NUCLEIC ACIDS COMPONENTS AND THEIR ANALOGUES. LXVI.* HYDROLYSIS OF 5-AZACYTIDINE AND ITS CONNECTION WITH BIOLOGICAL ACTIVITY

P. Piťhová, A. Pískala, J. Piťha and F. Šorm

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague

Received November 20th, 1964

Hydrolysis of 5-azacytidine (*I*) in neutral and basic media affords the following products: 1-β-D-ribofuranosyl-3-guanylurea (*III*), guanidine, α-D-ribofuro[1′,2′:4,5]-2-oxazolidone (*IV*) and D-ribose. As the intermediate, the N-formyl derivative of the compound *III* has been determined spectrophotometrically. In acidic media, the hydrolysis affords 5-azacytosine (*V*), 5-azauracil (*VI*) and D-ribose. In contrast to 5-azacytidine (*I*), the isolated products show a decreased biological activity. As shown by experiments and quantum chemical calculations, the biological activity of the compound *I* is probably due to the incorporation of a 5-azacytosine derivative into nucleic acids followed by a hydrolytic fission of the triazine ring.

In the previous paper[1] of this Series, a study has been presented about structure and tautomerism of 5-azacytidine (*I*), a compound of remarkable cancerostatic, bacteriostatic[2] and mutagenic properties. In connection with practical applications of 5-azacytidine, it was of interest to study its stability and the course of its hydrolytical destruction. Furthermore, two additional aspects have been taken into account: firstly, whether the biological activity is not due to some hydrolysis intermediate (as found in this Institute in the case of 5-azauracil[4]), and, secondly, whether the mutagenic properties are not caused by an eventual incorporation of 5-azacytosine derivatives into nucleic acids followed by a rapid hydrolysis of the instable heterocyclic nucleus. Finally, we have tried to compare the experimental results with results of simple quantum chemical calculations in the Hückel approximation and to use this method for determination of properties by extrapolation in those cases where the experimental determination would be difficult.

Experimental

Melting points were taken on a heated microscope stage and are corrected. The analytical samples were dried for ten hours at 0·3 mm Hg and room temperature.

The nuclear magnetic resonance spectra were measured in deuterochloroform on the apparatus 40 Mc of Czechoslovak origin equipped with superstabilisation. Calibration was performed

* Part LXV.: This Journal *30*, 2480 (1965).

CELGENE 2027
APOTEX v. CELGENE
IPR2023-00512

by the usual technique of side bands (inner standard TMS). Concentration: 100 mg per 0·5 ml of deuterochloroform. Infrared spectra were measured on a spectrophotometer Zeiss (Jena) model UR-10 as usual. Ultraviolet spectra were taken by the technique described in the previous paper[1] in a 0·1M borate buffer at 37°C. The quantum chemical calculations were performed with the use of parameters recommended in the literature[5,6].

Inhibition of the growth of *Escherichia coli* was determined as described previously[7].

Descending *paper chromatography* was performed on paper Whatman No 1 without any previous saturation in the following solvent systems: (S_1) isobutyric acid–water–aqueous ammonia (66 : 33 : 1·5), (S_2) 1-butanol–acetic acid–water (5 : 2 : 3), and (S_3 1-butanol–ethanol–water (40 : 11 : 19). The spots were detected (D_1) in ultraviolet light, (D_2) according to Reindel and Hoppe[8] and (D_3) with the sodium hydroxide–sodium nitroprusside–potassium ferricyanide reagent (by the latter reagent, the guanidine[9] derivatives are detected). The vicinal *cis*-diol system of the ribosyl derivatives was detected with potassium periodate–benzidine[10] (D_4). Some detections were performed with the use of radioactivity (D_5). Paper electrophoresis was carried out on paper Whatman No 1 according to Markham and Smith in a 0·05M borate buffer (pH 9·25) for the period of thirty minutes at 30 V/cm.

Isolation of the Hydrolysis Products

A) A solution of 5-azacytidine (*I*; 0·122 g; 0·5 millimole) in 1M aqueous ammonia (10 ml) was allowed to stand at 37°C for three hours and taken down under diminished pressure in a rotatory evaporator (bath temperature 30°C). The residual sirup was repeatedly coevaporated with three 10 ml portions of methanol, dissolved in methanol (2 ml) and precipitated with a solution of picric acid (0·120 g) in ethanol (2 ml) to deposit immediately the picrate of ribosylguanylurea *III* in the form of needles, m.p. 172—174°C (decomposition). The next day, the needles were collected and washed with ethanol; yield, 0·163 g (70%). For $C_{13}H_{17}N_7O_{12}$ (463·3) calculated: 33·70% C, 3·71% H, 21·17% N; found: 33·96% C, 3·55% H, 21·21% N. Paper electrophoresis of the crude reaction mixture: anodic mobility (2·7 cm) of the compound *III* was determined by detections D_2, D_3 and D_4 (no other compounds were found by these detections; 1-β-D-ribopyranosyl-3-guanylurea[11] did not move at all under analogous conditions). The compound *III* was obtained also by keeping 5-azacytidine (*I*; 0·122 g; 0·5 milimole) with saturated methanolic ammonia (10 ml) and water (0·2 ml) at room temperature for five days and working up as above; yield, 0·095 g (41%) of the picrate of compound *III*, m.p. 172—174°C (decomposition).

