ANOMALOUS NUCLEOSIDES

VII*. Synthesis Of 6-Azacytidine And Its Derivatives

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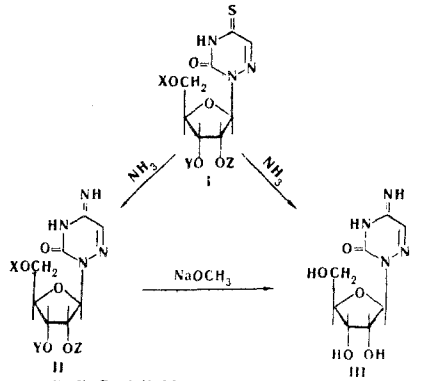
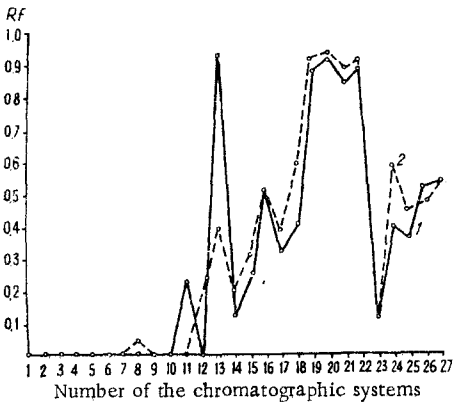
A preparative synthesis of the antimetabolite 6-azacytidine is described which involves the amination under mild conditions without the use of an autoclave of 2', 3', 5'-tri-O-acyl-4-thip-6-azauridines with the isolation of the intermediate 2', 3', 5'-tri-O-acyl-6-azacytidines and subsequent elimination of the protective groups at room temperature.

One of the most interesting and promising classes of anomalous nucleosides, antimetabolities of the biosynthesis of the nucleic acids, are the azapyrimidine nucleosides (6-azauridine, 6-azacytidine, etc.). In recent years they have been studied widely in many countries. The biosynthesis and later the chemical synthesis of 6-azauridine have been effected [1–4]. 6-Azacytidine [5–7] and other azapyrimidine nucleosides have been obtained by chemical methods. The biological study of the azapyrimidine nucleosides has shown that they possess a broad spectrum of biological activity. This has led to the realization in a number of countries of new variants of the synthesis of 6-azacytidine and other azanucleosides [8,9]. The methods for the synthesis of 6-azacytidine described in the literature are fairly complex; they require the prolonged use of high temperatures and high pressures or the chromatographic separation of condensation products and are unsuitable for the practical production of azanucleosides in large amounts. In this respect, the preparative synthesis of 6-azacytidine (III) and its derivatives that we carried out [11] by the following scheme is more convenient.



a X=Y=Z=C6H5CO–
b X=Y=Z=CH3CO–
c X=CH3CO–, Y+Z=(CH3)2C<
d X=H, Y+Z=(CH3)2C<
e X=Y=Z=H

As the starting material we took the anomalous nucleoside 6-azauridine, in which the hydroxy groups of the ribose residue were protected by acylation or by the acetonation of the vicinal hydroxyls and subsequent acylation of the primary hydroxyl. The protected azauridines were converted into thiones as described by Chernetskii et al [7] giving I. In order to simplify the synthesis of 6-azacytidine, we have carefully studied the limiting stage in the process of the amination of the thioazauridines and at the same time reinvestigated the splitting off of the protecting O-acyl and O-alkylidene groups.



Chromatographic spectra: 1) 6-azacytidine; 2) 6-azauridine (for the solvent systems, see Experimental part)

We effected the replacement of the mercapto group by an amino group by bubbling gaseous ammonia into a solution of I under various conditions (varying the solvent, the temperature, and the time of the reaction). The course of the exchange was studied chromatographically in various systems of solvents (see Experimental part). On the chromatograms spots corresponding to compounds I, II, and III were identified. It was established that the interaction of the thioazauridines I with ammonia first leads to the replacement of the mercapto group by the amino group with the formation of substituted 6-azacytidines (II) and then to the gradual splitting off of the O-acyl protecting groups. Consequently, it is not necessary to carry out the ammonolysis of I in an autoclave for a long time, since the substitution reaction takes place readily when ammonia is bubbled into aqueous solutions of the thioazauridines. Under these conditions, depending on

*For communication VI, see [10].

the temperature and the time of the reaction and the nature of the protecting groups, fully or partially substituted II and unsubstituted 6-azacytidine (III) are obtained. The optimum conditions for obtaining substituted azacytidines are created in most cases by carrying out the ammonolysis in boiling propanol. The use of other alcohols as solvent markedly retards the reaction.

