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Optimization of the Synthesis of 5-Amino-1,2,4-triazol-3-ylacetic Acid and Bis(5-amino-1,2,4-triazol-3-yl)methane

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Abstract—The influence of the molar ratio and concentration of the reactants and of the temperature and time of the synthesis on the yield of malonic acid guanylhydrazides in the reaction of aminoguanidine with malonic acid in acidic aqueous solutions was examined, and improved procedures for preparing 5-amino-1,2,4-triazol-3-ylacetic acid and bis(5-amino-1,2,4-triazol-3-yl)methane were suggested.

5-Amino-1,2,4-triazol-3-ylacetic acid **I** is a typical representative of aminoazolecarboxylic acids, which are extensively studied because of their capability to form heteroaromatic oligoamides and affect gene expression [1–4]. Compound **I** can be used for preparing antibiotics [5, 6] and biologically active [7] and energy-rich [8] substances. However, in contrast to its commercially produced homolog, 5-amino-1,2,4-triazole-3-carboxylic acid, acid **I** is yet relatively difficultly available. Two procedures for preparing this compound have been described in the literature: reaction of aminoguanidine hydrocarbonate **II** with ethoxycarbonylethyl acetimidate hydrochloride in acetic acid [9] and reaction of **II** with

malonic acid (MA) in aqueous solution [10, 11]. The latter procedure is more attractive because of the availability of the starting substances.

According to this procedure, first hydrocarbonate **II** is dissolved in an aqueous solution of MA to obtain a solution of aminoguanidine malonate **III**. On heating, salt **III** in solution is converted to malonic acid guanylhydrazide (GH) which, in turn, is converted without isolation to target product **I** by heating with an alkali, followed by acidification of the reaction mixture (Scheme 1).

It should be noted that, in synthesis of **I**, formation of a second product, bis(5-amino-1,2,4-triazol-3-yl)methane **IV**, could be expected because of comparable reactivity

Scheme 1.

$$+ H_2O + CO_2, \quad (1)$$

$$+ H_2O, \quad (2)$$

$$(3)$$

of both carboxy groups of MA. It was shown previously [12] that the reaction of aminoguanidinium (AG) cation with MA in acid solutions yields, along with GH, also malonic acid diguanylhydrazide (DGH), whose alkaline cyclization yields compound **IV** (Scheme 2).

A procedure for preparing compound **IV** in 33.5% yield by heating of aminoguanidine hydrochloride with MA in 2 : 1 molar ratio, followed by treatment of DGH with potassium carbonate, has been described in a patent [13]. Compound **IV** is used in production of polymers [14] and agents for corrosion protection of metals [15]. The reaction of AG with MA can become a convenient source of these compounds if an efficient procedure for their separation will be developed.

The goal of this study is optimization of the synthesis of **I** and **IV** from aminoguanidine and MA under the conditions of acid catalysis.

In our attempts to reproduce the published procedures for preparing **I** from **II** and MA, we failed to obtain a target product in a yield exceeding 15%, although, according to the published data [10, 11], the yield of **I** should reach 60–70%. Analysis of the reaction mixtures showed that low yield of acid **I** is due to low conversion of the starting compounds in the step of formation of the intermediate GH. According to the iodatometric titration data, the conversion of AG after heating a solution of salt **III** for 4 h according to [10] was 9–15%. Thus, to increase the yield of **I**, it is primarily necessary to optimize the step of GH preparation.

According to kinetic studies [12], the reaction of formation of GH from AG and MA is acid-catalyzed, and its rate is directly proportional to the H_3O^+ concentration. It is natural to assume that the low yield of GH is due to

Scheme 2.

$$(4)$$

$$(5)$$

low rate of reaction (2) at low concentrations of H_3O^+ ions (pH of the solution of salt **III** is 3.5–3.9). Indeed, after acidification of the reaction mixture to pH 0.5, the conversion of AG at 92–95°C reaches 80% in 20 min, after which it does not noticeably change.

Thus, it is advisable to perform the synthesis of GH in acid solutions (pH ≤ 1). In so doing, the possibility of DGH formation and the reversibility of reactions (2) and (4) [12] should be taken into account. As starting compounds we used **II**, MA, and HCl_{conc}.