B) 5-Azacytidine (*I*; 0·244 g; 1 millimole) was heated to 60°C in 10 ml of a borate buffer (pH 9·28) for five hours. The solution was taken down under diminished pressure in a rotatory evaporator, the residue dissolved in a small amount of water and the solution chromatographed in the solvent system S_3 on three sheets of paper Whatman No 3. By this technique, the mixture was separated into two bands (R_F values 0·09 and 0·31). The first broad band showed a weak absorption in ultraviolet light and could be detected by procedures D_2, D_4 and D_3 (violet coloration). This band was not homogeneous as shown by rechromatography (solvent system S_1) of the concentrated eluate and detection of the compound *I* (R_F value 0·58, a weak spot, detection procedure D_1) and *III* (R_F value 0·76, an intensive spot, detection procedures D_2, D_3 and D_4). The second band was narrow, did not absorb in ultraviolet light and could be detected by procedures D_2 (orange coloration) and D_3. Rechromatography of the concentrated eluate of this band revealed the presence of guanidine (R_F value 0·85, detection procedures D_2 and D_3) and D-ribose (R_F value 0·55, detection procedure D_4). Moreover, guanidine was identified in solvent systems S_2 and S_3 (R_F values 0·61 and 0·25, resp.) Precipitation with a solution of picric acid (0·050 g) in ethanol (1 ml) and keeping overnight afforded guanidine picrate which on recrystallisation from water melted at 333°C (decomposition) without depression on admixture with an authentic specimen. Yield, 0·018 g (6%). For $C_7H_8N_6O_7$ (288·2) calculated: 29·17% C, 2·80% H, 29·17% N; found: 29·45% C, 3·05% H, 28·94% N.

C) A solution of 5-azacytidine (*I*; 2·44 g; 0·01 mole) in water (100 ml) was refluxed for five hours and then taken down under diminished pressure in a rotatory evaporator. The residue was dissolved in methanol (100 ml) and treated with 50 g of moist Dowex 50 W(H^+) ion exchange resin, previously washed with water and methanol. After some minutes the resin was filtered off and washed with methanol. The filtrates were refiltered with charcoal (0·5 g) and evaporated under diminished pressure. The residual thick sirup was dissolved in boiling ethanol and the solution allowed to stand overnight. The crystals were collected (1·02 g) and the mother liquors concentrated to deposit additional 0·125 g of the same product. Total yield of the compound *IV* 1·245 g (65%); m.p. 169—170°C. For $C_6H_9NO_5$ (175·1) calculated 41·14% C, 5·18% H, 8·00% N; found: 41·12% C, 5·20% H, 8·18% N. In the carbonyl region of the infrared spectrum measured in a nujol suspension, a broad band at 1727 cm^{-1} with shoulders (1697 and 1747 cm^{-1}) was registered; no absorption band occurs in the amide II region. By the action of potassium periodate in a phosphate buffer (pH 6·8) at room temperature no consumption of the reagent was recorded in the course of eight hours. The compound reduced the Benedict solution at the boil. A short boiling with 1M aqueous potassium hydroxide (2—3 minutes) led to a deep decomposition (blackening). Chromatography of the hydrolysate in the solvent system S_3 revealed the presence of D-ribose (R_F value 0·20, detection procedure D_4). D-Ribose was found also in the mother liquors (solvent system S_3, R_F value 0·20, detection procedure D_4) after crystallisation of the compound *IV*. The collected ion exchange resin (*vide supra*) was washed with water and then eluted in a column with 3N-HCl (300 ml). The eluate was evaporated under diminished pressure and the residue dried over sulfuric acid and potassium hydroxide to afford crude crystalline guanidine hydrochloride. The crystals were dissolved in water (20 ml), the solution was filtered and poured into a stirred solution of potassium picrate (2·68 g) in hot water (60 ml) to afford guanidine picrate. The next day, the precipitate was collected and washed with water, ethanol and ether; yield, 2·23 g (77%). The recrystallised sample melted at 333°C (water; decomposition) without depression on admixture with an authentic specimen. For $C_7H_8N_6O_7$ (288·2) calculated: 29·17% C, 2·80% H, 29·17% N; found: 29·32% C, 2·93% H, 29·08% N. The above procedure was used also for the hydrolysis of 1-β-D-ribopyranosyl-5-azacytosine (0·244 g; 1 millimole) to give 0·096 g (55%) of the compound *IV*, m.p. 169—170°C, and 0·225 g (78%) of guanidine picrate. The mixture contained also D-ribose, as shown by chromatography (R_F value 0·20; detection procedure D_4) in the solvent system S_3.

