[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.

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

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UDC 547.972

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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Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 200-204, March-April, 1978. Original article submitted November 15, 1977.

0009-3130/78/1402-0162$07.50

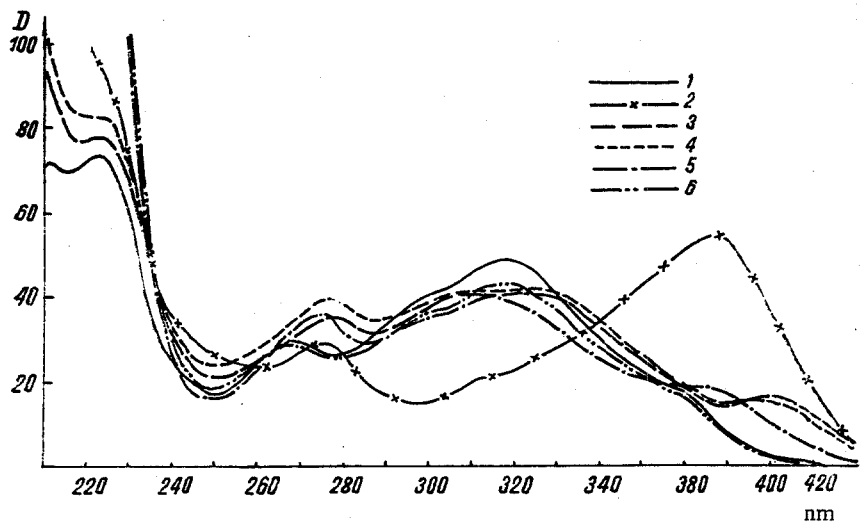

Fig. 1. UV spectra of 2"-O-p-coumaroyljuglanin (I): 1) MeOH 2) NaOMe; 3) $AlCl_3$; 4) $AlCl_3$ + HCl; 5) NaOAc; 6) NaOAc + H_3BO_3.

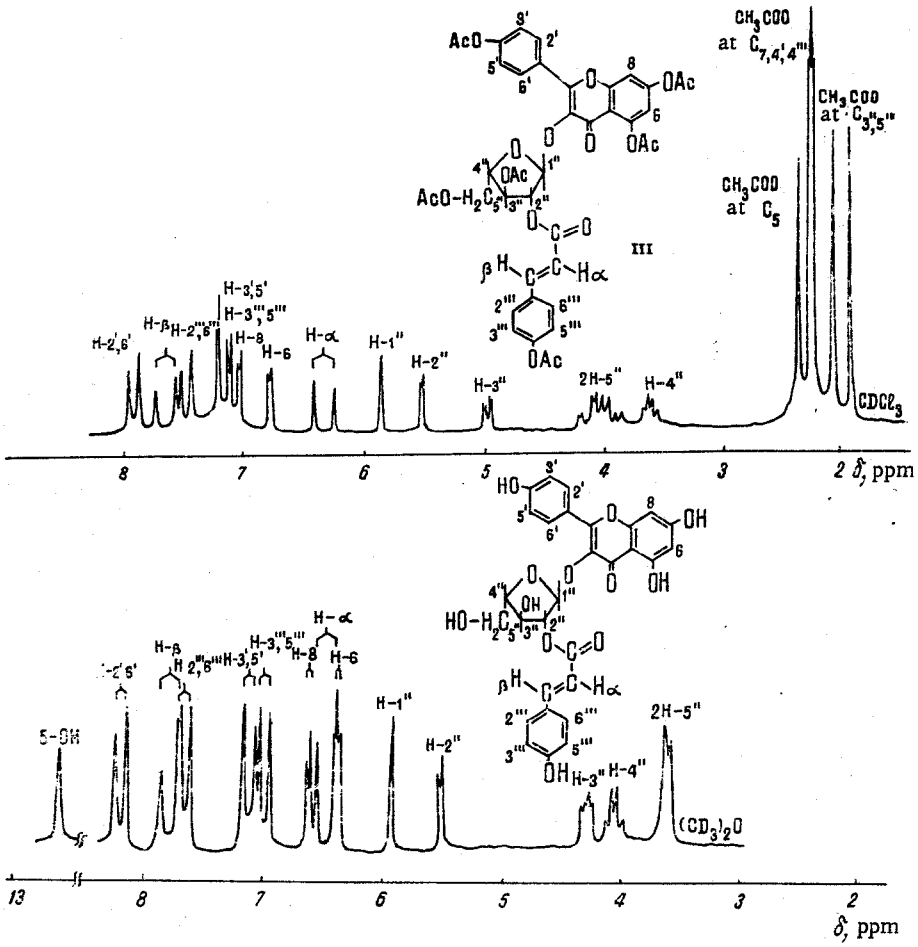

Fig. 2. PMR spectra of 2"-O-p-coumaroyljuglanin (I) in deuteroacetone and of its hexaacetate (II) in deuterochloroform (100 MHz, TMS).

