centrifuging. The extract obtained in this way (nitrogen of the albumin fraction 1.72%, globulin nitrogen 4.47%) was freeze-dried. The yield was 4% of the initial defatted meal in the cases both of the aqueous extract and the salt extract.

An electrophoretic investigation using the acrylamide gel micromethod [2] showed the presence in the total aqueous extract of 14 zones: two zones in the cathode region and twelve zones in the anode region (Fig. 1). An electrophoregram of the freeze-dried albumin fraction showed eight zones migrating in the direction of the anode.

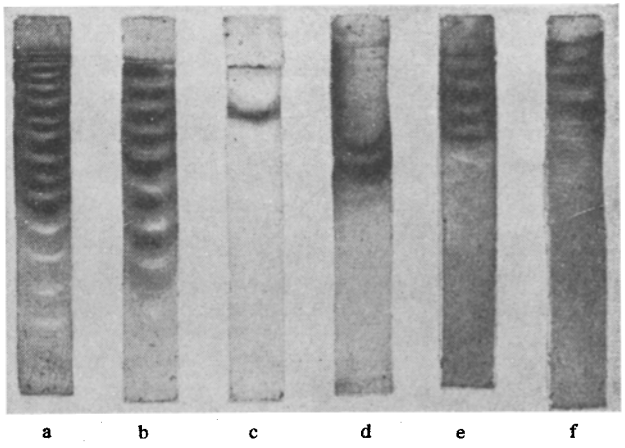

Fig. 1. Electrophoregrams in acrylamide gel. a) Total water-insoluble fraction; b) albumin fraction; c) second fraction from DEAE-cellulose; d) third; e) fourth; f) fifth fraction.

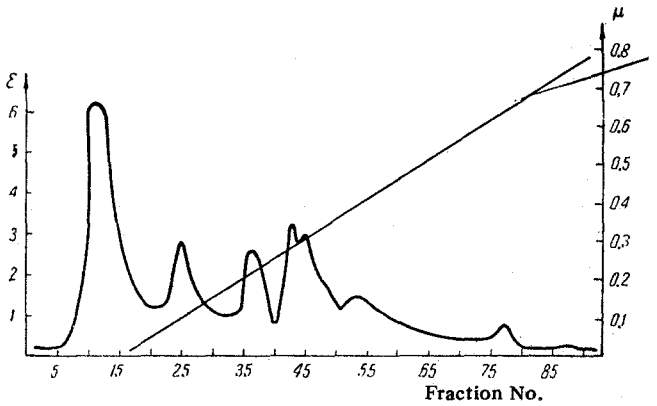

Fig. 2. Separation of the albumin fraction on DEAE-cellulose.

The separation of the total water-soluble fraction on DEAE-cellulose and on calcium phosphate gave similar results (column diameter 2.5 cm, rate of elution 20 ml/hr, 700 mg of protein; elution with phosphate buffer, pH 7.4, with NaCl gradient) (Fig. 2).

The fractions belonging to the individual peaks were combined, dialyzed, and freeze-dried. The dried fractions were subjected to electrophoresis in acrylamide gel. The fraction corresponding to the first peak proved to be nonprotein material. The second fraction (yield 14.1%) showed a single zone on the electrophoregram, the third (yield 17.8%) two zones, the fourth (yield 20.1%) four zones, and the fifth (yield 26%) two zones. Work on the isolation and further separation of the albumin fraction of the proteins is continuing.

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26 September 1967

Institute of the Chemistry of Plant Substances, AS UzSSR

UDC 577.17

SOME DATA ON THE STUDY OF BOVINE LUTEINIZING HORMONE

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Khimiya Prirodnykh Soedinenii, Vol. 4, No. 1, p. 66, 1968

We have previously described a complex method for isolating and purifying the hormones of the hypophysis [1]. Luteinizing hormone has been obtained from the cakes remaining after the extraction of bovine hypophyses with acidified acetone. By subsequent purification by salt and alcohol fractionation, a sample has been obtained which is ten times more active than the NIH-LH-SI standard. Further purification of the sample by chromatography on carboxymethylcellulose and diethylaminoethylcellulose did not raise the luteinizing activity. The activity of the sample was determined by the superovulation method. The content of follicle-stimulating hormone determined from the increase in weight of the mouse uterus was not more than 0.04%. The testing was carried out by A. A. Molodyk in the Laboratory of Hormone Therapy (Director Professor N. I. Lazarev) in the Institute of Experimental and Clinical Oncology, AMS USSR.

