TABLE 2. Biological Value and Amino Acid Score of the Alkali-Soluble Proteins of the Woody Verdure of the Sea Buckthorn

Essential amino acids	Amino acid score, % on the total amino acids				
	June	July	August	September	October
Lysine	69,09	84,73	110,55	88,00	88,55
Cystine + methionine	68,29	62,00	76,57	40,00	64,29
Threonine	178,25	150,25	136,25	127,00	251,00
Valine	59,80	134 00	98,40	82,00	77,20
Isoleucine	60,25	74,50	50,75	59,50	55,75
Leucine	101,71	209,29	172,71	120,60	137,14
Tyrosine + phenylalanine	145,83	130,00	125,33	125,70	146,17
BVP index	91,25	112,24	104,35	88,07	104,48

In the present communication we give the results of an amino acid analysis of the alkali-soluble protein of the woody verdure of the sea buckthorn (Table 1) and indices of the biological value of these proteins (BVP) calculated by the same methods as in [1].

The amino acid composition of the alkali-soluble proteins is largely similar to that of the water-soluble proteins. Characteristic for both groups of proteins is a predominance of monoamino bicarboxylic amino acids (aspartic and glutamic acid) over amino acids of basic nature (lysine, histidine, arginine). Both groups of proteins contain fairly small amounts of sulfur-containing amino acids (cystine and methionine).

The amino acid scores of the essential amino acids of the alkali-soluble proteins (Table 2) were calculated by the method recommended by the FAO in 1973 [2], and the BVP index by the procedure of Gruzdev et al. [3]. According to the BVP index, this group of proteins from the woody verdure of the sea buckthorn is superior not only to such fodder crops as alfalfa and clover [4], but also to the water-soluble proteins of the same woody verdure [1], exceeding the 100% level in some months.

LITERATURE CITED

1. G. G. Yushipitsina, N. A. Chuprova, and S. M. Repyakh, *Khim. Prir. Soedin.*, No. 3, 409 (1988).
2. FAO/WHO Ad Hoc Expert Committee on Energy Requirements, Rome, Paper 522 (1973).
3. A. G. Gruzdev, E. V. Sedova, and V. V. Lazarenko, *Izv. TSKhA*, No. 2, 85 (1974).
4. N. V. Turbin, A. G. Gruzdev, and É. A. Zhebrak, *Dokl. Akad. Nauk SSSR* **220**, No. 3, 743 (1975).

ISOLATION OF A TRYPSIN INHIBITOR FROM COTTON SEEDS

T. D. Kasymova, K. T. Abdurakhmanova, and P. Kh. Yuldashev

UDC 577.156

At the present time, great interest is being aroused by the question of the specific role of protease inhibitors in plant–pathogen interaction.

From healthy seeds of a cotton plant of the variety Tashkent 1 we have isolated a protein inhibitor which suppresses the activity of trypsin and protease C isolated for the same cotton seeds [1]. There are reports that some inhibitors isolated from plants suppress the activity of the proteases of microorganisms, and it is therefore possible that the plant protease inhibitors play an important role in their protection from phytopathogenic microorganisms [2]. In agreement with this, a positive correlation has been observed between the activity of inhibitors and the resistance of plants to fungal diseases. In an investigation of the proteolytic activity and amount of inhibitors in wheat it has been established that in a susceptible variety the activity of the enzyme was twice as great as in a resistant variety [3].

We have made a comparative study of the activities of the proteases of the seeds of a healthy and a wilt-affected plant and the relative resistance to wilt attack of the varieties Tashkent-1 and Tashkent-2. We studied the dependence of the change

Institute of Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.

Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 134-136, January-February, 1990. Original article submitted March 29, 1989; revision submitted July 12, 1989.

