggested on the basis of an analysis of their PMR spectra agree well with the results of chemical reactions, which gave a common product – a glycoside of p-hydroxybenzoic acid – and also 3-hydroxy-5-methoxybenzoic acid for (I) and 3,5-dihydroxybenzoic acid for compound (II).

The relative retention times (RRTs) of the TMS ethers of the compounds investigated were as follows:

Compound	RRT
Pinostilbene (Ia)	1*
Resveratrol (IIa)	1.56
p-Hydroxybenzoic acid	1†
3-Hydroxy-5-methoxybenzoic acid	1.09
3,5-Dihydroxybenzoic acid	2.11

The nature of the carbohydrate moiety can be determined from the CS and, particularly, the SSCC of the anomeric proton. The distance (6.5 Hz) between the components of the doublet of the anomeric proton due to the SSCC with the vicinal proton on the second carbon atom of the carbohydrate residue shows their diaxial configuration. This enables us to exclude the possibility of the existence of the carbohydrate moiety in the α-pyranose form, for which an SSCC of 3-5 Hz should be expected [7], and in the α- and β-furanose forms, since the characteristic SSCCs of the anomeric protons for the latter are 4-5 and 0-2 Hz, respectively [8]. The SSCCs that we obtained are characteristic for the β-D-anomers of a hexapyranose [9].

Pinostilbene and resveratrol were identified in the products of the enzymatic hydrolysis of the glycosides under investigation (by the GLC of their TMS ethers), and glucose was identified under the conditions described previously [10]. The β-D-pyranose form of the glucose in compounds (I) and (II) agrees with the value of the molecular rotation [11].

Thus, the structure of 3,4'-dihydroxy-5-methoxystilbene 4'-D-β-D-glucopyranoside has been established for pinostilbenoside and that of 3,4',5-trihydroxystilbene 4'-O-β-D-glucopyranoside for resveratroloside.

At the present time, six natural glycosidated stilbenes are known. In these compounds, glycosidation takes place at position 3 of ring A, and the biogenesis of this is based on the acetate-malonate route [12]. The compounds that we have isolated have a somewhat unusual type of glycosidation: they are glycosidated in the para position of ring B, the biosynthesis of which takes place by the shikimate route. This type of glycosidation is fairly widespread among the flavonoids. The results that we obtained agree well with the theory of the biogenetic relationship of the stilbenes and the flavonoids [12].

EXPERIMENTAL

The UV spectra were taken in methanol on a Unicam SP-8000 recording spectrophotometer, and the IR spectra in KBr on a UR-10 spectrophotometer. The PMR spectra were recorded on a Varian XL-100/12 spectrometer with a working frequency for ^1H nuclei of 100 MHz.

*The RT of the TMS ether of pinostilbene (10 min 45 sec) was taken as 1.00 in the calculation of the RRT of the stilbenes.

†The RT of the TMS ether of p-hydroxybenzoid acid (4 min 24 sec) was taken as 1.00 in the calculation of the RRT of the acids.

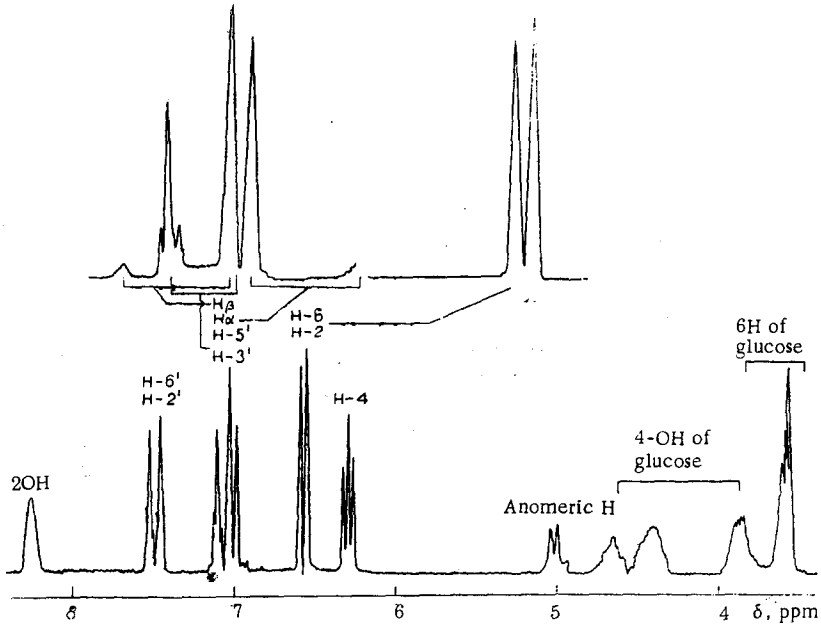
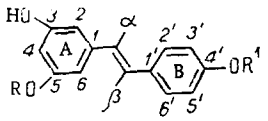


Fig. 2. PMR spectrum of resveratroloside [solvent $(CD_3)_2O$].

