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VERSICOSIDE — A NEW LIGNAN GLYCOSIDE FROM *Haplophyllum versicolor*

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A new lignin glyocoside has been isolated from *Haplophyllum versicolor* and has been called versicoside. It has been established by chemical and spectral methods that verscioside is (+)-epipinoresinol 4'-O-[O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside].

Continuing a study of components of the plant of the genus *Haplophyllum* A. Juss, we have investigated the epigeal part of *Haplophyllum versicolor* Fisch. et Mey. growing on the Ustyurt plateau. Chromatographing an ethanolic extract on a column of silica gel in the chloroform—methanol system led to the isolation of a new glycoside, which we have called versicoside. Versicoside (I) is an optically active phenolic compound with the composition $C_{32}H_{42}O_{15}$. Its IR spectrum contains absorption bands of hydroxy and methoxy groups, of aromatic C—C bonds, and of the C—O vibrations of glycosides. The UV spectrum of (I) has maxima at 230 and 280 nm, which shows the presence of hydroxybenzene rings in the molecule

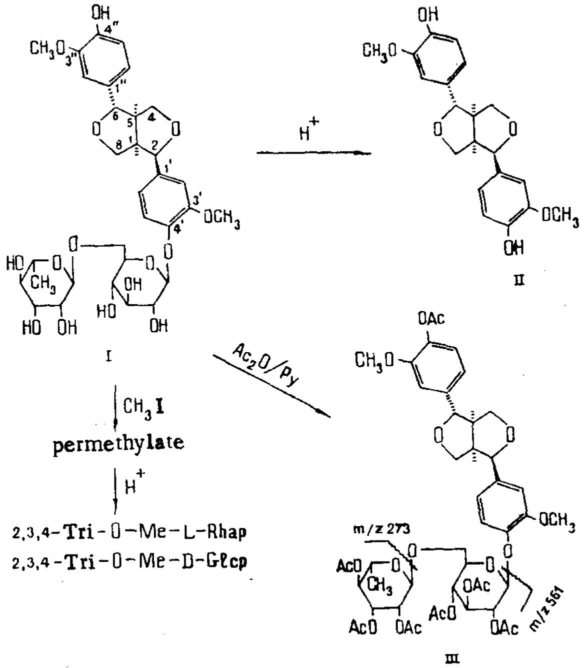

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TABLE 1. Chemical Shifts of the Carbon Atoms in the ^{13}C NMR Spectra of (+)-Epipinoresinol 4'-O-β-D-Glucopyranoside (A) and Versicoside (B) in DMSO-d_6 (δ, 0 — TMS)

Carbon atom and multiplicity	A [11]	B	Carbon atom and multiplicity	A	B
1 d	49,2	49,2	$\underline{C}H_3O$ q	55.7	55,5
5 d	53,6	53,7		55,8	55,7
4 t	70,4	70.3	*D*-Glcp		
8 t	68,7	68,7	1 d	100,4	100,4
2 d	81,1	81,0	2 d	73,2	73,1
6 d	86,8	86,8	3 d	76,7	76,6
1' s	132,4	132,2	4 d	69,8	69,8
1'' s	132,6	132,5	5 d	76.7	75,4
3' s	145,9	145,1	6 t	60.7	66.4
3'' s		145,7	*L*-Rhap		
4' s		148,4	1 d		100,0
4'' s		147,2	2 d		70,3
2' d	110,5	110 8	3 d		70,6
2'' d	115,2	110.8	4 d		71,9
5' d	115.3	115 0	5 d		68.2
5'' d	117,7	115,2	6 q		17,7
6' d	118,5	118,4			
6'' d		117,4			

The acid hydrolysis of versicoside formed the monosaccharides D-glucose and L-rhamnose and an aglycone with the composition $C_{20}H_{22}O_6$, M^+ 358 (II) — an optically active crystalline substance with mp 136-137°C. The formation on the acetylation of (II) of a diacetyl derivative (δ 2.23 ppm, 6 H, singlet in the PMR spectrum), gave grounds for considering that the aglycone contained two phenolic hydroxy groups. In addition, according to the PMR spectra (I) and (II) each contained two OCH_3 groups.

