NEW ACYLATED FLAVONE GLYCOSIDE FROM *Gnaphalium uliginosum*

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A new acylated flavone glycoside has been isolated for the first time from the herb marsh cudweed, and for it the structure of 6"-caffeyl-7-β-D-glucopyranosyloxy-4',5-dihydroxy-3',6'dimethoxyflavone has been established. In addition, the aglycone, identified as 4',5,7-trihydroxy-3',6-dimethoxyflavone has been isolated. The identifications were made on the basis of UV, IR, PMR, and mass spectra, the products of alkaline and acid hydrolyses, and the results of elementary analysis, melting points, and specific rotations.

Gnaphalium uliginosum L. (marsh cudweed) is an annual herbaceous plant of the family Compositae. The chemical composition of this plant has been studied inadequately. Galenical preparations from the epigeal part are used in folk medicine for the treatment of hypertonic disease and possess a wound-healing action [1].

In a study of the chemical composition of the herb marsh cudweed, we have isolated five compounds of flavonoid nature. The present communication gives the results of a study of two compounds. According to the results of IR and UV spectroscopy (λ_{max} 276, 344 nm), we assigned substance (I) to the flavone group. Analysis of its PMR spectrum showed that it contained substituents in the 3', 4', 5, 6, and 7 positions, two of them being methoxy groups. An investigation of the substance with the aid of UV spectroscopy in various media showed that it contained hydroxy groups in the 4', 5, and 7 positions. Thus, substance I has the structure of 4',5,7-trihydroxy-3',6-dimethoxyflavone, which was confirmed by its mass spectrum, showing the molecular peak M^+ 330 and the peaks of fragmentary ions with m/e 182, 148, and 151 corresponding to the structure [2, 3].

From the results of UV, IR, and PMR spectroscopy, substance (II) was a flavone glycoside. Hydrolytic cleavage yielded an aglycone identical with compounds (I) and glucose. According to spectral characteristics, the sugar was attached to aglycone through the hydroxyl at C_7 and had the β configuration of the glycosidic bond.

The IR spectrum of (II) had, in addition to the absorption of the functional groups characteristic of the class of flavonoids, two strong absorption bands at 1700 and 1685 cm^{-1}, permitting the assumption that an ester function was present in the molecule.

The alkaline saponification of substance (II) under mild conditions gave caffeic acid, which was identified by paper chromatography, and the saponification product – 7-β-glucopyranosyloxy-4',5-dihydroxy-3',6-dimethoxyflavone.

The question of the position of attachment of the caffeic acid was answered by an analysis of the PMR spectrum of (II) and its hexaacetate (Figs. 1 and 2). The PMR spectrum of the hexaacetate of (II) had signals of a hydroxy group at C_5 (s, 12.74 ppm) that had not taken part in the acylation reaction and the signals of three aromatic and three aliphatic acetyl groups.

It follows from the facts given that the caffeic acid is attached to the carbohydrate moiety of substance (II), since if the glycoside were acylated in the aglycone the spectrum of the acetate would show the signals of only two aromatic acetoxy groups.

The PMR spectra of substance (II) and its acetate contained the signals of 10 protons of aromatic parts of the molecules, which can be assigned to the aglycone and caffeic acid. The spin–spin coupling constant of 16 Hz shows the trans position of the α- and β-protons in the caffeic acid.

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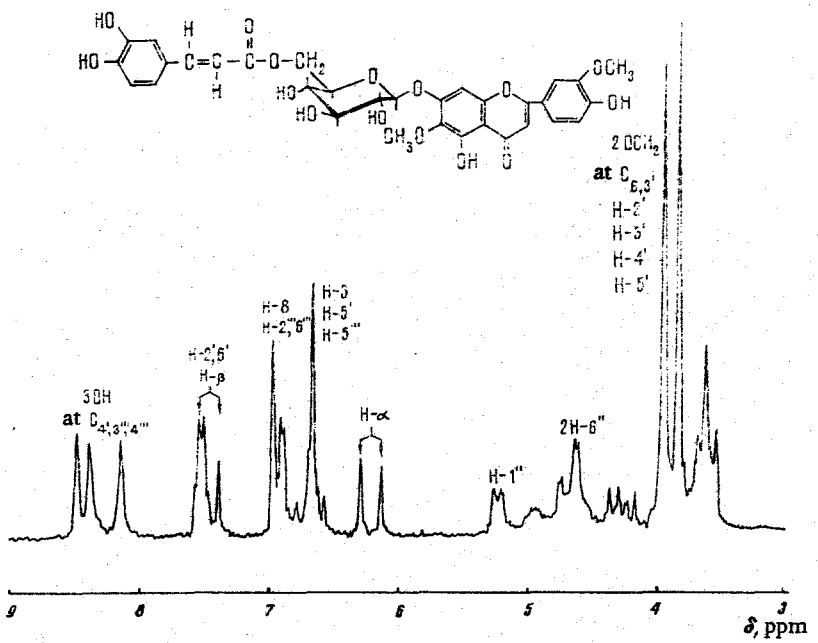