Analysis of the Hydrolytical Course by means of 5-Azacytidine-^{14}C

The labeled compound was dissolved (concentration 10^{-4}M) in a borate buffer[1] (0·1M), the solution allowed to stand at 37°C for the period given in Fig. 1 and the resulting mixture separated by paper chromatography in the solvent system S_1. The radioactive components of the mixture were determined either directly on the chromatographical paper or in the eluates. The direct measurement was performed in an automatic apparatus with the 4π geometry using the Geiger-Müller tube with a thin end-window. D-Ribose was determined in the eluates of radioactive zones by means of the orcinol reaction. The fission products (Table II) were identified by comparison with compounds obtained preparatively. In the quantitative determination, the radioactive zones were eluted and the eluates evaporated; radioactivity of the residues was measured on a 2π windowless proportional flow counter in an infinitely thin layer. As shown by repeated chromatography of eluates and reelution, no considerable hydrolysis occurred during the work-up: thus, *e.g.*, in the case of ribofuranosylguanylurea *III* and guanidine, 90 and 100% recovery, resp., was observed.

3′,5′-Di-O-acetyl-α-D-ribofuro[1′,2′:4,5]-2-oxazolidone (*IVa*)

A stirred and ice-cooled solution of the compound *IV* (0·350 g; 2 millimole) in dry pyridine (5 ml) was treated with acetic anhydride (0·82 g). The mixture was allowed to stand in a stop-

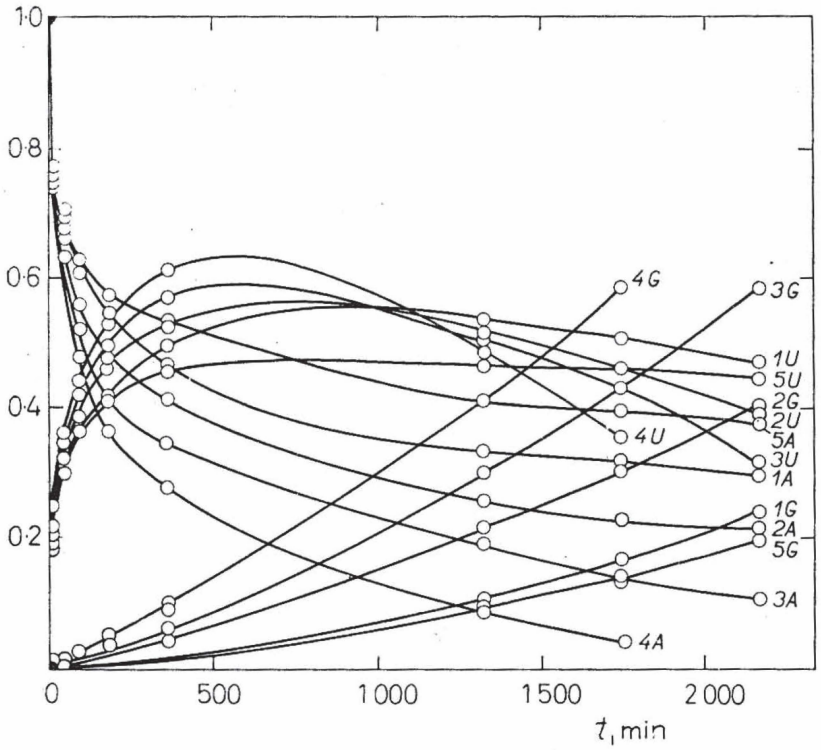


Fig. 1

Time Dependence of 5-Azacytidine-4-^{14}C Fission Products as Determined by Paper Chromatography

A 5-Azacytidine-4-^{14}C; *U* 1-Ribofuranosyl-3-guanylurea; *G* Guanidine; a borate buffer of the ionic strength 0·1—0·15; *1* pH 6·6; *2* pH 7·2; *3* pH 7·8; *4* pH 8·4; *5* water.

