

2. On the basis of spectral characteristics and some chemical transformation it has been established that confoline has the structure of (\pm)-N-formylconvoline.

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FRACTIONAL AND AMINO ACID COMPOSITIONS OF THE PROTEINS OF THE NEEDLES OF *Pinus sylvestris*

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UDC 581.134.4:630.892

Four fractions of soluble protein have been isolated by the method of successive extraction from the needles of *Pinus sylvestris*, and their quantitative ratios have been established. The quantitative amino acid compositions of the fractions isolated and of the unextracted protein, which amounts to about 55% of the total amount of protein in the needles, have been determined. The amount of crude protein in the initial material was 11% on the absolutely dry needles.

The degree to which the proteins have been studied in various plant tissues, and, in particular, in coniferous tissues, is far from commensurate with their physiological and practical importance [1-7].

In the present investigations we have considered the fractional composition of the proteins present in the needles of *P. sylvestris*, the amino acid composition of the total protein and of each protein fraction, and the amounts of the various forms of nitrogen in the needles.

The results that we have obtained on the total nitrogen, the protein nitrogen, and the nonprotein nitrogen permit the conclusion that the amount of protein (more than 11%) of pine needles is not inferior to many varieties of fodder herbs and cereals:

<u>Form of nitrogen</u>	<u>Nitrogen content, %</u>	
	<u>on the dry substance</u>	<u>on the total nitrogen</u>
Total	1.88	100
Protein	1.25	66.5
Nonprotein	0.63	33.5
Crude protein	11.72	-

The fractional (group) composition of the proteins of *P. sylvestris* needles is as follows:

Siberian Technological Institute, Krasnoyarsk. Translated from Khimiya Prirodnikh Soedinenii, No. 5, pp. 676-678, September-October, 1980. Original article submitted March 31, 1980.

Fraction	Content, % on the total protein
Albumins	12.03
Globulins	8.96
Prolamines	7.49
Glutelins	16.58
Unextractable protein	54.94

The proteins of all the groups isolated belong to the class of simple proteins. The soluble proteins, i.e., the albumins, globulins, prolamines, glutelins taken together, make up about 45% of the total amount of protein in the needles. The maximum amount of all the soluble proteins is represented by the albumins and glutamins which together make up 64% of all the soluble proteins of the needles.

More than half of all the proteins of the needles cannot be isolated even after extraction with borate buffer, which is considered one of the most effective extractants for plant proteins.

The amounts of amino acids in the total protein and in each of the total protein fractions are shown in Table 1.

The results of the study of the amino acid compositions of the proteins of the needles show that the water-soluble fraction (albumins) contains the maximum amount of so-called essential amino acids, these making up 42% of the total amount. The predominating amino acids in the albumin fraction are valine and leucine, and the limiting acids histidine and methionine. Of the other amino acids, the high content of aspartic and glutamic acids must be noted.

The glutelins, together with the albumins, form the fraction richest in essential amino acids. In this group of proteins, the essential amino acids make up 36.5% of the total of all the amino acids. Predominating here are valine, leucine, and phenylalanine, and limiting are histidine and methionine.

The protein fraction isolated by 80% ethanol — the prolamines — may be considered to be of the poorest quality (in relation to nutrient value). This fraction lacks methionine in determinable amounts and has a low lysine content, while methionine and glycine are two of the amino acids for animals of which there is the greatest shortage. It must be mentioned that all the fractions isolated the richest in lysine are the salt-soluble proteins — the globulins. This fraction also includes the largest amount of another essential amino acid — leucine.

TABLE 1. Amino Acid Composition of the Total Protein and the Protein Fractions of *Pinus sylvestris* Needles

Amino acid	Content, % of the total					
	total proteins	albumins	globulins	prol- amines	glutelins	unextractable protein
Lysine	4.26	6.86	7.03	2.92	3.60	4.03
Histidine	0.60	0.48	0.38	0.62	0.48	0.85
Arginine	5.07	2.72	2.52	1.39	6.36	4.29
Cystine/2	Traces	Traces	5.78	2.66	—	—
Aspartic acid	12.92	12.49	3.42	16.33	12.28	12.37
Threonine	4.62	5.59	3.44	2.38	3.67	4.10
Serine	7.14	4.11	5.05	6.30	6.46	5.06
Glutamic acid	14.13	13.72	10.54	8.76	13.48	14.59
Proline	11.18	6.30	8.31	16.14	7.08	9.51
Glycine	7.29	7.22	7.51	11.54	7.51	7.23
Alanine	6.92	5.57	6.42	5.34	5.48	6.75
Valine	5.69	9.71	8.45	9.37	10.80	9.28
Methionine	0.58	0.23	0.29	—	0.92	1.54
Isoleucine	2.80	6.16	6.31	2.62	4.03	4.52
Leucine	10.19	12.32	12.62	9.44	8.07	9.03
Tyrosine	1.76	5.67	1.30	7.24	1.67	1.25
Phenylalanine	4.80	1.03	7.94	0.99	5.08	5.69

EXPERIMENTAL

The *Pinus sylvestris* needles were collected in July from trees 15-20 years old and they were treated with acetone in a Soxhlet apparatus at 60°C for 10 h. This succeeded in eliminating the resinous substances and the bulk of the pigments from the material. The needles treated in this way were dried at 55-60°C and ground.

Determination of the Forms of Nitrogen. Total nitrogen was determined by an accelerated Kjeldahl method [8], and protein nitrogen by the method adopted in working with the vegetative parts of plants [9]. The amount of nonprotein nitrogen was found from the difference between the total nitrogen and the protein nitrogen.

Isolation of the Protein Fractions. The protein compounds of the *Pinus sylvestris* needles were separated into fractions according to their degree of solubility in various extrac-
tants, using the method proposed by B. P. Pleshkov [9], with some slight modifications.

Hydrolysis of the Proteins. The protein fractions purified by reprecipitation were subjected to acid hydrolysis with 6 N HCl in evacuated sealed tubes at 105°C for 24 h.

Amino Acid Analysis. The hydrolysates were freed from humins by filtration and were evaporated under vacuum at 40°C, and the dry residues were dissolved in aliquot amounts of citrate buffer, pH 2.2.

The quantitative determination of the amino acids was carried out on a model AAA-881 automatic amino acid analyzer (Czechoslovakia) using a standard mixture of amino acids as external standard.

SUMMARY

1. The amount of crude protein in *Pinus sylvestris* needles is about 11% on the absolutely dry weight of the needles.
2. Four groups of soluble proteins have been isolated and their quantitative ratio has been determined. The combined water- and alkali-soluble fraction of the proteins amount to 30% of the crude protein in the needles.
3. The amino acid composition of the groups of proteins isolated have been determined. The amino acid compositions of the proteins of the different fractions vary only slightly. The total amounts of essential amino acids in the fractions differ.

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