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O-ACYLATED FLAVONOID GLYCOSIDE OF THE NEEDLES OF *Picea obovata*

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The needles of plants of the family *Pinaceae* have recently attracted attention as a source of O-acylated flavonoid compounds [1-6]. It is interesting to note that in both the genera *Pinus* and *Picea* the acylating agents are phenolic acids that are typical for coniferous plants, particularly p-coumaric acid [2-5]. At the same time, a little-known form of acylation has been observed: simultaneous acylation by two p-coumaric acid residues [3, 4].

The present work was devoted to determining the structure of a new compound (I) which we have isolated from the needles of *Picea obovata* Ledeb. (Siberian spruce) and is of interest as an example of a flavonol glycoside acylated simultaneously by residues of two phenolic acids characteristic for spruce needles — p-coumaric and ferulic acids [7].

From the results of hydrolytic cleavage and its PMR spectrum (Fig. 1), compound (I) includes residues of kaempferol, glucose, and p-coumaric and ferulic acids. The substance is very stable under conditions of severe acid hydrolysis (25% HCl, 100°C), which does not go to completion even after boiling for 48 h. Kaempferol was detected among the hydrolysis products but none of its acyl derivatives.

From its IR spectrum the substance can be characterized as an ester (ν_{CO} 1690, 1710 cm^{-1}). The UV spectra measured by a standard method contain a very broad band with a maximum at 320 nm, which does not permit the free OH groups in kaempferol to be differentiated; a 5-OH group in (I) was revealed by its PMR spectrum.

The glycosidation of kaempferol at the 3-OH group was established on the basis of the UV spectra of the deacylated product (II), which was identical with astragalin (kaempferol 3-O-β-D-glucopyranoside). The mild saponification of compound (I) led to the appearance even in the first 10-15 min of two monoacylated compounds (III) and (IV), the latter practically disappearing after 20-30 min. From the products of alkaline hydrolysis we isolated astragalin (II), astragalin ferulate (III), and p-coumaric and ferulic acids (Scheme 1).

The β-orientation of the glycosidic bond of the D-glucopyranose in compounds (I-III) is shown by the optical rotation of these glycosides and by the diaxial coupling constant of the anomeric proton in their PMR spectra.

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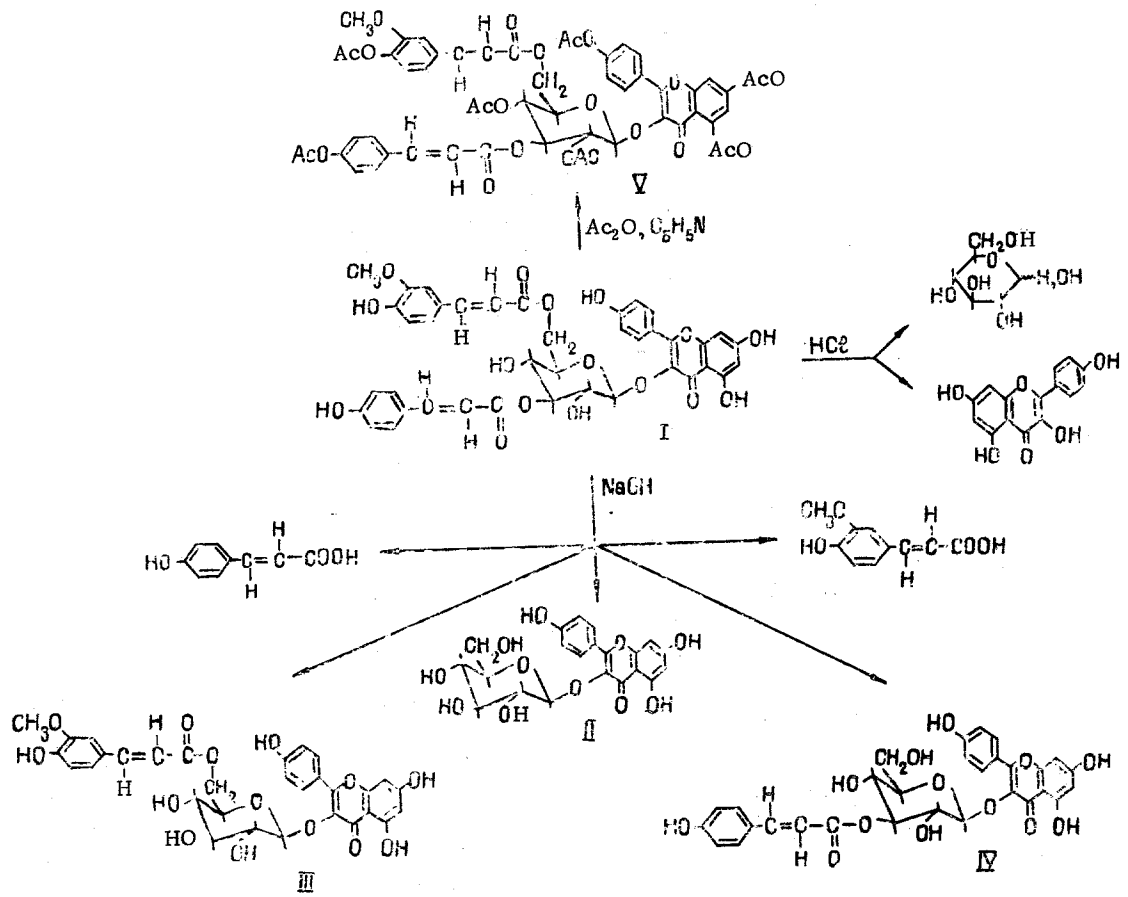

