SYNTHESIS OF β-(2-AMINOPURIN-9-YL)-α-ALANINES

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The alkylation of 2-amino-6-chloropurine and of 2-amino-6-methylthiopurine with 1-bromo-2,2-diethoxyethane in dimethylformamide in the presence of sodium hydride has yielded 2-amino-6-chloro-9-(2',2'-diethoxyethyl)purine and 2-amino-9-(2',2'-diethoxyethyl)-6-methylthiopurine. By further transformations of these acetals, 6-substituted 2-aminopurin-9-ylacetaldehydes have been prepared from which, by the cyanohydrin synthesis β-(2-amino-6-hydroxypurin-9-yl)-α-alanine (guanin-9-yl-α-alanine) and β-(2-amino-6-mercaptopurin-9-yl)-α-alanine have been obtained. The synthesis of guanin-9-yl-α-alanine has completed the preparation of a series of α-alanine derivatives of the five most important heterocyclic bases present in RNA and DNA.

Continuing a study of various routes for obtaining purin-9-yl-α-amino-acids, i.e., structures in which α-amino-acid residues are attached directly to the nitrogen heteroatom in position 9 of the purine ring, we undertook in this work a study of the possibility of synthesizing N_9-α-alanine derivatives of guanine and of 2-amino-6-mercaptopurine. In our opinion, these compounds are of independent interest, and they may also be valuable starting materials for the preparation of potential inhibitors of enzymes participating in the nucleic acid – protein metabolism in the organism.

The synthesis of a N_9-α-alanine derivative of guanine was of special interest in connection with the study of peptides constructed from residues of pyrimidinyl- and purinyl-α-amino acids that we are carrying out [1-3]. Such peptides are structural analogs of the most important biopolymers, and it is not difficult to foresee the possibility of extremely interesting, from our point of view, intermolecular interactions for peptide chains bearing in the lateral radicals aminoacid residues of heterocyclic bases forming components of RNA and DNA. The intramolecular interactions of such hybrid structures, which we shall hereafter call "nucleopeptides," must be subject to the principle of complementarity and lead to the appearance of hydrogen bonds between corresponding pairs of nitrogen bases. It is not excluded that such intermolecular interactions of the complementary type will exist both between individual nucleopeptide chains and between a nucleopeptide chain, on the one hand, and a polynucleotide chain on the other. Intramolecular interactions in the nucleopeptides mentioned must obey the general principles of the formation of hydrogen bonds within the limits of an ordinary polypeptide chain which, in particular, may lead to the formation of secondary structures of the α-helix type. However, to obtain these nucleopeptides one must have available the corresponding α-amino acid derivatives of all five heterocyclic bases entering into the composition of RNA and DNA (i.e., uracil, thymine, cytosine, adenine, and guanine). We have previously reported the synthesis of β-(uracil-1-yl)-α-alanine (willardiine) [4-9], β-(thymin-1-yl)-α-alanine [1, 10], β-(cytosin-1-yl)-α-alanine [1, 10], and β-(adenin-9-yl)-α-alanine [2, 11]. However, the synthesis of β-(guanin-9-yl)-α-alanine has not hitherto been effected.

After a series of preliminary experiments, we established that it is possible to use for the synthesis of β-(2-aminopurin-9-yl)-α-amino acids the method that we used previously to obtain a series of other β-(purin-9-yl)-α-amino-acids [2, 11]. This method is based on the alkylation of 6-substituted purines with 1-bromo-2,2-diethoxyethane by a modification of the Montgomery-Temple method [12] and the subsequent use of the Strecker-Zelinskii-Stadnikov cyanohydrin synthesis.

The starting materials in the present work were 2-amino-6-chloropurine and 2-amino-6-methylthiopurine. To obtain the 2-amino-6-chloropurine, we developed, in place of the method involving the chlorination of 2-amino-6-methylthiopurine described in the literature [13], the more convenient method of chlorinating 2-amino-6-mercaptopurine in analogy with the method of obtaining 6-chloropurine from 6-mercaptopurine [14]. 2-Amino-6-methylthiopurine was obtained by methylating 2-amino-6-mercaptopurine [15].

