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

II. 3"-O-p-COUMAROYLISOQUERCITRIN

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Among acylated flavonoid glycosides, p-coumaroyl derivatives of kaempferol 3-glucoside (tribuloside, tiliroside) are widely distributed [1-3]. As the result of a number of investigations it has been established that in tribuloside the acyl residue is attached to C-6" of the glucose moiety [1] but the question of the localization of this residue in tiliroside still remains open [2, 3]. It is interesting to note that until now no coumaroyl derivatives of another widely known compound — quercetin 3-glucoside — have ever been detected.

We have isolated compound (I) from the needles of the Scotch pine (*Pinus sylvestris* L.), and this, according to the results of acid hydrolysis and PMR spectroscopy, contains in equimolar ratio quercetin, glucose, and p-coumaric acid. The IR spectrum of compound (I) has, in addtion to ν_{CO} of quercetin at 1660 cm^{-1}, an absorption band of the carbonyl of an ester group at 1695 cm^{-1}. Under conditions of mild alkaline hydrolysis, (I) yielded p-coumaric acid and compound (II) gave quercetin 3-O-β-D-glucopyranoside (isoquercitrin).

The production of isoquercitrin enabled us to answer the question of the position of attachment of the carbohydrate residue to quercetin in (I), since it was difficult to draw a definite conclusion from the UV spectra of this compound (Fig. 1).

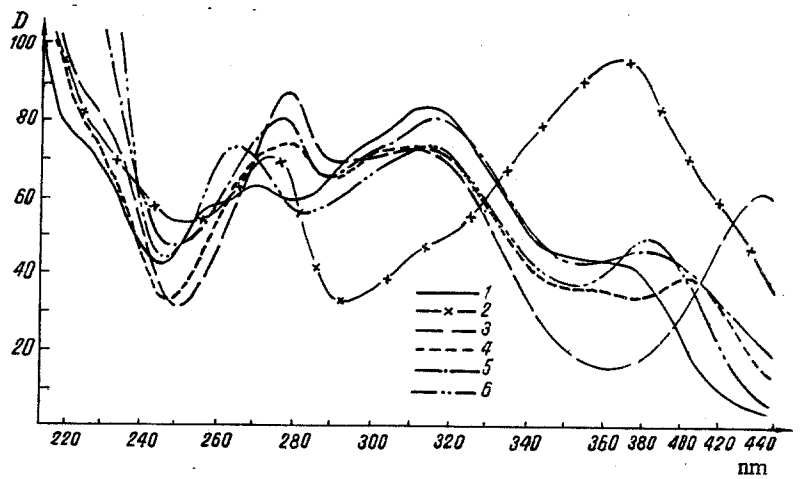

Fig. 1. UV spectra of 3"-O-p-coumaroylisoquercitrin (I): 1) MeOH; 2) NaOMe; 3) $AlCl_3$; 4) $AlCl_3$ + HCl; 5) NaOAc; 6) NaOAc + H_3BO_3.

The acetylation of (I) gave the octaacetate (III), in the PMR spectrum of which there were the signals of three aliphatic acetoxy groups. These facts, in combination with the presence of the signal of a hemiacyl proton in the PMR spectrum of compound (I) show the attachment of the p-coumaric acid to the carbohydrate part of the molecule. We showed the fine structure of compound (I) with the aid of proton magnetic resonance.

