

## FLAVONOIDS OF THE EPIGEAL PART OF *Kickxia elatine*

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*From the epigeal part of Kickxia elatine (L.) Dumort we have isolated demethoxycentaureidin 7-O-β-D-glucoside, pectolinarin, and acetylpectolinarin, and the new flavone glycoside demethoxycentaureidin 7-O-rutinoside, the structure of which was established on the basis of chemical transformations and spectral characteristics.*

Sharp-pointed fluellin, *Kickxia elatine* (L.) Dumort (fam. Scrophulariaceae), is an annual plant growing in the fields, wastelands, pastures, and plantations of Central Asia [1]. It is used in folk medicine as a sedative, wound-healing agent, and general tonic, and also in hemorrhages and lacrimation [1, 2]. In order to reveal the active principle and to find new biologically active substances, we have studied the flavonoids of this plant. The flavonoids of *K. elatine* have not been studied previously.

The epigeal part of the plant was gathered in the fruit-bearing period in the foothills of the Talas range. Four individual flavonoids were isolated from an alcoholic extract of the epigeal part.

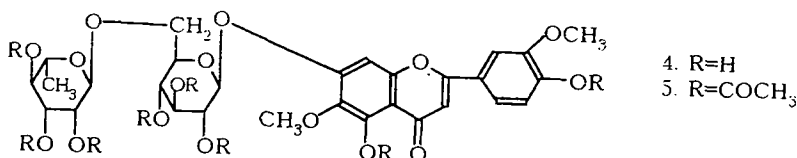
According to its spectral characteristics, compound (1) was a flavone glycoside, and on acid hydrolysis it split to form demethoxycentaureidin (4',5,7-trihydroxy-3',6-dimethoxyflavone) [3], and *D*-glucose. By a study of its UV, PMR, and mass spectra and chemical transformations, and also a comparison of its physicochemical properties with the literature, flavonoid (1) was identified as demethoxycentaureidin 7-O-β-*D*-glucoside [3].

On the basis of chemical transformations and spectral characteristics, compounds (2) and (3) were identified as the known flavone glycosides pectolinarin and acetylpectolinarin, respectively [4].

The UV spectrum ( $\lambda_{\max}$  274, 335 nm) of the new flavonoid (4) was characteristic for flavone derivatives [5]. Its PMR spectrum showed signals of the protons of two methoxy groups, of H-3, of a methyl group, and of two anomeric and other protons of a carbohydrate residue, and also of four aromatic protons and a chelate hydroxy group (5-OH). Its chromatographic mobility and its PMR spectrum showed the glycosidic nature of the compound under investigation. This was confirmed by the formation of demethoxycentaureidin and monosaccharides — *D*-glucose and *L*-rhamnose — on the acid hydrolysis of (4).

Acetylation of glycoside (4) gave the octaacetyl derivative (5), C<sub>45</sub>H<sub>50</sub>O<sub>24</sub>, the mass spectrum of which contained, in addition to the peak of the molecular ion with *m/z* 974, intense peaks of ions of an acylated biose with *m/z* 561 and of a terminal rhamnose with *m/z* 273, 213, and 153 [6]. Consequently (4) was a bioside of demethoxycentaureidin. This was confirmed by the formation of glycoside (4), demethoxycentaureidin 7-O-β-*D*-glucoside (1) on partial hydrolysis.

In order to establish the structure of its carbohydrate moiety, glycoside (4) was subjected to Hakomori methylation. In a hydrolysate of the methylation product, TLC revealed the presence of 2,3,4-tri-*O*-methyl-*L*-rhamnose and 2,3,4-tri-*O*-methyl-*D*-glucose. Thus in the (4) molecule, a terminal *L*-rhamnose residue is attached to a *D*-glucose residue by a 1→6 bond.



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The absence of a bathochromic shift in the UV spectrum of (4) in the presence of sodium acetate showed the glycosylation of the 7-OH group of the aglycon [5]. In the PMR spectrum of (4) the signals of the anomeric protons of *L*-rhamnose and *D*-glucose resonated at 5.37 and 5.65 ppm in the forms of a broadened singlet and a doublet with a SSCC of 6.5 Hz, respectively, and, consequently, the glycosidic center of the *D*-glucose residue had the  $\beta$ -configuration and that of *L*-rhamnose the  $\alpha$ -configuration [7, 8].

Thus, flavonoid (4) had the structure of 7-[*O*- $\alpha$ -*L*-rhamnopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -*D*-glucopyranosyloxy]-4',5-dihydroxy-3',6-dimethoxyflavone, or demethoxycentaureidin 7-*O*-rutinoside.

## EXPERIMENTAL

**General Observations.** The solvent systems used were chloroform—methanol (97:3) (1); (95:5) (2); and (3:1) (3); and butan-1-ol—pyridine—water (6:4:3) (4). TLC was conducted on Silufol UV-254 plates, and CC on type KSK silica gel with a grain size of 100-160  $\mu$ m. In TLC the substances were detected by treatment with ammonia vapor, iodine vapor, and 25% methanolic tungstophosphoric acid. Sugars were detected with the aid of paper chromatography (Filtrak No. 11) by spraying with aniline hydrogen phthalate followed by heating at 90-100°C for 3-5 min. Melting points were determined on an instrument of the Boëtius type with a RNMK 0.5 visual attachment.

