O-ACYLATED FLAVONOID GLYCOSIDES OF THE NEEDLES OF *Pinus sylvestris*

III. DI-p-COUMAROYLTRIFOLIN

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From the needles of the Scotch pine (*Pinus sylvestris* L.) we have isolated a new flavonol glycoside (I) acylated with two p-coumaric acid residues. Until now, this type of acylation has formed a rare case.

According to the PMR spectrum (Fig. 1) and to alkaline and acid hydrolysis, compound (I) includes residues of kaempferol, galactose, and p-coumaric acid in molar ration of 1:1:2. It must be mentioned that acid hydrolysis requires severe conditions (even after being boiled with 10% HCl for 24 h some of the starting material was still present).

The IR spectrum of compound (I) shows, in addition to the stretching vibrations of the CO group of kaempferol (1660 cm^{-1}) a broad band of ester groups with maxima at 1695 and 1705 cm^{-1}. The UV spectra of compound (I) with diagnostic additives (Fig. 2) does not make it possible to draw a definitive conclusion concerning the position of attachment of the carbohydrate moiety to the kaempferol, since a broad band at 315 nm that is characteristic for derivatives of cinnamic acid makes the long-wave maximum of the flavonoid moiety of the molecule. The bathochromic shift of the short-wave maximum on the addition of sodium acetate indicates the presence of a free 7-OH group, and a free 5-OH group is revealed by the PMR spectrum of (I) (singlet at 12.3 ppm, see Fig. 1). The conclusion that the galactose was attached to the 3-OH group of kaempferol was made on the basis of the UV spectra of the deacylated product (II), which was identified as trifolin (kaempferol 3-O-β-D-glactopyranoside).

To establish the complete structure of compound (I), it was necessary to answer the question of the particular part of trifolin — the carbohydrate, the flavonoid, or both simultaneously — to which the two p-coumaric acid residues were attached and to determine the nature of the acylation: either in the form of two monoacyl or in the form of one didepside residue. Stepwise alkaline hydrolysis yielded p-coumaric acid, trifolin (II), and trifolin monocoumaroate (III); the appearance of a rapidly-disappearing substance (IV) was also observed which, from its chromatographic behavior, was also probably a trifolin monocoumarate. On acid hydrolysis, only free kaempferol was obtained, and its acyl derivatives were not detected. The combination of these fact with the fact that the PMR spectrum of the full acetate (V) contains the signals of two aliphatic acetoxy groups (1.95 and 2.07 ppm) and of five aromatic acetoxy groups (2.42-2.17 ppm, 15 H, see Fig. 1) permits the deduction that both acyl residues are attached to the carbohydrate part of the molecule.

The positions of the acyl residues in the galactose were determined by an analysis of PMR spectra. The spectra of compound (I) and its heptaacetate (V) contain the signals of 14 aromatic and four olefinic protons, which are assigned to kaempferol and to two trans-p-coumaric acid residues ($J_{\alpha,\beta}$ = 16 Hz); in the spectrum of compound (II), the signals of the p-coumaric acid residues are absent, and in the spectrum of (III) there are characteristic signals of one trans-p-coumaric acid residue. The signal of the anomeric proton (doublet with J = 8 Hz), and also the values and signs of the optical rotations of compounds (I-III) permit them to be characterized as β-D-galactopyranosides in the C1 conformation. In the PMR spectrum of compound (I) in deuteroacetone, two of the seven carbohydrate protons resonate in the weak-field region. One signal we have assigned to H-1" (δ 5.32 ppm) and the second belongs to a hemiacyl proton (δ 4.96 ppm). The multiplicity of this signal (double doublet with J_1 = 3 Hz, J_2 = 11 Hz) is characteristic for a proton having the axial orientation and interacting with neighboring equatorial and axial protons. Such a signal of a hemiacyl proton can be realized only in the case of the acylation of the 3"-OH group of β-D-galactopyranose. The