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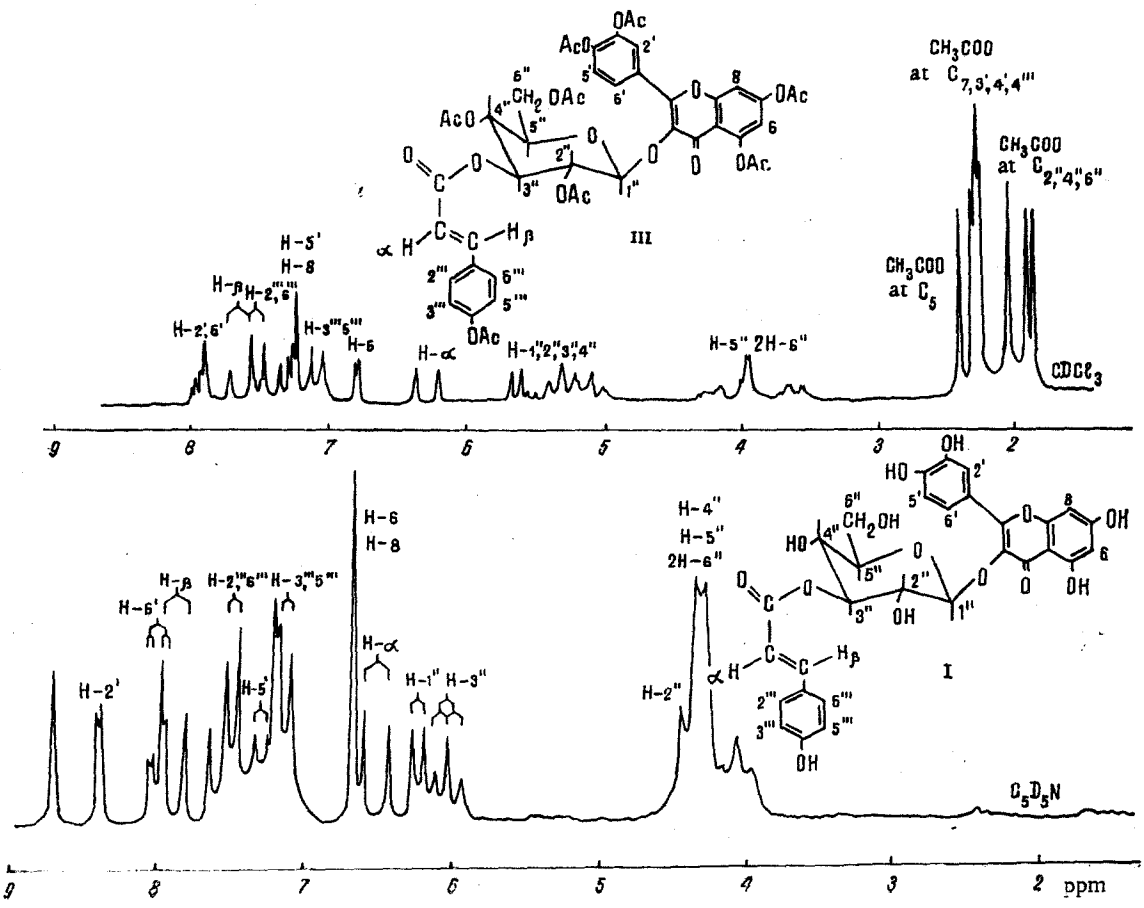

Fig. 2. PMR spectra of 3"-O-p-coumaroylisoquercitrin (I) in C_5D_5N and its octaacetate (III) in $CDCl_3$ (100 MHz, TMS).

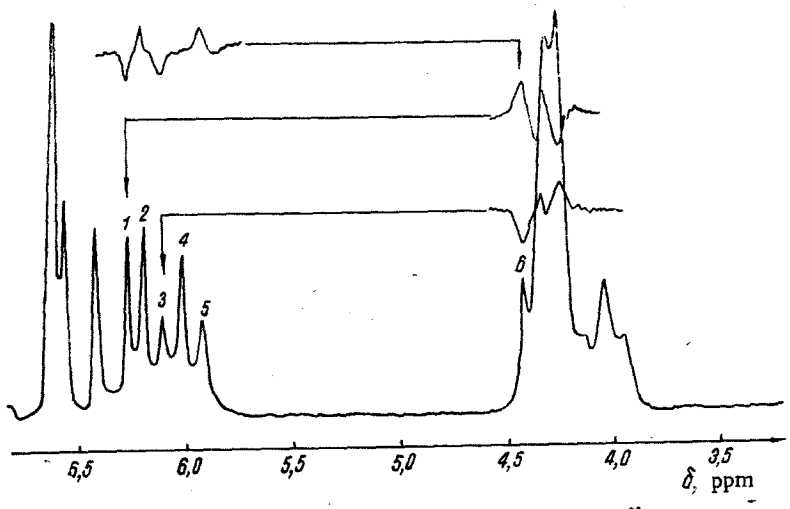

Fig. 3. Fragment of the PMR spectrum of 3"-O-p-coumaroylisoquercitrin (I) in C_5D_5N and INDOR spectra.

