FLAVONOIDS OF *Gnaphalium uliginosum*

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The herb low cudweed has yielded for the first time a new acylated flavone glycoside, for which the structure of 3',4',5,7-tetrahydroxy-6-methoxyflavone 7-O-(6"-O-caffeyl-β-D-glucopyranoside) has been established. In addition, 6-methoxyluteolin, 6-hydroxyluteolin 7-O-β-D-glucopyranoside, and scutellarein 7-O-β-D-glucopyranoside have been isolated. Identification was made on the basis of UV, IR, PMR, and mass spectra, the products of alkaline and acid hydrolyses, and the results of elementary analyses, melting points, and specific rotations.

We have previously reported a chemical study of two flavonoids from the herb *Gnaphalium uliginosum* (low cudweed) [1]. Continuing the investigation of the active substances of this plant, we have isolated another four flavonoids (I-IV).

According to UV spectroscopy, these compounds belong to the flavone group. The shift in the long-wave maximum on the addition of aluminum chloride in hydrochloric acid did not exceed 25 cm and therefore all four flavonoids have an oxygen-containing substituent in position 6 and a hydroxyl at C-5. Compounds (I-III) each have two maxima in the short-wave part of the spectrum, which is characteristic for 3',4'-disubstituted flavonoids.

A study of the PMR spectra of compounds (I), (III), and (IV) enabled them to be assigned to the group of monoglycosides, while (II) was assigned to the aglycones. The acid hydrolysis of (I), (III), and (IV) gave the same sugar — D-glucose. In the PMR spectra of each of these compounds, taken in CCl_4, the anomeric proton of the glucose resonated at about 5 ppm in the form of a doublet with a spin–spin coupling constant of 6.5 Hz. This signal is characteristic for glucose in the C1 conformation attached to the aglycone by a β-glycosidic bond through the hydroxyl in position 7. This was confirmed by an analysis of the UV spectra of the glycosides and their aglycones taken in various media.

Its IR spectrum showed that compound (I) was an ester (ν_{CO} 1750, 1695 cm^{-1}).

In the weak-field part of the PMR spectra of (I) taken in deuteromethanol, deuteropyridine, and CCl_4 (in the form of the silyl ether, Fig. 1) there are the signals of eight aromatic and two olefinic protons, the latter appearing in the form of doublets with J = 16 Hz. These signals permit the assumption that the acylating component is one of the cinnamic acids with the trans configuration of the α- and β-olefinic protons. Alkaline saponification under mild conditions gave an aromatic acid which was identified as caffeic acid by paper and thin-layer chromatography in the presence of a marker. In this case, the signal of the methoxy group had to be assigned to the aglycone.

Substance (I) was very stable under the conditions of severe acid hydrolysis and was not cleaved completely even on being boiled for 40 hours; consequently, the isolation of the aglycone from the reaction mixture required chromatographic separation on a column.

UV spectroscopy with additions of boric acid and sodium acetate showed that the aglycone of (I) contained an orthodihydroxy grouping in the 3',4' positions and, consequently, the methoxy group was present at the sixth carbon atom of the skeleton, and the aglycone was identified as 6-methoxyluteolin.

On analyzing the PMR spectra of the glycosides taken in deuteropyridine and CCl_4, it was possible to distinguish in the region of the signals of the carbohydrate component a two-proton multiplet located in a weaker field than the other signals of the sugar protons. These are the signals of protons geminal to an acyl residue. One of the spin–spin coupling