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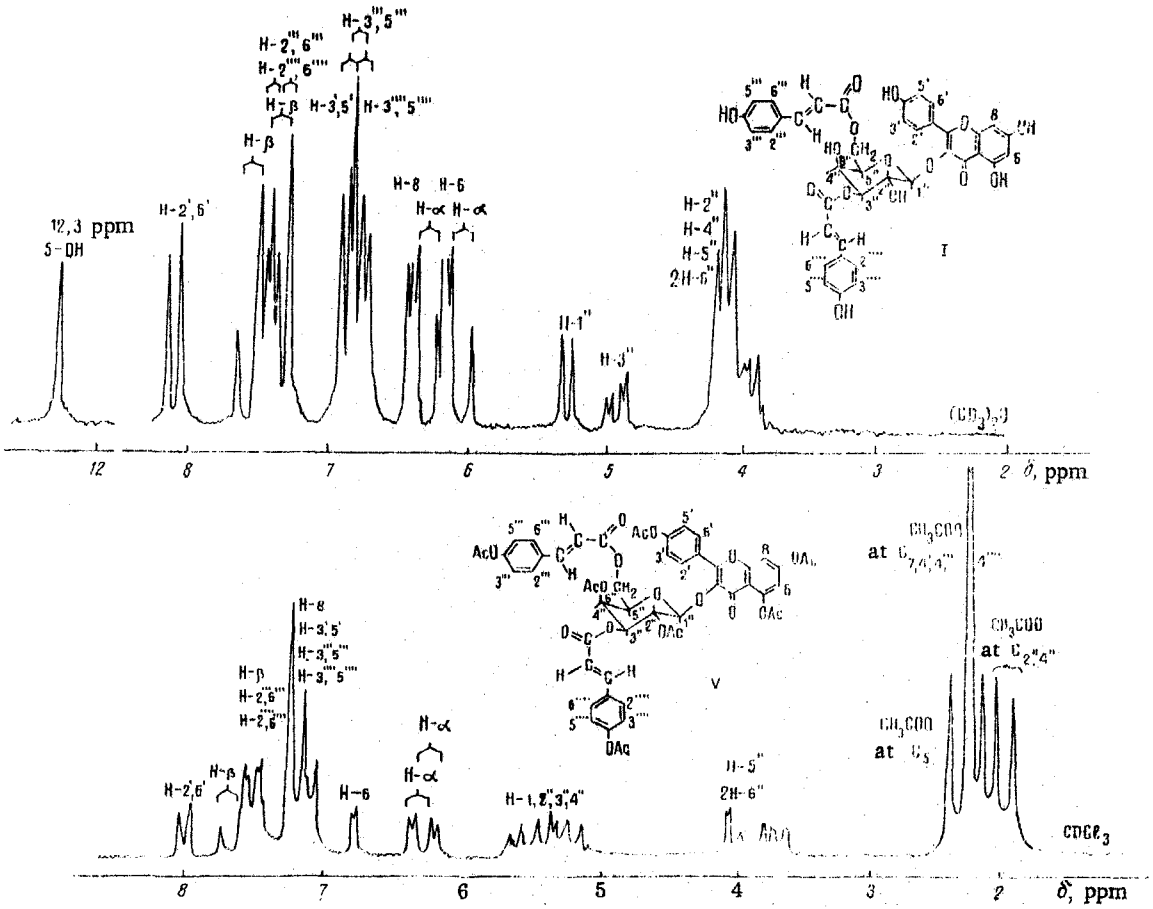

Fig. 1. PMR spectra of 3'',6''-di-O-p-coumaroyltrifolin (I) in deuteroacetone and of its heptaacetate (V) in deuterochloroform.