PMR spectra were taken on a Tesla BS-657A spectrometer in Py- $d_5$ . Mass spectra were obtained on a MKh-1310 instrument at an ionizing energy of 50 eV, IR spectra on a UR-20 instrument in KBr, and UV spectra on Specord UV-Vis and SF-26 spectrophotometers.

**Isolation of the Flavonoids.** The dried and comminuted epigeal part of the plant gathered in the fruit-bearing period on August 23, 1992 (Chimkent province, foothills of the Talas range) (0.4 kg) was extracted at room temperature with 90% ethanol 8 times. The alcoholic extracts were concentrated in vacuum to 0.8 liter and diluted with water to 1.6 liters. The aqueous alcoholic extract was shaken successively with petroleum ether (4  $\times$  0.5 liter), chloroform (6  $\times$  0.5 liter), ethyl acetate (8  $\times$  0.5 liter), and *n*-butanol (8  $\times$  0.5 liter). After the solvents had been distilled off, 8.0 g of petroleum ether fraction, 5.5 g of chloroform fraction, 10.0 g of ethyl acetate fraction, and 26.5 g of butanol fraction were obtained.

The ethyl acetate extract (10 g) was chromatographed on a column (3  $\times$  120 cm) of silica gel (250 g), with elution successively by chloroform and systems 1 and 2, fractions with a volume of 500 ml being collected. Individual fractions of the system 1 eluate yielded 0.3 g of flavone (1). Further elution of the column with system 2 gave 0.2 g of flavone (2), 0.29 g of flavone (4), and 0.32 g of flavone (3).

**Demethoxycentaureidin 7-*O*- $\beta$ -*D*-glucoside (1)**,  $C_{23}H_{24}O_{12}$ , mp 264-266°C,  $\lambda_{\max}$  (ethanol), nm: 256.5, 275, 346; +CH<sub>3</sub>COONa 258, 271, 348;  $\nu_{\max}$  (KBr),  $cm^{-1}$ : 3600-3400 (OH), 2930 (OCH<sub>3</sub>), 1662 (C=O  $\gamma$ -pyrone), 1615, 1565, 1510 (C=C bond), 1120, 1050, 1045, 1025 (C-O).

PMR spectrum (Py- $d_5$ ), ppm: 3.68 (s, -OCH<sub>3</sub>), 3.94 (s, -OCH<sub>3</sub>), 4.00-4.59 (glucose protons), 5.77 (d, 6.5 Hz, H-1''), 6.88 (s, H-3), 6.89 (d, 8.5 Hz, H-5'), 7.19 (s, H-8), 7.43 (dd, 2.0 and 8.5 Hz, H-6'), 7.75 (d, 2.0 Hz, H-2'), 13.54 (br.s, 5-OH).

The acid hydrolysis of glycoside (1) (5% hydrochloric acid, 4 h) formed *D*-glucose and demethoxycentaureidin with the composition  $C_{17}H_{14}O_7$  ( $M^+$  330), mp 217-219°C, PMR spectrum (Py- $d_5$ ): 3.60 (s, -OCH<sub>3</sub>), 3.85 (s, -OCH<sub>3</sub>), 6.83 (s, H-3), 6.87 (d, 8.0 Hz, H-5'), 7.13 (s, H-8), 7.41 (dd, 2.0 and 8.0 Hz, H-6'), 7.78 (br.s, H-2'), 13.73 (br.s, 5-OH).

The acetylation of glycoside (1) (acetic anhydride in the presence of pyridine) gave a hexaacetate with mp 119-121°C ( $M^+$  744 and the peaks of fragmentary ions of tetracetylglucose with  $m/z$  331, 271, and 169).

**Pectolinarin (2)**,  $C_{29}H_{34}O_{15}$ , mp 272-274°C,  $\lambda_{\max}$  (ethanol), nm: 276, 332;  $\nu_{\max}$  (KBr),  $cm^{-1}$ : 3580-3350 (OH), 2932 (OCH<sub>3</sub>), 1660 (C=O), 1617, 1570, 1515 (C=C bond), 1120, 1056, 1048, 1020 (C-O).

PMR spectrum (Py- $d_5$ ), ppm: 1.45 (d, 5 Hz, -CH<sub>3</sub>), 3.60 (s, -OCH<sub>3</sub>), 3.94 (s, -OCH<sub>3</sub>), 3.73-4.74 (protons of the carbohydrate moiety), 5.38 (br.s, H-1'''), 5.67 (m, H-1''), 6.78 (s, H-3), 7.17 (d, 8.4 Hz, H-3', 5'), 7.24 (s, H-8), 7.96 (d, 8.4 Hz, H-2', 6').