The alkylation of 2-amino-6-chloropurine and of 2-amino-6-methylthiopurine with 1-bromo-2,2-diethoxyethane was carried out in the presence of sodium hydride in dimethylformamide at an elevated temperature. The acetals obtained, after purification, were subjected to nucleophilic substitution reactions at position 6 of the purine ring. Thus,

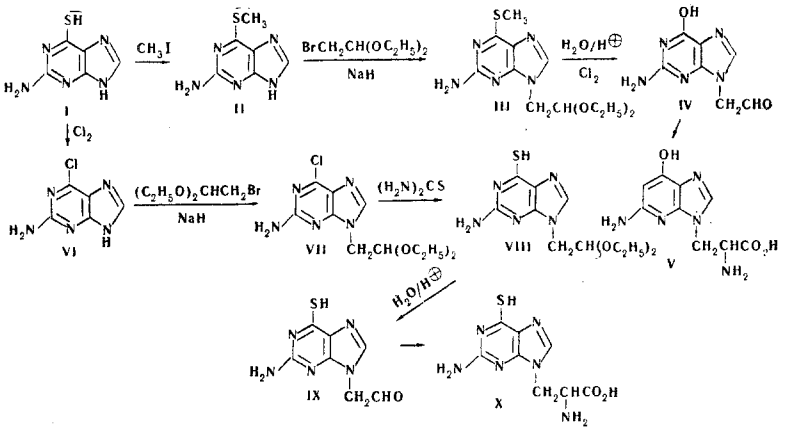
by the reaction of 2-amino-6-chloro-9-(2',2'-diethoxyethyl)purine with thiourea we obtained 2-amino-9-(2',2'-diethoxyethyl)-6-mercaptopurine, the subsequent hydrolysis of which with hydrochloric acid led to 2-amino-6-mercaptopurin-9-ylacetaldehyde.

By the action of chlorine on 2-amino-9-(2',2'-diethoxyethyl)-6-methylthiopurine and subsequent treatment of the substance with hydrochloric acid we likewise obtained 2-amino-6-hydroxypurin-9-ylacetaldehyde. It must be mentioned that an attempt to obtain this aldehyde by the hydrolysis of authentic 2-amino-6-chloro-9-(2',2'-diethoxyethyl)purine was unsuccessful.

The purin-9-ylacetaldehydes obtained were not subjected to further purification, because of their instability, but were used directly in the cyanohydrin synthesis by means of which from the 6-substituted 2-aminopurin-9-ylacetaldehydes mentioned above we obtained β-(2-amino-6-hydroxypurin-9-yl)-α-alanine (i.e., guanin-9-yl-α-alanine) and β-(2-amino-6-mercaptopurin-9-yl)-α-alanine.

The first phase of the cyanohydrin synthesis – the preparation of the corresponding amino nitrile – was effected by heating a mixture of a 6-substituted 2-aminopurin-9-ylacetaldehyde with an aqueous solution of potassium cyanide, ammonium chloride (taken in equimolar amounts), and ammonia. Then the amino nitriles were subjected to hydrolysis since we convinced ourselves that there was no possibility of previously isolating them from the reaction mixture. Hydrolysis was effected by heating the reaction mixture with 10 N hydrochloric acid.

All the reactions described are illustrated in the scheme.



The 6-substituted β-(2-aminopurin-9-yl)-α-alanines synthesized formed high-melting crystalline substances of amphoteric nature possessing strong absorption in the near UV region. The spectra of these compounds agreed completely with literature information on the UV absorption of authentic 6,9-disubstituted 2-aminopurines [16, 17], which shows the structure of 6-substituted 2-aminopurin-9-yl-α-alanines for the substances obtained. With ninhydrin, these compounds give a positive reaction for α-amino acids.

Thus, as a result of the investigation performed, methods for the synthesis of the previously unknown β-(2-amino-6-mercaptopurin-9-yl)-α-alanine and β-(2-amino-6-hydroxypurin-9-yl)-α-alanine, i.e., β-(guanin-9-yl)-α-alanine have been developed. The synthesis of the latter compound completes the preparation of the series of α-alanine derivatives of the five most important heterocyclic bases entering into the composition of RNA and DNA and has thereby created the necessary prerequisites for the synthesis of nucleopeptide structures including complementary nucleic bases of both the pyrimidine and the purine series.