The IR, UV, and mass spectra of the alycone were close to those of the lignans of the 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane series [1-3]. In order to establish the mutual positions of the OH and OCH_3 groups in the aromatic nuclei, (II) was oxidized with nitrobenzene in an alkaline medium. The resulting formation of vanillin showed that both the aromatic rings had the guaiacyl type of substitution.

On the basis of the facts given above it was possible to assume that the aglycone (I) must be identical with pinoresinol [4] or one of its isomers. In actual fact, the physical constants both of (II) and of its acetate coincided with those for (+)-epipinoresinol and its acetate [5]. The PMR spectrum of (II) and (+)-epipinoresinol [6] were also identical. Thus, versicoside is a glycoside of (+)-epipinoresinol.

The acetylation of (I) gave the heptaacetate $C_{46}H_{56}O_{22}$, M^+ 960 (III), the PMR spectrum of which showed the signals of the protons of seven acetoxy groups, one of which, from its chemical shift (δ 2.23 ppm) was assigned to the methyl of an Ar—OCO$\underline{CH_3}$ grouping. Consequently, versicoside is a bioside. In order to establish the structure of the carbohydrate moiety, compound [I] was methylated by Hakomori's method [7]. 2,3,4-Tri-O-methyl-L-rhamnopyranose and 2,3,4-tri-O-methyl-D-glucopyranoside were detected by GLC in a hydrolysate of the methylation product. Thus, versicoside contains L-rhamnose as its terminal sugar residue, and this is attached to a D-glucose residue by a 1 → 6 bond, both sugars having pyranose oxide rings.

In the PMR spectrum of (I), the resonance signals of the anomeric protons of the L-rhamnose and D-glucose residues appear at 5.21 and 5.32 ppm in the form of a broadened singlet with a half-width of ~2 Hz and a doublet with a spin-spin coupling constant of 6.5 Hz, respectively.

The facts given indicated that the anomeric center of the D-glucose residue has the β configuration and that of the L-rhamnose residue the α configuration [8]. It is known that the spatial positions of the guaiacyl groups in the molecule of (+)-epipinoresinol differ, i.e., they have the axial and equatorial orientations.

The question of to which guarcyl residue the carbohydrate moiety is attached was solved by a study of the ^{13}C NMR spectrum of versicoside.

It is known from the literature that in the ^{13}C NMR spectra of 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane lignans with different orientations of the aryl groups the signals of the

C-1 carbon of the axial aryl group and of the carbon atom of the heterocyclic ring to which it is attached (C-2 or C-6) appear in a stronger field than that for an equatorial aryl group [9-11]. For example, for epipinoresinol, the C-1' and C-1" signals have approximate CS values of δ 129.6 and 132.6 ppm, respectively [11]. On the alkylation or glycosylation of one of the phenolic hydroxy group, the C-1 signal of the corresponding benzene ring undergoes a paramagnetic shift by 1.5-3.0 ppm [11]. The closeness of the CS values for C-1' (δ 132.2 ppm) and C-1" (δ 132.5 ppm) in the spectrum of versicoside permits us to consider that the carbohydrate moiety in this molecule is attached to the axial aryl group.

A comparison of the CS values of the carbon atoms in the ^{13}C NMR spectra of (+)-epipinoresinol 4'-O-β-D-glucopyranoside [11] and versicoside confirmed our hypothesis (see Table 1).

It can be seen from the table that on passing from (+)-epipinoresinol 4'-O-β-D-glucoside to versicoside the C-6 and C-5 carbon atoms of the D-glucose residue experience a glycosylation effect (+5.7 ppm and −1.3 ppm, respectively), which confirms the 1 → 6 arrangement of the bond between the rhamnose and glucose residues [11].