Scheme 1. Chemical conversions of 3"-O-p-coumaroyl-6"-O-feruloylastragalin (I).

The acetylation of compound (I) gave the heptaacetate (V), in the PMR spectrum of which there were the signals of two aliphatic (1.95 and 2.06 ppm) and five aromatic acetoxy groups (2.42 ppm, 3H; 2.26 ppm, 9H; 2.16 ppm, 3H). The chemical reactions described show that both acyl residues are attached to the carbohydrate part of the molecule. Their positions in the glucose were determined by means of NMR.

The region of resonance of aromatic protons (6.1-8.3 ppm) in the spectra of compound (I) (see Fig. 1) and its heptaacetate (V) include the signals of 17 protons, which were assigned to kaempferol and to trans-ferulic and trans-p-coumaric acids.

In the spectrum of compound (III) in this region can be seen the signals of the protons H-2',6' (doublet, δ 8.37, J = 9 Hz) and H-6 and H-8 (singlet, 6.6 ppm, 2H) of kaempferol. Its other protons (H-3',5') form a narrow signal (7.20-7.10 ppm) with an intensity of 5H together with the three aromatic protons of the ferulic acid, the presence of which in the molecule of (III) is established by the two doublets of α- and β-olefinic protons with the trans configuration (7.76 and 6.46 ppm, $J_{\alpha,\beta}$ = 16 Hz) and the signal of a methoxy group at 3.8 ppm).

The fact that compound (III) is an ester (ν_{CO} 1690 cm^{-1}) and the absence of the signals of hemiacyl methine protons in the weak field permits the conclusion that the ferulic acid residue is attached to the CH_2OH group of glucose, the protons of which form a multiplet with its center at 4.86 ppm [there is an analogous signal of the two H-6" hemiacyl protons in the spectrum of compound (I) — see Fig. 1]. The anomeric proton appears in the spectrum of compound (III) in the form of a doublet with J = 7 Hz (6.14 ppm), which, in association with the facts given above, corresponds to the structure of kaempferol 3-O-(6"-O-feruloyl-β-D-glucopyranoside) for (III). No such compound has been described in the literature.

In the PMR spectrum of compound (I) in the region of resonance of the carbohydrate protons, in addition to the doublet of the anomeric proton (5.42 ppm, J = 8 Hz) and the multiplet of a CH_2-O-acyl group (4.34 ppm, J_{gem} = 12 Hz) there is also a signal in the form of a triplet (5.22 ppm, $J_1 = J_2$ = 9.5 Hz), the appearance of which in the weak field is due to the