Fig. 1. PMR spectrum of substance (II) in deuteroacetone.

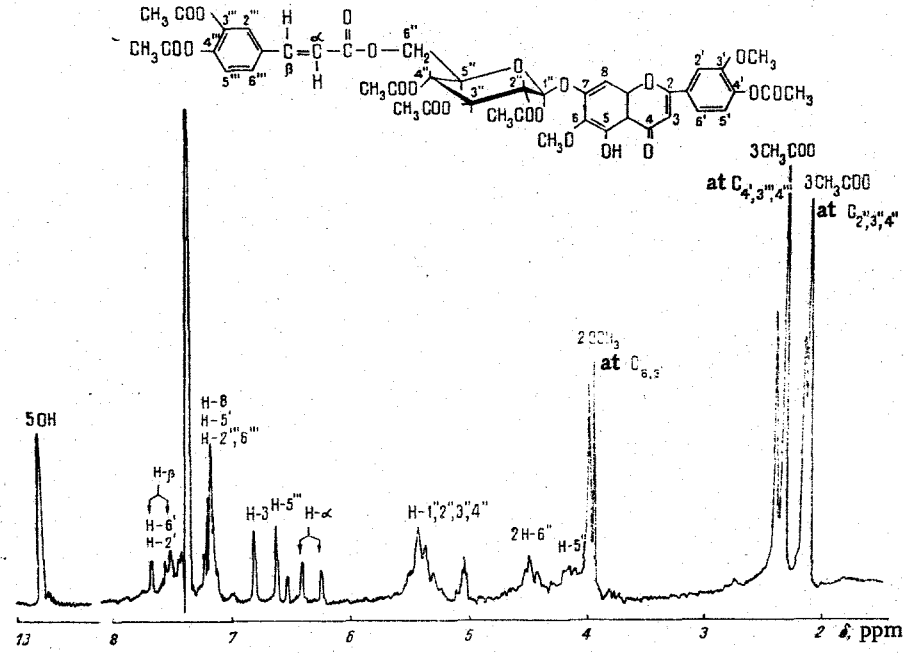

Fig. 2. PMR spectrum of the hexaacetate of (II) in $CDCl_3$.

The PMR spectrum of (II) had a group of signals in the 3.50-5.30 ppm region relating to the protons of the carbohydrate moiety of the molecule and to the two methoxy groups of the aglycone. At the same time, in addition to the signal of the anomeric proton, a two-proton multiplet was observed in the weak field (4.68 ppm), which can be explained by the acylation of the primary alcohol group in glucose (J_{gem} = 12 Hz).

Thus, the compound isolated has the structure of 6''-O-caffeyl-7-β-D-glucopyranosyloxy-4',5-dihydroxy-3',6-dimethoxyflavone and is a new compound. Compound (I) has been isolated previously from *Digitalis lanata* [4].

EXPERIMENTAL

The spectral characteristics were obtained on the following instruments: UR-20 (paraffin oil, IR), Hitachi EPS-3T (UV), Varian HA-100 at 100 MHz with tetramethylsilane as internal standard (PMR), and Varian CH-8 at 70 eV (mass spectra). Melting points were determined

on a Kofler block; elementary analysis was performed on a Hewlett-Packard 185B automatic CHN analyzer; angles of rotation were determined on a Polamat A polarimeter at 546 and 578 nm with recalculation to λ 598.3 nm. Chromatographic monitoring was carried out by TLC (Silufol) in systems 1) chloroform–methanol (8:2) and 2) chloroform, and by PC in systems 3) 15% CH_3COOH and 4) 60% CH_3COOH.

