where pK_{st} = pK_a for benzoic acid in the solvent under investigation, $E_{1/2st}$, potential of benzoic acid at the half-neutralization point, and $E_{1/2x}$, potential of the substance under investigation at the half-neutralization point.

SUMMARY

1. The indices of the relative acidity constants (pK_a) of 22 flavonoids have been determined in water, methanol, acetone, dimethylformamide, and dimethyl sulfoxide.

2. Linear equations of the relationsips between $pK_a^{H_2O}$ and pK_a in acetone, dimethylformamide, and dimethyl sulfxode are given.

3. The indices of the titration constants (pK_t) have been calculated and this has shown that an improvement in the conditions of titration of flavonoids take place on passing from water in the following sequence of organic solvents: methanol < DMFA < acetone < DMSO.

LITERATURE CITED

1. N. A. Tyukavkina, N. N. Pogodaeva, and V. I. Lutskii, Khim. Pri. Soedin., 24 (1970).
2. N. A. Tyukavkina and N. N. Pogodaeva, Khim. Prir. Soedin., 11 (1971).
3. N. A. Tyukavkina and N. N. Pogodaeva, Khim. Prir. Soedin., 176 (1972).
4. A. B. Blank and I. I. Mirenskaya, Zh. Obshch. Khim., 27, No. 11, 1883 (1972).
5. E. N. Nevskaya and V. A. Nazarenko, Zh. Obshch. Khim., 27, No. 9, 1669 (1972).
6. V. P. Georgievskii, Khim. Prir. Soedin., 303 (1979).
7. A. V. Luzanov and V. E. Umanskii, Opt. Spektrosk., 40, 201 (1976).
8. V. E. Umanskii and A. V. Luzanov, in: Computer Mathematics and Computer Technique [in Russian], Kharkov, No. 5 (1974), p. 10.
9. A. Gordon and R. Ford, The Chemist's Companion, Wiley, New York (1972).
10. I. P. Kovalev and E. V. Titov, Zh. Obshch. Khim., 33, No. 5, 1670 (1963).
11. V. P. Georgievskii and A. I. Rybachenko, Ukr. Khim. Zh., 41, No. 3, 289 (1975).
12. V. P. Georgievskii and A. I. Rybachenko, Zh. Prikl. Khim., 22, No. 4, 763 (1949).
13. V. P. Georgievskii, N. A. Kazarinov, and M. O. Karryev, Physiochemical Methods of Analyzing Biologically Active Substances of Plant Origin [in Russian], Ashkhabad (1976).

O-ACYLATED FLAVONOID GLYCOSIDES FROM THE NEEDLES OF *Picea obovata*

II. 3"- and 6"-ISOMERS OF p-COUMAROYLASTRAGALIN

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

UDC 547.972

Two monoacylated glycosides have been isolated from the needles of *Picea obovata* — the previously unknown 3"-p-coumaroylastragalin (I) and 6"-p-coumaroylastragalin (II). It has been established that under the conditions of mild saponification and acid hydrolysis isomerization of (I) into (II) and the splitting out of the acyl residue takes place. The position of the acyl substituent has been shown by PMR spectroscopy using the INDOR method. The physicochemical characteristics of the acylated glycosides and of their heptaacetates are given.

All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Irkutsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR, Moscow. I. M. Sechenov First Moscow Medical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 186-190, March-April, 1980. Original article submitted January 18, 1980.

0009-3130/80/1602-0141$07.50

We have previously reported the isolation from the needles of the Siberian spruce *Picea obovata* Ledeb. of a diacylated glycoside — 3"-O-p-coumaroyl-6"-O-feruloylastragalin [1]. A further study of the flavonoid components of the needles has led to the additional isolation of two monoacylated glycosides, and their structures are described in the present paper.

According to the results of hydrolytic cleavage and PMR spectroscopy (Figs. 1 and 2), both compounds (I) and (II) include residues of kaempferol, glucose, and p-coumaric acid, while their IR spectra contain the absorption band of an ester carbonyl group (1696 and 1688 cm^{-1}, respectively). In the case of compound (II), mild saponification, monitored by TLC (a) led to p-coumaric acid and astragalin (kaempferol 3-O-β-D-glucopyranoside (III)). The hydrolysis of (I) under these conditions led to the formation of p-coumaric acid and of two flavonoid compounds with R_f 0.25 (III) and 0.42 (IV), which were separated on a column. Compound (III) was identified as astragalin, while compound (IV) proved to be identical with compound (II).