pered flask at room temperature for 24 hours and taken down under diminished pressure in a rotatory evaporator (bath temperature 40°C). The residue was coevaporated with four 10 ml portions of methanol and then dissolved in chloroform (20 ml). The chloroform solution was washed subsequently with water, aqueous sodium hydrogen sulfate, water, a saturated solution of sodium hydrogen carbonate, dried over anhydrous sodium sulfate and evaporated under diminished pressure. The residual sirup was dissolved in benzene (5 ml) and the solution was precipitated with light petroleum (20 ml). The sirupous precipitate failed to crystallize. It was, therefore, dissolved in ether (50 ml), the solution concentrated and the concentrate treated with light petroleum (30 ml). The supernatant was decanted and the residue dried at 0·1 mm Hg to afford 0·385 g (74%) of a white foam. For $C_{10}H_{13}NO_7$ (259·2) calculated: 46·34% C, 5·06% H, 5·40% N; found: 46·67% C, 5·20% H, 5·30% N. Infrared spectrum (chloroform): ν(CO) 1749 cm^{-1} (five-membered cyclic urethane), ν(NH) 3462 cm^{-1} (a five-membered cyclic urethane). The known *cis*-1′,2′,3′,4′-tetrahydronaphtho-[1′,2′:4,5]-2-oxazolidone was measured for comparison under same conditions: ν(CO) 1762 cm^{-1} and ν(NH) 3455 cm^{-1}.

3′,5′-Di-O-benzoyl-α-ribofuro[1′,2′:4,5]-2-oxazolidone (*IVb*)

A stirred and cooled solution of the compound *IV* (0·175 g; 1 millimole) in dry pyridine (3 ml) was treated with a solution of benzoyl chloride (0·42 g) in dry pyridine (2 ml), the mixture allowed to stand at room temperature for 24 hours, poured into cold water (20 ml) and extracted with chloroform (30 ml). The chloroform solution was washed subsequently with water, aqueous sodium hydrogen sulfate, water, a saturated aqueous solution of sodium hydrogen carbonate,

Table I

Hydrolytical Products of 5-Azacytidine (*I*)
Detected by Paper Chromatography or Obtained Preparatively

Medium, Temperature, Time	Products (Yield)	Detection
Water, 37°C, 0—2 200 min	*I*, *III*, guanidine (Fig. 1)	D_1, D_5
10^{-1}M Borate Buffers, pH 6·6; 7·2; 7·8; 8·4; 37°C, 0—2 200 min	*I*, *III*, guanidine (Fig. 1)	D_1, D_5
1% Aqueous Ammonia, 37°C, 120 min	*III* (91%)	D_1, D_5
1% Aqueous Ammonia, 100°C, 30 min	*III* (43%), guanidine (57%)	D_1, D_5
1M-HCl, 100°C, 30 min	*I* (51%), *V* (49%)	D_1, D_5
6M-HCl, 100°C, 30 min	*I* (5%), *V* (72%), *VI* (20%)	D_1, D_5
Water, 100°C, 300 min	isolated: 65% of *IV*, 77% of guanidine, D-ribose	D_2, D_3, D_4
1M Aqueous Ammonia, 37°C, 180 min	isolated: 70% of *III*	D_2, D_3
Aqueous-Methanolic Ammonia, 37°C, 5 days	isolated: 41% of *III*	D_2, D_3
10^{-3}M Borate Buffer, pH 9·3, 60°C, 300 min	isolated: 6% of guanidine, *I*, *III*, D-ribose	D_1, D_2, D_3, D_4

dried over anhydrous sodium sulfate and evaporated under diminished pressure. The residual sirup was chromatographed on a thin layer (18 × 48 cm) of alumina (Brockmann activity II—III) in ethyl acetate. The zone (R_F value 0·65) absorbing in ultraviolet light was separated mechanically, eluted with ethyl acetate and the eluate evaporated. The residue which failed to crystallize was purified by precipitation of its solution in benzene (5 ml) with light petroleum (20 ml) followed by precipitation of the ethereal solution (10 ml) with light petroleum (40 ml). The final precipitate was dried at 0·1 mm Hg to afford 0·245 g (66%) of a solid foam. Infrared spectrum (chloroform): ν(CO) 1 726 cm^{-1} (benzoate) and 1 777 cm^{-1} (a five-membered cyclic urethane), ν(NH) 3 462 cm^{-1} (a five-membered cyclic urethane). The nuclear magnetic resonance spectrum (in deuterochloroform) showed a band at 5·46τ, characteristic for the grouping $CH_2OCOC_6H_5$. For $C_{19}H_{17}NO_7$ (371·3) calculated: 3·77% N; found: 3·46% N.