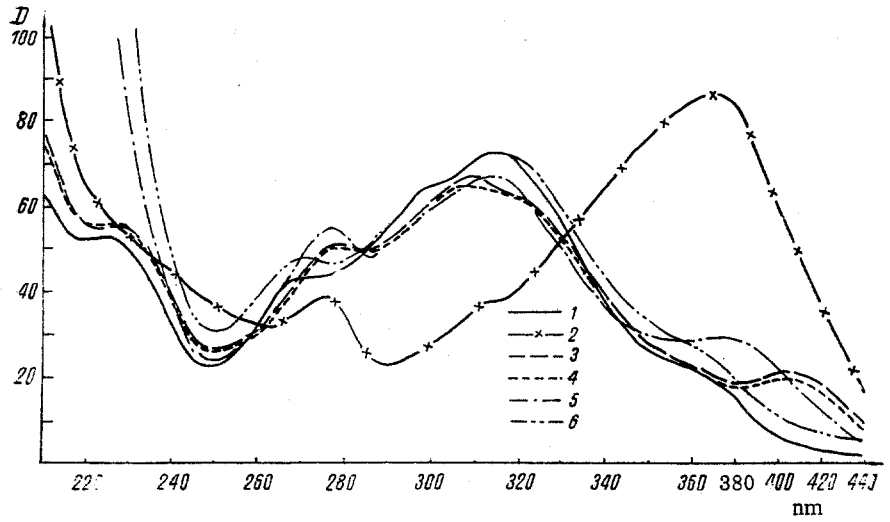

Fig. 2. UV spectra of 3",6"-di-O-p-coumaroyltrifolin (I): 1) MeOH; 2) NaOMe; 3) $AlCl_3$; 4) $AlCl_3$ + HCl; 5) NaOAc; 6) NaOAc + H_3BO_3.

position of attachment of the second acyl residue was found from a comparison of PMR spectra in [D]pyridine. Compounds (I) and (III) show similar two-proton multiplets with their centers at 4.8 ppm, which are characteristic for the protons of an acylated $-CH_2OH$ group. This fact shows that compound (III) has the structure of 6"-O-p-coumaroyltrifolin, and compound (I) corresponds to the structure of 3,4',5,7-tetrahydroxyflavone 3-O-(3",6"-di-O-p-coumaroyl-β-D-galactopyranoside).

The very small paramagnetic shift of the signals of the methylene hemiacyl protons explains the fact that in the PMR spectrum of compound (I) in [D]acetone the signals of the protons at C-6" are masked by the H-2", H-4", and H-5" signals.

EXPERIMENTAL

The spectra were obtained on the following instruments: UR-20 (paraffin oil, IR), Hitachi EPS-3T (UV), Varian HA-100D at 100 MHz, with tetramethylsilane as internal standard (PMR), and Varian CH-8 at 70 eV (mass spectra). The melting points were determined on a Kofler block; elementary analyses were carried out on a Hewlett–Packard 185B automatic CHN analyzer; angles of rotation were determined on a Polamat A polarimeter at 546 and 578 nm and recalculated to λ 589.3 nm. Chromatographic control was performed by TLC (Silufol) in the following solvent systems: 1) chloroform–methanol (4:1), 2) chloroform–methanol (9:1), and 3) benzene–acetone (3:1), and PC in the butanol–pyridine–water (6:4:3) system (descending).

Isolation. The ethereal fraction (32 g) obtained from 14.5 kg of Scotch pine needles (moisture content 51%) in the manner described previously [1] was chromatographed on polyamide in the chloroform–methanol system. At a 95:5 composition of the mixture, compound (I) was eluted from the mixture, its amount after repeated recrystallization from methanol being 200 mg.

3",6"-Di-O-p-coumaroyltrifolin (I). Light yellow acicular crystals, soluble in acetone and methanol, mp 263-266°C, composition $C_{39}H_{32}O_{15}\cdot H_2O$, $[\alpha]_D^{20}$ −77.4° (c 0.5; acetone); R_f 0.6 (system 1); ν_{CO} 1660, 1696, 1705 cm^{-1}; the UV spectra are given in Fig. 2: λ_{max} MeOH, nm (log ε): 268 (4.49), 300 sh (4.71), 3.15 (4.76), 3.60 sh (4.21).