Acyl migration [the conversion of (I) into (II)] and the splitting out of the acyl residue [conversion of (I) into (III)] was also observed under the conditions of the acid hydrolysis of (I): in addition to the initial substance (R_f 0.55) and kaempferol (R_f 0.8) the appearance was detected of small amounts of substances with R_f 0.42 and 0.25. Compound (II) hydrolyzes more readily than (I) and no such phenomena are observed during its hydrolysis.

The formation of (III) on alkaline hydrolysis answers the question of the position of the attachment of the glucose in (I) and (II), while their UV spectra do not permit definite conclusions to be drawn — a broad band at 315 nm interfers. The acetylation of (I) and (II) led to heptaacetates with different properties, the PMR spectra of which had the signals of three alcoholic acetoxy groups and, consequently, in both compounds the p-coumaric acid residue is attached to the carbohydrate moiety of the molecule.

The PMR spectra of (I) and (II) taken in deuteropyridine (see Fig. 2) are practically identical in the region of the resonance of aromatic protons where there are signals assigned to kaempferol and to trans-p-coumaric acid. However, the spectra of the carbohydrate fragments differ considerably. The signal of the anomeric proton in both cases forms a doublet with J = 8 Hz, which shows the β-configuration of the glycosidic bond of the D-glucopyranose. In the spectrum of compound (II) there are no signals of gem-acyl methine protons in the weak field, which indicates a possible attachment of the p-coumaric acid residue to the CH_2OH group of the glucose, the protons of which, in actual fact, form a multiplet at 4.8 ppm. Thus, compound (II) has the structure of kaempferol 3-O-β-D-(6"-O-coumaroyl)glucopyranoside. Two substances with such a structure have been described in the literature — tiliroside [2] and tribuloside [3] — differing in their physicochemical properties. The melting points of the compound (II) that we had isolated and of its acetate corresponded to those for tiliroside and its acetate.

In the spectrum of compound (I) measured in deuteropyridine, there is a signal in the weak field in the form of a triplet (6.0 ppm, $J_1 = J_2 = 9$ Hz) which can be explained by the acylation of the 3"-OH or the 4"-OH group of the D-glucopyranose residue in the C1 conformation. An unambiguous choice was made with the aid of the INDOR method, which we have previously used with success for establishing the structure of 3"-O-p-coumaroylisoquercitrin [4]. THE INDOR spectrum measured in deuteroacetone (Fig. 1) on line (1) of the doublet (5.37 ppm, $J_{1,2} = 8$ Hz) and on line (5) of the triplet (5.18 ppm, $\Sigma J = 18$ Hz) gave identical signals at the same position, which permits them to be ascribed to the H-2" proton (3.70 ppm, $J_{1,2} = 8$ Hz, $J_{2,3} = 10$ Hz). In the INDOR spectrum obtained on the weak-field line (6) of the H-2" quartet, the signals appeared above the lines of the doublet (δ 5.37) and of the triplet (δ 5.18). Thus, it was established that the triplet relates to the H-3" proton, i.e., the acyl residue is attached to the 3"-OH group of the glucose and compound (I) is the previously undescribed kaempferol 3-O-β-D(3"-O-p-coumaroyl)glucopyranoside. Its structure is shown in Fig. 1.

EXPERIMENTAL

For general information, see [1]. 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); 4) toluene—ethyl formate—formic acid (5:4:1); and 5) benzene—methanol (4:1), and by PC in the butanol—pyridine—water (6:4:3) system (descending).