Dimethylaminomethylenecyanoguanidine

A solution of cyanoguanidine (8·4 g; 0·1 mole) in hot absolute methanol (150 ml) was refluxed with dimethylformamide dimethylacetal (13·1 g; 0·11 mole) for six hours under exclusion of atmospheric moisture (potassium hydroxide safe-guard tube). In the course of heating, the product began to separate. The next day, the crystals (9·6 g) were collected, washed with methanol and dried under diminished pressure. Concentration of mother liquors afforded an additional crop (1·5 g). Total yield, 11·1 g (80%). The product melted (quick heating) at 194—196°C (decomposition). For $C_5H_9N_5$ (139·2) calculated: 43·15% C, 6·52% H, 50·33% N; found: 43·16% C, 6·75% H, 50·19% N.

N-Formyl-N′-cyanoguanidine

A solution of dimethylaminomethylenecyanoguanidine (5·56 g; 0·04 mole) in 2M-HCl (20 ml) was mechanically stirred at room temperature for 90 minutes. The reaction product separated from the solution. The mixture was cooled down in iced water and the product collected, washed with a little of cold water and dried in vacuo over concentrated sulfuric acid and potassium hydroxide; yield, 2·6 g. Evaporation of mother liquors afforded an additional crop (0·6 g) of the same product. Total yield 3·2 g (71%). When heated to a higher temperature, the compound is changed slowly without any melting (up to 350°C). R_F value: 0·71 (solvent system S_2, detection procedure D_1). Ultraviolet spectrum is given in Table II. For $C_3H_4N_4O$ (112·1) calculated: 32·14% C, 3·64% H, 49·99% N; found: 32·11% C, 3·65% H, 49·69% N.

Table II

Ultraviolet Spectra

Compound	Buffer, pH	λ_{max}, nm	log ε_{max}
I	6·6	244	3·8[a]
	7·2	244	3·8[a]
1-β-D-Glucopyranosyl-3-guanylurea	6·6	225	4·1[b]
	7·2	222	4·3[b]
	7·8	222	4·3[b]
	8·4	222	4·3[b]
III	6·6	225	[b]
Cyanoguanidine	water	215	4·17
N-Formyl-N′-cyanoguanidine	water	238	4·24

[a] Zero time extrapolation; [b] ε at 244 nm negligible.

Results and Discussion

Results of the isolation experiments are shown in Table I and Fig. 1; time dependence of the ultraviolet spectrum is shown in Fig. 2. On the basis of these results, the simplified Scheme 1 of the hydrolysis may be written. In acidic media, hydrolysis of the glycosidic bond and deamination takes place (*A*). In neutral and basic media, the reaction sequence *B* occurs. In analogy to 5-azauracil, the first stage of hydrolysis in neutral and basic media consists in an attack in position 6, as unequivocally shown by isolation of 1-β-D-ribofuranosyl-3-guanylurea (*III*). The furanoid structure of the compound *III* follows from its electrophoretical behaviour in a borate buffer. As expected, the corresponding 1-β-D-ribopyranosyl-3-guanylurea exhibits a lower electrophoretical mobility. The fact that the lactol ring structures remain unchanged in both above cases points to the probability that also the β-configuration at the glycosidic