We are continuing investigations on the chemistry of the purinyl-α-amino acids and on the synthesis of the corresponding peptides.

EXPERIMENTAL

2-Amino-9-(2',2'-diethoxyethyl)-6-methylthiopurine (III). To 3.62 g (0.02 mole) of 2-amino-6-methylthiopurine [15] were added 70 ml of dry dimethylformamide and 0.43 g (0.02 mole) of sodium hydride, and the mixture was stirred

at room temperature for 2 hr, after which 7.88 g (0.04 mole) of 1-bromo-2,2-diethoxymethane was added, and it was heated to 80°C and stirred at this temperature for 7 hr. Then the temperature was gradually raised over 1.5 hr to 140°C. After the solvent had been driven off, the viscous syrupy mass was extracted with ether (3 × 100 ml), the ethereal extract was twice washed with cold water and was then evaporated in vacuum, and the residue was crystallized from water. This gave 1.38 g (23.2%) of III in the form of colorless crystals with mp 125-126°C. Found %: C 48.68; H 6.34; N 23.39; S 10.53. $C_{12}H_{19}N_5O_2S$. Calculated %: C 48.47; H 6.44; N 23.55; S 10.78. R_f 0.96 (system 1)*, 0.98 (system 2). UV spectrum, λ_{max}, nm (log ε): 246 (3.95), 323 (3.97) (pH 1); 245 (4.11); 311 (4.19) (pH 7); 244 (4.17); 309 (4.13) (pH 13).

β-(2-Amino-6-hydroxypurin-9-yl)-α-alanine (V). With stirring, chlorine was passed into a suspension of 0.89 g (0.003 mole) of III in 9 ml of absolute ethanol, the mixture being cooled in such a way that during the first 20 min of the process its temperature did not exceed 0°C. Then the methanol was distilled off in vacuum, the residue was treated with 9 ml of 5 N HCl, the mixture was heated in the water bath for 15 min, the HCl was distilled off in vacuum, the last traces of the acid were eliminated by azeotropic distillation with small portions of water, the residue was dissolved in the minimum amount of water at 90°C, and the resulting solution was brought to pH 5 by the addition of aqueous ammonia. The precipitate of IV that deposited (0.2 g) was dissolved in 1 ml of water, after which 0.06 g of ammonium chloride, 0.07 g of potassium cyanide, and 1 ml of 25% aqueous ammonia were added and the resulting mixture was kept at 55-60°C for 5 hr. Then 2 ml of conc. HCl was added and the mixture was heated in the water bath for 1 hr, after which it was left at room temperature overnight. After this, the mixture was evaporated to dryness, the residue was treated with 1.2 ml of 10 N HCl, the mixture was boiled for 3 hr and was then evaporated to dryness in vacuum, and the last traces of HCl were eliminated by azeotropic distillation with water. The residue was dissolved in the minimum amount of water and the solution was brought to pH 5 with aqueous ammonia. The precipitate that deposited was separated off and recrystallized from water. This gave 0.11 g of V (41%, calculated on the aldehyde), mp 260°C (decomp.). Found %: C 35.57; H 4.35; N 30.29. $C_8H_{10}N_6O_3 \cdot 2H_2O$. Calculated %: C 35.04; H 5.15; N 30.07. R_f 0.19 (system 1), 0.07 (system 2). UV spectrum: λ_{max}, nm (log ε): 255 (4.06) (pH 1); 253 (4.09) (pH 7); 268 (4.00) (pH 13).

2-Amino-6-chloropurine (VI). A mixture of 6 ml of methanol and 18 ml of conc. HCl was saturated with hydrogen chloride at 0°C, and then the solution was cooled to −20°C and, with stirring, 6 g (0.086 mole) of I was added. After this, chlorine was passed into the mixture at such a rate that the temperature did not rise above −5°C for 1 hr. Then it was poured onto 44 g of ice, using additional external cooling. The resulting mixture was brought to pH 1.5-2 by the addition of aqueous ammonia and was filtered, and the residue was recrystallized from water. This gave 3.6 g (59.3%) of VI, mp above 275°C (decomp.). Found %: C 35.43; H 2.42; N 41.63; Cl 20.69. $C_5H_4ClN_5$. Calculated %: C 35.41; H 2.38; N 41.30; Cl 20.91, R_f 0.74 (system 1), 0.70 (system 2).