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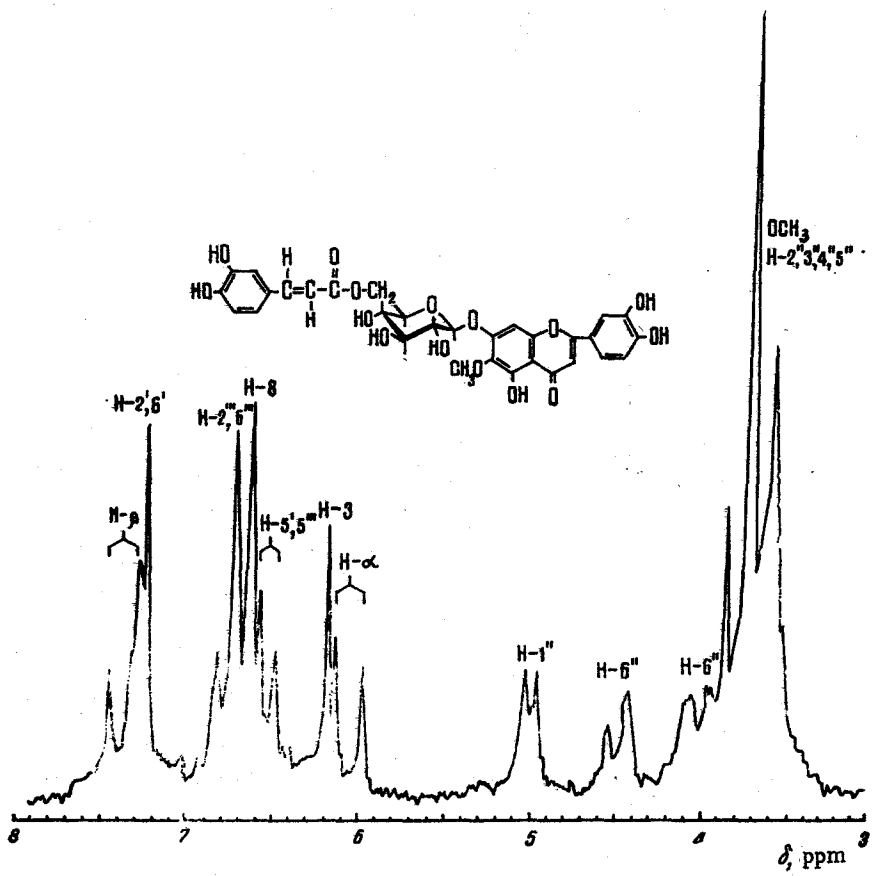

Fig. 1. PMR spectrum of the trimethylsilyl ether of compound (I) in CCl_4.

constants of this multiplet was 12 Hz, which permitted the signal to be assigned to the two geminal protons at C-6" of a glucose residue.

Thus, the glycoside under investigation has the structure of 3',4',5,7-tetrahydroxy-6-methoxyflavone 7-O-(6"-caffeyl-β-D-glucopyranoside). This compound has not been described previously.

Compound (II) proved to be identical with the aglycone of (I) (6-methoxyluteolin).

The PMR spectrum of substance (III) contained only the signals of skeletal protons at C-2',3,5',6',8 and the protons of the carbohydrate moeity. The UV spectrum showed the diagnostic shift of the long-wave maximum in the presence of boric acid and sodium acetate, which permitted the identification of a 3',4'-orthodihydroxy grouping.

Acid hydrolysis gave an aglycone the mass spectrum of which contained the peak of the molecular ion M^+ 302 and peaks of fragmentary ions with m/e 168 (a), 169 (a + 1), 140 (a − 28), 134 (b), 137 (c), and 109 (c − 28), corresponding to the structure of 6-hydroxyluteolin [2]. The identity of the aglycone as 6-hydroxyluteolin was confirmed by comparison with an authentic sample. On the basis of the facts given above, substance (III) is 6-hydroxyluteolin 7-O-β-D-glucopyranoside.

According to the result of PMR spectroscopy, compound (IV) is a 4',5,6,7-substituted flavone and has no substituents other than a glucose residue and hydroxy groups. The aglycone obtained by acid hydrolysis was identified as scutellarein. Consequently, compound (IV) is scutellarein 7-O-β-D-glucopyranoside.

Thus, the flavonoids of low cudweed are derivatives of scutellarein and of 6-hydroxy-, 6-methoxy-, and 3',6-dimethoxyluteolin — the aglycones, glucosides, and glucosides acylated with caffeic acid.

EXPERIMENTAL

For a description of the instruments and of the isolation of the compounds, see our previous paper [1]. The compounds were eluted from the column by mixtures of chloroform and ethanol in the following ratios: compound (II) 94:6; (I) 90:10; (IV) 86:14; (III) 80:20. The fractions were subjected to additional purification by chromatography on microcolumns of LS 100/250 silica gel (Czechoslovakia) and by recrystallization.

Chromatographic monitoring was performed by TLC (Silufol) in chloroform–methanol (8:2) (system 1) and by PC in 2% CH_3COOH (system 2), BAW (4:1:5) (3); 60% CH_3COOH (4); and butanol–pyridine–water (6:4:3) (5).