would lead to a paramagnetic shift of the signal of the H-4" proton (and to analogous shifts for all other methine protons) and the H-5"e signals remaining in the strong field would differ considerably in chemical shifts ($\Delta\delta$ = 0.60 ppm) [4].

The characteristics of the PMR spectra that have been given and also the magnitude and sign of the optical rotations of compounds (I) and (II) give grounds for assuming the furanose form of L-arabinose with the α configuration of the glycosidic bond.

In respect of its constants and by a direct comparison, compound (II) was identical with juglanin (kaempferol 3-α-L-arabinoside, mp 224-225°C, $[\alpha]_D^{20}$ [c 0.82; ethanol—pyridine (9:1)] [5]. Thus, our results enable the structure of juglanin to be refined as kaempferol 3-O-α-L-arabinofuranoside.

The position of attachment of the p-coumaric acid residue was shown by comparing the PMR spectra of the isolated compound (I) and its full acetate (III). The spectrum of (III) has the signals of two aliphatic acetoxy groups (three-proton signals at 2.06 and 1.92 ppm) (see Fig. 2), which shows the attachment of the p-coumaric acid to the carbohydrate part of the molecule. The signal of the hemiacyl proton in the PMR spectrum of (I) (5.53 ppm, d, J = 2 Hz) can be realized only if the 2"-OH group of the α-L-arabinofuranoside residue is acylated. Thus, the new natural compound (I) that we have isolated has the structure of kaempferol 3-O-α-L-(2"-O-p-coumaroylarabinofuranoside) (see Fig. 2).

This is the first time that such a compound has been isolated from needles of the genus *Picea*. There are reports in the literature on the detection of a kaempferol coumaroylarabinoside (bryophylloside) in *Bryophyllum daigremontianum* [6] and of kaempferol 3-p-coumaroylarabinoside in the needles of *Larix decidua* [7], but no constants or structural information was given.

EXPERIMENTAL

For general information, see p. 157.

Isolation. Freshly-collected needles of the Koyama spruce (16.3 kg, moisture content 15%) were extracted three times with methanol by steeping at room temperature. The methanolic extract was concentrated in vacuum, the residue was freed from chlorophyll by decantation, and it was treated with petroleum ether and diethyl ether. The evaporated and dried diethyl ether extract (36 g) was chromatographed on polyamide sorbent. First the column was washed with chloroform, and then chloroform—methanol (95:5) eluted compound (I). After repeated recrystallization from methanol its yield was 50 mg.

Kaempferol 3-O-α-L-(2"-O-p-coumaroylarabinofuranoside) (I) consisted of faintly yellowish acicular crystals soluble in acetone and methanol, mp 181-185°C; composition $C_{29}H_{24}O_{12}\cdot H_2O$; $[\alpha]_D^{20}$ −78.7° (c 0.57; methanol); R_f 0.60, TLC (system 1). ν_{CO} 1700, 1655 cm^{-1}.

The UV spectra are given in Fig. 1; λ_{max}, MeOH, nm (log ε): 268 (4.26), 318 (4.47); 360 sh. (4.02).

PMR spectrum (see Fig. 2) in $(CD_3)_2CO$ (ppm): 12.6 (s, 5-OH), 8.2 (d, 9 Hz, H-2',6'), 7.77 (d, 16 Hz, H-β), 7.66 (d, 9 Hz, H-2"',6"'), 7.12 (d, 9 Hz, H-3',5'), 6.98 (d, 9 Hz, H-3"',5"'), 6.62 (d, 2.5 Hz, H-8), 6.47 (d, 16 Hz, H-α), 6.38 (d, 2.5 Hz, H-6), 5.92 (s, H-1"), 5.53 (d, 2.5 Hz, H-2"), 4.3 (dd, 2.5 and 5.5 Hz, H-3"), 4.08 (m, H-4"), 3.64 (d, 4.5 Hz, 2 H-5").

