

(I) with diazomethane, isocorypalmine was isolated from the reaction products, which confirmed the presence of a hydroxy group at C₁ and of a methoxyl at C₂. The facts given for compound (I) agree well with literature information for stepholidine from Stephania glabra [4].

The base (II) was isolated in the form of the hydrochloride, mp 254-256°C (acetone-alcohol). Its physicochemical constants and spectral characteristics were close to those of apoglaziovine [5]. In actual fact, when N-methylcrotsparine was heated with 4 N hydrochloric acid (water bath, 2 h) a base was obtained that was identical with the alkaloid (II) that has been isolated (TLC and mass and IR spectra).

Thus, ten bases have been isolated from the leaves of L. tulipifera, and stepholidine and apoglaziovine have been found in this source for the first time.

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N-FORMYL-L-ASPARTIC ACID SYNTHESIS AND CHARACTERIZATION

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N-Formylamino acids have found use in the synthesis of peptides, which is due to their availability and the relative ease of elimination of the formyl group. Methods are known for obtaining formylamino acids that are based on heating amino acids in concentrated formic acid (I) [1] or the treatment of amino acids with the mixed anhydride of (I) and acetic acid [2, 3]. N-Formyl-L-aspartic acid (II), which has been used for the synthesis of peptides, including aspartame, has not hitherto been described in the pure form, apparently because of the complications caused by the autocatalytic splitting out of the formyl group. Thus, we have found that a preparation of (II) obtained by boiling aspartic acid (III) [sic] contained 15-30% of free (III) after repeated (up to 8 times) evaporation with water.

We have developed a method for obtaining (II) by treating a solution of (III) in (I) with acetic anhydride (IV). In the first stage the anhydride of (II) - (V) - is formed, and in the second stage the ring undergoes hydrolytic opening with the formation of (II). This method of synthesizing (II) differs from that proposed by Zumstein et al. [4] by the fact that, in the first place, we have substantially decreased the amount of (IV) used in the reaction [threefold excess of (IV), calculated on the (III), in place of an eightfold excess].

At 5-10°C, with stirring, 29 ml (0.3 mole) of (IV) was added dropwise to 13.3 g (0.1 mole) of (III) in 30 ml of 99.7% (I), and the reaction mixture was stirred at 20°C until the (IV) had dissolved completely and then for another 1.5 h. If the deposition of a precipitate was observed, the reaction mixture was heated in the water bath (40-45°C). After 1.5 h the solution was evaporated to dryness in vacuum (at a bath temperature not above 50°C). The (V) so formed was treated with 50 ml of water and, after it had dissolved, the solution was reevaporated in vacuum (at a bath temperature not above 50°C) until a transpar-

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ent yellowish syrupy mass had formed. The same operation was repeated once more after the addition of 25 ml of water.

The "syrup" was placed in the refrigerator for two days for complete crystallization, which takes place with the evolution of heat. Where necessary it is possible to initiate crystallization by the addition of a seed of (II). When working with large amounts of reactants [for example, with an increase in the amount of (III) used in the reaction to 200 g, with a corresponding increase in the amount of (I) and (IV)], it is more convenient to carry out crystallization in a polyethylene packet, which facilitates the subsequent extraction of the crystalline (III). The crystals obtained were dried in the air to constant weight. Yield 90-95%. mp 130-131°C (according to the literature, 127.5-128°C [5], 136-137°C [6]). pH of a 1% solution 2.85-2.90. R_f values on Silufol plates - 0.35 in the methyl ethyl ketone-acetic acid-pyridine-water (32:4:2:6) system, and 0.54 in the methanol-chloroform (3:7) system.

After additional purification of the (II) with the aid of reprecipitation by ethyl acetate, a sample of (II) containing, according to the results of amino acid analysis, 0.2-0.3% of free (III) was obtained. $[\alpha]_D^{20} +22.22^\circ$ (c 1; 15 N, HCOOH). Found, %: C 37.07; H 4.13; N 8.49. $C_5H_7O_5N$. Calculated, %: C 37.27; H 4.37; N 8.69.

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ISOLATION OF A THIRD COMPONENT OF STELLIN - A PROTAMINE FROM THE GONADS OF *Acipenser stellatus*

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We have previously reported the isolation and the determination of the structures of two protamines from the gonads of *Acipenser stellatus* - stellins A and B [1, 2]. By a combination of ion-exchange chromatography on CM-Sephadex G-25 and reversed-phase HPLC on a Zorbax C-8 column we have succeeded in isolating a third component of stellin - stellin C, which, from the results of amino acid analysis according to Kossel's classification [3] is a triprotamine. To determine the structure of the protein we used thermolysin hydrolysis and Edman degradation. Analysis of the amino acid sequence by Edman's method was performed on a solid-phase sequenator by the method described previously [4]. To separate the thermolysin peptides we used reversed-phase HPLC on a Zorbax ODS column. Eight peptides were isolated and their structures were established. The results obtained, taken together, enabled the complete amino acid sequence of stellin C to be established:

A comparison of the amino acid sequence of stellin C with that of stellin A showed that they practically coincide. The difference consists in the fact that stellin A contains an additional alanine as the N terminal amino acid residue. It is not yet clear whether the observed difference is connected with the functioning of two different genes or is due to

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