SYNTHESIS OF 4-AMINO(DESOXY)-10-METHYLPTEROYL-L-GLUTAMIC ACID

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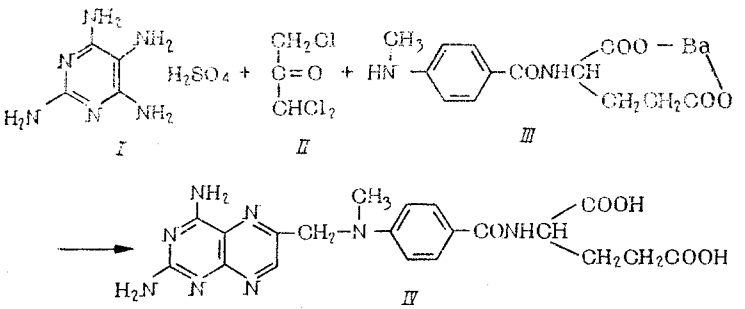
4-Amino(desoxy)-10-methylpteroyl-L-glutamic acid (amethopterin [1], methotrexate [2]) is an antimetabolite of pteroyl-L-glutaric (folic) acid. Inhibiting reactions associated with metabolism of nucleic acids in the organism the compound is used in cancer chemotherapy [3]. Its synthesis from 2, 4, 5, 6-tetraaminopyrimidine, p-methylaminobenzyl-L-glutamic acid, and 2,3-dibromopropionaldehyde is known [1], from which the intermediate dihydro form of pterine is formed; this condensation is accompanied by numerous side reactions.

We have synthesized 4-amino(desoxy)-10-methylpteroyl-L-glutamic acid (IV) by a one-step, three-component condensation of 2,4,5,6-tetraaminopyrimidine (I), the barium (or disodium) salt of p-methylaminobenzoyl-L-glutamic acid (III), and 1,1,3-trichloroacetone (II), the use of which as the three-carbon component of the condensation excludes formation of intermediate pterine dihydro forms and guarantees a more unambigous reaction. The condensation is carried out in the presence of sodium bisulfite.

Because of the large number of functional groups in the reacting compounds having different reactivities and a certain lability of compound (IV), that of the ability to be oxidized with cleavage at the C–N bond at the methylene bridge, there arose the necessity of studying the effect of different factors (temperature, pH, concentration of reactants) on its yield.



We have determined that the yield of compound (IV) at 50-60°C oscillates from 3 to 17%, with its content in the condensation product of up to 58%. The yield decreases upon increasing the reaction temperature above 75° (Fig. 1). Significant amounts of fluorescing impurities are found in the reaction product upon paper chromatography, which are simple pteridine compounds.

We have also shown that the optimum pH value for the condensation is 3.5. Upon decreasing the pH to 2.7-2.9, the content of compound (IV) in the condensation product is decreased to 60%, while the amount of fluorescing impurities of simple pteridines is increased. Upon increasing the pH to 4.5-4.7, the amount of impurity increases even more, and a shift of the main maximum (λ 305 mμ) of compound (IV) is shifted to the long wave region by 20 mμ. It should be noted here that the amino groups in positions 4 and 5 of the pyrimidine molecule possess different reactivities [4], and consequently, increasing the acidity of the medium will favor protonation of the more basic amino group in position 5, which will make the formation of the isomeric 4-amino(desoxy)-10-methyl(7)pteroyl-L-glutamic acid possible during the course of the reaction.

Considering the significant solubility of compound (IV) in the reaction medium, we chose (Fig. 2) as the optimum concentration of reactants a ratio of p-methylaminobenzoyl-L-glutamic acid (barium or disodium salt), tetraaminopyrimidine, and 1,1,3-trichloroacetone of 1:1.5:2.5.

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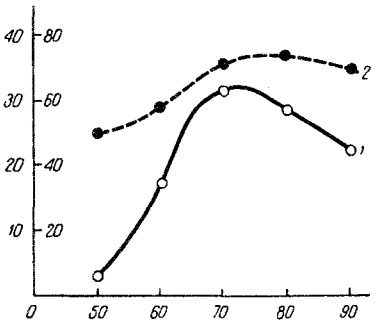

Fig. 1. Dependence of yield (1) and content (2) of 4-amino-(desoxy)-10-methylpteroyl-L-glutamic acid on temperature (pH 3.5, duration, 5 h). On the ordinate axis: on the left, yield (%); on the right, content (%); on the abscissa axis, temperature (deg).