2-Amino-6-chloro-9-(2',2'-diethoxyethyl)purine (VII). Some 2.3 g (0.013 mole) of VI and 0.31 g (0.013 mole) of sodium hydride were added to 22 ml of dry dimethylformamide and the resulting mixture was stirred at room temperature for 2 hr, after which 5.8 g (0.026 mole) of 1-bromo-2,2-diethoxyethane was added, and the reaction mixture was kept then at 70°C for 12 hr. After this, the temperature was gradually raised to 125°C over 1 hr 30 min, and then the mixture was evaporated to the state of a viscous syrup, which was extracted with ether (3 × 100 ml). The ethereal extract was washed with cold water three times and was evaporated to dryness in vacuum. The residue was recrystallized from water, giving 1.1 g (28.5%) of VII in the form of colorless crystals, mp 136°C. Found %: C 45.74; H 5.61; N 24.89. $C_{11}H_{16}ClN_5O_2$. Calculated %: C 46.24; H 5.64; N 24.51. R_f 0.90 (system 2). UV spectra, λ_{max}, nm (log ε): 241.5 (3.89), 311.5 (3.98) (pH 1); 245 (3.73), 307 (3.91) (pH 7); 246 (3.74), 306 (3.85) (pH 13).

2-Amino-9-(2',2'-diethoxyethyl)-6-mercaptopurine (VIII). A solution of 0.7 g (0.0025 mole) of VII and 0.21 g (0.0028 mole) of thiourea in 17 ml of n-propanol was boiled under reflux for 1 hr and, after cooling, the precipitate was filtered off and was crystallized from ethanol. This gave 0.5 g (72%) of VIII in the form of a yellow crystalline substance with mp above 200°C (decomp.). Found %: C 47.00; H 6.39; S 10.91. $C_{11}H_{17}N_5O_2S$. Calculated %: C 46.62; H 6.05; S 11.82. R_f 0.94 (system 1).

β-(2-Amino-6-mercaptopurin-9-yl)-α-alanine (X). A solution of 0.4 g (0.0014 mole) of VIII in 4 ml of 1 N HCl was heated in the water bath for 40 min and was then evaporated to dryness. The residue was dissolved in the minimum amount of water and the solution was brought to pH 5-6 by the addition of aqueous ammonia. The substance that precipitated was separated off and dried, and the IX obtained in this way (0.15 g), without futher purification, was subjected to the cyanohydrin synthesis by its addition to a solution of 0.05 g of ammonium chloride and 0.06 g of potassium cyanide in a mixture of 0.6 ml of 25% aqueous ammonia and 0.6 ml of water. The reaction mixture was kept at 55-60°C for 5 hr,

*System 1: $i\text{-}C_3H_7OH-NH_4OH-H_2O$ (7:1:2) (ascending chromatogram). System 2: $n\text{-}C_3H_7OH-NH_4OH-H_2O$ (6:3:1) (descending chromatogram). Paper: Leningrad medium.

and then 2 ml of conc. HCl was added and it was heated in the water bath for 1 hr and left at room temperature overnight. Then it was evaporated to dryness, the residue was treated with 1.2 ml of 10 N HCl, the resulting mixture was boiled for 3 hr, and then the HCl was distilled off in vacuum, the final traces of it being removed by azeotropic distillation with water as described above. The residue was dissolved in the minimum amount of water, the solution was brought to pH 5 by the addition of aqueous ammonia, and the precipitate that deposited was filtered off and recrystallized from water. This gave 0.1 g (49.8%, calculated on the aldehyde) of X in the form of a white substance with mp 270°C (decomp.). Found %: C 34.26; H 5.14; N 30.20. $C_8H_{10}N_6O_2S \cdot 1.5H_2O$. Calculated %: C 34.16; H 4.66; N 29.88. R_f 0.15 (system 1), 0.21 (system 2). UV spectra, λmax, nm (log ε): 258 (3.71), 343.5 (4.07) (pH 1); 248 (3.77), 340.5 (4.14) (pH 7); 252 (3.97), 317.5 (4.17) (pH 13).

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