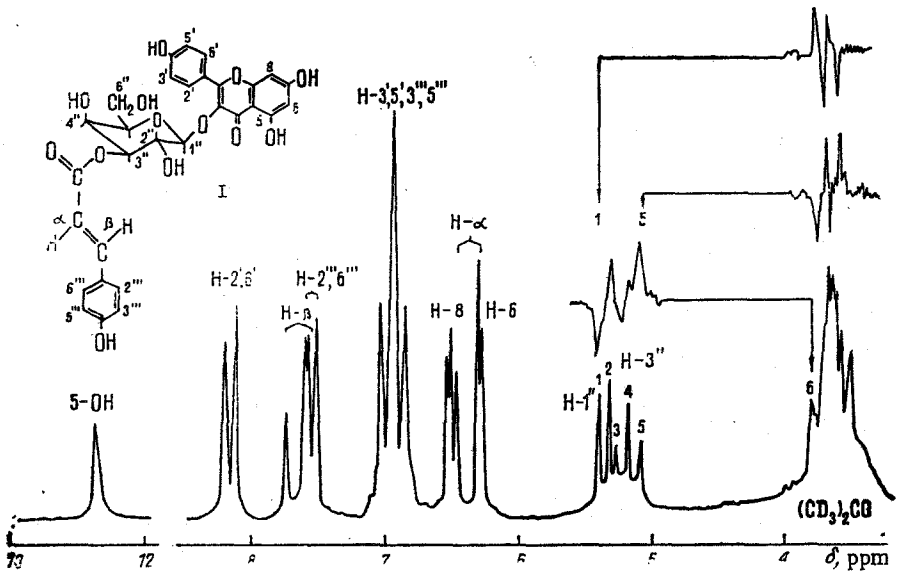


Fig. 1. PMR and INDOR spectra of 3"-O-p-coumaroylastragalin in deuteroacetone.

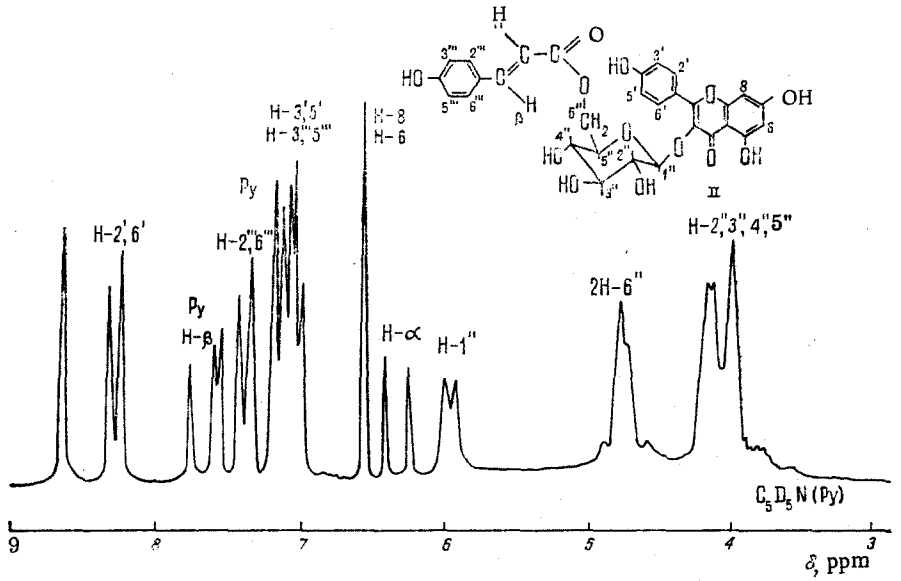


Fig. 2. PMR spectrum of 6"-O-p-coumaroylastragalin in deuteropyridine.

Isolation. When an ethereal extract from a methanolic extract of Siberian spruce needles [1] was chromatographed on polyamide in the chloroform–methanol (9:1) system and on silica gel in the benzene–acetone (4:1) system, three compounds were isolated: 200 mg of (I) (R_f 0.55; TLC, system 1), 120 mg of (II) (R_f 0.42), and 110 mg of a compound (R_f 0.65) the properties of which have been described in our previous paper [1].

3"-O-p-Coumaroylastragalin (I). Dark yellow amorphous substance soluble in acetone, methanol, and pyridine; mp 179-182°C; composition $C_{30}H_{26}O_{13}\cdot H_2O$, $[\alpha]_D^{20}$ −59.7° (s, 0.84, methanol); R_f 0.55 (TLC, system 1), 0.3 (TLC, system 4), 0.3 (TLC, system 5); ν_{CO} 1696, 1660 cm^{-1}; λ_{max}^{MeOH} 207, 318 nm.

PMR spectrum (Fig. 1) in $(CD_3)_2CO$ (ppm): 12.35 (s, 5-OH), 8.14 (d, 9 Hz, H-2',6'), 7.65 (d, 16 Hz, H-β), 7.55 (d, 9 Hz, H-2''', 6'''), 7.0 (d, 9 Hz, H-3',5'), 6.9 (d, 9 Hz, H-3''',5'''), 6.5 (d, 2 Hz, H-8), 6.38 (d, 16 Hz, H-α), 6.28 (d, 2 Hz, H-6), 5.37 (d, 8 Hz, H-1''), 5.18 (t, 9 and 9 Hz, H-3''), 4.0-3.3 (5 H of glucose).

PMR spectrum in C_5D_5N (ppm): 8.4 (d, 9 Hz, H-2', 6'), 7.8 (d, 16 Hz, H-β), 7.4 (d, 9 Hz, H-2''', 6'''), 7.2-7.0 (m, H-3', 5', 3''',5'''), 6.6 (s, H-6,8), 6.5 (d, 16 Hz, H-α), 6.3 (d, 8 Hz, H-1''), 6.0 (t, H-3''), 4.5-3.9 (5 H of glucose).