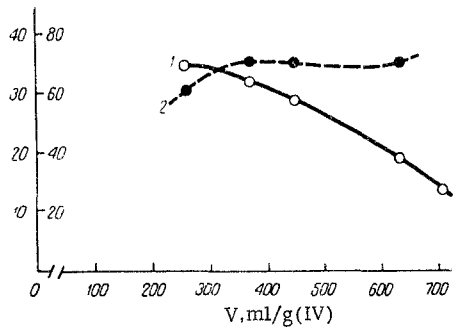

Fig. 2. Dependence of yield (1) and content (2) of 4-amino(desoxy)-10-methylpteroyl-L-glutamic acid on the concentration of reactants (pH 3.5; 70°). On the ordinate axis: on the left, yield (%); on the right, content (%). On the abscissa axis, volume of reaction mass (ml) per 1 g of 4-amino(desoxy)-10-methylpteroyl-L-glutamic acid.

Under optimum conditions, compound (IV) was obtained in a yield of 32% and as a content of 70% in the condensation product.

In the purification of compound (IV), we isolated a significant amount of the impurities as the magnesium, calcium, or sodium salts of the simple pteridine compounds, which are insoluble at pH 7.0-8.0. Chromatographic separation on cellulose in a phosphate buffer (pH 7.0) saturated with iso-amyl alcohol was also used.

The purified 4-amino(desoxy)-10-methylpteroyl-L-glutamic acid (IV) had R_f 0.60 (system 1: phosphate buffer, pH 7.0), literature data [5], R_f 0.48-0.62; R_f 0.69 (system 2: acetate buffer, pH 5.0), literature data [6], R_f 0.68. UV absorption spectrum in 0.1 N hydrochloric acid solution: λ_{max} 243, 305 mμ (Fig. 3); literature data [1], λ_{max} 244, 307 mμ.

1,1,3-Trichloroacetone, the three-carbon component of the condensation, was obtained by direct chlorination of acetone, initially at 35-40° (up to formation of 1,1-dichloroacetone), and then at 85-90°; after distillation of mono- and 1,1-dichloroacetone, 1,1,3-trichloroacetone was extracted from the chlorination product with water.

EXPERIMENTAL PART

1,1,3-Trichloroacetone (II). Into 50 ml of acetone at 35-40° was passed about 100 g of chlorine. The temperature of the reaction mixture was increased to 85-90° and about 90 g of chlorine was added. The mixture was then cooled to room temperature and flushed with air to remove the excess chlorine and hydrogen chloride. We obtained 90-100 g of chlorination product having a density of 1.46-1.48; it was distilled in vacuum and the fraction boiling up to 70° at 104 mm, consisting of a mixture of monochloroacetone and 1,1-dichloroacetone, was discarded. The residue containing 1,1,3-trichloroacetone was poured into 100 ml of water, stirred, and after clarification, the upper transparent layer was separated and the bottom oily layer was subjected again to two more extractions with water (50 and 30 ml). From the aqueous solution (200 ml) (II) was extracted with ether (200, 100, 50 ml). The ether was distilled, and dry benzene (20-25 ml) was added to the residue, which was then distilled in vacuum. We obtained 43 g of anhydrous 1,1,3-trichloroacetone (yield 40%). Bp 172° (in agreement with literature data [7]), n^{20} 1.4922 (literature data [8], n^{20} 1.4917).

For the synthesis of compound (IV), 1,1,3-trichloroacetone was used as an aqueous solution without extraction with ether.

4-Amino(desoxy)-10-methylpteroyl-L-glutamic Acid (IV). A. A suspension of 5.4 g of the barium salt of p-methylaminobenzoyl-L-glutamic acid [9], 4.95 g of 2,4,5,6-tetraaminopyrimidine sulfate [10], and 6 g

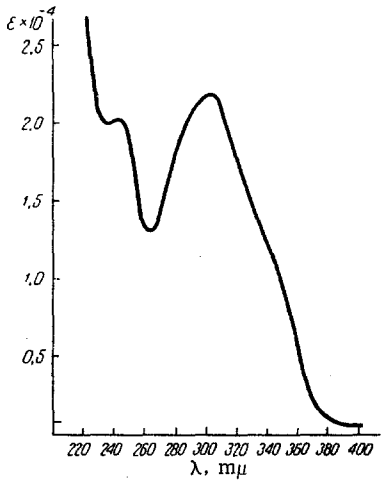

Fig. 3. UV spectrum of 4-amino (desoxy)-10-methylpteroyl-L-glutamic acid in 0.1 N hydrochloric acid solution (SF-4 spectrophotometer).

