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

I. O-ACETYLATED DERIVATIVES OF FLAVONOL GLYCOSIDES

G. G. Zapesochnaya, S. Z. Ivanova,
S. A. Medvedeva, and N. A. Tyukavkina

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In the field of well-studied flavonoid glycosides, a new group of compounds has appeared relatively recently — their acylated derivatives. The acylating acids are more frequently hydroxyaromatic acids (p-coumaric, ferulic) and more rarely aliphatic acids (acetic, malonic) [1]. The most disputed question has been that of the position of the acyl residue. At the present time it has been established that the following sequence of binding of the fragements exists: heterocycle (flavonoid) — carbohydrate — acid. A similar sequence of binding is known in the field of nitrogen-containing heterocycles (mononucleotides).

There is no information on the presence of O-acylated flavonoid glycosides in coniferous woody plants, with the exception of a recent report of the detection of such compounds in larch needles [2].

From the needles of the Scotch pine (*Pinus sylvestris* L.) we have isolated three compounds (I-III) that are O-acetylated derivatives of flavonoid glycosides.

From the results of hydrolytic cleavage, (I) contains isorhamnetin and glucose, II contains isorhamnetin and galactose. and the components of (III) are quercetin and glucose. Compounds (I-III) differ chromatographically from the corresponding 3-glycosides though they have IR spectra identical with them.

The acetylation of the flavonol glycosides can be judged from their IR and PMR spectra. The IR spectra of compounds (I-III) contain an additional band, as compared with the IR spectra of the corresponding flavonol 3-glycoside, of an acetate carbonyl at 1720 (I), 1730 (II), and 1705 (III) cm^{-1}. The PMR spectra of compounds (I-III) include three-proton singlets at 1.75, 1.61, and 1.70 ppm, respectively, which shows the presence of a CH_3COO group in the carbohydrate moiety of each molecule. This conclusion is also confirmed by the fact that the acetylation of (I) gave an acetate identical with isorhamnetic 3-O-β-D-glucopyranoside heptaacetate; the full acetate of (III) was identical in composition, melting point, and PMR spectrum with isoquercetin octaacetate, and the product of the alkaline saponification of (II) was identical in respect of its UV spectrum and chromatographic behavior with isorhamnetin 3-O-β-D-galactoside [3].

The question of the position of the acetyl residue in the molecule has been solved mainly on the basis of PMR spectra. The PMR spectra of compounds (I-III) in [D]pyridine and of their TMS ethers in CCl_4 contain in the weak field, in addition to the signal of the anomeric proton, two-proton multiplets the origin of which can be explained only by the acetylation of a primary alcohol group of a glucose or galactose residue [4]. If the secondary hydroxyls at C-2", C-3", or C-4" in the molecule had been acetylated, one-proton signals in the form of a triplet or doublet of doublets would have been observed in the weak field. Thus, we

All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Irkutsk Institute of Organic Chemistry, Siberian Branch of the Academy of Sciences of the USSR. I. M. Sechenov I Moscow Medical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 193-196, March-April, 1978. Original article submitted November 11, 1977.

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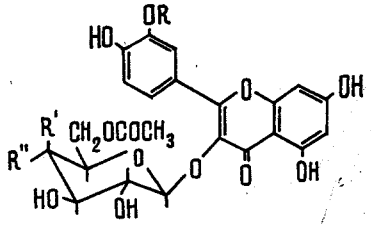
assigned the position of the acetate group to C-6" and have established the following structures for the compounds isolated:



I. R=CH_3, R′=H, R″=OH;
II. R=CH_3, R′=OH, R″=H;
III. R=H, R′=H, R″=OH.

Compounds (I) and (II) are new, while (III) — quercetin 3-(6"-O-acetyl-β-D-glucopyranoside) [mp 212-216°C, $[\alpha]_D^{20}$ +10° (ethanol)] has been isolated previously from *Peucedanum ostruthium* [5].

EXPERIMENTAL

The spectra were obtained on the following instruments: UR-20, in paraffin oil (IR); Hitachi EPS-3T (UV); Varian HA-100D at 100 MHz, with tetramethylsilane as internal standard (PMR); Varian CH-8 at 70 eV (mass spectra). The melting points were determined on a Kofler block. Elementary analysis was carried out on a Hewlett—Packard 185B automatic CHN analyzer, and the angles of rotation were determined on a Polamat A polarimeter at 546 and 578 nm with recalculation to λ 589.3 nm.

