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CHEMICAL COMPOSITION OF *Silene viridiflora*

N. E. Eshmirzaeva, N. K. Khidyrova, M. Khodzhaeva, L. G. Mezhlumyan, and Kh. M. Shakhidoyatov

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Neutral substances, carbohydrates, and microelements from the aerial part of Silene viridiflora *in addition to the protein content and its amino-acid composition were determined.*

Key words: *Silene viridiflora*, sterols, polyisoprenoids, amino acids, carbohydrates, microelements.

The literature indicates that the genus *Silene* (Caryophyllaceae) has the highest content of ecdysteroids [1-8]. The maximum amount of phytoecdysteroids in the aerial part of plants of the genus *Silene* L. accumulates during budding and flowering [9]. The aerial part of *S. viridiflora* L. (budding phase) is a promising source of phytoecdysteroids for practical application.

We carried out a comprehensive investigation of the chemical composition of the principal classes of natural compounds in *S. viridiflora* (ripening phase) collected in Tashkent at the experimental plot of the S. Yu. Yunosov ICPS of the ASRU [10].

Fresh raw material was dried in the shade at 20-25°C to a residual moisture content of 2.7%. Ground leaves and roots were extracted with $(C_2H_5)_2O$, $CHCl_3$, C_2H_5OH, and CH_3OH to isolate the extracable substances (ES). The ES content in the leaves was 4.2, 3.8, 12.6, and 17.3% whereas the values in the roots were 2.4, 3.2, 6.0, and 13.1%, respectively. TLC of the neutral part identified [11] the following compounds: sitosterol, stigmasterol, polyisoprenoids, and α-tocopherol, the contents of which were 0.08, 0.02, 0.01, and 0.005% of the air-dried mass (ADM) of plant material.

Proteins were extracted under alkaline conditions by borate buffer (0.2 M, pH 9.0) as before [12]. The protein content was determined by the Kjeldahl method [13]. The protein yield was 5.88 wt. %.

The amino-acid composition of the isolated protein was determined after acid hydrolysis on an amino-acid analyzer. A total of 17 amino acids was observed in the hydrolysate. Table 1 lists the results. The quantitative determination of amino acids in the protein hydrolysate from *S. viridiflora* showed that the protein contained essential amino acids - histidine, isoleucine, leucine, lysine, phenylalanine, methionine, threonine, valine, and arginine.

The content of organic acids was determined as before [14]. It made up 8.32% calculated as malic acid in the absolute dry raw material.

Remaining raw material was boiled with alcohol (80°) to determine the sugars soluble in alcohol (SSA). SSA extracts were purified with lead acetate (10%) and Na_2SO_4. Purified SSA solutions were evaporated to dryness. Paper chromatography (PC) (system 1) detected the following sugars that were characteristic of all plant organs: galactose, glucose, arabinose, and rhamnose in various ratios. Water-soluble polysaccharides (WSPS-1 and WSPS-2) were extracted by treating the raw material with water at room temperature and on a water bath at 70°C. Pectinic substances (PS) were obtained using oxalic-acid solution (0.5%). Table 2 gives the percent content of carbohydates and shows that the amount of free sugars is highest in leaves. WSPS-1 accumlated in roots; PS, in stems.

Solutions of WSPS-1 and WSPS-2 were condensed and precipitated with alcohol (1:3). Dried samples were cream-colored powders that were very soluble in water. Hydrolysates of WSPS-1 from seeds and leaves contained galactose, glucose, arabinose, and rhamnose according to PC (system 1); from stems, galactose, glucose, and arabinose; from roots, galactose and glucose. Hydrolysates of WSPS-2 from all organs contained (PC, system 1) only glucose. Aqueous solutions of WSPS-2 give a positive reaction with iodine, which is consistent with the presence of starch.

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75, e-mail: Nilufar80@rambler.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 365-366, July-August, 2005. Original article submitted May 8, 2005.