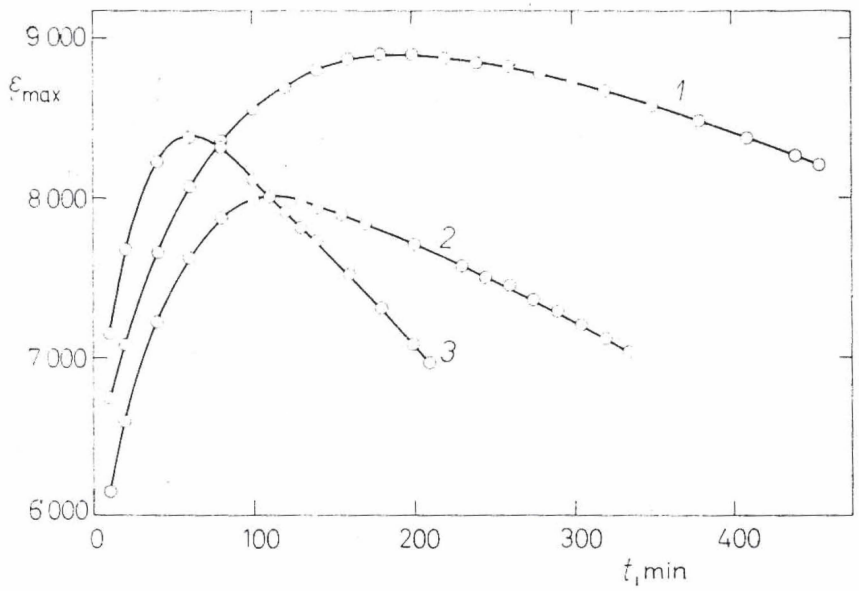
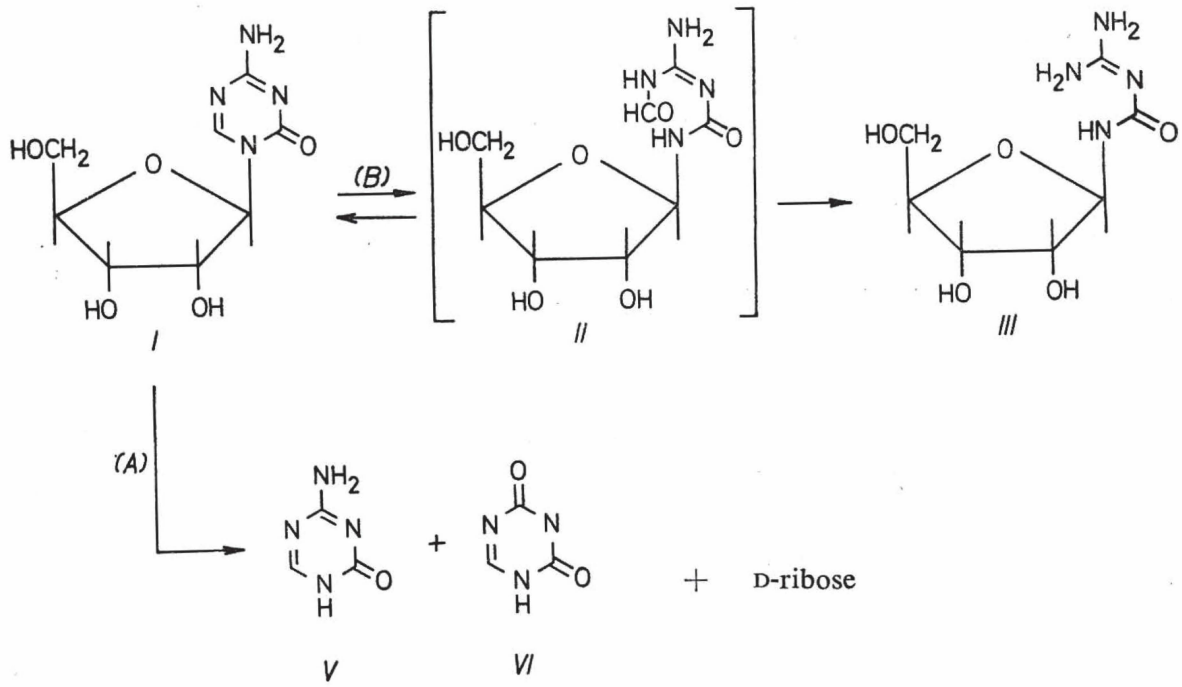
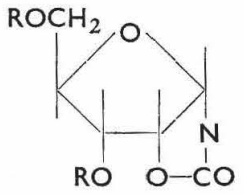


Fig. 2
Time Dependence of the Molar Absorptivity ε_{max} of 5-Azacytidine (*I*) at 244 nm
1 pH 6·6; *2* pH 7·2; *3* pH 7·8.



centra does not change. The N-formyl derivative *II* is assumed as intermediate of the hydrolysis $I \rightarrow III$. Isolation or identification of this intermediate by paper chromatography failed, but the time dependence of the ultraviolet spectrum points to its presence. It may be noted (Fig. 2) that in the first stage of the hydrolysis an increase of absorbance at 244 nm occurs. This increase can not be due to the formation

of the compound *III* in view of the spectral properties of the compound *III* and the analogous glucosyl derivative. On the other hand, it is very probable that compounds of the type *II* would strongly absorb at the above wave length (*cf.* comparison of cyanoguanidine and N-formyl-N′-cyanoguanidine dates in Table II). Attempts to synthesize the compounds of the type *II* were fruitless; all the formylation experiments with guanylurea led to the recovery of the starting compound or to the cyclic intermediate *V*. Notwithstanding, isolation of the compound *V* points to the reversibility of the reaction $I \rightleftarrows II$. The compound *III* as a relatively strong base is highly protonated in approximately neutral solutions.



IV, R = H
IVa, R = CH_3CO
IVb, R = C_6H_5CO

Hydrolysis of the compound *I* in boiling water afforded (in addition to guanidine and D-ribose) a good yield of α-D-ribofuro[1′,2′:4,5]-2-oxazolidone (*IV*), *i.e.*, a cyclic N-glycosyl urethane. Such a type of compounds has not been described yet. The structure of the compound *IV* is suggested on the basis of elemental analysis, infrared spectra of the derivatives *IVa* and *IVb* (a five-membered cyclic urethane), potassium periodate oxidation (absence of a vicinal diol) and nuclear magnetic resonance spectra (presence of the grouping $C_6H_5COOCH_2$ in the benzoate *IVb*). Under analogous hydrolytic conditions, the same product *IV* has been obtained also from 1-β-D-ribopyranosyl-5-azacytosine under isomerisation of the sugar moiety.