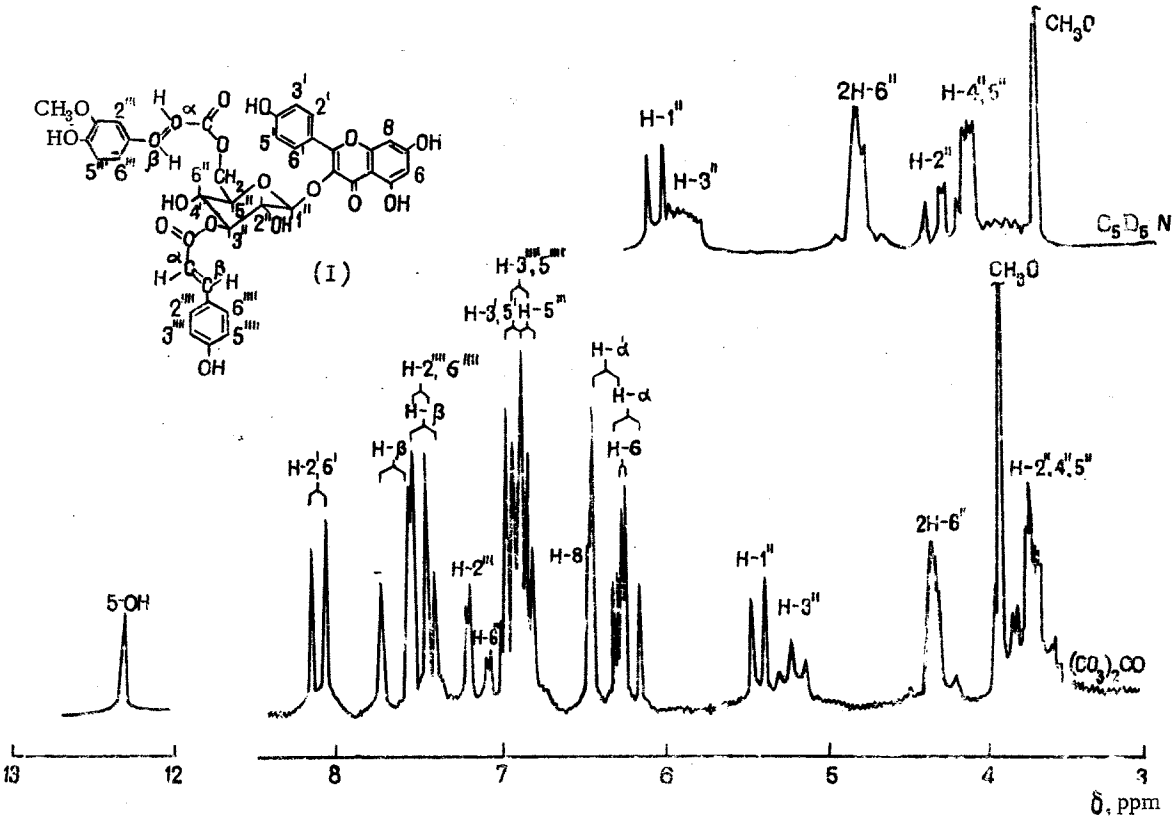

Fig. 1. PMR spectrum of 3''-O-p-coumaroyl-6''-O-feruloylastragalin (I) in deuteroacetone and deuteropyridine.

acylation of the 3''-OH or the 4''-OH group of glucose. An attempt to make an unambiguous choice with the aid of the INDOR method was unsuccessful. The INDOR signals obtained on the lines of the doublet (δ 5.42) and the triplet (δ 5.22) appeared in the same position of the spectrum (3.7 ppm). However, in this region (3.9-3.55 ppm) there are the overlapping signals of three carbohydrate protons and it is impossible to reveal lines belonging to the individual protons directly from the spectrum obtained in deuteroacetone.

The assignment of the signal of the proton at the acyloxy group (triplet, 5.22 ppm) was made from a comparison of the spectra of compound (I) in deuteroacetone and deuteropyridine (see Fig. 1). Attention is attracted by the fact that the components of the triplet are considerably broadened. This broadening depends on the nature of the solvent (in deuteropyridine it forms an unresolved multiplet) and cannot be a consequence of the existence of long-range coupling constants, which are close to zero for glucose.

It is known [8, 9] that in a system of nuclei consisting of three protons, A, B, X, the form and multiplicity of the X signal depends not only on the values of the coupling constants J_{AX} and J_{BX} but also on the ratio $\Delta\nu_{AB}/J_{AB} \leqslant 1$ (where $\Delta\nu_{AB}$ is the difference of the chemical shifts of the A and B protons in Hz). In glucose, the vicinal constants of all the protons amount to 8-10 Hz, and therefore overlapping of the H-3'' and H-2'' signals ($\Delta\nu \approx 10$ Hz) must be caused by the broadening not only of the components of the H-4'' triplet but also of the H-1'' doublet, which is not observed in the spectra. Consequently, the multiplicity of the 5.22-ppm signal is affected by the closeness of the H-4'' and H-5'' chemical shifts; i.e., the signals under consideration must be assigned to the H-3'' proton. Also in favor of this assignment is the fact that in the spectrum taken in deuteropyridine the intensities of the lines of the H-2'' quartet (4.32 ppm, $J_{1,2} = 8.0$, $J_{2,3} = 9.5$ Hz) are approximately the same. This indicates that the signals of both the protons interacting with them (H-1'' and H-3'') are located far into the weak field [9, 10].