3',4',5,7-Tetrahydroxy-6-methoxyflavone 7-O-(6"-Caffeyl-β-D-glucopyranoside). Yellow prismatic crystals soluble in ethanol, methanol, and pyridine, mp 174-178°C, $[\alpha]_D^{20}$ −310° (c 0.2; ethanol). IR spectrum (cm^{-1}): 3600-4100 (OH group), 1750, 1695 (C=O in an ester), 1660 (C=O in a γ-pyrone), 1610, 1575, 1525, 1490 (C=C bond). UV spectrum (nm): λ_{max} (MeOH) 252, 280, 340, (+NaOAc) 259, 273, 349, (+$AlCl_3$) 277, 417, (+$AlCl_3$+HCl) 278, 355, (+H_3BO_3+NaOAc) 265, 371, (+NaOMe) 267, 390.

PMR spectrum in deuteromethanol (ppm): 7.35 (d, 16 Hz, H-β), 7.2 (m, H-2',6'), 6.55-6.80 (H-8,6"' 2"'), 6.4 (d, 8 Hz, H-5',5"'), 6.44 (s, H-3), 6.05 (d, 16 Hz, H-α), 5.04 (d, 6.5 Hz, H-1"), 3.82 (s, OCH_3), 3,3-3.7 (6 H of glucose).

PMR spectrum in deuteropyridine (ppm): 8.4 (s, OH), 7.83 (d, 16 Hz, H-β), 7.78 (d, 2.5 Hz, H-2'), 7.44 (q, 2.5 and 8 Hz, H-6'), 7.15 (m, H-2"', 6"'), 6.8-7.0 (two doublets, 8 Hz, H-5',5"'), 7.05 (s, H-8), 6.68 (s, H-3), 6.36 (d, 16 Hz, H-α), 5.6 (d, 6.5 Hz, H-1"), 4.75-5.05 (m, J_{gem} = 12 Hz, 2H-6"), 4.1-4.3 (m, 4 H of gluclose), 3.95 (s, OCH_3).

PMR spectrum of the trimethylsilyl derivative in CCl_4 (ppm): 7.36 (d, 16 Hz, H-β), 7.15-7.3 (m, H-2',6'), 6.77 (m, 2.5 and 8.5 Hz, H-2"',6"'), 6.63 (s, H-8), 6.42 (d, 8.5, H-5',5"'), 6.21 (s, H-3), 6.05 (d, 16 Hz, H-α), 5.01 (d, 6.5 Hz, H-1"), 3.75 (OCH_3), 4.5 and 4.02 (2 q, J_{gem} = 12 Hz, 2H-6"), 3.5-4.0 (m, 4 H of glucose).

Acid Hydrolysis of (I). A mixture of 8 mg of compound (I) and 5 ml of 25% hydrochloric acid was heated in the boiling water bath. The course of the reaction was monitored by TLC (system 1) and PC (system 3). After 40 hours' heating, the initial compound was still detected in the reaction mixture together with the aglycone. The mixture was cooled, the precipitate that deposited was filtered off, washed with distilled water, and dissolved in ethanol, and the solution was mixed with a small amount of LS 100/250 silica gel (Czechoslovakia) and was chromatographed on a small column (d 0.5, h 8 cm) using mixtures of chloroform and methanol as eluents. Chloroform–methanol (8:2) eluted the aglycone, which, after the elimination of the solvent, was recrystallized from ethanol.

The acid filtrate after the separation of the precipitate was evaporated, with the addition of water several times at the end of the evaporation. D-Glucose was identified in the evaporated residue by PC (system 5).

Alkaline Hydrolysis of (I). A solution of 20 mg of (I) in 6 ml of 0.5% caustic soda was left at room temperature for 10 min. Then it was acidified with 7% hydrochloric acid and extracted with ether (4 × 10 ml). The ethereal extract was washed with water to neutrality and was evaporated to dryness. Caffeic acid was identified in the residue by chromatography on paper (system 2) and on Silufol (system 1) in the presence of markers.

3',4',5,7-Tetrahydroxy-6-methoxyflavone, $C_{16}H_{12}O_7$ (the aglycone of (I)). Mass spectrum: 315 ($M^+ - 1$), 300 ($M^+ - 1 - 15$), 272 ($M^+ - 1 - 15 - 28$). UV spectrum (nm): λ_{max} (MeOH) 255, 265, 350, (+NaOAc) 278, 370, (+$AlCl_3$) 277, 436, (+$AlCl_3$+HCl) 372, (+H_3BO_3+NaOAc) 267, 382, (+NaOMe) 272, 409.