For the quantitative study of the hydrolysis, sufficient numerical data are not available. The compound *II* could not be detected either by chromatography or autoradiography. As suggested by the time dependence of ultraviolet spectra (*vide infra*), the compound *II* is present in a concentration exceeding 10%. The failure in isolation of the compound *II* is probably due to its instability: a decomposition takes place with the formation of compounds *I* and *III* in an unknown ratio. Results of concentration measurements of compounds *I* and *III* shown in Fig. 1 constitute merely the upper limit of concentrations present in the reaction mixture. Since the attempted preparation of the compound *III* in a free state and sufficient purity failed, the actual composition of the reaction mixture could not be estimated precisely. With the assumption that the compound *II* possesses log ε_{max} 4·18 and that the amount of compounds *III* and *IV* is negligible at the begin of hydrolysis, the rate of the pseudomolecular reaction $I \rightarrow II$ was estimated. As shown on comparison with data published in the previous paper[12], the hydrolysis $I \rightarrow II$ proceeds (pH 6·6, calculated ε_{max} of the compound *II* 15400, $k = 3{\cdot}4 \cdot 10^{-3}$ min^{-1}; pH 7·22, ε_{max} 15200, $k =$

$= 4 . 10^{-3}$ min^{-1}) just a little faster than the analogous hydrolysis stage of 5-azauracil.

As shown by the tests with *Escherichia coli* (the technique, *cf.*[12]), the compounds *III*, *IV* and guanidine are considerably less active than 5-azacytidine[2] which inhibits the growth by 50% at the concentration of 0·25 μg/ml (a 400 fold concentration is necessary in the case of the compound *III* and guanidine).

Quantum chemical calculations[5,6] indicate a low density of π electrons in position 6 (0·744) of 5-azauracil; the hight reactivity of this position towards hydroxylic ions[1,6] is in agreement with this finding. The electron density in position 6 of 5-azacytosine is a little higher than in 5-azauracil, but considerably lower than in cytosine (Table III). The rate of hydrolysis of 5-azacytidine is comparable with the decomposition rate of 5-azauracil. It may be expected in analogy with 5-azauracil derivatives that substitution with D-ribose causes an increase of the rate of hydrolysis. Different values of hydrolytical rates of various 5-azauracil and 5-azacytosine derivatives are therefore in agreement with the considerably low density of π electrons.

Table III

Static Indices[a]

Compounds	q_i							
	1	2	3	4	5	6	7	8
Cytosine[b]	1·639	0·796	1·438	0·828	1·169	0·835	1·492	1·803
V	1·620	0·790	1·441	0·800	1·320	0·746	1·484	1·798
V (in *VII*)	1·623	0·783	1·516	0·789	1·323	0·738	1·480	1·747
V[c]	1·642	0·765	1·708	0·820	1·333	0·742	1·456	1·535
V (in *VIII*)	1·634	0·771	1·649	0·799	1·331	0·736	1·462	1·616

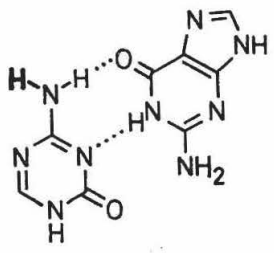
Compounds	p_{ij}								F_i	S_i
	12	23	34	45	56	61	27	48		
Cytosine[b]	0·375	0·433	0·636	0·525	0·758	0·529	0·778	0·470	0·455	—
V	0·364	0·429	0·643	0·508	0·719	0·572	0·785	0·483	0·441	0·254
V (in *VII*)	0·360	0·420	0·597	0·506	0·718	0·572	0·791	0·539	0·442	0·244
V[c]	0·355	0·372	0·445	0·478	0·731	0·553	0·816	0·717	0·448	0·260
V (in *VIII*)	0·357	0·387	0·495	0·490	0·724	0·562	0·809	0·660	0·446	0·247

[a] q_i, electron density of the position *i*; p_{ij}, order of the bond *ij*; F_i free valence, S_i superdelocalisibility for the position 6; [b] *cf.*[5]; [c] in the imino form.