Isolation. The air-dry raw material was extracted three times with hot ethanol, and the extract was evaporated to dryness. The residue was diluted with distilled water and was extracted successively with chloroform and ethyl acetate. The ethyl acetate extract was subjected to chromatographic separation on columns of polyamide in chloroform–ethanol systems. Substance (I) was eluted from the column at a ratio in the mixture of 97:3, and substance (II) at 92:8. Both substances were recrystallized from ethanol.

4',5,7-Trihydroxy-3',6-dimethoxyflavone (I) formed yellow crystals soluble in ethanol and methanol, mp 226-227°C, composition $C_{17}H_{14}O_7$.

IR spectrum (cm^{-1}): 3450-3100 (OH groups), 1665 (C=O of a γ-pyrone), 1625, 1610, 1580, 1520, 1500 (C=C bonds in rings), 1465, 1430, 1375, 1310, 1280, 1260, 1220, 1165, 1130, 1110, 1095, 1045, 1030, 1010, 950, 900, 870, 850-820, 795, 720, 710, 690.

UV spectrum, nm λ_{max} (MeOH): 252 sh, 276, 344; (+NaOAc) 290, 390; (+$AlCl_3$) 263, 285, 380; (+$AlCl_3$+HCl) 262, 281, 360; (NaOAc+H_3BO_3) 280, 355; (+MeONa) 265, 280, 420.

PMR spectrum (in deuteroacetone): m, 7.60 ppm, J_1 = 2.5 Hz, J_2 = 8.5 Hz, 2 H (H-2',6'); d, 7.0 ppm, J = 8.5 Hz, 1 H (H-5'); s, 6.70 ppm, 1 H (H-8); s, 6.64 ppm (1 H, H-3); s, 4.00 ppm, 3 H, s, 3.88 ppm, 3 H — two OCH_3 groups.

6"-O-Caffeyl-7-β-D-glucopyranosyloxy-4',5-dihydroxy-3',6-dimethoxyflavone (II) formed pale yellow tabular crystals soluble in ethanol and methanol, with mp 161-163°C, $[\alpha]_D^{20}$ −29.5° (c 1.0; dimethylformamide).

IR spectrum (cm^{-1}): 3600-3100 (OH group), 1700, 1685 (c=O of an ester), 1658 (C=O of a γ-pyrone ring), 1630, 1600, 1590, 1560, 1512 (C=C bonds in rings) 1490, 1460, 1430, 1412, 1375, 1350, 1320, 1290-1270, 1215, 1175, 1155, 1140, 1130, 1120, 1085, 1040, 1015, 1005, 990, 980, 950, 880, 860, 850, 830, 810, 790-770, 730, 720.

UV spectrum, nm; λ_{max} (MeOH): 251 sh, 278, 338; (+NaOAc) 270 sh, 340, 408; (+$AlCl_3$) 261, 370; (+$AlCl_3$+HCl) 285, 354; (+NaOAc+H_3BO_3) 256, 349; (+MeONa) 265, 396.

PMR spectrum (in deuteroacetone): ss, 8.46, 8.36, 8.13 ppm, 1 H each (4'-OH, 3'''-OH, 4'''-OH); d, 7.46 ppm, J = 16 Hz, 1 H (H-β); m, 7.48-7.60 ppm, 2 H (H-2',6'); s, 6.96 ppm, 1 H (H-8); m, 6.88-6.98 ppm, J_1 = 2.5 Hz, 2 H (H-2''',6'''); s, 6.65 ppm, 1 H (H-3); mm, 6.56-6.74 ppm, 2 H (H-5', H-5'''); d, 6.2 ppm, J = 16 Hz, 1 H (H-α); d, 5.24, J = 7 Hz, 1 H (H-1"); q, 4.68 ppm, J_1 = 2.5 Hz, J_{2gem} = 12 Hz, 2 H (2 H-6"); ss, 3.93, 3.82 ppm, 3 H each (2-OCH_3 at C_6 and C_3'); m, 4.4-3.5 ppm, 4 H (the other protons of the carbohydrate moiety).

