GLYCOSIDES FROM *LINARIA VULGARIS MILL*

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Abstract: A new flavonol glycoside, 5,4′-dimethylkaempferol 3-O-β-D-(6″-α-L-rhamnopyranosyl)-glucopyranoside, together with three known compounds were isolated from the n-butanolic soluble fraction of underground and aerial parts of *Linaria vulgaris Mill*, collected on the territory of Moldova. The characterisation of these compounds was achieved by various chromatographic and spectroscopic methods (IR, UV, ^{13}C-NMR, ^{1}H-NMR and MS).

Keywords: *Linaria vulgaris Mill*; flavonol glycoside; linaroside V; NMR analysis.

Introduction

Linaria vulgaris Mill is widely spread on the territory of Europe as well as in the Republic of Moldova. The plant is used in traditional, folk medicine and in homeopathy due to its contents of biologically active substances. In medicine, the liquid extract of *L.* vulgaris *Mill* is used as purgative [1], diuretic, stimulator of the gall-bladder secretion [2, 3]. The ointment made from it is useful in the case of skin disease [1], while the tinctures in dentistry [4]. This plant has antibacterial and fungicidal properties and it is used for treating liver and kidney diseases, tonsillitis, asthma, dermatomes, etc [5].

The previous phytochemical investigation of *L.* vulgaris *Mill* has been revealed the presence of alkaloids, flavonoids, triterpenoids, steroids and iridoid glycosides [6 – 13].

A new compound **IV** and three known compounds **I-III** have been isolated from the butanol watery extract of the plants of *L.* vulgaris *Mill*. By comparison of physical and spectroscopic properties (m.p., IR, UV, ^{1}H-NMR, ^{13}C-NMR spectra), the known compounds were identified as antyrrinoside (**I**) [9], benzyl alcohol O-β-D-glycopyranoside (**II**) [14] and benzyl alcohol β-D-(2′-O-β-xylopyranosiyl)-glycopyranoside (**III**) [15, 16]. In this article, we present the isolation and structural determination of the new compound, called linaroside V, and give its ^{13}C- and ^{1}H-NMR data, which have not been reported previously.

Results and Discussion

The n-butanol soluble fractions of *L. vulgaris* were fractionated on SiO_2 columns. Further separation and purification was achieved by combining chromatographic methods (silica columns, Sephadex LH-20, HPLC) to yield compounds **I-IV** in a pure form.

Compound **IV,** named linaroside V, was obtained as yellow amorphous powder (m.p. 188-193 ^{0}C). The ESMS spectroscopy of **IV** resulted in the quasimolecular ion $[M+H]^+$ at m/z 623, indicating the molecular formula of $C_{29}H_{34}O_{15}$. The IR spectrum showed strong absorption bands at 3420 (-OH group), 2965 (C-H bonds), 1650 (C=C aromatic ring), and 1620 cm^{-1} (C=O), confirming the flavonoid nature of **IV**. The structure of linaroside V was elucidated by ^{13}C-NMR and ^{1}H-NMR spectroscopy (see Table 1). The ^{13}C- and ^{1}H-NMR spectra showed signals for aromatic ring at δ C 122.5-128.4 ppm and δ H 6.92-7.15 ppm, while the signals at δ C 60.0; 55.3 ppm and δ H 3.76; 3.85 ppm indicated two methoxy substitutions in the aglycone. The ^{1}H-NMR spectrum suggested that **IV** is a disaccharide on the basis of two signals in the sugar region at δ 5.12 (d, J = 7.1 Hz) and 4.56 ppm (s), corresponding to the anomeric proton of β-glucose and to the anomeric proton of the α-rhamnose, respectively (rhamnose methyl group generates bonds at δ 17.7 in ^{13}C-NMR and 1.05 ppm in ^{1}H-NMR). The α-rhamnose was concluded to be attached to C-6 of β-glucose moiety, which was confirmed by its ^{13}C-NMR spectrum and ^{1}H–^{1}H COSY experiment. The HMBC spectra showed a correlation between the H-1‴-rhamnosyl proton (δ 4.56) and C-6″ glucose unit (66.8 ppm), H-1″glucosyl proton at δ 5.12 and aglycone C-3 (δ 142.4 ppm). The findings defined the disaccharide as a 3-O-rutinoside [17].

The ^{13}C- and ^{1}H-NMR spectral dates of **IV** have been compared with those previously described in the literature for nicotiflorin (kaempferol 3-O-β-D-(6″-α-L-rhamnopyranosyl)-glucopyranoside) [17, 18]. The coincidence of signals of the sugar moieties has been revealed, except for the signals corresponding to the flavonol aglycone, due to the presence of two methyl groups in the spectra of **IV** (Table 1). Different NMR experiments indicated C-5 and C-4′ methoxy substitutions in the aglycone. In HMBC experiment, the protons signals at δ 3.76 (3H, OCH_3) and 3.85 (3H, OCH_3) were correlated, with C-5 at 132.2 and C- 4′ at 162.5 ppm, respectively. Thus, the aglycone of **IV** is 5, 4′-dimethylkaempferol. Considering all the data, the structure of the new flavonol glycoside - linaroside V is determined to be the 5, 4′-dimethylkaempferol 3-O-β-D-(6″-α-L-rhamnopyranosyl)-glucopyranoside (Fig.1).