Thus, of the two possible sites of attachment of the p-coumaric acid (3'' or 4''), the facts given above indicate the 3'' position, which means that the complete structure of compound (I) corresponds to kaempferol 3-O-(3''-O-p-coumaroyl-6''-O-feruloyl-β-D-glucopyranoside).

EXPERIMENTAL

The spectra were obtained on the following instruments: UR-20, paraffin oil (IR); Hitachi EPS-3T (UV); Varian HA-100D at 100 MHz, internal standard tetramethylsilane (NMR); and Varian CH-8 at 70 eV (mass spectra). The melting points were determined on a Kofler block; the elementary analyses on a Hewlett–Packard 185B automatic CHN analyzer; and the angles of rotation on a Polamat A polarimeter at 546 and 578 nm with recalculation to λ = 589.3 nm. Chromatographic monitoring was carried out by GLC (Silufol) in the following systems: a) chloroform–methanol (4:1), b) chloroform–methanol (9:1), and c) benzene–acetone (3:1), and PC in the butanol–pyridine–water (6:4:3) system (descending).

Isolation. Specially collected Siberian spruce needles (7.2 kg, moisture content 13%) were exhaustively extracted with methanol by steeping at room temperature. The methanolic extract was concentrated in vacuum, and the residue was freed from chlorophyll by decantation and was treated with petroleum ether and diethyl ether. The evaporated diethyl extract (55 g) was chromatographed repeatedly on polyamide in the chloroform–methanol (9:1) system and on silica gel in the benzene–acetone (4:1) system. Compound (I) was isolated in an amount of 110 mg.

3"-O-p-Coumaroyl-6"-O-feruloylastragalin (I). Slightly yellowish amorphous substance, soluble in acetone, methanol, and pyridine; mp 168–171°C, composition $C_{40}H_{34}O_{16}\cdot H_2O$, $[\alpha]_D^{20}$ −32.3° (c 0.93, acetone), R_f 0.65 (TLC) (a) ν_{CO} 1710, 1690, 1655 cm^{-1}; λ_{max}^{MeOH}, nm (log ε) 270 (4.49), 300 sh. (4.66), 320 (4.69).

PMR spectrum (see Fig. 1) in $(CD_3)_2CO$ (ppm): 12.6 (s, 5-OH), 8.12 (d, 9 Hz, H-2', 6'), 7.65 (d, 16 Hz, H-β), 7.52 (d, 16 Hz, H-β), 7.5 (d, 9 Hz, H-2"", 6""), 7.22 (d, 2 Hz, H-2"'), 7.04 (dd, 2 and 9 Hz, H-6"'), 6.94 (d, 9 Hz, H-3', 5'), 6.88 (d, 9 Hz, H-3"", 5""), 6.84 (d, 9 Hz, H-5"'), 6.45 (d, 2 Hz, H-8), 6.36 (d, 16 Hz, H-α), 6.26 (d, 2 Hz, H-6), 6.17 (d, 16 Hz, H-α), 5.42 (d, 8 Hz, H-1"), 5.22 (t, 9.5 and 9.5 Hz, H-3"), 4.34 (m, 2H-6"), 3.9 (s, CH_3O), 3.9-3.55 (3H of glucose).

PMR spectrum in C_5D_5N (ppm): 8.27 (d, 9 Hz, H-2', 6'), 7.81 (d, 16 Hz, H-β), 7.71 (d, 16 Hz, H-β), 7.4 (d, 9 Hz, H-2"", 6""), 7.22-6.97 (m, H-3', 5'); H-3"", 5"", H-2"', 5"', 6"'), 6.53 (s, H-8, H-6), 6.46 (d, 16 Hz, H-α), 6.40 (d, 16 Hz, H-α), 6.06 (d, 8.5 Hz, H-1"), 5.9 (m, H-3"), 4.8 (m, 2H-6"), 4.32 (q, 8 and 9.5 Hz, H-2"), 4.2-3.8 (H-4", 5"), 3.7 (s, CH_3O). After elimination of the pyridine and drying of the sample, the PMR spectrum in deuteroacetone was measured again and found to be identical with that given above.

Acid Hydrolysis of (I). A mixture of 5 mg of compound (I) and 3 ml of 20% HCl was heated on a boiling-water bath. The course of the reaction was followed by TLC (a). After the mixture had been heated for 48 h, in addition to the aglycone that had been formed, the initial compound could still be detected. No other products of flavonoid nature were detected during the reaction. In the precipitate formed, kaempferol was identified by TLC (a, b) and by mass spectrometry (M^+ 286), and in the neutralized and evaporated aqueous residue glucose was identified by the PC method.