Acid Hydrolysis of (II). A mixture of 100 mg of (II) and 40 ml of 10% HCl was heated in the boiling water bath. The course of the reaction was checked by PC (systems 3 and 4). After 40 hours' heating, compound (II) had hydrolyzed completely. The precipitated aglycone was filtered off, washed with water to neutrality, and purified on a column of polyamide sorbent. The ethanolic eluate containing the aglycone was evaporated to dryness, and the residue was recrystallized from ethanol. This gave 40 mg of aglycone which was shown to be identical with substance (I) by UV, IR, and PMR spectroscopy, mass spectrometry (M^+ 330), and comparative chromatography.

D-Glucose was detected in the neutralized and evaporated aqueous residue by the PC method.

Alkaline Hydrolysis of (II). A solution of 40 mg of (II) in 6 ml of 1% NaOH was left at room temperature for half an hour. The course of the reaction was followed by TLC (system 1), and it yielded one saponification product of flavonoid nature. The mixture was neutralized with 7% HCl and extracted with diethyl ether (6×10 ml). The ethereal extract was washed with water, evaporated, and purified on a column of polyamide. Water eluted compound (III), which was identified by PC as caffeic acid. From the aqueous extract butanol (5×15 ml) extracted a saponification product of flavonoid nature (IV). After the butanol had been distilled off, compound (IV) was purified by column chromatography on polyamide. It was crystallized from ethyl acetate with the addition of ethanol.

Saponification Product (IV). IR spectrum (cm^{-1}): 3100, 3600 (OH groups), 1660 (C=O of a γ-pyrone), 1605, 1570, 1520 (C=C bonds in rings), 1490, 1465, 1435, 1380, 1290, 1275, 1210, 1135, 1100, 1080, 1050, 1020, 960, 850, 825, 800, 780, 720.

Acetylation of (II). A mixture of 83 mg of compound (II), 0.2 ml of pyridine, and 15 ml of acetic anhydride was heated in the water bath at 60-70°C for 1 h. Then it was poured into 200 ml of ice water and the mixture was left with stirring for 2 h. The precipitate that had formed was filtered off, washed with water, and dried. After recrystallization from methanol cream-colored crystals of the hexaacetate of (II) — compound (V) — with mp 105-108°C were obtained.

UV spectrum, nm, λ_{max} (MeOH): 278, 328 sh, (+NaOAc) 280; (+$AlCl_3$) 257 sh, 288 sh, 342; (+$AlCl_3$ + HCl) 333; (+NaOAc + H_3BO_3) 280, 333; (+MeONa) 272, 395.

PMR spectrum (in $CDCl_3$): s, 12.74, 1 H (OH at C_5); d, 7.58 ppm, J = 16 Hz, 1 H (H-β); q, 7.50 ppm, J_1 = 2.5 Hz, J_2 = 8 Hz, 1 H (H-6'); d, 7.44 ppm, J = 2.5 Hz, 1 H (H-2') s, 7.16 ppm, 1 H (H-8); m, 7.1-7.24 (H-5', 2''', 6'''); s, 6.8 ppm, 1 H (H-3); d, 6.58 ppm, J = 8 Hz, 1 H (H-5'''); d, 6.32 ppm, J = 16 Hz, 1 H (H-α); m, 5.0-5.5 ppm, 4 H (H-1", 2", 3", 4"); m, 4.4-4.55, 2 H (2 H-6"); m, 4.1-4.2 ppm, 1 H (H-5"); ss, 3.95, 4.00, 3 H each (two OCH_3 groups at C-3', 6); ss, 3.3-3.55 ppm, 9 H (three aromatic CH_3COO groups at C-3''', 4', 4'''); ss, 3.1-3.2 ppm, 9 H (three aliphatic CH_3COO groups at C-2", 3", 4").

SUMMARY

A new acylated flavone glycoside has been isolated from the herb marsh cudweed for the first time, and the structure of 6"-caffeyl-7-β-D-glucopyranosyloxy-4',5-dihydroxy-3',6-dimethoxyflavone has been established for it. In addition, the aglycone, identified as 4',5,7-trihydroxy-3',6-dimethoxyflavone has been isolated.

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