The spectral data of compounds **I-III**, including UV, ^{13}C- and ^{1}H-NMR, were verified by comparison with those previously described in the literature [9, 14, 15, 16].

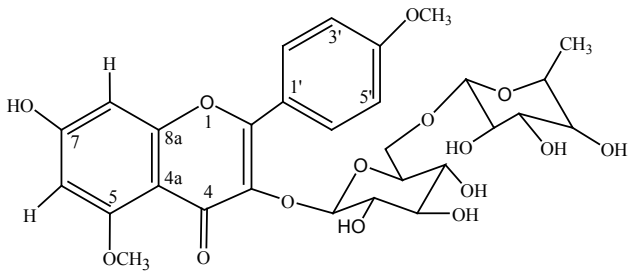

Fig. 1. Structure of linaroside V

Table 1

^{13}C- and ^{1}H- NMR spectral data for compound IV (175 MHz, 300 MHz, CD_3OD, DMSO-d_6)

Position	δ C, ppm	δ H, ppm, J (Hz)
Aglycon		
2	161.8	
3	142.4	
4	182.7	
5	132.2	
6	102.1	6.92 d (2.0)
7	152.2	
8	94.0	6.93 d (2.5)
9	156.7	
10	105.9	
1′	122.5	
2′	128.4	8.03 d (8.8)
3′	114.5	7.15 d (8.2)
4′	162.5	
5′	114.5	7.15 d (8.4)
6′	128.4	8.03 d (8.6)
Glc		
1″	100.1	5.12 d (7.1)
2″	73.1	3.33 m
3″	76.3	3.32 m
4″	69.5	3.18 t (8.6)
5″	75.5	3.62 m
6″	66.8	3.47 d (10.8)
		3.89 dd (6; 9)
Rha		
1‴	100.3	4.56 s
2‴	70.3	3.66 m
3‴	70.7	3.46 m
4‴	71.9	3.14 m
5‴	68.5	3.41 m
6‴	17.7	1,05 d (6.5)
MeO-C_5	60	3.76 s
MeO-$C_{4'}$	55.3	3.85 s

Conclusion

The new flavonol glycoside called linaroside V has been isolated from *Linaria vulgaris Mill* and its chemical structure has been established by various spectroscopic methods.

Experimental

Plant material

The plants of *Linaria vulgaris Mill* were collected in the Rebublic of Moldova in august-september 2006 and were identified by Professor Vasilii Florea (Laboratory of Medicinal Plants, Academy of Sciences of Moldova).

General Experimental Procedures

Spectra were recorded using the following instruments: IR – on Specord 71-IR spectrophotometer, KBr; UV – on Specord UV-VIS spectrophotometer (MeOH, c=1). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX-spectrometer (300 MHz, 175 MHz); solvents CD_3OD, DMSO-d_6; TMS as internal standard. The mass-spectra were obtained on ESMS in the positive ion mode instrument. The chromatography was performed on silica gel (60-100 μm Merck); GPC: Sephadex LH-20 (Pharmacia). Preparative HPLC: Varian ProStar 210, Varian 350 refractive index detector, Luna C-18 Phenomenex column 250 cm x 10 mm i.d. x 10 μm, 50% MeOH, 2.0 ml min^{-1}.

Extraction and Isolation

The air-dried plants (2 kg) were extracted with 70% ethanol under reflux (4 l x 3) for 5 h each time. The total extract was concentrated and extracted with $CHCl_3$ and n-BuOH. The n-BuOH soluble fraction was dried in vacuum (to afford 50 g), purified by crystallization and fractionated by combining chromatographic methods: columns (60 mm x 30 mm) on silica gel (40 x 100 μm, Merk) with a solvent system of $CHCl_3$-MeOH-H_2O (95:5:0 → 300:120:30 v/v/v), and Sephadex LH-20 using MeOH. The fractions (5 ml) were collected, characterized by TLC on Silufol. The fractions with similar R_f values were recombined and further purified by reversed-phase HPLC. Four compounds (I-IV) were obtained: **I** -50 mg; **II** – 25 mg; **III** – 27 mg; **IV** – 120 mg.

Linaroside (**IV**) – yellow amorphous powder, m.p. 188-193^0 C, R_f = 0.54 ($CHCl_3$-MeOH-H_2O; 76:14:3); IR, ν_{max} KBr cm^{-1}: 3420 (oh); 2695 (C-H); 1650 (C=C); 1620(C=O). ESMS, m/z 623 (calcd for $C_{29}H_{34}O_{15}$ $[M+H]^+$); 476 $[M+H\text{-}Rha]^+$; 314 $[M+H\text{-}Rha\text{-}Glc]^+$. ^{13}C- and ^{1}H-NMR see in the Table 1.

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