trum showed the peaks of ions with m/z 58 (100%), 365, 383, 407, 424, and M^+ 440, characteristic for the mass-spectrometric fragmentation of cycloprotobuxine bases [5]. From its spectral characteristics, this alkaloid belongs to bases of the 9β,19-cyclo-5α-pregnane type, which is different from the alkaloids of the genus *Buxus* isolated previously.

Thus, from *Buxus sempervirens* L. from a growth site not studied previously we have isolated cyclobuxine-D, cyclovirobuxine-D, cycloprotobuxine-A, cycloprotobuxine-B, and a base with mp 245-247°C.

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CYANOGEN BROMIDE HYDROLYSIS OF RAT TRANSFERRIN

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The transferrins form a group of homologous glycoproteins capable of reversibly binding to ferric iron ions in two centers present in the protein molecule [1]. The results of investigations on the structure of protein homologs of the transferrin type give grounds for assuming that the transferrin molecules with two centers for the reversible binding of iron ions have as their ancestor a protein molecule with one center for binding iron ions and that this phenomenon is the result of a doubling of the gene of the transferrin precursors taking place in the course of evolution.

We have made an attempt to obtain cyanogen bromide fragments of rat transferrin obtained by a method described previously [2] with the aim of their subsequent separation and study.

According to the results of analysis of amino acid composition of native transferrin, showing the presence of methionine residues in the protein, one may theoretically expect the production of five fragments after hydrolysis with cyanogen bromide. Hydrolysis with cyanogen bromide was carried out in the following way: 70 mg (1 μmole) of the protein was dissolved in 3 ml of 70% formic acid, and 3 ml of a solution of cyanogen bromide (1 g of cyanogen bromide/10 ml of 70% formic acid) containing a 100-fold excess of cyanogen bromide with respect to the methionine was added. The reaction was carried out for 48 h in an atmosphere of nitrogen in the dark at +4°C with gentle stirring. The reaction mixture was evaporated in a rotary evaporator to a volume of ~3 ml. An aliquot of the reaction mixture was subject to electrophoresis in polyacrylamide gel (PAAG) by Weber and Osborn's method [3]. The electrophoretogram showed the presence of five bands corresponding to five cyanogen bromide fragments.

The fragments obtained after hydrolysis were separated on a column of Sephadex G-50 equilibrated with 35% acetic acid at a rate of flow of 15 ml/h. Fractions with a volume of 5 ml were collected and the peptide material in an eluate was recorded from its absorption at 280 nm on a Gilson spectrophotometer (France). The results of separation are shown in Fig. 1. The peptide fractions present in the peaks were evaporated in a rotary evaporator to a volume of ~1 ml. The chromatographic peaks were denoted, respectively, BF-1, BF-2, BF-3, BF-4, and BF-5.

Each peptide fragment was checked for homogeneity by electrophoresis in PAAG in the presence of 1% of sodium dodecyl sulfate. The pattern of electrophoresis of aliquots from the fractions obtained on gel filtration indicated substantial aggregation in fractions BF-1,

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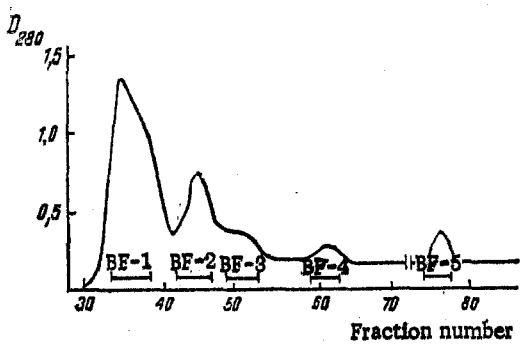

Fig. 1. Gel filtration of a cyanogen bromide hydrolysate of rat transferrin on a column (2.5 × 100 cm) of Sephadex G-50. Elution with 35% acetic acid at a rate of flow of 15 ml/h with collection of 5-ml fractions.

BF-2, and BF-3. As can be seen from Fig. 1, BF-5 was eluted in the form of an individual symmetrical peak. On electrophoresis we observed here an individual peptide fragment with a molecular weight of 6000 dalton. Fraction BF-1 was rechromatographed. Analysis by electrophoresis in PAAG of an aliquot from fraction BF-1 subjected to rechromatography showed the presence of a peptide with a molecular mass of about 26,000 dalton. Fraction BF-1 was saturated with iron ions with the aid of a soltuion of FeCl# and with the addition of trace amounts of NaHCO#. An aliquot from this fraction was subjected to electrophoresis, and after electrophoresis the gels were treated by a method proposed by Kh. S. Rafikov [4] for determining iron-containing proteins. The presence of a brown band in the gel is confirmation of the fact that the component with a molecular mass of 26,000 dalton contains an iron-binding center.

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LIPOLYTIC ENZYMES OF THE VENOM OF *Vespa orientalis*

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The venom of the giant hornet *Vespa orientalis* possesses lipolytic activity [1, 2]. We now give information on the isolation and purification of three lipolytic enzymes from the venom.

The fractionation of the whole venom was carried out in the first stage by gel filtration on Sephadex G-50 under the conditions given previously [1]. The fraction with phospholipase activity after demineralization and lyophilization was subjected to chromatography on CM-cellulose CM-52 in Tris-HCl buffer, pH 7.25, using a linear NaCl gradient (up to 0.3 M). Phospholipase activity was possessed by three fractions (CM-II, CM-III, and CM-V) out of the five. CM-III and CM-V catalyzed the hydrolysis mainly of lecithin, and CM-II that of lysolecithin. Electrophoretic analysis [2] showed the inhomogeneity of these fractions. Individual proteins were obtained after the twofold chromatography of CM-II on CM-cellulose under the same conditions, and after the rechromatography of CM-III and CM-V according to the results of disc electrophoresis in 15% polyacrylamide gel and analysis of the terminal amino acids. The yields of CM-II, CM-III, and CM-V were approximately 1.5 and 1.5-2%, and the degrees of purification 100-, 20-, and 70-fold, respectively. Below, we give some properties of the puri-

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