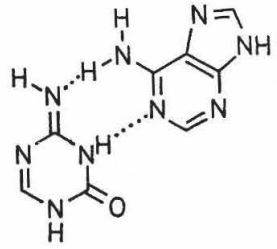
In acidic media, 5-azacytidine is readily cleaved with the formation of D-ribose, again in agreement with the quantum chemical calculations. As indicated by results of *A.* and *B.* Pullman[5,13], hydrolysis of the glycosidic bond occurs the easier the lower

is the density of π electrons on the nitrogen atom. Comparison of values in Table III shows that an increased hydrolysis rate may be expected with the 5-azacytidine derivatives.

The ready hydrolysis of 5-azacytidine (scheme 1, mechanisms *A* and *B*) may serve for explanation of the interesting biological properties of 5-azacytidine which acts as a cancerostatic agent as well as mutagen. As shown in the previous paper[1] on the basis of results of calculations in the Hückel approximation, the mutagenic properties are not caused by the amino–imino tautomerism; furthermore, the calculations indicated that formation of pairs with purine bases was energetically favourable. Incorporation of the aza analogue into pairs may modify the chemical properties of the heterocycle; no considerable change of reactivity by the pair formation, however, is indicated on comparison of static indices of 5-azacytosine (*V*) in the amino and imino form[1] with those of the pairs 5-azacytosine–guanine (*VII*) and 5-azacytosine–adenine (*VIII*) (Table III). Incorporation of 5-azacytosine derivatives into nucleic acids followed by a rapid hydrolytical cleavage of the 5-azacytosine nucleus may lead to destruction of certain information units. Moreover, the hypothesis concerning the biological action of 5-azacytosine derivatives is supported by comparison of quantum chemical indices of 5-azacytosine and cytosine (energetical indices were shown in the previous paper[1], static indices are listed in Table III): it may be seen that the most remarkable difference between both compounds consists in the character of position 6 important for the reactivity of the heterocyclic nucleus towards nucleophilic agents.



VII



VIII

The authors wish to thank Dr Z. Samek (Department of Molecular Spectroscopy of this Institute) for measurement and interpretation of nuclear magnetic resonance spectra. Analyses were performed in the Analytical Department (Head Dr J. Horáček) of this Institute by Mrs V. Rusová, Mrs E. Šípová and Mr V. Štěrba.

References

1. Piťhová P., Pískala A., Piťha J., Šorm F.: This Journal *30*, 1626 (1965).
2. Šorm F., Pískala A., Čihák A., Veselý J.: Experientia *20*, 202 (1964).
3. Piťhová P., Fučík V., Zadražil S., Šormová Z., Šorm F.: This Journal, in the press.
4. Čihák A., Škoda J., Šorm F.: This Journal *28*, 3297 (1963).
5. Pullman B., Pullman A.: *Quantum Biochemistry*. Interscience Publishers, New York 1963.
6. Zahradník R., Koutecký J., Jonáš J., Gut J.: This Journal *28*, 1499 (1963).

7. Gut J., Morávek J., Párkányi C., Prystaš M., Šorm F.: This Journal *24*, 3154 (1959).
8. Reindel F., Hoppe W.: Chem. Ber. *87*, 1103 (1954).
9. Roche J., Thosi N. Van, Hatt J. V.: Biochim. Biophys. Acta *14*, 71 (1954).
10. Viscontini M., Hoch D., Karrer S.: Helv. Chim. Acta *38*, 642 (1955).
11. Pískala A., Šorm F.: This Journal, in the press.
12. Piťhová P., Pískala A., Piťha J., Šorm F.: This Journal *35*, 90 (1965).
13. Pullman A. in the book: *Electronic Aspects of Biochemistry* (B. Pullman, Ed.). Academic Press, New York 1964.

Translated by J. Pliml.

Note added in proof: In formulae *IV*, *IVc* and *IVb*, N should read NH.

Резюме

П. Питьгова, А. Пискала, И. Питьга и Ф. Шорм: *Компоненты нуклеиновых кислот и их аналоги. LXVI. Гидролиз 5-азацитидина и его связь с биологической активностью.* Гидролиз 5-азацитидина (*I*) в нейтральной и щелочной средах приводит к следующим продуктам: 1-β-D-рибофуранозил-3-гуанилмочевине (*III*), гуанидину, α-D-рибофуро[1′,2′:4,5]-2-оксазолидону (*IV*) и D-рибозе. В качестве промежуточного продукта было спектрофотометрически обнаружено N-формильное производное соединения *III*. В кислой среде были получены 5-азацитозин (*V*), 5-азаурацил (*VI*) и D-рибоза. В противоположность 5-азацитидину (*I*), выделенные продукты обладают пониженной биологической активностью. Как показано опытами и на основании квантово-химических вычислений, биологическая активность соединения *I* связана, по-видимому, с включением производного 5-азацитозина в нуклеиновые кислоты и с последующим расщеплением триазинового кольца.