CHEMISTRY

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1961

SovietRxiv

Abstract

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CHEMISTRY

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6-AZACYTIDINE AND ITS DERIVATIVES

Synthetic nucleotides containing anomalous bases, analogs of the purine and pyrimidine constituents of nucleic acids (6-azauracil, mercaptopurine, etc.), are of considerable interest both for the study of the biosynthesis of nucleic acids and as potential cancerostatic and virostatic preparations. Such compounds are being intensively studied at the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences (1). In 1957, 6-azauridine (I) (2), possessing high antitumor activity, was obtained by biosynthesis. It was of interest to synthesize 6-azacytidine (II), since it is known that cytidine is incorporated into nucleic acids much more intensively than uridine (3), and it could be expected that 6-azacytidine would be a stronger antimetabolite—an inhibitor of nucleic-acid synthesis.

For the synthesis of 6-azacytidine, the high mobility of the sulfur atom in 4-thio derivatives of pyrimidine nucleosides was used (4). The starting material was 6-azauridine, which is now produced in the Czechoslovak Socialist Republic on an industrial scale. 6-Azauridine was converted by acylation into peracyl derivatives (tribenzoyl (III)- and triacetyl (IV)-6-azauridines). Treatment of I with a large excess of acetic anhydride led to the formation of tetraacetyl-6-azauridine (V). Peracyl-6-azauridines with phosphorus pentasulfide were converted into peracyl-4-thio-6-azauridines (VI and VII). Ammonolysis of the substituted 4-thio-6-azauridines gave 6-azacytidine (II) and its derivatives (IX, X, XII).

On heating tribenzoyl (VI)- and tetraacetyl (VII)-4-thio-6-azauridines with ammonia, 6-azacytidine (II) was obtained (m.p. 217–219°, with decomposition; R_f in system A (water-saturated n-butanol) 0.1; in system B (isopropanol—ammonia—water (7 : 1 : 2)) 0.5).

Found, %: C 39.68; H 4.95; N 22.88
$C_8H_{12}O_5N_4$. Calculated, %: C 39.33; H 4.92; N 22.93

6-Azacytidine was also obtained as a result of ammonolysis of 4-thio-6-azauridine (VIII), obtained by debenzoylation of tribenzoyl-4-thio-6-azauridine (VI).

According to the literature, when uridine is treated with phosphorus pentasulfide, only one hydroxyl group in the 4-position is replaced by a sulfur atom (4). We obtained spectrophotometric confirmation of this fact also for derivatives

Reaction scheme: derivatives I–XII of 6-azauridine, showing conversions via BzCl, Ac_2O, P_2S_5, CH_3ONa, NH_3, NH_2NH_2, NH_2OH, and n-$C_4H_9NH_2$.

Figure 1: Reaction scheme: derivatives I–XII of 6-azauridine, showing conversions via BzCl, Ac_2O, P_2S_5, CH_3ONa, NH_3, NH_2NH_2, NH_2OH, and n-$C_4H_9NH_2$.

of 6-azauridine. The spectrum of thioazauridine (VIII), obtained by debenzoylation of tribenzoyl-4-thio-6-azauridine (VI), has λ_{max} 244 and 332 mμ, which, according to the data of J. Jónaš, exactly corresponds to the spectrum of 4-thio-6-azauracil, whereas 2-thio-6-azauracil has λ_{max} 266 mμ.

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Heating tribenzoyl-4-thio-6-azauridine (VI) with *n*-butylamine gave N′-butyl-6-azacytidine (IX) (a white, noncrystallizing foam, R_f in system A 0.70, in system B 0.78).

$C_{12}H_{20}O_5N_4$. Found %: C 47.42; H 7.01; N 18.77
Calculated %: C 48.00; H 6.71; N 18.66

The reaction of tribenzoyl-4-thio-6-azauridine (VI) with hydrazine, depending on the conditions, led to various products. The following were obtained: 1) N′-amino-6-azacytidine (X) (a yellow, noncrystallizing foam, R_f in system B 0.34, in system C (*n*-butanol—water—acetic acid (5:3:2) 0.39, in system D (methylcellosolve—water (8:2)) 0.78).

$C_8H_{13}O_5N_3$. Found %: C 35.58; H 5.13; N 25.40
Calculated %: C 35.60; H 4.88; N 27.15

2) N′-amino-6-azacytosine (XI) (yellow needles, decomposing above 240°, R_f in system B 0.24, in system C 0.82).

$C_3H_5ON_5$. Found %: C 28.19; H 4.17; N 54.48
Calculated %: C 28.30; H 3.94; N 55.10

On interaction of paracetyl-4-thio-6-azauridine with hydroxylamine, N′-oxy-6-azacytidine (XII) was formed (white needles, mp 234–235.5°; R_f in system A 0.14, in system B 0.43).

Found, %: C 37.18; H 4.54; N 21.38
$C_8H_{12}O_6N_4$. Calculated, %: C 36.92; H 4.64; N 21.53

6-Azacytidine and its derivatives, when boiled in an acidic medium, are gradually converted into 6-azauridine (I). N′-amino-6-azacytosine under analogous conditions is converted into 6-azauracil.

Preliminary experiments were carried out to investigate the effect of azacytidine on experimental tumors. Tests were performed on mice with ascitic cancer and Ehrlich carcinoma. In experiments with ascitic cancer, 6-azacytidine was administered intraperitoneally in physiological saline for 8 days beginning on the second day after transplantation, at a dose of 250 mg/kg, to 10 mice. As with 6-azauridine, a slight prolongation of the life span of mice treated with 6-azacytidine was found in comparison with the control—by 17%. In experiments with Ehrlich carcinoma, 6-azacytidine was administered in an analogous manner for 9 days, beginning on day 4 after inoculation, to a group of 10 mice, which were dissected on day 15 after inoculation. The average tumor weight was 345 mg versus 379 mg in the untreated control. In another experiment, 6-azacytidine was administered in a similar manner together with thiosemicarbazide ($9 \times 250\ \gamma$/kg). The average tumor weight was 261 mg and, by the t-test, was significantly statistically reduced. Under the action of 6-azauridine in an analogous experiment with thiosemicarbazide, the tumor weight decreased only to 314 mg. As is evident from the experiments, 6-azacytidine in combination with thiosemicarbazide markedly retards the growth of Ehrlich carcinoma. In comparison with it, the synergistic effect of 6-azauridine with thiosemicarbazide was considerably smaller. Like 6-azauridine, 6-azacytidine has very low toxicity.

A more detailed report on the study of 6-azacytidine and its derivatives will be published in the journal *Collection of Czechoslov. Chem. Commun.*

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Received
4 III 1961

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Note: Figure translations are in progress. See original paper for figures.