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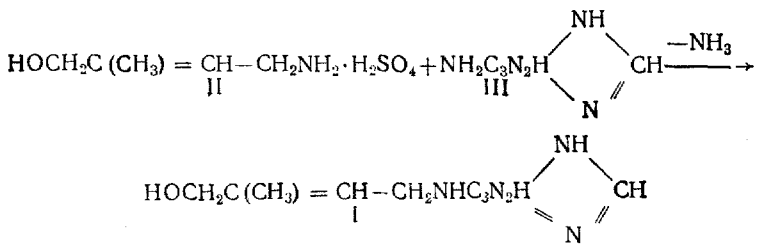
Khimiya Prirodnykh Soedinenii, Vol. 4, No. 1, p. 67, 1968

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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15 September 1967

Institute of the Chemistry of Plant Substances, AS UzSSR

UDC 547.972+547.982+547.992

# POLYPHENOLS OF GERANIUM COLLINUM. II

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Khimiya Prirodnykh Soedinenii, Vol. 4, No. 1, pp. 67-68, 1968

We have studied the leaves of Geranium collinum Steph. (upland geranium) [1] collected at the beginning of July in the little Alma-Ata gorge of the Trans-Ili Ala-Tau. The freshly collected material was treated with methanol. The extract was concentrated under vacuum at 40-50° C, acidified with acetic acid to pH 3, and exhaustively treated first with petroleum ether and chloroform to eliminate the essential oils and resins, and then with ether to extract the polyphenols. Concentration of the ethereal extract yielded a precipitate with mp 350° C which proved, on the basis of its IR spectrum, elemental analysis, and qualitative reactions, to be ellagic acid [2]. The solution was subjected to partition in the ether—water system. The ethereal fraction was evaporated to dryness, and the residue was dissolved in methanol and chromatographed on Kapron. The first fractions, which contained two phenolic acids, were rechromatographed on Kapron in water and the following acids were isolated: gallic with mp 236-238° C, and 3-methoxygallic with mp 197-199° C. The identification of the latter was confirmed by qualitative reaction [3], elemental analysis, IR spectrum, and a determination of the equivalent.

On further chromatography of the ethereal extract, methanol eluted quercetin (mp 303-305° C), kaempferol (mp 278-280° C), and 3,7,8,4'-tetrahydroxyflavone (mp 310-315° C) [4]. The substances were identified from the results of alkaline fusion, a spectroscopic and chromatographic study of the anthocyanidin derivatives, and the UV spectra with ionizing and complex-forming additives. 3,7,8,4'-Tetrahydroxyflavone has $\lambda_{max}$ 370, 268 mμ. The spectra with additives showed that it contained OH groups at C-3 and C-7 and ortho- and C-3, 4'-di-OH groupings [5] and lacked a C-5-OH [6]. The methylation of 3,7,8,4'-tetrahydroxyflavone with dimethyl sulfate gave the tetramethyl ether with mp 145-147° C. The identification was confirmed by the results of elemental analysis and by the IR spectrum.

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2 February 1967

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UDC 547.985 + 547.914

# A CHEMICAL STUDY OF EUPHORBIA SERAWCHANICA

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Khimiya Prirodnykh Soedinenii, Vol. 4, No. 1, p. 68, 1968

The milky juice and hypogeal organs of some species of Euphorbia contain compounds specific for these plants (euphorbone, euphorbol, and biglandulinic and ferganic acids) [1-3].

We have studied Euphorbia serawchanica Rgl. [4]. A qualitative analysis showed the presence in it of glycosides, flavonoids, and coumarins.

The roots and epigeal part of E. serawchanica were steeped in 8% sulfuric acid and the acid extract was shaken with ether; the ethereal extract was evaporated to dryness. The residue was treated with ether and the ether-insoluble part was dissolved in acetone. The acetone solution was passed through a layer of alumina. This gave crystals with mp