In the weak-field region of the PMR spectrum of (I) (Fig. 2) can be seen the signals of 11 aromatic protons, which are assigned to quercetin and to p-coumaric acid (the constants J = 16 Hz indicate the trans position of the α and β protons in the p-coumaric acid). The glucose protons give overlapping signals in the 3.9-4.5 ppm region (5 H) and two one-proton signals in a weaker field: a doublet relating to the anomeric proton of β-D-glucopyranose (6.23 ppm, J = 8 Hz) and a triplet (6.01 ppm, $J_1 = J_2 = 9$ Hz) belonging to the H-3" or H-4" hemiacyl proton (in the case of acylation of the 2"-OH group, one of the spin–spin coupling constants would be $J_{1,2} = 8$ Hz).

In order to assign this signal to one of these protons – H-3" or H-4" – we used the INDOR method, which shows the positions and structures of the signals of protons on neighboring carbon atoms. If the signal concerned related to H-3", then H-1" and H-3" would interact with the same proton, H-2". If, however, the signal related to H-4", then H-1" and H-4" would interact with different protons and the structures of their signals would differ.

The INDOR spectrum of compound (I) in deuteropyridine (Fig. 3) was obtained on the weak-field lines (1) of the doublet (6.23 ppm, $J_{1,2} = 8$ Hz) and (3) of the triplet (6.01 ppm, ΣJ = 18 Hz). In the two cases, the signals appeared at the same positions of the spectrum. Consequently, the INDOR spectrum relates to the H-2" proton (δ = 4.33, $J_{1,2} = 8$ Hz, $J_{2,3} = 10$ Hz).

As was to be expected, in the INDOR spectrum obtained on the weak-field line (6) of the H-2" quartet signals appeared above the lines of the doublet (6.23 ppm) and the triplet (6.01 ppm). Thus, it may be considered that the triplet relates to the H-3" proton and, consequently, the p-coumaric acid acylates the 3"-OH group of the glucose. Thus, compound (I) is the previously undescribed 3"-O-p-coumaroylisoquercitrin (see Fig. 2).

EXPERIMENTAL

For general information, see p. 157.

Isolation. The ether-soluble fraction (32 g) obtained from 14.5 kg of Scotch-pine needles (moisture content 51%) by the method described previously [4] was chromatographed on polyamide in the chloroform–methanol system. With a composition of the mixture of 9:1, compound (I) was eluted from the column, and its yield after three recrystallizations from methanol and washing with acetone was 55 mg.

3"-O-p-Coumaroylisoquercitrin (I) formed light yellow crystals with mp 175-178°C; composition $C_{30}H_{26}O_{14}\cdot H_2O$; $[\alpha]_D^{20}$ −36.6° (c 0.4; methanol); R_f 0.47, TLC (system 1). ν_{CO} 1695, 1660 cm^{-1}. λ_{max}^{MeOH}, nm (log ε): 257 sh (4.39), 270 (4.42), 300 sh (4.51), 314 (4.55), 360 (4.25).

PMR spectrum in C_5D_5N (see Fig. 2): 8.37 (d, 2.5 Hz, H-2'), 7.97 (dd, 2.5 and 9 Hz, H-6'), 7.84 (d, 16 Hz, H-β), 7.46 (d, 9 Hz, H-2"',6"'), 7.28 (d, 9 Hz, H-5'), 7.10 (d, 9 Hz, H-3"',5"'), 6.64 (s, H-6 and H-8), 6.48 (d, 16 Hz, H-α), 6.23 (d, 8 Hz, H-1"), 6.01 (t, 9 and 9 Hz, H-3"), 4.5-3.9 ppm (5 H of glucose).

Acid Hydrolysis of (I). A mixture of 5 mg of (I) and 2 ml of 10% HCl was heated at 100°C for 3 h. After cooling, the precipitated aglycone was filtered off, and was identified by TLC in system 2 and also by UV and mass spectroscopy as quercetin. The aqueous solution was evaporated to dryness, and in it glucose was identified by PC and p-coumaric acid by mass spectrometry (M^+ 164) and by TLC in system 2.

Alkaline Hydrolysis of (I). A mixture of 15 mg of (I) and 2 ml of 0.5% NaOH was heated at 60°C for 30 min. Then the mixture was neutralized with 2% HCl and was chromatographed on polyamide in chloroform–methanol. A 95:5 mixture eluted p-coumaric acid (TLC, system 2), and an 85:15 mixture eluted isoquercitrin (II), mp 228-230°C, $C_{21}H_{20}O_{12}$, $[\alpha]_D^{20}$ −32° (c 0.4; formamide); R_f 0.17, TLC (system 1). λ_{max}^{MeOH} 257, 360 nm.