The assignment of the carbon signals in the ^{13}C NMR spectrum of versicoside was made on the basis of an experiment with complete and incomplete decoupling of C—H interactions and by a comparison of the ^{13}C CSs with literature figures for the ^{13}C NMR spectra of (+)-epipinoresinol 4'-O-β-D-glucopyranoside [11] and other lignans of the 2,6-diaryl-3,7-dioxabicyclo[3.3.0] octane series [13, 14].

Thus, versicoside has the structure of (+)-epipinoresinol 4'-O-[O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside.

Lignans of the 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane series (eudesmin and pluviatilol) have been found previously in other species of *Haplophyllum*.

EXPERIMENTAL

General Observations. Column chromatography was performed on silica gel L 100/160 (Czechoslovakia). The lignans were detected by spraying plates with concentrated H_2SO_4 followed by heating at 110°C for 5-10 min. Mass spectra were measured on a MKh-1310 instrument at an ionizing voltage of 50 V. IR spectra were taken on a UR-20 spectrometer in KBr, and PMR spectra in C_5D_5N and $CDCl_3$ on JNM-4H-100 and JNH-C60-H instruments, and in DMSO-d_6 on a Varian XL-200 instrument with TMS as internal standard, δ scale.

Isolation of Versicoside. The dried and comminuted epigeal part (5 g) of the plant *Haplophyllum versicolor* Fisch. et Mey., collected on June 2, 1981 on the Ustyurt plateau* was extracted with ethanol at room temperature five times. After evaporation of the solvent, 68.0 g of total extractive substances was obtained. Part of this total (65.0 g) was chromatographed on a column in the solvent system chloroform—methanol (99:1-90:10). This system in a ratio of 93:7 eluted 3.17 g of versicoside.

Versicoside (I) — $C_{32}H_{42}O_{15}$, mp 223-224°C (acetone), $[\alpha]_D^{20}$ − 8.8° (c 0.61 ; ethanol); $\lambda_{max}^{ethanol}$ 230, 280 nm (log ε 4.22, 3.75); ν_{max}^{KBr} (cm^{-1}): 3513-3310 (OH), 1618, 1606, 1528 (aromatic C═C bonds).

PMR in C_5D_5H (ppm): 1.44 (3 H, d, 5 Hz, —CH_3); 2.82 (1 H, m, H-1); 3.29 (1 H, m, H-5); 3.56, 3.62 (3 H, s, each; 2 × OCH_3); 3.25-4.39 (protons of the sugar moiety and 2 H-4 and 2 H-8); 4.44 (1 H, d, 7 Hz, H-6); 4.70 (1 H, d, 5.5 Hz, H-2); 5.21 (1 H, br.s, H-1 of Rha); 5.32 (1 H, d, 6.5 Hz, H-1 of Glc); 6.80-7.08 (6 H, Ar-H).

PMR in DMSO-d_6: 1.12 (3 H, d, 6.2 Hz, —CH_3); 2.84 (1 H, m, H-1); 3.04-3.96 (H-5, 2 H-4, 2 H-8 and the protons of the sugar moiety); 3.77, 378 (3 H, s, each, 2 × OCH_3); 4.09 (1 H, d, 8 Hz, H-6), 4.33 (1 H, d, 6 Hz, H-2); 4.58 (1 H, br.s, H-1 of Rha); 4.68-4.90 (H-1 of Glc and OH); 5.19, 5.29 (OH groups); 6.74-7.11 (6 H, Ar—H); 8.94 (1 H, br.s, Ar—OH).

Acid Hydrolysis of Versicoside. Versicoside (174 mg) was hydrolyzed with 20 ml of 5% sulfuric acid in the water bath in an atmosphere of nitrogen for 2 h. The precipitate that had deposited was filtered off, washed with water, dried, and chromatographed on a column of Sephadex LH-20. On elution with chloroform—methanol (9:1), 63 mg of aglycone was obtained.