We found that the equilibrium yield of GH and DGH can be increased by performing the synthesis at a low water content of the reaction mixtures, when the initial water concentration $c(H_2O)$ is comparable with the concentrations of the other reactants. By $c(H_2O)$ in this paper we understand the concentration of water introduced as solvent and with HCl solution and released in the reaction of hydrocarbonate **II** with HCl. We found that, with a decrease in $c(H_2O)$, the equilibrium yield of DGH increases to a greater extent than that of GH (Fig. 1).

This fact becomes understandable if we consider the equilibrium constants of reactions (2) and (6): K_{GH} and K_{DGH}, respectively. It follows from Eqs. (7) and (8) that the equilibrium concentration of GH is inversely proportional to $[H_2O]$, and that of DGH is inversely proportional to $[H_2O]^2$:

Fig. 1. Yield ω of (*1*) GH and (*2*) DGH as a function of the H_2O concentration $c(H_2O)$ at 70°C and MA : **II** : HCl molar ratio of 1 : 1 : 1.15.

$$2AG + MA \overset{H_3O^+}{\rightleftarrows} DGH + 2H_2O \qquad (6)$$

$$K_{GH} = \frac{[GG]\,[H_2O]}{[MA]\,[AG]} \qquad (7)$$

$$K_{DHG} = \frac{[DHG]\,[H_2O]^2}{[MA]\,[AG]^2} \qquad (8)$$

Table 1. Equilibrium yield of GH and DGH at MA : **II** : HCl molar ratio of 1 : 1 : 1.3. Initial MA concentration 4.085 M

Compound	Yield, %, at indicated temperature, °C			
	60	70	80	94
GH	52	51	50	47
DGH	45	43	40	36

where [GH], [DGH], [MA], [AG], and [H_2O] are the equilibrium concentrations of the corresponding reactants, M.

Furthermore, with a decrease in $c(H_2O)$ below 28 wt %, DGH starts to gradually crystallize from the reaction mixture. This process leads to a still greater shift of the equilibrium toward reaction (6) (Fig. 1, dashed lines). Thus, a decrease in $c(H_2O)$ below 28 wt % is not appropriate.

An increase in the temperature leads to a decrease in the equilibrium yields of GH and DGH (Table 1) owing to exothermicity of reactions (2) and (4) [12]. Furthermore, above 70°C the side reaction of MA decarboxylation noticeably accelerates [16, 17], which also leads to a de-

crease in the yields of GH and DGH. Below 60°C, the reaction becomes noticeably slower. Thus, the temperature range 60–70°C can be considered as optimal.

The **II** : HCl molar ratio does not noticeably affect the equilibrium yields of GH and DGH but significantly affects the rate at which the equilibrium is attained (Fig. 2). Because in the system under consideration two consecutive reactions (2) and (4) occur, under definite conditions the yield of the intermediate product, GH, should reach a maximum. At the molar ratio **II** : HCl = 1 : 1.3 and a temperature of 70°C, the equilibrium is attained within 25–30 min (Fig. 2), but the rates of reactions (2) and (4) are so high that the process selectivity cannot be controlled kinetically (Fig. 3). At the molar ratio **II** : HCl = 1 : 1.15, both reactions are slower.

As seen from Fig. 4, the maximal GH yield (60%) is attained in 50–70 min, when the maximum possible conversion of AG is practically reached. Then the GH yield decreases to the equilibrium level (51%) owing to an increase in the DGH yield. As the **II** : HCl molar ratio is changed to 1 : 1.05, the reaction rate decreases considerably. Thus, the optimal conditions for preparing GH are as follows: molar ratio **II** : HCl = 1 : 1.15, reaction time 50–70 min.

With an increase in the MA : **II** molar ratio to 2 : 1, the equilibrium yield of GH based on **II** increases to 87%, whereas the yield of DGH decreases to 10%. However, in so doing, expensive MA is lost, which is undesirable from the economic viewpoint. Since compound **IV** is also of commercial value, it is appropriate to perform the synthesis of **I** at the equimolar ratio of MA and **II**, with simultaneous preparation of compounds **I** and **IV**.

