ing to its mass spectrum, melting point, and $[\alpha]_D$, the base was identical with (−)-oridine (oreoline) [4, 6].

Base (V) had mp 190-192°C (acetone); $[\alpha]_D$ −61 ± 3° (c 0.52; chloroform). Mass spectrum: m/e 303 (45), 302 (100), 260 (70). On the basis of its mass spectrum and melting point, base (V) was identified as N-methyloridine [6] (N-methyloreoline) [4].

The other bases, which formed a minor fraction of the total alkaloids, could not be identified.

We are the first to have isolated these alkaloids from *P. lisae*.

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A STUDY OF THE STRUCTURE OF THE 11S GLOBULIN FROM COTTON SEEDS

XIII. CHYMOTRYPTIC PEPTIDES OF SUBUNIT C

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In order to establish the sequence of amino acids in subunit C of the 11S globulin [1], we have performed its cleavage with chymotrypsin (Worthington). The enzyme was added to a suspension of 0.5 g of the reduced and carboxymethylated protein in a small volume of 0.2 M ammonia-acetate buffer, pH 8.8 (enzyme:substrate ratio 1:50). The mixture was incubated at 37°C for 16 h. A small aliquot of the solution was deposited on a plate (20 × 20 cm) coated with cellulose to obtain peptide maps. Chromatography was performed in the butan-1-ol—pyridine—acetic acid—water (15:10:3:12) system. Electrophoresis was performed in pyridine—acetate buffer, pH 6.5; 800 V, 40 min (Fig. 1).

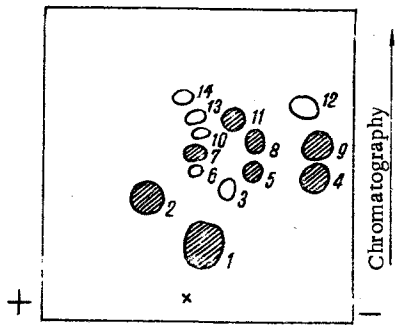

Fig. 1. Peptide map of a chymotryptic hydrolyzate of subunit C. The tryptophan-containing peptide 11 was detected after spraying the plate with Ehrlich's reagent.

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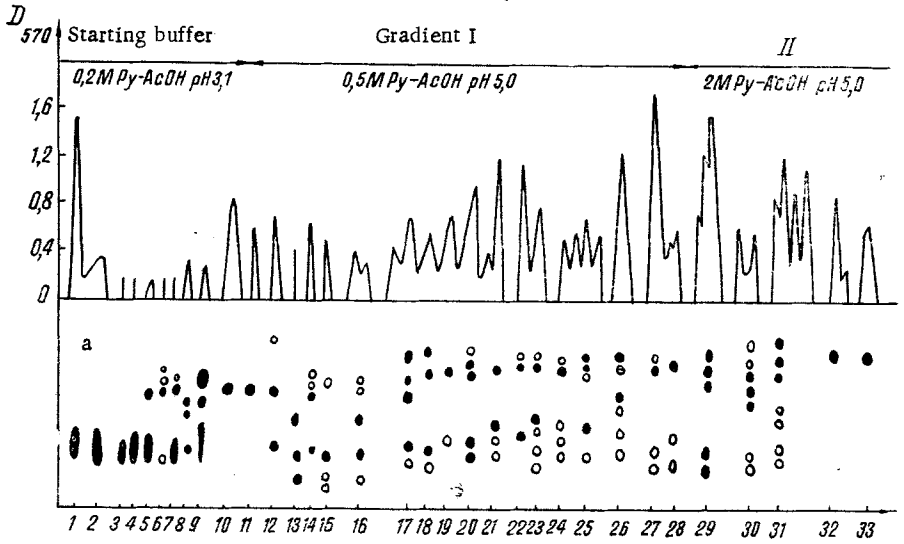

Fig. 2. Chromatography of a chymotryptic hydrolyzate of subunit C on a column of Dowex 50 × 4; thin-layer chromatography of the combined fractions on cellulose (a).

To purify the peptides, the dried mixture was dissolved in pyridine—acetate buffer, pH 2.2. The solution was centrifuged, and the supernatant liquid was deposited on a column (1.2 × 145 cm) of Dowex 50 × 4 (400 mesh). Chromatography was carried out by gradient elution using pyridine—acetate buffers with different molarities and pH values. Fractions with a volume of 10 ml were collected and were analyzed by the ninhydrin reaction after alkaline hydrolysis [2] (Fig. 2).

The purity of the combined fractions was checked by thin-layer chromatography on cellulose plates (6 × 9 cm). Fractions 1, 2, 3, 4, 10, 11, 19, 32, and 33 were obtained in the pure form. The other fractions were chromatographed on paper (FN 17 "Filtrak," GDR), and then electrophoresis was performed in a thin layer of cellulose. The chromatography on plates and on paper, and also the electrophoresis were performed in the same systems as for the peptide maps.

In this way, we obtained 16 homogeneous peptides the N-terminal amino acids and compositions of which are given below:

XT2 Thr (Ser, Asp_2, Glu_3, Pro, Gly, Ala, Val, Leu)
XT3 Thr (Ser, Asp_2, Glu_3, Pro, Gly, Ala, Val, Leu)
XT4 Thr (Ser, Asp_2, Glu_3, Pro, Gly, Ala, Val, Leu)
XT10 Val (Asp_2, Glu_2, Gly, Ala, Ileu, Leu, Arg, Pro)
XT11 Val (Asp_2, Glu_3, Gly, Ala, Ileu, Leu, Arg, Pro)
XT17-2-3 His (Glu, Trp)
XT17-3 Val, Asx
XT17-5 Leu, Tyr
XT18-2 Gly (Glu_2, Asp, Ser, Ala, Val, Arg)
XT19 Val (Arg, Ala, Gly, Pro, Glu_2, Asp, Leu)
XT27-1 Ala (Asp_3, Ser, Glu_4, Gly, Val, Leu, Lys, Arg)
XT27-3 Clx (Ileu, Asp, Gly, Phe)
XT29-3 Val (Asp_2, Ser, Pro, Lys, Arg_2, Glu_3, His)
XT29-5 Lys (Asp, Glu_2, Arg, Ileu)
XT32 Arg, Leu
XT33 Arg, Clx

The compositions of the peptides were determined after hydrolysis with 5.7 N HCl on a type LKB 4101 analyzer. The tryptophan was determined by the qualitative reaction with Ehrlich's reagent.

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