PMR spectrum in C_5D_5N (ppm): 8.32 (d, 9 Hz, H-2',6'), 7.82 (d, 16 Hz, H-β), 7.48 (d, 9 Hz, H-2"',6"'), 7.24 (d, 9 Hz, H-3',5'), 7.08 (d, 9 Hz, H-3"',5"'), 6.68 (m, H-6,8), 6.6 (s, H-1"), 6.44 (d, 16 Hz, H-α), 6.18 (d, 2.5 Hz, H-2"), 4.9 (dd, 2.5 and 5.5 Hz, H-3"), 4.6 (m, H-4"), 4.04 (d, 4.5 Hz, 2 H-5").

Acid Hydrolysis of (I). A mixture of 5 mg of (I) and 2 mg of 10% HCl was heated at 100°C for 3 h. After cooling, the precipitate of aglycone was filtered off (yield 45%), and it was identified by TLC in system 2, and also by UV and mass spectroscopy (M^+ 286), as kaempferol. In the evaporated aqueous solution arabinose was identified by PC, and p-coumaric acid by mass spectrometry (M^+ 164) and 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. The mixture was neutralized with 2% HCl and chromatographed on polyamide in the chloroform—methanol system. At a composition of the mixture of 95:5, p-coumaric acid was eluted (TLC, system 2), and the 85:15 mixture eluted juglanin (II) with the composition

$C_{20}H_{18}O_{10}$; mp 213-216°C; $[\alpha]_D^{23°}$ −177.3° (c 0.25; methanol); R_f 0.45, TLC (system 1); λ_{max}, MeOH, nm: 268, 351; NaOMe 275, 326, 400; NaOAc 274, 303, 370; NaOAc + H_3BO_3 268, 352; $AlCl_3$ and $AlCl_3$ + HCl 275, 305, 350, 402. PMR spectrum in C_5D_5N (ppm): 8.24 (d, 9 Hz, H-2',6'), 7.14 (d, 9 Hz, H-3',5'), 6.6 (s, H-6 and H-8), 6.3 (s, H-1"), 5.03 (d, 2 Hz, H-2"), 4.7 (dd, 2 and 5 Hz, H-3"), 4.53 (m, H-4"), 3.95 (d, 4.5 Hz, 2H-5").

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). The addition of ice water led to the formation of a precipitate, which was washed with water and purified by chromatography on a column of 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 hexaacetate (II) was obtained with mp 91-94°C, composition $C_{41}H_{36}O_{18}\cdot H_2O$ $[\alpha]_D^{20°}$ −82.9° (c 1.63; acetone); R_f 0.7, TLC (system 3). ν_{CO} 1750, 1740, 1650 cm^{-1}.

The PMR spectrum in $CDCl_3$ is given in Fig. 2; PMR spectrum in $(CD_3)_2CO$ (ppm): 8.04 (d, 9 Hz, H-2',6'), 7.7 (d, 16 Hz, H-β), 7.68 (d, 9 Hz, H-2",H-6"), 7.36 (d, 2.5 Hz, H-8), 7.3 (d, 9 Hz, H-3',5'), 7.12 (d, 9 Hz, H-3",5"), 6.9 (d, 2.5 Hz, H-6), 6.13 (d, 16 Hz, H-α), 5.96 (s, H-1"), 5.5 (d, 2 Hz, H-2"), 5.0 (dd, 2 and 5.5 Hz, H-3"), 4.09 (dd, 3 and 12 Hz, H-5"), 3.97 (dd, 5 and 12 Hz, H-5"), 3.6 (m, H-4"), 2.24 (s, $2CH_3COO$), 2.21 (s, CH_3COO), 2.18 (s, CH_3COO), 2.03 (s, CH_3COO), 1.87 (s, CH_3COO).

The sample of juglanin was kindly provided by Prof. Naokata Morita (Toyama University, Japan).

SUMMARY

1. The needles of the Koyama spruce have yielded a new acylated flavonoid glycoside for which the structure of 3,4',5,7-tetrahydroxyflavone 3-O-α-L-(2"-O-p-coumaroylarabinofuranoside) has been established.

2. The structure of juglanin (kaempferol 3-α-L-arabinoside) has been refined as kaempferol 3-O-α-L-arabinofuranoside.

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