6-Methoxyluteolin (II), $C_{16}H_{12}O_7$. UV spectrum (nm): λ_{max} (MeOH) 254, 264, 350, (+NaOAc) 275, 364, (+$AlCl_3$) 277, 430, (+$AlCl_3$+HCl) 370, (+H_3BO_3+NaOAc) 266, 380, (+NaOMe) 270, 408.

The PMR spectrum in deuteroacetone was identical with that of the aglycone (I). On combined chromatography on paper (systems 3 and 4) and Silufol (system 1), the R_f values and the nature of the fluorescence of (II) and the aglycone of (I) coincided.

3',4',5,6,7-Pentahydroxyflavone 7-O-β-D-Glucopyranoside (III). Yellow crystals soluble in methanol, ethanol, and pyridine, mp 200-202°C. Mass spectrum: 302 (M^+), 301, 168, 169, 140, 137, 134, 109. UV spectrum (nm): λ_{max} (EtOH) 259, 283, 350, (+NaOAc) 260, 282, 350, (+$AlCl_3$) 379, (+$AlCl_3$+HCl) 370, (+H_3BO_3+NaOAc) 369, (+NaOMe) 400.

PMR spectrum in deuteromethanol (ppm): 7.25 (m, H-2',6'), 6.87 (s, H-8), 6.83 (d, 8 Hz, H-5'), 6.46 (s, H-3), 3.7-3.9 (protons of glucose). The other signals overlapped.

PMR spectrum in CCl_4 of the substance in the form of the complete silyl ether and the silyl ether with a free OH group in position 5 (ppm): 7.36 (q, 2.5 and 8 Hz, H-6'), 7.26 (d, 2.5 Hz, H-2'), 6.83 (d, 8 Hz, H-5'), 6.57 and 6.49 (s, H-8), 6.40 and 6.28 (s, H-3), 5.05 and 4.97 (d, 6.5 Hz, H-1"), 3.5-3.8 (m, six protons of glucose).

3',4',5,7-Tetrahydroxyflavone 7-O-β-D-Glycopyranoside (IV). Light yellow crystals soluble in ethanol and pyridine. IR spectrum (cm^{-1}): 3600, 3370 (OH group), 1665 (C=O of a γ-pyrone), 1610, 1595, 1575, 1550, 1500 (C=C in conjugated systems). UV spectrum (nm): λ_{max} (MeOH) 289, 340, (+NaOAc) 307, 345, (+$AlCl_3$) 305, 371, (+$AlCl_3$+HCl) 305, 365, (+H_3BO_3+NaOAc) 306, 340, (+NaOMe) 382. PMR spectrum in deuteropyridine (ppm): 8.97 (s, OH group), 8.39 (s, OH group), 7.82 (d, 8.5 Hz, H-2',6'), 6.92 (s, H-8), 6.80 (s, H-3), 5.78 (d, 6.5 Hz, H-1"), 4.2-4.5 (m, 6 H of glucose).

Acid Hydrolysis of (III) and (IV). After the PMR spectra of (III) and (IV) had been recorded, the solvent was distilled off. The trimethylsilyl ether of (III) was treated with aqueous ethanol and left for a day at room temperature, and the mixture was then evaporated to dryness. The residues were each treated with 6 ml of 20% hydrochloric acid and heated in the boiling water bath for 5 h. After the reaction mixtures had been cooled, the aglycones were extracted with ether and chromatographed on paper (systems 3 and 4) and on Silufol (system 1) in the presence of markers. From its R_f values and its reddish brown fluorescence, the aglycone of (III) was identified as 6-hydroxyluteolin, and the aglycone of (IV) as scutellarein.

SUMMARY

A new acylated flavone glycoside has been isolated for the first time from the herb low cudweed, and its structure has been established as 3',4',5,7-tetrahydroxy-6-methoxyflavone 7-O-(6"-O-caffeyl-β-D-glucopyranoside).

In addition, 6-methoxyluteolin, 6-hydroxyluteolin 7-O-β-D-glucopyranoside, and scutellarin 7-O-β-D-glucopyranoside have been isolated.

LITERATURE CITED

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