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TABLE 1. Amino-Acid Composition of Proteins from *Silene viridiflora*, wt. %

Amino acid	Content, %	Amino acid	Content, %
Asparagine	0.94	Methionine*	0.77
Leucine*	0.69	Isoleucine*	0.66
Threonine*	0.79	Tyrosine	1.33
Serine	0.59	Phenylalanine*	1.63
Glutamine	0.76	Histidine*	1.18
Proline	0.29	Lysine*	0.62
Alanine	0.46	Arginine*	0.60
Glycine	0.37	Cysteine	0.53
Valine*	0.59		

*Essential amino acids.

TABLE 2. Carbohydrate Content in Various Organs of *Silene viridiflora*

Plant organ	PhE + SLN	WSS	WSPS-1	WSPS-2	PS
	% of air-dried raw material				
Seeds	4.3	1.4	0.7	0.3	0.43
Leaves	4.1	1.7	2.5	1.7	2.9
Stems	3.5	1.0	1.3	1.1	4.4
Root	3.0	1.1	6.5	3.2	1.3

PhE - Phytoecdysteroids.
SLN - Substances of lipophilic nature.

Extracts of PS were dialyzed against distilled water, condensed, and precipitated with alcohol (1:2). The hydrolysates of PS from all organs contained (PC, system 1) sugars such as galactose, arabinose, rhamnose, and galacturonic acid, which are characteristic of pectins [15].

The concentrations of microelements were determined by atomic absorption [16] and gave the following values (mass fraction, %): Ba, 0.0041; V, 0.00084; Bi, 0.00059; Ge, 0.00012; Co, 0.000062; Mn, 0.12; Cu, 0.00014; Mo, 0.0012; Ni, 0.000003; Sr, 0.000028; Pb, 0.00012; Ag, 0.000020; Ti, 0.016; Zn, 0.00046; Au, >0.00030.

Thus, a systematic study of the chemical composition of the aerial part of *S. viridiflora* found isoprenoids, proteins, carbohydrates, microelements, and organic acids.

EXPERIMENTAL

Extractable substances were isolated from ground raw material (10 g) by $(C_2H_5)_2O$, $CHCl_3$, C_2H_5OH, and CH_3OH (3:1, 0/0 v/v) three times with shaking at room temperature every 6 h. Extracts were combined. Solvent was removed in a rotary evaporator at temperatures below 40°C until the solid was dry. Yields of extractable substances from leaves were 4.2, 3.8, 12.6, and 17.3%; from roots, 2.4, 3.2, 6.0, and 13.1%, respectively. The neutral part was isolated as before [17]. TLC was performed on Silufol UV-254 plates using benzene:ethylacetate (24:1) with development by I_2 vapor, H_2SO_4:vanillin, and Emmerie—Engel reagent. Sitosterol, stigmasterol, polyisoprenoids, and α-tocopherol were detected and determined by the literature method [11].

Proteins were isolated as before [12]. The protein content was analyzed quantitatively using the Kjeldahl method [13].

The amino-acid composition of the protein was determined on a T339 amino-acid analyzer (Czech Rep.) after hydrolyzing samples three times in HCl (6 N) at 110°C for 24 h. After the hydrolysis was complete, HCl was quickly removed and the hydrolysate was analyzed.

The content of free organic acids calculated as malic acid per absolute dry weight in percent was calculated using the formula

$$X = (V\ 0.0067 \times 250 \times 100 \times 1000)/M \times 10(100 - W)$$

where X = 8.32, 0.0067 is the amount of malic acid corresponding to NaOH solution (1 mol, 0.1 M) in g, V is the volume of NaOH solution (0.1 M) used in the titration in mol; m is the mass of raw material in g; and W is the mass loss on drying the raw material in %.

SSA, WSPS, and PS were isolated as before [15]. WSPS-1, WSPS-2, and PS were hydrolyzed by H_2SO_4 (2 N) at 100°C for 14, 10, and 48 h, respectively.

Paper chromatography used $C_4H_9OH:C_5H_5N:H_2O$ (6:4:3) with anilinium phthalate developer.

The concentrations of microelements were determined by atomic absorption spectrophotometry (Perkin—Elmer 403) using the analytical line at 283.3 nm.

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