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 (with monitoring by TLC in system 3). On the addition of ice water a precipitate deposited, and this was washed with water and 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 ether, a white amorphous powder of the octaacetate (III) was obtained with mp 100-103°C, composition $C_{46}H_{42}O_{22}$, $[\alpha]_D^{20}$ −81.1° (c 0.55; acetone), R_f 0.65, TLC (system 3). ν_{CO} 1765, 1755, 1720, 1630 cm^{-1}. PMR spectrum in

[D]acetone: 8.1 (m, H-2', 6'), 7.70 (d, 9 Hz, H-2"', 6"'), 7.66 (d, 16 Hz, H-β), 7.46 (d, 2.5 Hz, H-8), 7.43 (d, 9 Hz, H-5'), 7.16 (d, 9 Hz, H-3"',5'"), 6.95 (d, 2.5 Hz, H-6), 6.42 (d, 16 Hz, H-α), 5.85-5.0 (H-1",2",3",4"), 4.3-3.8 (m, H-4", 2H-6"), 2.38 (3H), 2.32 (6 H), 2.30 (3 H), 2.26 (3 H) (four singlets of five aromatic acetoxy groups), 2.08, 1.9, 1.8 (three singlets of three aliphatic acetoxy groups).

SUMMARY

A new acylated flavonoid glycoside has been isolated from Scotch pine needles and the structure of 3',4',5,7-tetrahydroxyflavone 3-O-β-D-(3"-O-p-coumaroylglucopyranoside) has been established for it.

LITERATURE CITED

1. S. P. Bhutani, S. S. Chibber, and T. R. Seshadri, Phytochemistry, 8, 299 (1969).
2. L. Horhammer, L. Stich, and H. Wagner, Arch. Pharmazie, 294/66, 43 (1961).
3. J. B. Harborne, Phytochemistry, 3, 151 (1964).
4. S. A. Medvedeva, S. Z. Ivanova, N. A. Tyukavkina, and G. G. Zapesochnaya, Khim. Prirodn. Soedin., 650 (1977).

AN O-ACYLATED FLAVONOID GLYCOSIDE FROM THE NEEDLES OF *Picea koraiensis*

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Flavonoid compounds of needles of the genus *Picea* (family Pinacaea) have been studied very little. There are only reports on the detection of kaempferol [1] and of taxifolin 3'-glucoside [2].

We have previously established the presence of a number of flavonoids in the needles of the Yeddo spruce [3]. On investigating a methanolic extract of the needles of the Koyama spruce (*Picea koraiensis* Nakai) we have isolated for the first time an acylated flavonoid glycoside (I) the hydrolytic decomposition of which led to equimolar amounts of kaempferol, arabinose, and p-coumaric acid.

The UV spectrum of compound (I) in methanol (Fig. 1) has two bands at 268 and 318 nm, the latter being very broad. A free 5-OH group in the molecule of (I) was detected from its PMR spectrum (singlet at 12.6 ppm, Fig. 2). The presence of an ester group in (I) was confirmed by a band at 1700 cm^{-1} in the IR spectrum and also by the production from (I) by saponification under mild conditions of kaempferol 3-arabinoside (II) and p-coumaric acid. The attachment of the arabinose to the 3-OH group of kaempferol was shown on the basis of the UV spectra of compound (II) with diagnostic reagents.

The PMR spectra of compound (I) and its full acetate (III) (see Fig. 2) contained the signals of 12 protons of the aromatic part of the molecule, which were assigned to kaempferol and to trans-p-coumaric acid ($J_{\alpha,\beta}$ = 16 Hz). The carbohydrate protons in the spectra of (I-III) also form distinct signals the multiplicity and chemical shifts of which enable their assignments to be made: the anomeric proton forms a singlet, the proton at C-2" a doublet with $J_{2,3}$ = 2 Hz, the proton at C-3" a doublet of doublets ($J_{2,3}$ = 2 Hz, $J_{3,4}$ = 5 Hz), and the proton at C-4" forms a multiplet appearing in the form of a quartet. The signals of the methylene protons at C-5" in the spectra of (I) form a doublet; in the spectrum of the acetate (III) the signals of these protons appear in the form of two quartets (with a difference between the chemical shifts $\Delta\delta$ = 0.14 Hz) and undergo a paramagnetic shift, while the H-4" signal is displaced upfield. This means that in compound (I) there is no hydroxy group at C-4". This nature of the H-4" and 2H-5" signals enables substances (I-III) to be characterized as arabinofuranosides. In the case of arabinopyranosides, acetylation

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