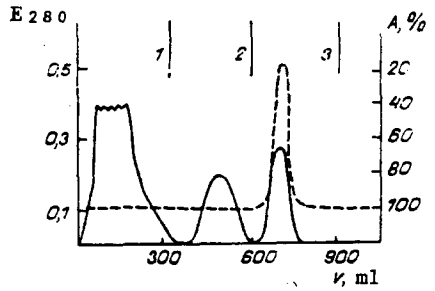

Fig. 1. Affinity chromatography of the trypsin inhibitor from cotton seeds: conditions of elution: 1) 0.1 M acetic acid brought with ammonia to pH 8.2; 2) 0.1 M ammonium acetate containing 0.3 M NaCl; 3) 0.2 M acetic acid, pH 2.5. A) Residual activity; V) volume.

in the proteolytic activity of the cotton seeds on the degree of attack by wilt [4]. The amount of total protein obtained from healthy seeds and from seeds 50% affected by wilt were relatively close, but their proteolytic activities differed sharply. In the seeds resistant to attack by wilt of the variety Tashkent-1 a fall in proteolytic activity of up to 90% was observed. Consequently, in response to the appearance of the pathogen in the plant there is an increase in the amount of protease inhibitors which, above all, inhibit their own proteases, and this explains the sharp decrease in proteolytic activity. It is likely that the higher the resistance of the plant to wilt, both greater is the amount of inhibitors that is synthesized in it at the moment of entry of the pathogen and the lower the activity of the proteolytic enzymes.

We have found a difference in the protein spectrum of healthy and wilt-affected seeds by carrying out an electrophoretic investigation of extracts of buffer-soluble proteins from seeds of the variety Tashkent-1 [4]. The distinct protein components detected on an electrophoretogram of the protein extract from wilt-affected seeds of the variety Tashkent-1 are probably inhibitors of proteolytic enzymes.

In order to answer this question, we have developed a scheme for the isolation of a protease inhibitor from the seeds of a cotton plant of the variety Tashkent-1. The stages of purifying the inhibitor included: extraction with 0.1 M phosphate buffer, pH 7.5, precipitation with ammonium sulfate to 80%, isoelectric precipitation by dialysis against acetate buffer, pH 5.0, and gel filtration on Sephadex G-100. The inhibitor preparation that had been isolated suppressed the activity of proteolytic enzymes: trypsin by 90% and protease C by 70%. The purified inhibitor preparation was deposited on a trypsin–agarose affinity sorbent, and 100% sorption of the protein was observed. The inhibitor was eluted with a 0.2 M solution of acetic acid, pH 2.5.

Subsequently, to purify the inhibitor we used an affinity sorbent in the initial stages of purification (Fig. 1). After isoelectric precipitation, the protein was dissolved in 0.1 M acetic acid, and the solution was brought with ammonia to pH 8.2 and was passed through a column of the sorbent equilibrated with the same buffer. Nonspecifically sorbed proteins were washed out with 0.1 M ammonium acetate containing 0.3 M NaCl. The elution of the inhibitor has been described above. The purity of the inhibitor preparation obtained was determined by electrophoresis in 7% PAAG in Tris-HCl buffer containing 0.1% of Na SDS. The inhibitor was detected on the chromatogram in the form of a diffuse colored band, the electrophoretic mobility of which corresponded to a protein with a molecular mass of 15,000 Da. The amino acid composition of the inhibitor was determined. It was characterized by a high content of aspartic acid, glycine, and alanine residues.

Menegatti et al. have isolated from white mustard a trypsin inhibitor with a molecular mass of 18,000 Da which also contained a high level of aspartic acid, glycine, and serine residues [5]. The trypsin inhibitor that we have isolated from dormant cotton seeds is very similar in its properties to the trypsin inhibitors detected in kidney beans [6] and soybeans [7].

LITERATURE CITED

1. T. D. Kasymova, M. A. Kuchenkova, and P. Kh. Yuldashev, *Khim. Prir. Soedin.*, 276 (1985).
2. V. V. Mosolov, *Vopr. Med. Khim.*, No. 5, 52 (1987).
3. A. M. Yamaleev and R. F. Isaev, *Dokl. VASKhNIL*, No. 7, 22 (1980).
4. M. I. Gartsbein, T. D. Kasymova, M. A. Kuchenkova, P. Kh. Yuldashev, K. Kh. Akhmedov, R. G. Kim, and Yu. T. Uzakov, *Khim. Prir. Soedin.*, No. 5, 704 (1987).
5. E. Menegatti, S. Palmieri, P. Walde, and P. L. Luisi, *J. Agr. Food Chem.* **33**, No. 5, 784 (1985).
6. V. V. Mosolov and N. V. Fedurkina, *Biokhimiya* **39**, No. 3, 624 (1974).
7. V. Frattali and R. F. Steiner, *Biochemistry*, No. 7, 521 (1968).