In spite of its very high biological activity, the material isolated was not homogeneous. Thus, by gel filtration in 0.05 M pyridine—acetate buffer with pH 5.5 on Sephadex G-100 it yielded three fractions; the second and third fractions were active with respect to superovulation, while the third fraction was free from follicle-stimulating activity. When the same preparation (6 months from the time of its production) was subjected to gel filtration carried out under analogous conditions, the first fraction, which was homogeneous on electrophoresis in a starch gel, proved to be active. This might possibly be connected with the aggregation of the protein during storage.

Electrophoresis of the initial material in starch gel in tris-borate buffer at pH 8.0 showed the presence of several bands located in both the cathode and anode sections of the gel. By preparative electrophoresis in starch gel, four fractions were isolated, three of which possessed luteinizing activity to different extents. In the initial sample, in a determination of the N-terminal amino acid by dinitrophenylation with subsequent identification of the DNP-derivatives by two-dimensional thin-layer chromatography on silica gel, we found DNP-threonine (the bulk of the mixture), DNP-serine in considerable amounts, and also small amounts of DNP-glutamic and DNP-aspartic acids.

On ultracentrifuging, the sedimentation coefficient was S20, w = 3.46 S, the diffusion coefficient 0.953 × 10^{-6}, and the molecular weight 32 000. The amino acid composition calculated to 32 000 was as follows: lysine 9.4, histidine 2.3, ammonia 10, arginine 6.4, aspartic acid 14, threonine, 12, serine 13.4, glutamic acid 22.4, proline 19, glycine 18, alanine 16, cysteine 5, valine 11, isoleucine 6, leucine 12, tyrosine 6, phenylalanine 6, methionine 7, glucosamine 3, galactosamine 1, tryptophan 0.5 (determined after alkaline hydrolysis by a modified glyoxylic method), sialic acid 0.4 (determined by the reaction with thiobarbituric acid). The content of sugars was 2.4% (determined by the anthrone method). Mannose, galactose, and fucose were identified by paper chromatography.

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31 July 1967

Institute of the Chemistry of Natural Compounds, AS USSR

UDC 577.17 631.8111

A NEW METHOD FOR OBTAINING THE PHYTOHORMONE ZEATIN

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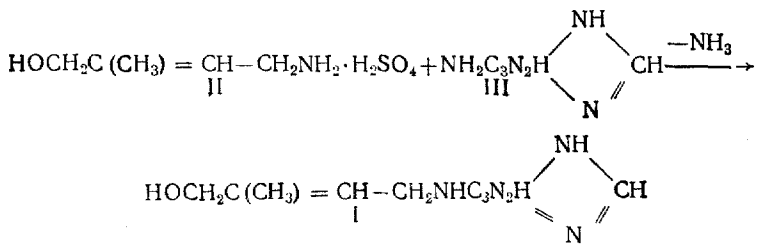
Khimiya Prirodnykh Soedinenii, Vol. 4, No. 1, p. 67, 1968

The structure of a natural kinin, zeatin (I), has recently been established and its multistage synthesis has been developed. The final stage of this synthesis is the condensation of trans-4-amino-2-methyl-2-buten-1-ol (II) with methylthiopurine.

We have modified this method. Instead of methylthiopurine we used the more accessible adenine (III). The alkylation of adenine by II with the splitting out of ammonia was carried out in a sealed tube (160° C). The reaction product, after recrystallization from water, melted at 207–208° C and gave no depression of the melting point with an authentic sample of zeatin. The yield of the latter was 80% of theoretical.



The intermediate α-hydroxy-α-methylbutyronitrile for the synthesis of II was obtained by the cyanohydrin method [2], which differs from the method described by Shaw et al. [1].

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