TABLE 1. Chemical Shifts (δ) in the PMR Spectra of the Compounds Investigated *



Compound	R	R'	Chemical shift (δ) in ppm											
			H-2	H-4	H-6	H_α	H_β	H-2'	H-3'	H-5'	H-6'	H-R	H-R'	OH
I	CH_3	Glc	6,69	6,36	6,67	7,03	7,10	7,49	7,10	7,10	7,49	3,79	5,02†	9,37‡
Ia	CH_3	H	6,70	6,38	6,68	6,98	7,08	7,44	6,89	6,89	7,44	3,79	8,46	8,32
II	H	Glc	6,57	6,30	6,57	6,98	7,03	7,49	7,06	7,06	7,49	8,19	5,02†	8,18
IIa	H	H	6,58	6,31	6,58	6,86	7,01	7,41	6,86	6,86	7,41	8,26	8,52	8,26

*Solution in $(CD_3)_2CO$, c 15%, temperature 30°C, internal standard tetramethylsilane.

†Chemical shift of the anomeric proton

‡Solution in $(CD_3)_2SO$, c 15%, since in $(CD_3)_2CO$ not all the signals of the OH group can be seen because of broadening.

Treatment of the Extract. Because of the lability of stilbene compounds, all the operations relating to isolation and identification were performed in an atmosphere of CO_2 containing traces of SO_2. For the same reason, before the chemical reaction of the stilbenes the hydroxy groups were protected by acetylation. The freshly collected phloem of the Siberian pine (0.7 kg; moisture content 47%) was extracted with a mixture of methanol and water (1 : 1 by volume). After the methanol had been distilled off, the extract (59.4 g) was exhaustively reextracted with chloroform (which dissolved out 1.8 g of material) and with diethyl ether (which dissolved out 6.1 g of material).

Chromatographic Separation. By chromatography on silica gel impregnated with 2% of sodium metabisulfite in the chloroform–methanol system, compound (Ia) and (IIa) were isolated from the ether-soluble extract [0.07 and 0.3% of the absolutely dry bark (a.d.b.), respectively]. The stilbenes were purified on alumina using diethyl ether as the eluant. By column chromatography on polyamide (with water–methanol as the eluant), the ether-insoluble extract yielded a fraction of stilbene glycosides (15.7 g; 4.2% of the a.d.b.). By a combination of repeated chromatography on polyamide and silica gel, from this fraction we isolated the individual compounds (I) and (II) with the latter predominating.

Pinostilbene formed pale pink needles with mp 117-118°C (methanol–chloroform). A mixture with an authentic sample [1] gave no depression of the melting point.

Resveratrol formed pale pink needles with mp 246°C (decomp; methanol–chloroform). A mixture with an authentic sample [1] gave no depression of the melting point.

Pinostilbenoside (I) formed white needles with mp 182-183°C (methanol–chloroform); $[\alpha]_D^{22}-52.5°$ (c 2.0; acetone; UV spectrum λ_{max} 235, 303, 315 nm (log ε 4.37, 4.42, 4.41); IR spectrum: 3400 (OH), 1620, 1510 cm^{-1} (C_6H_5).

The acetate of (I) formed white needles with mp 152-153°C (methanol); $[\alpha]_D^{22}-20.0°$ (c 2.0; acetone); UV spectrum: λ_{max} 227, 235, 305, 315 nm (log ε 4.32; shoulder; 4.57; 4.56); IR spectrum 1772 cm^{-2} (C = O of an acetate group).

Resveratroloside (II) formed white needles with mp 240°C (methanol–chloroform); $[\alpha]_D^{22}-54.0°$ (c 2.0; acetone); UV spectrum; λ_{max} 235, 303, 318 nm (log ε 4.22, 4.32, 4.30); IR spectrum: 3330 (OH), 1620, 1532 cm^{-1} (C_6H_5).

The acetate of (II) formed white needles with mp 169-170°C (methanol) $[\alpha]_D^{22}-21.0°$ (c 2.0; acetone); UV spectrum: λ_{max} 228, 236, 303, 315 nm (log ε 4.30, shoulder, 4.54, 4.53); IR spectrum: 1771 cm^{-1} (C = O of an acetate group).

Acetylation. A fraction enriched in the component under investigation was acetylated with acetic anhydride in pyridine [1].

Permanganate Oxidation. The acetylated glycoside (0.02 g) was dissolved in acetone (2 ml), and $KMnO_4$ was added until there was no further decoloration. The excess of $KMnO_4$ was eliminated with oxalic acid. The reaction mixture was filtered and evaporated. The reaction products after oxidation were heated with 0.1 N NaOH (5 ml) in the water bath for 2 h. The reaction mixture was neutralized, extracted with diethyl ether, and analyzed by GLC in the presence of acids.