Chromatographic monitoring was carried out by TLC (Silufol) in the following systems: 1) chloroform—methanol (4:1); 2) chloroform—methanol (9:1); 3) benzene—acetone (3:1) and by PC in the butanol—pyridine—water (6:4:3) system (descending).

Isolation. An evaporated methanolic extract from 14.5 kg (moisture content 51%) of Scotch pine needles freed from chlorophyll and treated with petroleum and diethyl ether as described previously [6] was extracted with butanol. The butanolic extract (200 g) was chromatographed on a polyamide sorbent (800 g), which was washed first with water and then with methanol. The methanolic fractions (40 g) were chromatographed on polyamide sorbent with elution by chloroform—methanol. At a composition of the mixture of 9:1, compounds (I) and (II) were eluted from the column, and at 8.5:1.5 compound (III) was obtained, and all these compounds were repeatedly recrystallized from methanol.

Isorhamnetin 3-O-β-D-(6"-O-Acetylglucopyranoside) (I). Yield 100 mg; composition $C_{24}H_{24}O_{13}\cdot 2H_2O$; R_f 0.45, TLC (system 1); mp 156-160°C; $[\alpha]_D^{20}$ 0.00° (c 0.31; methanol); ν_{CO} 1720, 1650 cm^{-1}; λ_{max}, MeOH, nm (log ε): 255 (4.28), 265 sh, 357 (4.19); NaOMe 273, 333, 418; NaOAc 276, 323, 386; NaOAc + H_3BO_3 256, 265 sh, 359; $AlCl_3$ 268, 303, 360sh, 406; $AlCl_3$ + HCl 268, 303, 361, 404 nm.

PMR in C_5D_5N (ppm): 8.2 (d, 2 Hz, H-2'), 7.75 (dd, 2 and 9 Hz, H-6'), 7.1 (d, 9 Hz, H-5'), 6.6 (m, 2 H, H-6, H-8), 6.1 (d, 7 Hz, H-1"), 4.6 (m, 2 H-6"), 4.3-3.9 (m, 4 H of glucose), 3.7 (s, CH_3O),1.62 (s, CH_3CO); TMS ether in CCl_4 (ppm): 7.6 (H-2'), 7.3 (H-6'), 6.76 H-5'), 6.4 (H-8), 6.06 (H-6), 5.7 (H-1"), 4.3-4.0 (2H-6"), 3.9-3.3 (4H of glucose), 3.9 (CH_3O), 1.75 (CH_3CO).

The hexaacetate (I) had the composition $C_{36}H_{36}O_{19}$; R_f 0.63, TLC (system 3); mp 97-100°C (amorphous compound); $[\alpha]_D^{20}$ −70.8° (c 2.57; acetone); ν_{CO} 1755, 1630 cm^{-1}. The PMR ($CDCl_3$) had the signals of four aliphatic acetoxy groups (1.84, 1.91, 1.94, and 2.03 ppm) and of three aromatic acetoxy groups (2.25, 6 H, and 2.38 ppm).

Isorhamnetin 3-O-β-D-(6"-O-Acetylgalactopyranoside) (II). Yield 20 mg; composition $C_{24}H_{24}O_{13}$; R_f 0.5, TLC (system 1); mp 230-232°C; $[\alpha]_D^{20}$ +8.86° (c 0.33; methanol); ν_{CO} 1730, 1655 cm^{-1}. The UV spectrum was similar to that of (I).

PMR spectrum in C_5D_5N (ppm): 8.4 (d, 2 Hz, H-2'), 7.7 (dd, 2 and 9 Hz, H-6'), 7.1 (d, 9 Hz, H-5'), 6.6 (m, H-6, H-8), 6.17 (d, 8 Hz, H-1"), 4.6 (m, 2 H-6"), 4.3-3.8 (m, 4 H of galactose), 3.84 (s, CH_3O), 1.61 (s, CH_3CO).