Pentacetate of (I). Amorphous substance with mp 122-125°C; R_f 0.5 (TLC, system 3); ν_{CO} 1770 sh., 1760, 1725 sh., 1632 cm^{-1}. PMR spectrum in $CDCl_3$ (ppm): 8.1 (d, 9 Hz, H-2', 6'), 7.72 (d, 16 Hz, H-β), 7.58 (d, 9 Hz, H-2'',6''), 7.34-7.12 (H-3',5',3''',5''',8), 6.86 (d, 2 Hz, H-6), 6.3 (d, 16 Hz, H-α), 5.7-5.0 (H-1'',2'',3'',4''), 4.1-3.7 (H-5'', 2H-6''), 2.40 (s, 3H), 2.26 (s, 6 H), 2.23 (s, 3 H), 2.02 (s, 3 H), 1.88 (s, 3 H), 1.85 (s, 3 H).

6''-O-p-Coumaroylastragalin (II). Yellow crystals soluble in methanol and pyridine and insoluble in acetone. mp 259-261°C, $C_{30}H_{26}O_{13}$, $[\alpha]_D^{20}$ −70° (s 0.8, methanol), R_f 0.42 (TLC, system 1), 0.22 (TLC, system 4), 0.25 (TLC, system 5), ν_{CO} 1688, 1660 cm^{-1}; λ_{max}^{MeOH} 268, 320 nm.

PMR spectrum (Fig. 2) in C_5D_5N (ppm): 8.3 (d, 9 Hz, H-2', 6'), 7.7 (d, 16 Hz, H-β), 7.4 (d, 9 Hz, H-2''', 6'''), 7.1 (d, 9 Hz, H-3', 5'), 7.0 (d, 9 Hz, H-3''', 5'''), 6.5 (s, H-6,8); 6.3 (d, 16 Hz, H-α), 6.0 (d, 8 Hz, H-1''), 4.8 (m, 2H-6''), 4.3-3.9 (4 H of glucose).

Heptaacetate of (II). Colorless cyrstlas with mp 180-182°C, R_f 0.4 (TLC, system 3), ν_{CO} 1775, 1743, 1725, 1630 cm^{-1}. PMR spectrum in $CDCL_3$ (ppm): 8.06 (d, 9 Hz, H-2', 6'), 7.58 (d, 16 Hz, H-β), 7.56 (d, 9 Hz, H-2''', 6'''), 7.3-7.1 (H-3', 5', 3''', 5''', 8), 6.8 (d, 2 Hz, H-6), 6.3 (d, 16 Hz, H-α), 5.6-5.0 (H-1'', 2'', 3'', 4''), 4.2-3.8 (H-4'', 2H-6''), 2.38 (s, 3 H), 2.2 (s, 6 H), 2.12(s, 3 H), 2.05 (s, 3 H), 1.90 (s, 6 H).

Acid Hydrolysis. A mixture of 7-10 mg of compound (I) or (II) with 5 ml of 10% HCl was heated on the boiling water bath for 2-3 h. The course of the reaction was followed by TLC (system 1). The hydrolysis of compound (I) took place with greater difficulty than that of (II), and in the course of it substances appeared the R_f values of which coincided with those of compound (II) and of astragalin (III), but then they disappeared completely and the same products were identified for both compounds: in the precipitate that deposited kaempferol was detected (TLC, UV, and mass spectra), and in the evaporated hydrolysate glucose (PC and TLC) and p-coumaric acid (TLC, mass spectrum).

Alkaline Hydrolysis. A mixture of 20 mg of compound (I) (or (II)) with 3 ml of 0.5% NaOH was heated at 50-60°C for 20 min (1 h). The course of the reaction was followed by TLC (system 1). The hydrolysis of (II) gave only compound (III) with R_f 0.25, while on the hydrolysis of (I) the formation of two flavonoid compounds was observed with R_f 0.25 (III) and 0.42 (IV), which were separated after neutralization by chromatography on polyamide in the water–methanol system. At a 90:10 composition of the mixture p-coumaric acid was eluted, this being identified by TLC (system 2), and 85:15 mixture eluted compound (III), and a 60:40 mixture eluted 4 mg of compound (IV).

Kaempferol 3-O-β-D-Glucopyranoside (III). mp 191-193°C, the UV and PMR spectra were identical with those of an authentic sample of astragalin.