**Acetylpectolinarin (3)**,  $C_{31}H_{36}O_{16}$ , mp 240-242°C,  $\lambda_{\max}$  (ethanol), nm: 277, 328. PMR spectrum (Py- $d_5$ ), ppm: 1.23 (d, 5 Hz, -CH<sub>3</sub>), 1.78 (s, -OCOCH<sub>3</sub>), 3.60 (s, -OCH<sub>3</sub>), 3.65 (s, -OCH<sub>3</sub>), 3.74-4.76 (protons of the carbohydrate moiety), 5.37 (br.s, H-1'''), 5.61 (t, 9.0 Hz, H-4'''), 5.65 (m, H-1''), 6.76 (s, H-3), 7.18 (d, 8.5 Hz, H-3', 5'), 7.23 (s, H-8), 7.96 (d, 8.5 Hz, H-2', 6').

The alkaline hydrolysis of glycoside (3) (0.5% KOH, 30 min at room temperature) gave pectolinarin (2).

**Acid Hydrolysis of (3).** Compound (3) (30 mg) was heated with 15 ml of 5% hydrochloric acid in the boiling water bath for 4 h. The precipitate that deposited was filtered off, recrystallized from ethanol, and identified as pectolarigenin with the composition  $C_{17}H_{14}O_6$ ,  $M^+$  314, mp 213-216°C [9]. *D*-Glucose and *L*-rhamnose were found in the evaporated hydrolysate by the PC method (system 4).

**Heptaacetate from (2) and (3).** A mixture of 35 mg of (2) or (3), 1 ml of pyridine, and 3 ml of acetic anhydride, was left at room temperature for 4 h. On the addition of ice water a precipitate deposited, and this was recrystallized from ethanol to give a whitish amorphous powder. The heptaacetates from (2) and (3) were identical, with mp 123-125°C, composition  $C_{43}H_{48}O_{22}$  ( $M^+$  916 and intense peaks of fragmentary ions with  $m/z$  561, 273, 213, and 153).

**Demethoxycentaureidin 7-O-rutinoside (4),**  $C_{29}H_{34}O_{16}$ , mp 198-200°C,  $\lambda_{max}$  (ethanol), nm: 274, 335; +  $CH_3COONa$  273, 336;  $\nu_{max}$  (KBr),  $cm^{-1}$  3600-3450 (OH), 2932 (OCH<sub>3</sub>), 1660 (C=O), 1614, 1560, 1505 (C=C-bond), 1120, 1040, 1035, 1020 (C-O of glycosides).

PMR spectrum (Py- $d_5$ ), ppm: 1.44 (d, 5 Hz, -CH<sub>3</sub>), 3.59 (s, -OCH<sub>3</sub>), 3.93 (s, OCH<sub>3</sub>), 3.80-4.74 (protons of the carbohydrate moiety), 5.37 (br.s, H-1'''), 5.65 (d, 6.5 Hz, H-1''), 6.84 (s, H-3), 6.93 (d, 8.0 Hz, H-5'), 7.17 (s, H-8), 7.38 (dd, 2.0 and 8.0 Hz, H-6'), 7.71 (br.s, H-2'), 13.55 (5-OH).

**Acid Hydrolysis of Glycoside (4).** A solution of glycoside (4) (35 mg) in 10 ml of methanol was treated with 20 ml of 5% hydrochloric acid, and the mixture was boiled on the water bath for 4 h. Then the methanol was distilled off in vacuum, and the precipitate that deposited was filtered off and recrystallized from ethanol. This gave 15 mg of demethoxycentaureidin with mp 217-219°C. *D*-Glucose and *L*-rhamnose were detected in the evaporated eluate by PC (system 4).

**The Octaacetate (5) of (4).** Glycoside (4) (30 mg) was acetylated with 3 ml of acetic anhydride in 1 ml of pyridine at room temperature for 4 h. After working up by a generally adopted method and recrystallization from ethanol, 19 mg of octaacetate with mp 114-116°C was obtained.

Mass spectrum,  $m/z$ :  $M^+$  974, 561, 273, 213, and 153.

**Partial Hydrolysis of (4).** Compound (4) (60 mg) was heated in the water bath with 6 ml of 10% acetic acid. The course of the reaction was monitored by TLC in system 3. After 6 h, the reaction mixture was neutralized with 10% sodium carbonate solution and was evaporated in vacuum. The residue was dried and chromatographed on a column of silica gel in system 2, which led to the isolation of 20 mg of demethoxycentaureidin 7-O- $\beta$ -*D*-glucoside (1) with mp 264-266°C.

**Determination of the Structure of the Carbohydrate Moiety.** Glycoside (4) was methylated by Hakomori's method. After the usual work-up, 35 mg of methylation product was obtained. This was hydrolyzed with 6% methanolic hydrochloric acid in the boiling water bath for 4 h. After appropriate working up, 2,3,4-tri-O-methyl-*L*-rhamnose and 2,3,4-tri-O-methyl-*D*-glucose were identified by TLC.

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