Chemistry of Natural Compounds, Vol. 48, No. 3, July, 2012 [Russian original No. 3, May-June, 2012]

POLYPRENOLS FROM LEAVES AND STEMS OF THE PLANT *Althaea officinalis*

N. K. Khidyrova,* E. V. Van, R. Kh. Shakhidoyatov, Kh. M. Bobakulov, N. D. Abdullaev, and Kh. M. Shakhidoyatov

UDC 547.315.2+582.796

Polyprenols from the aerial part of the plant Althaea officinalis *were studied. It was shown that polyprenols from leaves were polyprenol homologs with 9–13 isoprene units where undecaprenol dominated. The polyprenol contents in leaves and stems and the component composition of polyprenols of this plant were determined.*

Keywords: *Althaea officinalis*, total extracted substances, unsaponified fraction, polyprenols.

Roots of *Althaea officinalis* are widely used in medical practice as an expectorant, encapsulant, emollient, and anti-inflammatory agent for diseases of the respiratory tract such as bronchitis, tracheitis, pertussis, and bronchial asthma [1].

Rutin, phytosterol, tannin, phosphates, pectinic substances, vitamins, and mucous substances, the hydrolysis of which formed glucose, galactose, arabinose, rhamnose, and starch, were isolated from the plant roots. Mucus, essential oil, and ascorbic acid were observed in leaves and flowers [2]. Although the chemical composition of *A. officinalis* roots is rather well studied, until now the aerial part of this plant has not been investigated.

We studied earlier polyprenols (PPs) of various lines and varieties of cotton [3], *A. armeniaca* [4], and other plants of the family Malvaceae. An analysis of the literature and our research showed that PPs were distributed mainly in the green parts of the plants and exhibited a broad spectrum of biological activity [5].

In continuation of research on the chemical composition of plants of the family Malvaceae, herein we present results from a study of PPs from leaves and stems of *A. officinalis*. The results showed that the PP content in leaves peaked during fruiting (Table 1). This was noted earlier for *A. armeniaca* [6].

Therefore, we decided to study PPs from leaves and stems namely during this vegetative period. We established in previous studies that the yield of total extracted substances (TES) was greatest for *A. armeniaca* ground to 2.0–3.0 mm [4]. Ground leaves of sizes 0.5–1.0, 2.0–3.0, 4.0–5.0, and 6.0–7.0 mm were extracted in order to determine the optimum conditions for isolating TES. The extractant was alcohol at ratios 1:4, 1:3, 1:3, and 1:3. The duration of the extraction was 10, 9, 8, and 6 h, respectively. Table 2 presents the results.

The results showed that the yield (8.68%) of TES was highest for size 2.0–3.0 mm. Finer (up to 1.0 mm) particles hindered the extraction by clogging pores during filtration. Larger (from 4.0 to 7.0 mm) particles and extraction of intact leaves required the use of a large volume of solvent. On the other hand, the yield of TES decreased because the solvent used for the extraction contacted insufficiently the particles.

The PP content in plant stems was comparatively less than that in leaves and for other plants of this family. Thus, the yield of TES and unsaponified substances was 8.68 and 6.8 vs. 2.21 and 1.7% of the air-dried mass (ADM) for alcohol extraction from leaves and stems, respectively. The PP content in leaves (1.37%) was also significantly (by 3.9 times) greater than that in stems (0.35%).

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences, Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75, e-mail: nhidirova@yandex.ru. Translated from *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2012, pp. 326–328. Original article submitted October 10, 2011.

 0009-3130/12/4803-0358

TABLE 1. Change of PP Content of *Althaea officinalis* Leaves by Vegetative Phases, %

Vegetative phase	TES yield	Polyprenol content	
	of ADM	of ADM*	of TES**
4–5 actual leaves	8.21	0.408	8.08
Budding	14.27	0.670	10.40
Flowering	7.22	0.839	11.7
Fruiting	8.68	1.37	20.2
Ripening	9.79	0.991	14.28
Leaf shedding	12.70	0.944	12.12

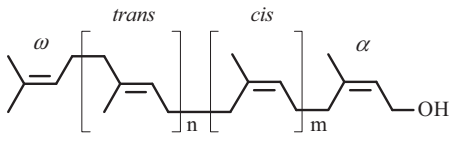
*ADM, air-dried mass; **TES, total extracted substances.

