

AMINO ACIDS FROM *Pentaphylloides fruticosa* AND *P. parvifolia*

I. G. Nikolaeva

UDC 615.43

Pentaphylloides fruticosa (L.) and *P. parvifolia* (Fisch. ex Lehm.) are widely distributed in eastern and western Siberia and the Far East [1, 2], are very common in the wild, and have been used for ages in folk and traditional medicine [3].

The goal of our work was to study the amino-acid composition of runners of *P. fruticosa* and *P. parvifolia* (Rosaceae).

Plants were collected during mass flowering, *P. fruticosa* in the Baikal region of Buryatia (beginning of July 2006) and *P. parvifolia* in Mukhorshibir region of Buryatia (end of July 2006). The protein content in samples of the studied species was determined beforehand using the Lowry method of protein determination [4]. The method is based on formation of a colored complex by reaction of protein with an alkaline solution of copper sulfate and sodium tungstate and molybdate. The protein content in *P. fruticosa* was 1.12%; in *P. parvifolia*, 0.68%.

Amino acids were detected using TLC. Samples were ground and extracted with water for 1 h. The resulting extracts were filtered and purified over a column of Al_2O_3 . The effluent was used for descending chromatographic determination on Silufol plates using *n*-BuOH:HOAc:H₂O (1, 4:1:2), PrOH:H₂O (2, 70:30), and EtOH (96%):conc. NH₄OH (3, 16:4.5). The chromatograms were developed by spraying with alcoholic (0.5%) ninhydrin followed by heating at 100°C for 5-6 min [5, 6]. Three spots of weak violet color with R_f 0.42, 0.51, and 0.64 were observed in both samples using system 1; four spots of pink color with R_f 0.38, 0.45, 0.55, and 0.62 using system 2; and five spots of orange-pink color with R_f 0.09, 0.13, 0.30, 0.42, and 0.50 using system 3. The color intensity of the spots was different. Thus, both samples contained at least five free amino acids.

We continued the investigation of the amino-acid composition by using an AAA 339 amino-acid analyzer (Czech Rep.). For this, runner samples (accurate weights) were extracted exhaustively with hot water, filtered, and evaporated to dryness in vacuo. The dry solids were treated with lithium citrate buffer (pH 2.2). Proteins were precipitated by sulfosalicylic acid solution (0.1 mL, 30%). The samples were centrifuged and analyzed. The qualitative amino-acid composition in the studied samples was determined from retention times using a standard mixture consisting of 24 amino acids as a standard. The quantitative amino-acid content was calculated in μ g/g of sample. Peak areas were used for the calculations. Table 1 gives the results.

A total of 19 free amino acids were observed in runners of *P. fruticosa* and *P. parvifolia*. Of these, 6 were essential (threonine, methionine, isoleucine, leucine, phenylalanine, and lysine) and were found in both species. Another essential amino acid, valine, was found in *P. parvifolia*. Aspartic acid was not found in the sample of *P. parvifolia*. The quantitative amino-acid contents were different. Significant contents of asparagine and proline were found in both samples.

The amino acids occurred in the plants in both the free and bound states (Table 1) as polypeptides. Bound amino acids were determined after acid hydrolysis by HCl (6 N) at 110°C for 24 h. A total of 16 amino acids was observed in both samples, including the essential amino acid valine in *P. fruticosa* and aspartic acid in *P. parvifolia*.

TABLE 1. Contents of Amino-Acids and Bound Amino-Acids (After Acid Hydrolysis) in Runners of *Pentaphylloides fruticosa* and *P. parvifolia*

Acid	Content of amino acids in runners, µg/g				Acid	Content of amino acids in runners, µg/g			
	<i>P. fruticosa</i>		<i>P. parvifolia</i>			<i>P. fruticosa</i>		<i>P. parvifolia</i>	
	AA	BAA	AA	BAA		AA	BAA	AA	BAA
Asn	279.51	6020.11	-	404.62	Met*	126.82	189.86	141.00	153.68
Thr*	203.07	332.89	64.91	78.31	Ile*	144.65	165.31	67.90	93.48
Ser	236.48	361.00	40.99	66.74	Leu	48.54	101.35	13.45	41.00
Asp	6127.79		1618.89		Tyr	16.31	154.93	20.84	45.30
Gln	179.46	692.11	76.86	137.91	Phe*	187.50	227.56	110.27	134.64
Glu	50.44		56.65		α-Amino-	334.56		29.125	
Pro	1019.50	463.57	368.90	71.94	butyric				
Gly	82.42	201.83	27.23	47.69	γ-Amino-	136.87		109.80	
Ala	149.91	185.77	89.55	110.48	butyric				
Val*	-	359.50	88.70	89.29	Lys*	48.61	1491.24	14.26	26.32
Cys	391.09		107.54		His	58.20	1002.59	17.46	18.06
					Arg		203.81		11.32

* Essential amino acids; AA, amino acid; BAA, bound amino acids.

REFERENCES

1. *Flora of the USSR* [in Russian], Vol. 10, Acad. Sci. USSR, Moscow-Leningrad (1936).
2. *Flora of Central Siberia* [in Russian], Vol. 2, Nauka, Novosibirsk (1979).
3. *Plant Resources of the USSR. Flowering Plants, Their Chemical Composition and Use. Hydrangeaceae-Haloragaceae Families* [in Russian], Nauka, Leningrad (1987).
4. O. H. Lowry, N. G. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
5. R. M. C. Dawson, D. C. Elliot, W. H. Elliot, and K. M. Jones, *Data for Biochemical Research*, Clarendon Press, Oxford (1986).
6. M. Sharshunova, V. Shvarts, and Ch. Mikhalets, *Thin-Layer Chromatography in Pharmacy and Clinical Biochemistry* [in Russian], Vol. 2, Mir, Moscow (1980).