The removal of the protecting benzoyl and acetyl groups was carried out under mild conditions by treating the appropriate triacylazacytidines with sodium methoxide in methanolic solution at room temperature. The pH of the reaction medium was maintained between 8.5 and 9.5, and under these conditions the splitting off of the protecting groups was complete in 2.5–3.5 hr, while there was practically no replacement of the amino group by a hydroxy group, i. e. 6-azacytidine was fairly stable under the conditions mentioned. The isopropylidene protection was removed by heating 2',3'-isopropylidene-6-azacytidine with 10% acetic acid for 30–45 min.

The synthesis of tribenzoylazacytidine (II) was also carried out via the chloro derivative of 6-azapyrimidin-2-one riboside [8]. However, the method described in the literature was somewhat simplified: the dropwise addition of a previously prepared 30% methanolic solution of ammonia was replaced by the direct bubbling of gaseous ammonia into a benzene solution of the chloro derivative in the cold.

The investigations performed have led to the development of preparatively convenient methods for obtaining 6-azacytidine and its O-acyl derivatives, and a number of such compounds have been obtained and processed for the performance of biological tests.

In the isolation and purification of the substances synthesized, the chromatographic spectra of 6-azacytidine and its derivatives in a considerable number of chromatographic systems arranged roughly in increasing polarity were used. The use of chromatographic spectra for the identification of antibiotics is described in the literature [12, 13]. A method of the distribution of the spots of the nitrogen bases forming components of the main nucleosides in eight systems of solvents was used even earlier in the chemistry of the nucleic acids. This scheme may be the precursor of chromatographic spectra. The figure shows the chromatographic spectra that we obtained for 6-azauridine and 6-azacytidine.

EXPERIMENTAL

Paper chromatography. For chromatographic analysis and the preparation of chromatographic spectra we used type B paper of the Leningrad No. 2 paper mill. Chromatography was carried out in the following set of solvent systems: 1) dry benzene; 2) dry ethyl acetate; 3) dry butyl acetate; 4) petroleum ether saturated with water; 5) carbon tetrachloride saturated with water; 6) benzene saturated with water; 7) chloroform saturated with water; 8) ethyl acetate saturated with water; 9) butyl acetate saturated with water; 10) nitromethane saturated with water; 11) dioxane + 10% of water; 12) dry acetone; 13) tetrahydrofuran + 10% of water; 14) n-butanol saturated with water; 15) isopropanol + 10% of water; 16) acetone + 10% of water; 17) dry methanol; 18) methanol + 10% of water; 19) water saturated with n-butanol; 20) dimethylformamide + 10% of water; 21) water; 22) ammonium chloride, 3% aqueous solution; 23) n-butanol saturated with water + 2% of piperidine; 24) n-butanol–pyridine–water (1.0:0.6:1.0); 25) n-butanol–acetic acid–water (5:2:3); 26) n-butanol–acetic acid–water (2:1:1); 27) isopropanol–aqueous ammonia–water (7:1:2). The spots of the substances on the chromatograms were detected under an ultraviolet lamp with UFS-1 filters of various thicknesses (2–5 mm).

The UV spectra were taken on a SF-4 spectrophotometer in the range from 210 to 300 nm. The solvents were water and 96% ethanol. The concentration of the solutions was 10^{-5} M and the layer thickness 1 cm.

2',3',5'-Tri-O-benzoyl-6-azacytidine (IIa). a) Dry ammonia was passed into a boiling solution of 0.36 g (0.63 nm) of **Ia** in 4.5 ml of propanol for 2 hr whereupon a white crystalline precipitate slowly deposited. The reaction mixture was cooled to 0° C. The precipitate was filtered off, washed with cold ethanol, and dried. This gave 0.3 g of substance. An additional 0.04 g of substance was obtained from the mother liquor. The total yield was 98%. White prisms (from ethanol) with mp 218–220° C. λ_{max} 229, 267 nm (in ethanol). R_f (system: 0.87 (14), 0.92 (25). Found, %: C 62.45; H 4.23; N 10.22. Calculated for $C_{29}H_{24}N_4O_8$, %: C 62.57; H 4.35; N 10.07.

b) Ammonia was bubbled through a cooled solution of 0.6 g (1nM) of the 4-chloro derivative of an azanucleoside [8] in 10 ml of benzene for 2 hr. The temperature of the reaction mixture was kept at 5–7° C. After the absorption of ammonia had ceased and the mixture had been allowed to stand for 30 min, the solvent was distilled off in vacuum. The residue was treated several times with water and was dried. This gave 0.5 g (86%) of a substance with mp 215–217° C.