PMR spectrum (see Fig. 1) in $(CD_3)_2CO$ (ppm): 12.3 (s, 5-OH), 8.13 (d, 9 Hz, H-2', 6'), 7.60 (d, 16 Hz, H-β), 7.48 (d, 8.5 Hz, H-2''', 6'''), 7.40 (d, 16 Hz, H-β'), 7.36 (d, 8.5 Hz, H-2'''', 6''''), 6.88 (d, 9 Hz, H-3', 5'), 6.84 (d, 8.5 Hz, H-3''', 5'''), 6.78 (d, 8.5 Hz, H-3'''', 5''''), 6.42 (d, 2.5 Hz, H-8), 6.32 (d, 16 Hz, H-α), 6.20 (d, 2.5 Hz, H-6), 6.10 (d, 16 Hz, H-α'), 5.32 (d, 8 Hz, H-1''), 4.96 (dd, 3 and 11 Hz, H-3''), 4.3-3.8 (5H of galactose), in C_5D_5N: 8.32 (d, 9 Hz, H-2', 6'), 7.86 (d, 16 Hz, H-β), 7.74 (d, 16 Hz, H-β), 7.44 (d, 8 Hz, H-2''', 6'''; H-2'''', 6''''), 7.16 (d, 9 Hz, H-3', 5'), 7.06 (d, 8 Hz, H-3''', 5'''; H-3'''', 5''''), 6.58 (s, H-6, H-8), 6.50 (d, 16 Hz, H-α), 6.40 (d, 16 Hz, H-α), 6.16 (d, 8 Hz, H-1''), 5.65 (dd, 3 and 11 Hz, H-3''), 4.8 (m, 2H-6''), 4.34 (dd, 8 and 11 Hz, H-2''), 4.15 (m, H-4'', H-5'').

Acid Hydrolysis of (I). A mixture of 10 mg of compound (I) and 4 ml of 10% HCl was heated on a boiling-water bath. The course of the reaction was followed by TLC (system 1). After the mixture had been heated for 24 h, in addition to the aglycone that had been formed, compound (I) was still detected, and it was hydrolyzed completely only after heating for 36 h. No other products of flavonoid nature were detected during the reaction. After the reaction mixture had cooled, the precipitate that had formed was filtered off, and kaempferol was identified in it by TLC (system 1) and mass spectrometry (M^+ 286). The filtrate was treated with diethyl ether and the extract was found by TLC (system 2) to contain p-coumaric acid, while galactose was identified by PC in the neutralized and evaporated aqueous residue.

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 1 h. The course of the reaction was followed by TLC (system 1), which showed the formation of three products of flavonoid nature: (II), (III), and the rapidly disappearing compound (IV) with R_f 0.45 [TLC (system 1)]. The mixture was neutralized with 2% HCl and chromatographed on polyamide in the water–methanol system. A mixture with the composition 90:10 eluted p-coumaric acid, which was identified by TLC (system 2) and by mass spectrometry (M^+ 164); and 85:15 mixture eluted compound (II), and a 60:40 mixture compound (III) with traces of (IV). Compounds (II) and (III) were then additionally purified by chromatography on polyamide in the chloroform–methanol system. The composition of the mixture was 85:15 for (II) and 90:10 for (III). After crystallization from methanol, compound (II) was obtained with a yield of 5 mg and compound (III) with a yield of 7 mg.