of sodium metabisulfite in 450 ml of water was heated to 70-75° and a solution of 5.2 g of 1,1,3-trichloroacetone in 40 ml of water was added. The reaction was carried out at the same temperature and a pH 3.4-3.7 for 5 h; the pH was maintained in the necessary interval with a 1 N solution of sodium hydroxide. The hot reaction mixture was then filtered and the mother solution was left at 5-10° for 10-12 h. The precipitated yellow-orange solid was filtered, and washed with water, alcohol, and ether. We obtained 2.6 g of a reaction product containing 70% of compound (IV); yield 32%. The content was determined spectrophotometrically, measuring the absorption at λ_{max} 305 mμ [2]. Paper chromatography (ascending chromatogram) showed an absorbed spot having R_f 0.60 in a phosphate buffer, pH 7.0 (system 1) and R_f 0.69 in an acetate buffer, pH 5.0 (system 2) corresponding to compound (IV), and a series of spots possessing blue fluorescence having R_f 0.10, 0.18, 0.31 (system 1) and R_f 0.04, 0.20, 0.38 (system 2), which evidently belong to the simple pteridine compounds.

B. A suspension of 4.45 g of the disodium salt of p-methylaminobenzoyl-L-glutamic acid (I), 4.95 g of 2,4,5,6-tetraaminopyrimidine sulfate, and 6 g of sodium metabisulfite in 450 ml of water was heated to 70-75° and a solution of 5.2 g of 1,1,3-trichloroacetone in 40 ml of water was added. The synthesis conditions are the same as in A. We isolated 2.2 g (content 68%) of compound (IV) as a yellow-orange powder in a yield of about 25%. Paper chromatographic data are the same as in A.

Purification of 4-Amino(desoxy)-10-methylpteroyl-L-glutamic Acid (IV). A. We dissolved 2.9 g of (IV) (70% content) in 60 ml of a 0.5 N solution of sodium hydroxide, neutralized the solution to pH 7.0-7.5 with a 1 N solution of hydrochloric acid, and heated the mixture with activated carbon for 20 min at 50-60°. The hot solution was filtered from impurities and after cooling, acidified with a 1 N solution of hydrochloric acid to pH 3.0-4.0. We isolated 1.2 g containing 85% of (IV). Yield 50%.

B. We mixed 5 g of cellulose with a 0.1 M phosphate buffer (pH 7.0) saturated with iso-amyl alcohol. After 30 min, the mixture was transferred to a column (25 × 3 cm). The column was filled with cellulose to a height of 20 cm and washed with 50 ml of buffer solution. To the column was added 100 mg of (IV) (70% content) as a solution in 2 ml of a 0.5 N solution of sodium hydroxide and eluted with the same buffer solution. The obtained fractions were investigated with paper chromatography in system 1. Fractions containing (IV) were combined and acidified with a 1 N solution of hydrochloric acid to pH 3.0-4.0. We obtained compound (IV) as a yellow powder of 90% content. Yield 42%.

To prepare an analytically pure sample of (IV), the material was precipitated two times from a basic solution and then consecutively passed through columns containing cellulose and activated carbon. R_f 0.60 in system 1, literature data [5], R_f 0.48-0.62; R_f 0.69 in system 2, literature data [6], R_f 0.68 (absorption spot, ascending chromatogram). UV absorption spectrum in a 0.1 N solution of hydrochloric acid; λ_{max} 243, 305 mμ, ε 2.11 × 10^4; literature data [1]: λ_{max} 244, 303 mμ. Found, %: C 51.30, 51.26, H 5.07, 5.09, $C_{20}H_{22}N_8O_5 \cdot H_2O$. Calculated, %: C 50.84, H 5.12.

CONCLUSIONS

4-Amino(desoxy)-10-methylpteroyl-L-glutamic acid was synthesized by the condensation of p-methylaminobenzoyl-L-glutamic acid (as the barium or disodium salt), 2,4,5,6-tetraaminopyrimidine, and 1,1,3-trichloroacetone.

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