Based on the results obtained, we developed an improved procedure for one-pot synthesis of 5-amino-
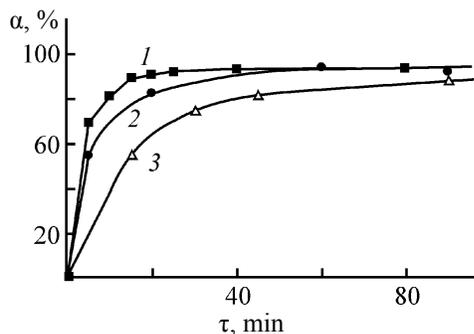
Fig. 2. Degree of AG conversion α as a function of time τ at 70°C. Initial MA concentration 4.085 M. Molar ratio MA : **II** : HCl: (*1*) 1 : 1 : 1.3, (*2*) 1 : 1 : 1.15, and (*3*) 1 : 1 : 1.05.
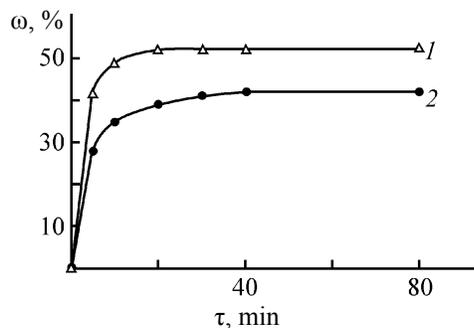
Fig. 3. Yield ω of (*1*) GH and (*2*) DGH as a function of time τ at 70°C. Molar ratio MA : **II** : HCl = 1 : 1 : 1.3, initial MA concentration 4.085 M.
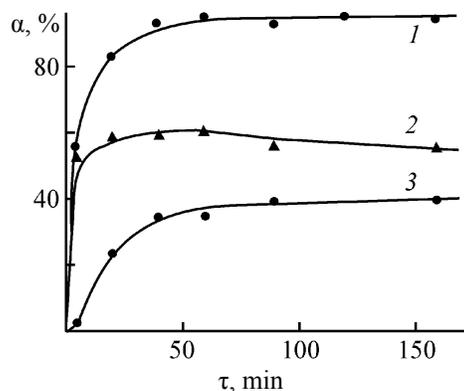
Fig. 4. (*1*) Degree of AG conversion α and yields ω of (*2*) GH and (*3*) DGH as functions of time τ at 70°C. Molar ratio MA : **II** : HCl = 1 : 1 : 1.15, initial MA concentration 4.085 M.

1,2,4-triazol-3-ylacetic acid **II** and bis(5-amino-1,2,4-triazol-3-yl)methane **IV** from aminoguanidine hydrocarbonate **II**, MA, and HCl$_{conc}$. At the molar ratio MA : **II** : HCl = 1 : 1 : 1.15, temperature of 70°C, and first step duration of 60–70 min, the yield of GH and DGH, according to HPLC of the reaction mixture, is 58–60 and 35–37%, respectively, at the AG conversion as high as 94–95%. Subsequent treatment of the reaction mixture with alkali leads to quantitative conversion of GH and DGH to compounds **I** and **IV**.

Isolation of **I** and **IV** from the reaction mixture involves certain problems. Both compounds have close solubility in water at temperatures below 60°C, which prevents their separation by crystallization (Table 2). In addition, compounds **I** and **IV** are amphoteric and form salts both with strong acids and with bases. Therefore, the acidity of the reaction mixture strongly affects the yield of **I** and **IV** in the course of isolation. Compound **I** in aqueous solutions is characterized by pK_a 4.85± 0.03 (deprotonation) and pK_a(BH$^+$) 2.71 ± 0.05 (deprotonation of the protonated form of **I**). Compound **IV** is a diacidic base, and it can be expected that pK_a(BH$^+$) of its monoprotonated form will be about 4.0–4.7, as for the majority of other 5-amino-3-R-1,2,4-triazoles [18, 19]. The above-noted acid–base properties prevent simultaneous isolation of **I** and **IV** in the free form.

Both reaction products are precipitated most completely if the reaction mixture after alkaline cyclization of guanylhydrazides is acidified with a concentrated HCl solution to pH 2.5–2.7. According to HPLC, the degree of isolation of acid **I** is 90–92%, and that of **IV**, 90–95%. In the process, acid **I** is isolated in the free form, and acid **IV**, in the form of hydrochloride despite the fact that the latter is well soluble in water. Apparently, the solubility of the hydrochloride considerably decreases in the presence of NaCl formed by neutralization of the reaction mixture. On heating the mixture of **I** and **IV** in water, hydrochloride of **IV** dissolves, and virtually pure acid **I** remains in the precipitate. Compound **IV** is then isolated in the free form by alkalization of a solution of its hydrochloride with sodium carbonate to pH 9–10. The yield of purified **I** and **IV** based on MA is 44–51 and 26–29%, respectively.