Kaempferol 3-O-β-D-Galactopyranoside (II). Composition $C_{21}H_{20}O_{11}\cdot 2H_2O$, mp 241-242°C, $[\alpha]_D^{20}$ −40.1° (c 0.2; methanol), R_f 0.25 [TLC (system 1)]; λ_{max}, nm, MeOH: 267, 300 sh, 352; NaOMe: 271, 326, 404; NaOAc: 275, 305, 380; NaOAC + H_3BO_3: 272, 300 sh, 355; $AlCl_3$ and $AlCl_3$ + HCl: 275, 305, 350, 400. PMR spectrum of (II) in deuteropyridine (ppm): 8.2 (d, 9 Hz, H-2', 6'), 7.1 (d, 9 Hz, H-3', 5'), 6.52 (d, 2 Hz, H-8), 6.46 (d, 2 Hz, H-6), 6.02 (d, 7 Hz, H-1''), 4.8-3.9 (m, 6 H of galactose).

6''-O-p-Coumaroyltrifolin (III). Composition $C_{30}H_{26}O_{13}$, mp 269-271°C, $[\alpha]_D^{20}$ −65.7° (c 0.28; methanol), R_f 0.4 [TLC (system 1)], ν_{CO} 1655, 1687 cm^{-1}; λ_{max}, nm (log ε), MeOH: 268 (4.31), 300 sh (4.37), 315 (4.41), 360 (4.08); NaOMe: 276, 370; NaOAc: 276, 312, 370; NaOAc + H_3BO_3: 268, 300 sh, 315, 360 sh; $AlCl_3$ and $AlCl_3$ + HCl: 276, 308, 320 sh, 404. PMR spectrum in deuteropyridine (ppm): 8.3 (d, 9 Hz, H-2', 6'), 7.68 (d, 16 Hz, H-β), 7.40 (d, 8 Hz, H-2''', 6'''), 7.09 (d, 9 Hz, H-3', 5'), 7.03 (d, 8 Hz, H-3''', 5'''), 6.56 (s, H-8 and H-6), 6.32 (d, 16 Hz, H-α), 5.95 (d, 8 Hz, H-1''), 4.74 (m, 2H-6''), 4.3-3.9 (4 H of galactose).

Acetylation of (I). A mixture of 10 mg of (I) 0.2 ml of pyridine, and 1.0 ml of acetic anhydride was left at 20°C for 24 h. The course of the reaction was followed by TLC (system 3). On the addition of ice water, a precipitate deposited which was washed with water and was purified by chromatography on silica gel in the benzene–acetone (4:1) system. After the solvent had been distilled off and the residue had been triturated in petroleum either, a white amorphous powder was obtained of the heptaacetate (I)-(V). Composition $C_{53}H_{46}O_{22}$, mp 118-120°C, $[\alpha]_D^{20}$ −70.5° (c 1.29; acetone), R_f 0.7 [TLC (system 3)], ν_{CO} 1767, 1757, 1720, 1632 cm^{-1}. PMR spectrum (see Fig. 1) in deuterochloroform (ppm): 8.00 (d, 9Hz, H-2', 6'), 7.66 (d, 16 Hz, H-β), 7.52 (d, 8.5 Hz, H-2''', 6'''), 7.50 (H-2'''', 6'''' and H-β'), 7.2-7.05 (H-3', 5'; H-3''', 5''', H-3'''', 5''''; H-8), 6.8 (d, 2.5 Hz, H-6), 6.32 (d, 16 Hz, H-α), 6.26 (d, 16 Hz, H-α'), 5.6-5.0 (H-1'', 2'', 3'', 4''), 4.1-3.6 (H-5'', 2H-6''), 2.42 (s, CH_3COO), 2.26 ($3CH_3COO$), 2.17 (s, CH_3COO), 2.07 (s, CH_3COO), 1.95 (s, CH_3COO).

SUMMARY

A new diacylated flavonol glycoside has been isolated for the first time from Scotch pine needles, and for it the structure of 3,4',5,7-tetrahydroxyflavone 3-O-(3'',6''-di-O-p-coumaroyl-β-D-galactopyranoside) has been established.

In the course of the chemical study of the compound isolated, the previously undescribed 6''-O-p-coumaroyltrifolin was isolated and characterized.

LITERATURE CITED

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