molecule, the coordination of the N(16) molecule is plane-trigonal. The lengths of the C(15)—O and C(15)—N(16) bonds are 1.22 and 1.32 Å, respectively (accuracy of the determination of the interatomic distances not worse than 0.015 Å).

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THE GLOBULINS OF COTTON SEEDS

XV. PRIMARY STRUCTURE OF A GLYCOPEPTIDE OF SUBUNIT C11 OF THE S-GLOBULIN

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Previously, from a tryptic hydrolysate of subunit C we obtained a fraction of peptides insoluble at pH 2.2 [1]. In the present paper we give information on the separation and purification of this fraction and the determination of the primary structure of a glycopeptide. To separate the peptides we used gel filtration on a column (2 × 70 cm) of Sephadex G-50 (fine) equilibrated with 50% acetic acid containing 6 M urea. The eluate was collected in 2.5 ml fractions and the absorption of the solution was measured at 280 nm. The results are given in Fig. 1.

The purity of the combined fractions was checked by TLC and PC under the conditions described previously [1] and by the determination of the N-terminal amino acids. Fraction 1 was homogeneous, and the presence of sugars in it was shown by the reaction with a 0.2% solution of anthrone in concentrated H_2SO_4. Fractions 2-5 of the peptides were separated by PC and electrophoresis as described previously in [1].

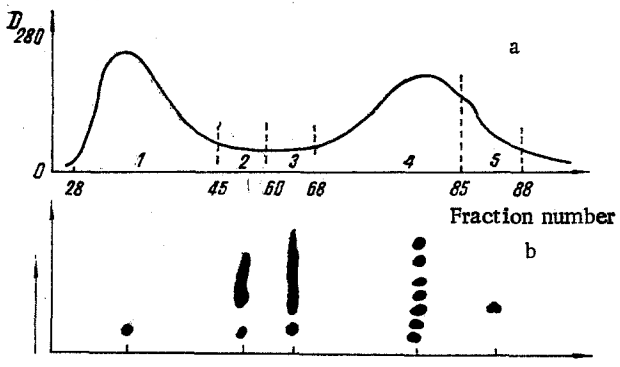

Fig. 1. Chromatography of the acid-insoluble tryptic peptides on a column of Sephadex G-50 (fine) (a) and thin-layer chromatography of the combined fractions (b).

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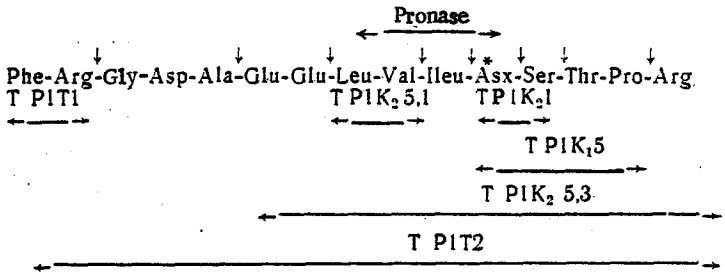
The glycopeptide isolated dissolved only in urea solutions and in 50% pyridine and had the following amino acid compositions: Asp_2, Thr, Ser, Glu_2, Pro, Gly, Ala, Val, Ileu, Leu, Phe, Arg_2. The N-terminal amino acid was Phe. The presence of two Arg's in the glycopeptide showed incomplete cleavage by trypsin. In fact, in determining the sequence of the peptide by the direct Edman method [2] it was possible to effect the four degradations Phe-Arg-Gly-Asp-... Since Arg was present in the second position, we performed an additional cleavage of the peptide with trypsin. Digestion was carried out for 24 h. The peptides were separated by PC under the conditions given above. This gave two fragments T P1T1 and T P1T2 (T P represents precipitated peptides).

The sequence of peptide T P1T2 was determined for only five residues, and therefore we carried out additional cleavage of the glycopeptide with 5.7 N HCl at 105°C for 10 min (K_1) and for 60 min (K_2). The peptideswere separated by the method described above. Thus, as a result of tryptic and two partial acid hydrolyses of the glycopeptide we isolated 14 different fragments. The N-terminal sequences were determined for six of them:

T R1T1 Phe-Arg
T P1T2 Gly-Asp-Ala-Glu-Glu-...
T P1$K_1$5 Asp-Ser-Thr-Pro
T P1$K_2$1 Asp-Ser
T P1K_2 5,1 Leu-Val
T P1K_2 5,3 Glu-Glu-Leu-Val-(Ileu, Asx, Ser, Thr, Pro, Arg).

On analyzing the results obtained, and also those given in our previous paper [3], we found the following sequence of amino acids in the glycopeptide (* — position of attachment of the carbohydrate):



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