SYNTHESIS OF CYSTAMINE

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Several methods are given in the literature for the preparation of cystamine (I) [bis(β-aminoethyl) disulfide dihydrochloride], starting from β-chloroethylamine hydrochloride (II). By the first method, the starting compound is treated in an aqueous alkaline solution with carbon disulfide, the 2-mercaptothiazoline obtained is hydrolyzed in an acid medium to the key intermediate – β-mercaptoethylamine (III) [3] – which is then oxidized into I by various agents [13, 17, 18]. Compound III, which also exhibits antitumorigenic activity (the mercamine preparation) [5], can be obtained by alkylation of sodium thiosulfate with β-chloroethylamine, followed by acid hydrolysis of the salt of the β-aminoethyl ester of thiosulfuric acid (IV) formed, the so-called Bunte salt [4, 7, 8]. Methods have been described for the preparation of I directly from II and sodium disulfide [16], II and β-aminoethyl sulfonate [10], and also by the reaction of ethyleneimine with hydrogen sulfide [15]. Taking into account the availability of the raw materials and solvents, cleanliness of the working area, simplicity of the technology, and other factors, the most acceptable scheme is synthesis of I from II and sodium thiosulfate via IV, which has been adopted in industry:

$$\underset{\text{II}}{ClCH_2CH_2NH_2\cdot HCl} \xrightarrow[-2NaCl]{Na_2S_2O_3} \underset{\text{IV}}{NH_2CH_2CH_2SSO_3H} \xrightarrow[(-H_2SO_4)]{H_2O,\ [H^+]} \underset{\text{III}}{NH_2CH_2CH_2SH} \xrightarrow{[O]} \underset{\text{I}}{NH_2CH_2CH_2SSCH_2CH_2NH_2}$$

The process is carried out without the intermediate isolation of III, in practically a single operation. However, as we have found, compound I obtained by this method contains several impurities, which can be determined chromatographically. First of these is monoethanolamine (R_f 0.14) which forms due to hydrolysis of II during the alkylation of $Na_2S_2O_3$. Moreover, according to our data, the cystamine preparation contains polysulfides having the structure of $NH_2CH_2-CH_2-S_x-CH_2CH_2NH_2$ or $-SCH_2CH_2NH-S_x-NHCH_2CH_2S-$, appearing on the chromatogram in the form of spots (R_f 0.68), lying above the spot of the main compound I (R_f 0.35). The apparance of these compounds is due to the reaction of I with elemental sulfur, formed during the decomposition of $Na_2S_2O_3$ in acid medium [12, 14]. An indirect proof for the formation of polysulfides from I is the fact that impurities appear on heating an aqueous solution of chromatographically pure I with sulfur, which according to the TLC data are identical with the by-products present in a technical grade cystamine, obtained from II when intermediate compounds are not isolated. Unfortunately, the polysulfides could not be isolated and characterized, because of their instability, as is known from the literature data [6]. It should be noted that it is impossible to eliminate monoethanolamine and polysulfides by a drastic change of the alkylation conditions: the pH, temperature, process duration, concentration and ratio between the reagents. Thus, for example, increase in the charge of $Na_2S_2O_3$ above the stoichiometric amount leads to increase in the content of polysulfides, in I. If, however, an excess of II is used, then the content of monoethanolamine in the desired end product increases sharply. The presence of the above impurities in I negatively affects the quality parameters such as the melting point and the transparency of the aqueous solution. The latter may be due to the decomposition of polysulfides with separation of a sulfur precipitate.

On the basis of the hypothesis that most of the impurites in the preparation are formed at the first stage of the technological process, we carried out the synthesis of I with separation of a crystalline IV after the first stage, which made it possible to eliminate monoethanolamine and excess $Na_2S_2O_3$. A chromatographically pure cystamine was thus obtained having a melting point 4-5°C higher than in the case of samples of the preparation synthesized by the

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known technology. To develop the improved method for the preparation of I, two problems had to be solved: to establish conditions for the alkylation process ensuring the maximal yield of IV, and to determine the conditions for the separation of this intermediate from the reaction mixture with the least loss.

We found that the reaction of sodium thiosulfate and II in water at the boiling point of the mixture proceeds at a high rate and even after 15-20 min of holding, 1-2% of $Na_2S_2O_3$ (of the charged amount) remains in the reaction mixture; its content can be determined by known methods [1]. After cooling the mixture, a thick white precipitate of the Bunte salt appears, which is readily separated from the mother liquor by filtration. Compound IV crystallizes from highly concentrated aqueous solutions (more than 50%), and also from a saturated aqueous solution of sodium chloride, modeling the aqueous-salt system, formed as a result of the alkylation reaction. The solubility of IV in the above salt solution is very strongly dependent on the temperature: thus, the content of IV in 100 g of a solution at 5°C is 1.285 g, at 20.7°C – 4.612 g, at 40.2°C – 5.595, and at 54.4°C – 24.21 g.