When the goal of the synthesis is preparation of **IV**, it is preferable to perform the reaction at the molar ratio MA : AG = 1 : 2.

The dependence of the AG conversion α on the initial H$_2$O concentration c(H$_2$O) in the reaction mixture passes

Table 2. Solubility of **I** and **IV**

Compd.	Solubility, g/100 g H$_2$O, at indicated temperature, °C				
	1.0	33.5	61.5	75.0	91.0
I	0.280 ± 0.006	0.635 ± +0.012	0.82 ± +0.02	2.12 ± +0.07	3.27 ± +0.03
IV	0.260 ± 0.006	0.847 ± 0.003	1.63 ± 0.02	4.24 ± 0.02	6.89 ± 0.07

through a maximum, as shown below (70°C, molar ratio MA : aminoguanidine hydrochloride : HCl = 1 : 2 : 0.33, reaction time 3 h):

c(H$_2$O), wt %	3	12	18	23
α	73	95	93	89

This is due to the following facts: At c(H$_2$O) < 3 wt %, GH and DGH formed in the process rapidly crystallize, and then the reaction proceeds very slowly. By the moment of crystallization, the AG conversion does not exceed 73%. Only on heating this mixture for 2 days, the AG conversion exceeding 90% can be attained. At the same time, with an increase in c(H$_2$O) to 23 wt %, the equilibrium conversion decreases to 89%.

The optimal value of c(H$_2$O) is about 12 wt %. This value is attained when using as starting reactants aminoguanidine hydrochloride and 36% HCl, with slight dilution of the reaction mixture with water. Under these conditions, the AG conversion in 2.5 h reaches 94–95%, with the DGH yield reaching 83–85% and the GH yield not exceeding 14%. The use of **II** as reactant is not appropriate, because the amount of H$_2$O released in its reaction with HCl is too large, which decreases the equilibrium yield of **IV**. After alkaline cyclization, neutralization of the reaction mixture, and recrystallization, the yield of **IV** is as high as 71–80%, at the main substance content ≥98% according to HPLC.

EXPERIMENTAL

Compound **II** (Merck) contained no less than 98% main substance. MA was of pure grade, and the other chemicals, of analytically pure grade.

The temperature of the reaction mixture was maintained with an accuracy of ±0.5°C using an oil bath placed on a magnetic stirrer with a heater (IKA RCT basic), equipped with an external thermocouple. The solution acidity was determined with a pH-150M device using an ESL-63-07 glass electrode and an EVL-1M3 reference electrode. The AG concentration in reaction mixtures was determined

by iodatometric titration, and the concentrations of GH, DGH, **I**, and **IV**, chromatographically by known procedures [12]. The ionization constants of **I** were determined by potentiometric titration [20].

Experiments on studying the effect of reaction conditions on the yield of GH and DGH were performed in 15-ml glass test tubes. Each tube was charged with a mixture of 0.7651 g (0.07353 mol) of MA, the required amount of **II** or aminoguanidine hydrochloride, 36% HCl, and, if necessary, H_2O. The test tubes were placed in a thermostat and heated with stirring until the reactants fully dissolved and the CO_2 evolution ceased. After that, the test tubes were stoppered with rubber plugs and kept at a required temperature for a required time. Then the test tubes with the reaction mixture were quickly cooled with ice-cold water, and the contents were analyzed for AG, GH, and DGH.