A mixture of the products from experiments (a) and (b) melted without depression.

6-Azacytidine (III). a) In drops, a 1 N solution of sodium methoxide in methanol was added to a suspension of 2 g (3.6 nM) of **IIa** in 55 ml of dry methanol, the pH of the reaction mixture being kept at 8.5–9.5. After stirring for 30 minutes, the **IIa** had dissolved completely and soon a crystalline precipitate of 6-acacytidine began to deposit. The mixture was stirred for another 2 hr and then the precipitate was filtered off and washed with chloroform, ethanol, and ether. This gave 0.67 g of 6-azacytidine. The methanolic filtrate was neutralized with acetic acid. The precipitate that deposited on cooling to 0° C was filtered off and washed as described above. This yielded another 0.07 g of substance. The methanolic mother liquor was distilled in vacuum, and similar treatment yielded a further 0.08 g of 6-azacytidine. The total yield was 90%. White prisms (from water) with mp 220–222° C. A mixture with a sample obtained by the method of Chernetskij et al. [7] gave no depression of the melting point. λ_{max} 263 nm (in water).

b) Ammonia was passed into a solution of 0.58 g (1.5 nM) of **Ib** in 9 ml of methanol at room temperature for 2 hr, and then the solution was kept in the cold for 12 hr. After this time practically all the **Ib** had been converted into 6-azacytidine. The methanol was distilled off in vacuum to half bulk, and the residue crystallized on cooling. The precipitate that deposited was washed as described above, and the mother liquor was evaporated and allowed to stand for further crystallization. The total yield of 6-azacytidine was 0.34 g (93%) in the form of white prisms (from water) with mp 218–220° C. The sample was identical with a reference sample.

c) A mixture of 50 mg of **IId** and 1 ml of 10% acetic acid was heated in the boiling water bath for 30 min. After the end of hydrolysis, 2 ml of methanol was added to the solution and it was evaporated in vacuum to dryness. This operation was repeated three times. This yielded 35 mg (80%) of white prisms (from aqueous ethanol) with mp 216–218° C. A mixture with products (a) and (b) melted without depression.

d) With heating, 50 mg of **Ie** was dissolved in 1.5 ml of propanol, and ammonia was bubbled into the boiling solution for 30 min. After the usual isolation procedure, 40 mg (86%) of white prisms (from water) was obtained with mp 216–218° C. The sample was identical with those obtained previously.

2',3'-O-Isopropylidene-5'-O-acetyl-6-azacytidine (IIc). Ammonia was passed into a boiling solution of 0.52 g (1.5 nM) of **Ic** in 25 ml of propanol for 1.5 hr. The solvent was rapidly distilled off in vacuum,

and the dry residue was treated with ethanol, filtered off, and washed with ethanol, with a mixture of ethanol and ether, and with ether. This gave 0.44 g (96%) of clusters of white needles (from ethanol) with mp 173°–175° C. λ_{max} 268 nm (in ethanol). R_f (system): 0.77 (14), 0.90 (25). Found, %: N 17.33; 17.37. Calculated for $C_{13}H_{18}N_4O_6$, %: N 17.19.