TABLE 2. Effect of Degree of Grinding of Leaves on Yield of Extracted Substances, % of ADM

Size, mm	1st decant.	2nd decant.	3rd decant.	4th decant.	Total yield
0.5–1.0	1.96	1.85	1.49	0.91	6.21
2.0–3.0	2.97	2.35	1.99	1.37	8.68
4.0–5.0	2.09	1.70	1.31	1.01	6.11
6.0–7.0	1.99	1.59	0.59	0.35	4.52

PPs were isolated in yields of 1.05 and 0.19% of ADM by column chromatography of total unsaponified substances of leaves and stems, respectively. The purity of the PPs was monitored by TLC compared with that of standard PP samples that were isolated from leaves of cotton line L-4. The PP content was 96–98% [5]. The quantitative PP content was determined by high-performance TLC (HPTLC) using toluene:EtOAc (19:1).

According to analytical results, PPs of *A. officinalis* leaves and stems consisted of polymeric homologs with n = 9–13 and decaprenol content 5.68 and 30.32%; undecaprenol, 63.68 and 36.68; and dodecaprenol, 29.65 and 23.24, i.e., undecaprenol dominated in them. Nonaprenol and tridecaprenol occurred in trace quantities in leaves. The nonaprenol content in stems was 9.72%. Tocopherols and their oxidized species were isolated as ballast substances. PPs of *A. officinalis* were identified chemically by comparing spectral properties (IR and PMR spectra) with those in the literature [5, 7, 8].



n = 3; m = 4 - 8

The PMR spectrum of the PPs exhibited singlets for methyl protons at 1.45 and 1.50 ppm that belonged to inner *trans*- and *cis*-isoprenoid units. Protons of methyls on the ω- and α-terminuses of the chain resonated as singlets at 1.48 and 1.64 ppm, respectively. Two broad triplets (J = 6.8 Hz) corresponding to methylene protons of the isoprenoid chain were observed at 2.00 and 2.08 ppm. A doublet characteristic of methylene protons adjacent to a hydroxyl was found at weaker field (3.88). The olefinic protons of the chain midsection (*cis*- and *trans*-) formed a broad multiplet at 5.18. The olefinic proton of the chain terminus resonated as a triplet at 5.3 ppm.

The IR spectrum of the PPs contained absorption bands at 838 cm^{-1} that corresponded to C–H bending vibrations of trisubstituted olefins. Characteristic C–O vibrations of a primary allyl alcohol appeared at 1002 cm^{-1}. The region near 1381 cm^{-1} belonged to methyl C–H bending vibrations. Absorption bands corresponding to methyl and methylene C–H bending vibrations were observed at 1448. Vibrations of C=C bonds appeared at 1667; C–H vibrations of CH_2 and CH_3 groups, at 2849, 2919, and 2958 cm^{-1}. These values agreed with the literature data [8, 9].

Thus, PPs from the aerial part of *A. officinalis* were studied for the first time. It was shown that they were polyprenol homologs with 9–13 isoprene units in which undecaprenol dominated. The qualitative compositions of polyprenol homologs of leaves and stems did not differ. However, their quantitative contents differed substantially.

EXPERIMENTAL

PMR spectra were recorded in C_6D_6 on a Unity 400 Plus spectrometer. IR spectra were taken in mineral oil on a Perkin–Elmer Model 2000 Fourier IR spectrometer. The quantitative content of PPs was determined by HPTLC on a Camag instrument (Switzerland) under the published conditions [10]. TLC used Silufol plates (AL SIL G/UV, Germany, 20 × 20) and solvents alcohol (96%), $CHCl_3$, and C_6H_6. The solvent systems for column chromatography were $CHCl_3$ and $CHCl_3$:hexane (12:1, 3:1, 2:1, 1:1, 1:2, 1:12). A column (18 × 98 cm) packed with silica gel sorbent (KSK, 100/250 mesh) was used.

