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FLAVONOIDS FROM THE AERIAL PART OF *Calamagrostis epigeios*

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Calamagrostis Adans. is a genus of grasses (Poaceae Barnh.) that numbers about 200 species. Seven species of these grow in Ukraine. *C. epigeios* L. Roth. (bushreed) is a perennial herbaceous wild plant [1] that is not the official species despite the fact that its raw material base is rather large.

We studied previously the fatty-acid and polysaccharide compositions of bushreed [2]. We studied phenolic compounds of bushreed grass in order to write a national monograph in the State Pharmacopoeia of Ukraine for this type of raw material. Ground raw material was extracted with EtOH (70%). The extract was evaporated and chromatographed (PC) using *n*-BuOH:AcOH:H_2O (4:1:2) (direction I) and AcOH (15%) (direction II). Analysis of the chromatograms in visible and UV light using specific reagents enabled up to 20 compounds of phenolic nature to be found in the grass. Several of them were assigned as hydroxycinnamic acids and coumarin-type compounds (11). The others were flavonoids (9) [3, 4].

Air-dried raw material (1.0 kg, ground to particle size 2.5–3 mm) that was collected during flowering in Kharkov Oblast (Ukraine) in 2011 was extracted exhaustively with EtOH (70%) (1:10 raw material:extractant ratio) in order to isolate compounds and establish their structures. The resulting EtOH:H_2O extract was evaporated *in vacuo* in a rotary evaporator to an aqueous residue that was worked up successively with $CHCl_3$, EtOAc, and *n*-BuOH. The solvents were distilled off to afford $CHCl_3$ (16.1 g), EtOAc (12.3), and BuOH (17.4) fractions.

The evaporated concentrated EtOAc extract was placed on a polyamide column and eluted by $CHCl_3$ and its mixtures with EtOH of gradually increasing concentration of the latter by 10% per 1 L. Fractions of 100 mL were collected and monitored by PC using the indicated solvents. Identical fractions were combined, evaporated to dryness, dissolved in the minimum amount of EtOH (96%) by adding several drops of H_2O, and left to crystallize. As a result, nine pure compounds were isolated. These were identified using UV, IR, and PMR spectra; results of chemical transformations; and comparison with authentic standard samples.

Apigenin (5,7,4′-trihydroxyflavone) (1). $C_{15}H_{10}O_5$, yellow crystals, soluble in EtOH, EtOAc, Me_2CO; insoluble in $CHCl_3$; mp 343–346°C. UV spectrum (EtOH, λ_{max}, nm): 272, 343; +CH_3COONa: 275, 365; +CH_3COONa + H_3BO_3: 272, 345. IR spectrum (KBr, ν_{max}, cm^{-1}): 3520–3100 (OH), 1665–1635 (γ-pyran C=O), 1625–1440 (aromatic C=C) [3].

Luteolin (5,7,3′,4′-tetrahydroxyflavone) (2). $C_{15}H_{10}O_6$, yellow needle-like crystals, soluble in Me_2CO, EtOH; insoluble in $CHCl_3$, C_6H_6; mp 328–330°C. UV spectrum (EtOH, λ_{max}, nm): 260, 272, 356; +CH_3COONa: 272, 368; +CH_3COONa + H_3BO_3: 272, 376. IR spectrum (KBr, ν_{max}, cm^{-1}): 3450–3300 (OH), 1665–1635 (γ-pyran C=O), 1612–1580 (aromatic C=C). PMR spectrum (100 MHz, C_5D_5N, δ, ppm, J/Hz): 6.61 (1H, d, J = 2.0, H-6), 6.73 (1H, d, J = 2.0, H-8), 6.78 (1H, s, H-3), 7.09 (1H, d, J = 8.0, H-5′), 7.53 (1H, br.s, H-2′), 7.60 (1H, dd, J = 2.0, 8.0, H-6′) [4].

The structure of **2** was confirmed by constants of the acetyl and methoxy derivatives. The compound was acetylated by acetic anhydride in the presence of Py to form the tetraacetate with mp 226–228°C.

Cinaroside (luteolin-7-*O*-β-D-glucopyranoside) (3). $C_{21}H_{20}O_{11}$. Quantitative acidic and enzymatic hydrolysis was performed in order to confirm the glycosidic nature of the compound. This produced equimolar amounts of the aglycon and the carbohydrate part. PC using Me_2CO:*n*-BuOH:H_2O (7:2:1) found D-glucose in the acid-hydrolysis products of **3**. The aglycon was identified as **2** based on physicochemical properties. The position of the carbohydrate unit was established using chemical and spectral methods. A bathochromic shift of absorption maximum I in the UV spectrum of **3** was not observed upon adding NaOAc. Therefore, it was assumed that the carbohydrate was located on C-7. The IR spectrum contained absorption bands for hydroxyls (3480–3300 cm^{-1}), γ-pyran carbonyl (1665 cm^{-1}), aromatic C=C (1558, 1510 cm^{-1}), and glycoside C–O (1100, 1020 cm^{-1}).

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The PMR spectrum (100 MHz, C_5D_5N, δ, ppm, J/Hz) exhibited proton resonances at 3.92–4.10 (sugar protons), 5.60 (1H, d, J = 7.0, H-1′, D-glucose anomeric proton), 6.73 (1H, d, J = 2.5, H-6), 6.78 (1H, s, H-3), 6.83 (1H, d, J = 2.5, H-8), 7.25 (1H, d, J = 8.0, H-5′), 7.40 (1H, dd, J = 8.0, 2.5, H-6′), 7.72 (1H, d, J = 2.5, H-2′) [5].

Acid hydrolysis of **3** produced luteolin and D-glucose. Acetylation of the compound by acetic anhydride in Py gave the heptaacetyl derivative of formula $C_{35}H_{34}O_{18}$, mp 121–123°C.

Quercetin (3,5,7,3′,4′-pentahydroxyflavone) (4). $C_{15}H_{10}O_7$, mp 310–312°C. UV spectrum (EtOH, λ_{max}, nm): 256, 268, 375; +CH_3COONa: 270, 406. IR spectrum (KBr, ν_{max}, cm^{-1}): 3385–3300 (OH), 1665 (γ-pyran C=O), 1612, 1560, 1518 (aromatic C=C). PMR spectrum (100 MHz, C_5D_5N, δ, ppm, J/Hz): 6.54 (1H, d, J = 2.5, H-6), 6.73 (1H, d, J = 2.5, H-8), 7.25 (1H, d, J = 8.5, H-5′), 7.85 (1H, dd, J = 8.5, 2.5, H-6′), 8.35 (1H, d, J = 2.5, H-2′), 11.80 (1H, br.s, 3-OH), 13.72 (1H, br.s, 5-OH) [6].

Compounds **1**–**4** were isolated for the first time from *C. epigeios* (L.) Roth.

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