2'3'-O-Isopropylidene-6-azacytidine (IId). a) A mixture of 0.69 g (2 nM) of **Ic** and 10 ml of methanolic ammonia, prepared by mixing liquid ammonia with absolute methanol (cooled with solid carbon dioxide and acetone), was sealed into a tube and heated in an autoclave at 100° C for 38 hr. The reaction mixture was evaporated in the vacuum of a water pump and then in the vacuum of a diffusion pump in the water bath (35–40° C). This gave 0.47 g (73%) of white needles with mp 202–203° C (decomp.) after recrystallization from methanol. λ_{max} 263 nm (in water). R_f (system): 0.56 (14), 0.77 (25). Found, %: C 46.39; H 5.61; N 19.69. Calculated for $C_{11}H_{16}N_4O_5$, %: C 46.48; H 5.63; N 19.72.

b) Ammonia was bubbled through a boiling solution of 50 mg of **Id** in propanol for 2 hr. Distillation of the solvent and treatment of the dry residue with ethanol gave 40 mg (92%) of small prisms (from ethanol) with mp 198–199° C. The substance was identical with that obtained previously.

c) A solution of 50 mg of **IIc** in 10 ml of methanol saturated with ammonia at 0° C was kept in the cold for a day. After a working up process analogous to that described above, 43 mg (88%) of white prisms was obtained with mp 196–198° C (from ethanol). A mixture with the product from experiment (a) melted without depression.

2', 3', 5'-Tri-O-acetyl-6-azacytidine (IIb). Dry ammonia was passed through a solution of 0.75 g of **Ib** in 8 ml of methanol at room temperature for 15 min. Then the solvent was rapidly evaporated in vacuum and the resulting syrup was separated by means of preparative paper chromatography (system 14). From 200 mg of syrup was obtained 47 mg of cream-colored powder with mp 162–164° C. λ_{max} 267 nm (in water). R_f (system): 0.63 (14), 0.71 (25). Found, %: N 15.59; 15.62. Calculated for $C_{14}H_{18}N_4O_8$, %: N 15.13.

N_4-Acetyl-2', 3', 5'-Tri-O-benzoyl-6-azacytidine. To a solution of 0.28 g (0.5 nM) of **IIa** in 1 ml of pyridine was added 0.5 ml of acetic anhydride and the mixture was kept at room temperature for 12 hr. With stirring, the solution was poured into ice-water, and the viscous mass was triturated in a mortar with ice. The precipitate was filtered off and washed successively with ice water, 50% ethanol, and ether. This gave 0.24 g (80%) of a white microcrystalline substance (from a mixture of ethanol and acetone) with mp 202–204° C. λ_{max} 215, 277 nm (in ethanol). R_f (system): 0.89 (14), 0.95 (25). Found, %: N 9.11; 9.14. Calculated for $C_{31}H_{26}N_4O_9$, %: N 9.33.

N_4-Acetyl-2', 3', 5'-tri-O-acetyl-6-azacytidine [16]. To a suspension of 2.44 g (10 nM) of **III** in 10 ml of pyridine was added 4.3 ml of acetic anhydride, and the mixture was heated in the water bath (50°C) until the solid matter had dissolved completely. Then the solution was cooled and kept for several hours at room temperature, after which the solvent was distilled off in vacuum. The white paste of crystals was treated with 15 ml of water, and the solid matter was filtered off, washed with water, and dried. This gave 3.98 g (96%) of white elongated needles (from aqueous ethanol) with mp 148–150° C. λ_{max} 267 nm (in ethanol). R_f (system): 0.82 (14), 0.86 (25). Found, %: N 13.30; 13.49. Calculated for $C_{16}H_{20}N_4O_9$, %: N 13.59.

2'3'-O-Isopropylidene-5'-O-acetyl-4-thio-6-azauridine (Ic) [17]. A mixture of 16.4 g (50 nM) of 2', 3'-O-isopropylidene-5'-O-acetyl-6-azauridine [15], 12.0 g (54 nM) of finely ground phosphorus pentasulfide, and 16 ml of pyridine was boiled under reflux for 3 hr. At the end of this time the pyridine was distilled off in vacuum. The dark brown residue was dissolved in 300 ml of chloroform and the chloroform solution was extracted five times with 100-ml portions of water and was then filtered and evaporated in vacuum. The red-brown residue was dissolved in absolute methanol with heating, and the solution deposited crystals after standing in the cold for several days. This gave 7.9 g (46%) of light brown needles with mp 145–148° C. The mother liquor yielded an additional 7.13 g of thio derivative. The total amount was 15.03 g (87.5%) of clusters of golden orange needles (from methanol) with mp 153–154° C. R_f (system): 0.85 (14), 0.9 (27). Found, %: C 44.99; H 5.08; N 12.65; S 9.10. Calculated for $C_{13}H_{17}N_3O_6S$, %: C 45.51; H 4.99; N 12.25; S 9.34.

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