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*T. Sdykov took part in the collection of the plant.

The acid hydrolysate was neutralized with $BaCO_3$. In the evaporated residue D-glucose and L-rhamnose were detected by TLC in the presence of authentic specimens.

(+)-Epipinoresinol (II). $C_{20}H_{22}O_6$, M^+ 358, mp 136-137°C (the diacetate had mp 143-145°C), $[\alpha]_D^{20} + 129.6°$ (c 2.0 ; acetone); $\lambda_{max}^{ethanol}$ 229, 284 nm (log ε 4.22, 3.85).

PMR spectrum in $CDCl_3$ (ppm): 2.70-4.05 (2 H, m, H-1, H-5); 3.85 (6 H, s, 2 × OCH_3); 4.34 (1 H, d, 7 Hz, H-6); 4.72 (1 H, d, 4.5 Hz, H-2); 5.59 (br.s, OH); 6.73-6.79 (6 H, Ar—H).

Oxidation of (+)-Epipinoresinol. A mixture of 30 mg of (II), 0.3 ml of nitrobenzene, and 0.25 ml of a 2 N solution of NaOH was heated in a sealed tube at 180°C for 1 H. The reaction product was diluted with water and extracted with ether. The residue after the solvent had been distilled off was separated by preparative TLC. A substance with mp 79-80°C was isolated which was identified as vanillin by TLC and a mixed melting point.

Acetylation of Versicoside. Versicoside (100 mg) was acetylated with acetic anhydride (3 ml) in the presence of pyridine (2 ml) at room temperature. After the usual working up of the reaction mixture, 115 mg of the pentaacetate (III) was obtained with the composition $C_{46}H_{56}O_{22}$, mp 74-75°C; ν_{max}^{KBr} (cm^{-1}): 1757, 1610, 1519, 1255, 1232.

PMR in $CDCl_3$ (ppm): 1.15 (3 H, d, 5.5 Hz, $-CH_3$); 1.99-2.06 (18 H, 6 × $OCOCH_3$); 2.23 (3 H, s, Ar—$OCOCH_3$); 2.83 (1 H, m, H-1); 3.21 (1 H, m, H-5); 3.46-5.22 (H-2, 4, 6, 8, and the protons of the sugar moiety), 3.75 (6 H, s, 2 × OCH_3); 6.63-7.02 (6 H, Ar—H).

Mass spectrum, m/z (%): 960 (M^+ 0.1), 918 (M—42, 0.1), 561(6), 517(1.2), 501(0.6), 415(0.7), 400(5.3), 383(1.4), 369(1.2), 358(8), 331(6.8), 312(6.4), 289(1.8), 274(42.8), 273(100); 257(5), 231(6.4), 227(6). 215(5.7), 213(78.6), 205(5.3), 197(5), 171(78.6), 169(71.4), 155(42.8), 153(78.5), 151(46.4), 145(17.8), 137 (35.7).

Determination of the Structure of the Sugar Moiety. Versicoside (60 mg) was methylated by Hakomori's method [7]. After the usual working up, 67 mg of methylation product showing no absorption bands of hydroxy groups in the IR spectrum was obtained. The methylation product was hydrolyzed with a 6% methanolic solution of sulfuric acid in the water bath for 4 h. After the appropriate working up, 2, 3, 4-tri-O-methyl-L-rhamnose and 2, 3, 4-tri-O-methyl-D-glucose were identified in the carbohydrate moiety of the hydrolysate by GLC.

SUMMARY

A new lignan glycoside has been isolated from the epigeal part of the plant *Haplophyllum versicolor* and has been identified as (+)-epipinoresinol 4'-O-[O-α-L-rhamnopyranosyl-(1→6)-O-β-D-glycopyranoside].

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