The aqueous residue after extraction with diethyl ether was heated with 10% HCl (4 ml) on the water bath for 2 h and was extracted with diethyl ether, and the extract was analyzed by GLC for its acid content. The aqueous residue was neutralized on AV-17 anion-exchange resin and was analyzed for the presence of carbohydrates.

Enzymatic Hydrolysis. A stilbene glycoside (0.02 g) was thermostated at 32-34°C in acetate buffer (pH 5.36) with the enzyme from Aspergillus oryzae for 48 h. The hydrolyzate was extracted with diethyl ether and analyzed by GLC in the presence of stilbenes, and the aqueous residue was analyzed in the presence of carbohydrates.

GLC Analysis. The stilbene aglycones and the aromatic acid aglycones were analyzed in the form of their TMS ethers [10] on a "Tsvet-4" instrument with a flame-ionization detector using a 300 × 0.3 cm column filled with 5% of SE-30 on Chromatom N-AW-HMDS at a rate of flow of helium of 29 ml/min and column temperatures of 264°C for the stilbenes and 195°C for the acids, the evaporator temperatures being 350 and 250°C, respectively. The results of the analysis have been given above.

SUMMARY

In addition to pinostilbene and resveratrol, two new stilbene glycosides have been isolated from the phloem of Pinus sibirica R. Mayr, and their structures have been established as 3,4'-dihydroxy-5-methoxystilbene 4'-β-D-glycopyranoside (pinostilbenoside) and 3,4',5-trihydroxystilbene 4'-β-D-glycopyranoside (resveratroloside).

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PARTIAL METHYLATION OF METHYL GLYCOSIDES

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We have previously studied the partial methylation of methyl β-D-xylopyranoside by various methods [1]. At the present time in view of the use of micropreparative gas–liquid chromatography (GLC) for separating the methyl ethers of methyl β-xylopyranoside from the mixtures formed in the partial methylation of methyl β-xylopyranoside [2], it appeared of interest to expand the possibilities of the method. With this aim, we have performed a partial methylation of a number of methyl glycosides by Purdie's method [3]. Using standard mixtures of methyl ethers it has been shown that the amounts of the individual methyl ethers are proportional to the areas of the peaks, and therefore no calibration coefficients were used.

On the partial methylation of the two anomers of methyl D-xylopyranoside, the monomethyl ether fraction contains all the possible derivatives, with the 2-O-methyl ether in predominating amount, which shows the high relative reactivity of the hydroxyl at C_2 (Tables 1 and 2). On the partial methylation of methyl β-xylopyranoside, the dimethyl ether fraction contains practically no 3,4-di-O-methyl ether, while it is formed in appreciable amounts from the methyl α-xylopyranoside. On partial methylation of the anomers of methyl L-arabinopyranoside (Tables 3 and 4), a considerable difference is observed in the amounts of monomethyl ethers; the 2-O-methyl ether predominates, its proportion reaching 50% in the

TABLE 1. Partial Methylation of Methyl α-D-Xylopyranoside

Reaction time, min	Initial glycoside, %	Methyl ether, %						
		2	3	4	2,3	2,4	3,4	2,3,4
5	91,9	3,5	2,4	2,2	—	—	—	—
10	89,5	4,4	3,4	2,7	—	—	—	—
20	75,6	9,8	7,9	5,5	0,3	0,6	0,3	—
30	60,9	15,9	12,3	7,7	0,8	1,6	0,8	—
45	44,0	26,9	16,3	8,9	1,1	2,0	0,8	—
60	23,9	27,6	21,7	8,4	5,8	8,8	3,4	0,4
90	11,7	27,8	21,0	5,0	12,7	12,7	7,0	2,1
120	7,5	28,9	21,2	4,0	13,5	14,7	5,9	4,3
180	4,3	25,5	17,8	2,4	20,0	16,3	8,5	5,2
240	3,3	22,6	17,4	2,0	19,8	17,7	7,4	9,8

TABLE 2. Partial Methylation of Methyl β-D-Xylopyranoside

Reaction time, min	Initial glucoside, %	Methyl ether, %						
		2	3	4	2,3	2,4	3,3	2,3,4
5	66,2	11,5	9,3	13,0	—	—	—	—
10	37,5	19,1	14,4	16,4	1,0	11,6	—	—
20	25,7	24,6	18,6	21,7	—	9,3	—	—
30	13,9	26,3	20,1	18,2	2,7	18,8	—	—
45	5,8	24,9	18,4	13,3	6,0	31,6	—	—
60	3,1	19,9	18,1	10,8	8,3	37,2	0,1	2,5
90	2,3	15,2	14,4	4,4	12,1	45,6	0,4	5,6
120	1,8	12,0	10,1	2,5	13,8	45,5	—	14,3

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