Isolation of TES. Leaves or stems (100 g each) were ground to size 2.0-3.0 mm and extracted (4×) with EtOH (96%, 1 L each) by soaking. The EtOH extracts were combined and evaporated in a rotary evaporator at 40°C to afford TES (8.68 and 2.21 g with quantitative PP contents 1.37 and 0.35% of ADM).

Alkaline Hydrolysis. The EtOH extract of leaves or stems (300 mL) was treated with KOH (12 g) and H_2O (24 mL), stirred on a magnetic stirrer for 3 h, treated with H_2O (300 mL), transferred to a separatory funnel, and extracted (3×) with C_6H_6 (120 mL). The C_6H_6 extracts were combined and washed with H_2O until the pH was 7. The solvent was evaporated in a rotary evaporator. The yield of the unsaponified fraction was 6.8% of the ADM for leaves and 1.7% for stems.

Isolation of PPs. Total unsaponified substances of leaves (1.0 g) were separated into fractions by column chromatography using a column (30 × 135 cm) packed with KSK silica gel (100/250 mesh) at a sorbent:extract ratio of 60:1 with elution by hexane:$CHCl_3$ of gradually increasing polarity. A total of 30 fractions (200 mL each) was collected. Fractions 23–25 contained PPs (0.155 g). Analogously, unsaponified substances of stems (1 g) afforded a PP fraction (0.112 g). The yields of PPs were 1.05 and 0.19% of the ADM.

ACKNOWLEDGMENT

The work was supported by Grant GNTP-10 "Development of highly effective agents from local natural raw materials for treating diseases of the gastro-intestinal tract."

REFERENCES

1. M. D. Mashkovskii, *Drugs* [in Russian], Vol. **1**, Abu Ali Ibn Sino, Tashkent, 1998.
2. *Plant Resources* [in Russian], Vol **2**, Nauka, Leningrad, 1986.
3. N. K. Khidyrova and Kh. M. Shakhidoyatov, *Khim. Prir. Soedin.*, 87 (2002).
4. N. K. Khidyrova, E. V. Van, R. Kh. Shakhidoyatov, N. M. Mamatkulova, and Kh. M. Shakhidoyatov, *Khim. Prir. Soedin.*, 387 (2007).
5. A. M. Rashkes, N. K. Khidyrova, Ya. V. Rashkes, U. Mirkhodzhaev, U. K. Nadzhimov, and Kh. M. Shakhidoyatov, "Method for preparing polyprenols" [in Russian], RUzb Pat. No. 1543 (1993); *Byull. Izobret.*, No. 1, 121 (1995).
6. O. Kh. Saitmuratova, A. M. Rashkes, N. K. Khidyrova, V. B. Leont′ev, and Kh. M. Shakhidoyatov, *Khim. Prir. Soedin.*, 65 (1998).
7. A. R. Wellburn, J. Stevenson, F. W. Hemming, and R. A. Morton, *Biochem. J.*, **102**, 313 (1967).
8. K. J. Stone, A. R. Wellburn, F. W. Hemming, and J. F. Pennock, *Biochem. J.*, **102**, 325 (1967).
9. T. P. Kukina, L. I. Demenkova, V. A. Raldugin, B. I. Maksimov, O. S. Chizhov, V. V. Veselovskii, and A. M. Moiseenkov, *Sib. Khim. Zh.*, **6**, 89 (1991).
10. E. V. Van, R. Kh. Shakhidoyatov, N. K. Khidyrova, N. I. Mukarramov, and Kh. M. Shakhidoyatov, *Khim. Prir. Soedin.*, 653 (2009).