5-Amino-1,2,4-triazol-3-ylacetic acid I and bis(5-amino-1,2,4-triazol-3-yl)methane IV. MA (45.9 g, 0.441 mol) and **II** (60 g, 0.441 mol) were mixed in a 0.5-l round-bottom flask. To this mixture, we carefully added with stirring 51.7 g (42 ml, 0.510 mol) of 36% HCl. The resulting mixture was heated with stirring at 70°C to complete homogenization, after which it was kept at this temperature for 60–70 min and then cooled to 20°C. To the resulting viscous mass we added 185 ml of 6.1 N NaOH (1.129 mol). The mixture was stirred and then heated at 90–95°C for 40 min. The resulting solution was cooled to 50–60°C, carefully acidified with 36% HCl to pH 2.5–2.7, and cooled to 5°C. After 30 min, the precipitate that formed was filtered off and dried. The solid precipitate obtained (68–71 g) contained, according to HPLC data, about 55% **I** and 35% bis(5-amino-1,2,4-triazol-3-yl)methane hydrochloride. To separate the components, the mixture was suspended in 350 ml of H_2O, heated to boil with stirring, boiled for 3–5 min, and then cooled to 20°C. The precipitate was filtered off and dried at 120–130°C. Compound **I** (27.6–31.9 g, 44–51%) was obtained; mp 187°C with decomposition (mp 190–192°C [11]). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.59 s (2H, CH$_2$), 5.68 br.s (2H, NH$_2$), 11.63 br.s (1H, NH). Mass spectrum, m/z (I_{rel}, %): 142 (19) [M]$^+$, 98 (100), 68 (49), 57 (36), 43 (86), 42 (69), 41 (83).

Found, %: C 34.00, H 4.19, N 39.18.

$C_4H_6N_4O_2$.

Calculated, %: C 33.81, H 4.26, N 39.42.

The mother liquor after separating acid **I** was heated to boil, alkalized with 11–13 g of Na_2CO_3 to pH 9–10, evaporated to a volume of 170–200 ml, and cooled to 3–5°C. The resulting precipitate was filtered off, recrystallized from 150 ml of H_2O, and dried at 120–130°C. Compound **II** (10.3–11.5 g, 26–29%) was obtained; mp 287–293°C (mp 293°C [13]). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.35 s (2H, CH$_2$), 5.68 br.s (4H, 2NH$_2$), 11.63 br.s (2H, 2NH). Mass spectrum, m/z (I_{rel}, %): 180 (36) [M]$^+$, 124 (36), 98 (100), 68 (19), 67 (20), 57 (40), 43 (89).

Found, %: C 33.05, H 4.50, N 62.45.

$C_5H_8N_8$.

Calculated, %: C 33.33, H 4.48, N 62.19.

Bis(5-amino-1,2,4-triazol-3-yl)methane IV. MA (40.0 g, 0.385 mol), 36% HCl (12.9 g, 10.5 ml, 0.127 mol), H_2O (15.6 ml), and aminoguanidine hydrochloride (85 g, 0.769 mol) were mixed in a 0.5-l round-bottom flask. The resulting mixture was heated with stirring at 70°C to complete homogenization, after which it was kept at this temperature for 3 h. The reaction mixture crystallized, and 150 ml of 6.1 N NaOH was added. The mixture was heated with stirring to dissolution and then stirred at 90–95°C for 40 min. Then the mixture was cooled to 5°C and allowed to stand at this temperature for 30 min, after which the precipitate was filtered off and dissolved in 500 ml of water. Sodium hydrogen carbonate was added to this solution to pH 9–10, and the solution was evaporated to 250 ml and cooled to 3–5°C. The precipitated crystals were filtered off and dried at 120–130°C. Compound **IV** (49.2–55.4 g, 71–80%) was obtained; mp 289–293°C (mp 293°C [13]). Samples of **IV** obtained by both procedures are identical in spectral properties.

CONCLUSIONS

(1) It is appropriate to perform the synthesis of 5-amino-1,2,4-triazol-3-ylacetic acid from malonic acid and aminoguanidine hydrocarbonate jointly with the preparation of bis(5-amino-1,2,4-triazol-3-yl)methane. The yield of the target products is determined by the yield of the intermediate malonic acid guanylhydrazides.

(2) The following conditions are suggested for preparing malonic acid guanylhydrazides: acid medium, catalysis by 36% HCl, 70°C, molar ratio malonic acid : aminoguanidine hydrocarbonate : HCl = 1 : 1 : 1.15, reaction time 60–70 min.

(3) If the goal of the synthesis is preparation of bis(5-amino-1,2,4-triazol-3-yl)methane, it is appropriate to

perform the synthesis from malonic acid and amino-guanidine hydrochloride, with catalysis by 36% HCl, at the molar ratio malonic acid : aminoguanidine hydrochloride : HCl = 1 : 2 : 0.33, temperature of 70°C, and initial concentration of water in the reaction mixture of about 12%.

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