Quercetin 3-O-β-D-(6"-O-Acetylglucopyranoside) (III). Yield 500 mg; composition $C_{23}H_{22}O_{13}\cdot H_2O$; R_f 0.31, TLC (system 1); mp 217-219°C; $[\alpha]_D^{20}$ +7.21° (c 0.416; methanol); ν_{CO} 1705, 1660 cm^{-1}. λ_{max}, MeOH, nm (log ε): 257 (4.33), 265 sh, 360 (4.26); NaOMe 273, 330, 416; NaOAc 275, 325, 382; NaOAc + H_3BO_3 263, 300, 380; $AlCl_3$ 274, 300 sh, 434; $AlCl_3$ + HCl 270, 301, 360, 402 nm.

PMR spectrum in C_5D_5N (ppm): 8.2 (d, 2 Hz, H-2'), 7.9 (dd, 2 and 9 Hz, H-6'), 7.12 (d, 9 Hz, H-5'),6.6 (d, 2 Hz, H-8), 6.54 (d, 2 Hz, H-6), 6.0 (d, 8 Hz, H-1"), 4.76-4.4 (m, 2 H-6"), 4.3-3.9 (m, 4 H of glucose), 1.68 (s, CH_3CO); TMS ether in CCl_4 (ppm): 7.3 (H-2', 6'), 6.76 (H-5'), 6.4 (H-8), 6.06 (H-6), 5.66 (H-1"), 4.2-3.9 (2H-6"), 3.8-3.2 (4H of glucose),1.7 (CH_3CO).

The pentaacetate (III) had the composition $C_{37}H_{36}O_{20}$; R_f 0.53, TLC (system 3); mp 170-172°C; $[\alpha]_D^{20}$ −87.14° (c 0.7; acetone); ν_{CO} 1755, 1630 cm^{-1}. The PMR spectrum ($CDCl_3$) had the signals of four aliphatic acetoxy groups (1.84, 1.92, 1.95, 2.04 ppm) and four acetoxy groups (2.24, 2.26, 2.28, 2.38 ppm).

The trimethylsilyl ethers of compounds (I-III) were obtained by a standard method [3].

Acid Hydrolysis. A 3- and 5-mg sample of compound (I), (II), or (III) was heated with 1-2 ml of 10% HCl in the boiling water bath for 2-3 h. After cooling, the aglycone that had crystallized out was filtered off, and it was analyzed by GLC in system 2 and also by UV and mass spectrometry. The aqueous residue was neutralized on AV-17 ion-exchangers resin and evaporated, and the carbohydrate was identified by PC.

Alkaline Hydrolysis. A 3- to 5-mg sample of compound (I), (II), or (III) was heated with 1-2 ml of 0.5% NaOH in the water bath at 60°C for 0.5 h. Then the mixture was neutralized with 2% HCl and was chromatographed on polyamide, being eluted with water—methanol (8.5:1.5). Under these conditions, compound (I) yielded isorhamnetin 3-glucoside with R_f 0.28, TLC (system 1); compound (II) yielded isorhamnetin 3-galactoside with R_f 0.28, TLC (system 1); and compound (III) yielded quercetin 3-glucoside with R_f 0.17, TLC (system 1). To identify the compounds obtained we also used UV spectrosocopy and acid hydrolysis.

Acetylation. A mixture of 5 mg of (I) or (III), 0.3 ml of pyridine, and 0.8 ml of acetic anhydride was left at room temperature for 24 h. On the addition of ice water, a precipitate deposited which was washed with water and recrystallized from ethanol [acetate of (I)]. The acetate of (III) was chromatographed on silica gel in the benzene—acetone (4:1) system and after the solvent had been distilled off and the residue had been triturated in petroleum ether, a white amorphous powder was obtained.

SUMMARY

O-Acetylated flavonol glycosides have been isolated for the first time from the needles of the Scotch pine and the following structures have been established for them: 3,4'5,7-tetrahydroxy-3'-methoxyflavone 3-O-β-D-(6"-O-acetylglucopyranoside), 3,4',5,7-tetrahydroxy-3'-methoxyflavone 3-O-β-D-(6"-O-acetylgalactopyranoside), and 3,3',4',5,7-pentahydroxyflavone 3-O-β-D-(6"-O-acetylglucopyranoside). The first two compounds have not previously been described in the literature.

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