Compound (IV). mp 250-256°C. This was identical in its chromatographic behavior and spectral characteristics with compound (II).

Acetylation. A mixture of 15 mg of (I) or (II), 0.3 mg of pyridine, and 1 ml of acetic anhydride was left at 20°C for 48 h (with monitoring by TLC in system 3). The addition of ice water led to a precipitate, which was washed with water and was recrystallized from ethanol [the acetate of (II)]. The acetate of (I) was chromatographed on silica gel in the benzene–acetone (4:1) system, and after the solvent had been distilled off trituration in petroleum ether gave a white amorphous powder.

SUMMARY

From the needles of the Siberian spruce have been isolated two isomeric p-coumaroylastragalins for which the structures of 3,4',5,7-tetrahydroxyflavone 3-O-β-D-3''-O-p-coumaroylglucopyranoside and 3,4',5,7-tetrahydroxyflavone 3-O-β-D-6''-O-p-coumaroylglucopyranoside have been established. The first compound has not been described in the literature while the constants of the second correspond to those of tiliroside.

LITERATURE CITED

1. G. G. Zapesochnaya, S. Z. Ivanova, V. I. Sheichenko, N. A. Tyukavkina, and S. A. Medvedeva, Khim. Prir. Soedin., 570 (1978).
2. W. M. Chari, M. Jordan, and H. Wagner, Planta Medica, 34, 93 (1978).
3. S. P. Bhutani, S. S. Chibber, and T. R. Seshadri, Phytochemistry., 8, No. 1, 299 (1969).
4. S. Z. Ivanova, G. G. Zapesochnaya, V. I. Sheichenko, S. A. Medvedeva, and N. A. Tyukavkina, Khim. Prir. Soedin., 196 (1978).

XANTHONES FROM THE ROOTS OF *Swertia iberica*

O. A. Denisova, V. I. Glyzin, A. V. Patudin, and D. A. Fesenko

UDC 547.972

From the roots of *Swertia iberica*, together with the previously known swertiaperennin decussatin, gentiakochianin, and norswertianin, we have isolated two new xanthones — isogentiakochianin and swertiaiberin. On the basis of UV, IR, PMR, and mass spectroscopy, the structure of 1,3,8-trihydroxy-7-methoxyxanthone is proposed for isogentiakochianin, and that of 1,2,3-trihydroxy-7,8-dimethoxyanthone for swertiaiberin.

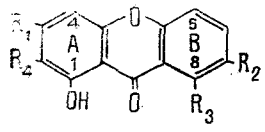
Plants of the genus *Swertia (Gentianaceae)* are of interest as a rich natural source of xanthone compounds. The chemical study of the xanthones in the plants of this genus began comparatively recently [1]. The increasing interest in xanthones is due to their pharmacological activity (stimulating action on the CNS [2] and cardiotonic [3] and tuberculostatic [4, 5] effects), and also to the fact that the qualitative composition and quantitative amount of xanthones are an important chemotaxonomic characteristic [6]. Within the framework of the study of domestic species of the genus *Swertia* we have undertaken a phytochemical investigation of *Swertia iberica* F. Fisch. et Mey, growing on the territory of the Georgian SSR.

On investigating a methanolic extract of the roots of *S. iberica* we isolated six individual substances belonging to the xanthone group (I-VI), two of which — (IV) and (V) — were new, and we have established the structures of these compounds.

Compound (I), from its composition ($C_{15}H_{12}O_6$), melting point (186-188°C), and UV-, IR, and PMR-spectroscopic characteristics corresponded to 1,8-dihydroxy-3,7-dimethaxyxanthone, which has been described previously under the names of swertiaperennin [7] and methylswertianin [8].

Compound (II), with the composition $C_{16}H_{14}O_6$, mp 156-158°C, according to spectroscopy, had the structure of 1-hydroxy-3,7,8-trimethoxyanthone and was identical with decussatin [7].

Compound (III), with the composition $C_{14}H_{10}O_6$, mp 223-226°C, according to spectroscopy,



I. R_1= OH, R_2=R_3=OCH_3, R_4=H
II. R_1= R_2= R_3= OCH_3, R_4=H
III. R_1= OCH_3, R_2= R_3= OH, R_4=H
IV. R_1= R_3=OH, R_2=OCH_3, R_4=H
V. R_1= R_4=OH, R_2= R_3= OCH_3
VI. R_1= R_2=R_3=OH, R_4=H

All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 190-195, March-April, 1980. Original article submitted October 9, 1979.

0009-3130/80/1602-0145$07.50