The separated salt IV was hydrolyzed with dilute sulfuric acid. As a result of this reaction, compound III was formed, and also I as a result of its partial oxidation by air. However the degree of the III → I conversion is low, and varies over fairly wide limits (from 5 to 20%). It should be noted that on cooling the reaction mixture, compound III partially crystallizes in the form of a sulfate, and can be separated by filtration. The oxidation of III into I was carried out using perhydrol in an acid medium. The process proceeds rapidly and with evolution of heat, and is readily monitored, either from the presence of the unoxidized thiol III in the mixture (a probe with potassium iodate), or of excess hydrogen peroxide (a probe with potassium iodide).

Compound I in the form of a base (oil) was extracted from the reaction mixture after alkalinization with n-butanol, and was converted into a hydrochloride by various methods: addition of an alcoholic solution of hydrogen chloride to a butanolic solution of I, followed by filtration of the desired end product; extraction of I by concentrated hydrochloric acid, evaporation of the extract and precipitation of I by adding isopropanol or butanol to the residue; addition of concentrated hydrochloric acid and azeotropic distillation of water from the mixture.

In all cases, high-quality samples of cystamine were obtained in similar yields (within 66-68%, based on II).

In the course of the investigations we took the PMR spectra of compounds I, III, IV (in D_2O). In all the cases, the pattern of the proton signals was typical for a system of two pairs of magnetically nonequivalent nuclei interacting with one another. Thus, the position and form of the signals depend on the pH of the solution. At pH 5.5 (the natural pH value of solution of I in water) there are two triplet signals of equal intensity in the spectrum of cystamine with centers at 3.41 ppm (4H) and 3.02 ppm (4H), corresponding to the resonance of the methylene group protons of the H_2NCH_2- and $-CH_2S-$ fragments, respectively. On acidification of this solution (to pH 1-2), the second triplet does not change its form, and the value of its chemical shift also does not change. The first triplet signal becomes broader, and shifts to some extent to a stronger field region (center at 3.39 ppm). On alkalinization of the solution of I by potassium hydroxide, the triplet signals approach one another, partially change their form and are shifted in the strong field direction. Thus, for example, at pH 8.5, the chemical shifts of the centers of the triplet signals are equal to 3.05 and 2.92 ppm, while at pH 12.0 the two signals form a symmetrical multiplet with a center at 2.85 ppm (8H).

In the PMR spectra of III and IV, the resonance signals of all four protons of $-CH_2-CH_2-$ fragment appear in the form of a singlet having an irregular form and with a chemical shift of 3.4 ppm (4H). After alkalinization of the solution, the singlet signal acquires a regular form and is shifted to some extent to the strong field region (3.35 ppm).

EXPERIMENTAL

The PMR spectra were run in D_2O, on a JNM-FX-90 Q Jeol spectrometer (Japan) with a working frequency of 90 MHz using CH_3CN as internal standard. The thin layer chromatography was carried out on Silufol UV-254 plates in a $CHCl_3$–ethanol–25% ammonia solution (5:10:1) system of solvents, with development of the chromatograms by a 1% solution of ninhydrin in acetone.

β-Aminoethyl Ester of Thiosulfuric Acid (IV). A mixture of 100 g (0.40 mole) of $Na_2S_2O_3 \cdot 5H_2O$ and 110 g of II (46.75 g, 100%; 0.40 mole), used in the form of a 42.5% aqueous solu-

tion) was boiled for 30 min, then cooled to 0-5°C, allowed to stand for 1.5 h, and made alkaline with a 40% aqueous solution of NaOH to pH 5.35. The crystals of IV and inorganic salts that separated out were filtered off, and washed with isopropanol (2 × 20 ml). The product was analyzed for content of the main compound by a known method of determination of organic disulfide [II]. The technical grade IV contains 76-78% of the main compound. Yield 64.2 g (78.1% based on II). After two recrystallizations from water mp 200-202°C (dec.). $C_2H_7O_3NS_2$.

Hydrochloride of Bis-(β-aminoethyl) Disulfide (I). A mixture of 62.2 g of technical grade IV (49.4 g, 100%), 100 ml of water, and 15 ml of concentrated H_2SO_4 was boiled for 30 min. The cooled reaction mixture was analyzed for content of III (by titration with an iodine [9] or potassium iodate [2] solution in the presence of potassium iodide or starch as indicator). From the thus determined amount of thiol, the amount of perhydrol required for the oxidation was calculated. The oxidation process was carried out by slowly adding the perhydrol and stirring the mixture at a temperature not higher than 20°C. The mixture was allowed to stand for 10 min and then was analyzed for the presence of excess H_2O_2 (darkening of an iodine-starch paper). In the case of a positive result of the analysis, the mixture was made alkaline (to pH 12-13) with a 40% solution of NaOH, base I was extracted with n-butanol (5 × 90 ml), the extract was dried over K_2CO_3 and clarified by activated carbon. The content of I in the solution was determined by titration of a sample with 0.1 N hydrochloriric acid in the presence of methyl orange. The yield of base I was 21.6 g (90.4% based on 100% IV). Compound I was precipitated by adding to the base a calculated amount of 30% HCl solution in isopropanol with cooling (0-5°C) and stirring for 1.5-2 h. After filtration and washing with isopropanol (2 × 20 ml), 31.2 g (67.1%, based on II) of chromatographically pure I, mp 219-220°C, was obtained.

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