Alkaline Hydrolysis of (I). A mixture of 30 mg of compound (I) and 3 ml of 0.5% NaOH was heated at 60°C for 2 h. The course of the reaction was followed by TLC (a), which showed the formation of three products of flavonoid nature: (II) (R_f 0.25), (III) (R_f 0.4), and the rapidly disappearing compound (IV) (R_f 0.55). The mixture was neutralized with 2% HCl and was chromatographed on polyamide in the water–methanol system. At a 90:10 composition of the mixture, p-coumaric and ferulic acids were eluted, these being identified by TLC (b) and mass spectrometry (M^+ 164 and 194); the 85:15 mixture eluted compound (II) and the 60:40 mixture eluted compound (III). After recrystallization from methanol, 6 mg of compound (II) and 7 mg of compound (III) were obtained.

Kaempferol 3-O-β-D-Glucopyranoside (II). Composition $C_{21}H_{20}O_{11}\cdot H_2O$, mp 193–195°C, $[\alpha]_D^{20}$ −26° (c 0.2; methanol); λ_{max} (nm): MeOH 267, 305 sh., 352; NaOMe 277, 326, 403; NaOAc 276, 307, 386; NaOAc + H_3BO_3 267, 355; $AlCl_3$ and $AlCl_3$ + HCl 275, 305, 352, 403. PMR spectrum in deuteropyridine (ppm) 8.38 (d, 9 Hz, H-2', 6'), 7.15 (d, 9 Hz, H-3', 5'), 6.66 (s, H-6,8), 6.2 (d, 6 Hz, H-1"), 4.4-3.8 (6H of glucose).

6"-O-Feruloylastragalin (III). Composition $C_{31}H_{28}O_{14}$, mp 170–174°C, $[\alpha]_D^{20}$ −50° (c 0.6; methanol), ν_{CO} 1690, 1665 cm^{-1}; λ_{max}, nm (log ε): MeOH 267 (4.28), 300 sh. (4.36), 330 (4.40); NaOMe 275, 388; NaOAc 276, 318, 378; NaOAc + H_3BO_3 268, 300 sh., 330; $AlCl_3$ and $AlCl_3$ + HCl 277, 307, 338, 404. PMR spectrum in deuteropyridine (ppm): 8.37 (d, 9 Hz, H-2', 6'), 7.76

(d, 16 Hz, H-β), 7.20-7.10 (m, 5H), 6.6 (s, H-6, 8), 6.46 (d, 16 Hz, H-α), 6.08 (d, 7 Hz, H-1"), 4.86 (m, 2H-6"), 4.4-3.9 (4H of glucose), 3.8 (s, CH_3O).

Acetylation of (I). A mixture of 15 mg of (I), 0.3 ml of pyridine, and 1.0 ml of acetic anyydride was left at 20°C for 30 h, the course of the reaction being followed by TLC (c). When ice water was added, a precipitate deposited and this was washed with water and dissolved in ethanol, the solution was evaporated to dryness, and the residue was triturated in petroleum ether. This gave a white amorphous powder of the heptaacetate of (I) — (V) — with the composition $C_{54}H_{48}O_{23}\cdot 0.5H_2O$, mp 120-122°C, $[\alpha]_D^{20}$ −65.6° (c 0.86; acetone); ν_{CO} 1770, 1768, 1722, 1630 cm^{-1}. PMR spectrum in deuterochloroform (ppm): 8.00 (d, 9 Hz, H-2', 6'), 7.64 (d, 16 Hz, H-β), 7.55 (d, 16 Hz, H-β), 7.5-7.0 (m, 8H), 6.75 (d, 2.5 Hz, H-6), 6.3 (d, 16 Hz, H-α), 6.26 (d, 16 Hz, H-α), 5.7-5.1 (m, 4H of glucose), 4.1 (m, 2H-6"), 3.86 (s, CH_3O), 3.9-3.6 (m, H-5"), 5.42 (s, CH_3COO), 2.26 ($3CH_3COO$), 2.16 (s, CH_3COO), 2.06 (s, CH_3COO), 1.95 (s, CH_3COO).

SUMMARY

From the needles of the Siberian spruce we have isolated a new diacylated flavonoid glycoside (I) for which we have established the structure of 3-(3"-O-p-coumaroyl-6"-O-feruloyl-glucopyranosyloxy)-4',5,7-trihydroxyflavone.

The new acylated glucoside 6"-O-feruloylastragalin (III) has been obtained and characterized for the first time.

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