ALKYLATION OF NUCLEIC ACIDS AND THEIR COMPONENTS

IV.* ALKYLATION OF NUCLEOSIDES BY 4-[N-(β-CHLOROETHYL)-N-METHYLAMINO]BENZALDEHYDE

A. M. Belikova, N. I. Grineva, and G. G. Karpova

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Guanosine, cytidine, inosine, and thymine react with 4-[N-(β-chloroethyl)-N-methylamino]-benzaldehyde to give 7-alkylguanosine, 3-alkylcytidine, 1-alkylinosine, and 1-alkylthymine, respectively. The order of reactivities of the nucleosides with respect to 4-[N-(β-chloroethyl)-N-methylamino]benzaldehyde in 36% aqueous dioxane at 50°C and pH 5-6 is guanosine > inosine > cytidine > thymine. Inosine, cytidine, and thymine are 36%, 21%, and 13% as reactive as guanosine. Adenosine and uridine do not react under these conditions. The ratio of the rate constant for the alkylation of guanosine by 4-[N-(β-chloroethyl)-N-methylamine]-benzaldehyde and the rate constant for the hydrolysis of the latter in 17% aqueous dioxane at 50° and pH 5-6 is 10.5 ± 0.5 mole^{-1}. The alkylation of guanosine at pH 7.5 is accompanied by cleavage of the imidazole ring of the 7-alkylguanosine, which proceeds at a higher rate than the rate of the limiting step – ionization of 4-[N-(β-chloroethyl)-N-methylamino]benzaldehyde. The transformations of the alkylated nucleosides in acids and alkalis were studied, and the rate constants of these transformations were determined.

The alkylation of t-RNA by 4-[N-(β-chloroethyl)-N-methylamino]benzaldehyde (XIV) [1, 2] and its acetals [3, 4] leads to modification of t-RNA without cleavage of the polynucleotide chain [2, 4] and opens up the possibility for studying the effect of alkylation on the biological functions of t-RNA. In this connection, in the present research we have studied the alkylation of nucleosides by XIV to ascertain the reactivities of the bases in nucleic acids with respect to XIV and its acetals.

The alkylation of guanosine by XIV gives 7-{β-[N-methyl-N-(4-formylphenyl)amino]ethyl}guanosine (I) [5]. In 17% aqueous dioxane at 50°C in the presence of a tenfold excess of XIV, 9% I is formed by the time the major portion of the XIV is consumed. It might have been expected that other nucleosides would react even more slowly with XIV. In order to observe the reaction and obtain the reaction products, the alkylation of the nucleosides was carried out at a higher XIV concentration (50 mmole) in 36% dioxane. Under these conditions, the efficiency of the alkylation proved to be greater than in 17% dioxane [5], despite a reduction in the ionization constant of XIV by a factor of about three [6]. Under these conditions, 25% I is formed, while 11.4% I is formed after 3 days. In addition to guanosine, cytidine, inosine, and thymine react with XIV under such conditions. The reaction of XIV with uridine and adenosine was not detected by chromatography of the reaction mixture on Sephadex A-25 and paper or by analysis of the acid hydrolysis products on Dowex-50 (H^+); the starting compounds were quantitatively recovered from the reaction mixture.

*See [26] for communication III.

Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk.
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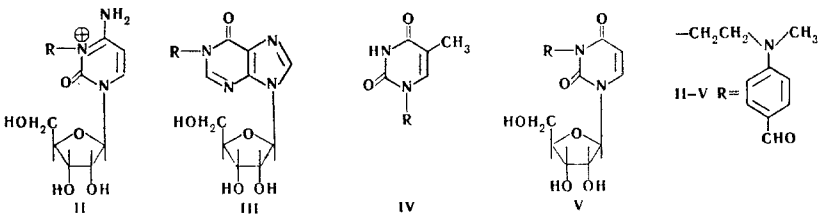
TABLE 1. R_f Values of the Starting Materials and Products

Compound	Systems				
	1	2	3	4	5
II	0,71	0,68	0,70	0,44	—
V	0,65	0,61	0,51	—	—
III	0,66	—	0,80	0,76	—
IX	0,64	0,47	—	0,76	—
IV	0,87	0,67	0,61	—	0,75
XIII	—	0,44	—	0,54	—
I	0,50	0,40	—	0,44	0,67
XII	0,44	0,44	—	0,32	—
Guanine	0,22	0,22	—	0,29	—
Cytidine	0,51	0,49	0,60	0,44	—
Uridine	0,44	0,65	0,64	0,44	0,82
Inosine	0,43	0,35	0,64	0,33	—
Thymine	0,64	0,74	0,71	—	—
Adenosine	0,54	—	0,59	0,38	—
XIV	0,89	0,94	0,82	0,90	—

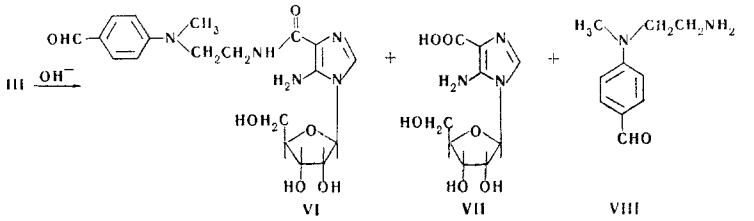
TABLE 2. UV Spectra of the Compounds Obtained

Compound	pH	λ_{max}, nm	$\varepsilon_{max} \cdot 10^{-3}$	λ_{min}, nm	$\varepsilon_{min} \cdot 10^{-3}$	ε_{260}	ε_{260}^{250}	ε_{260}^{270}	ε_{260}^{280}	ε_{260}^{290}	ε_{260}^{350}
II	1	285; 350	12,8; 25,0	260; 308	6,45; 6,8	6,45	1,06	1,39	1,89	1,89	3,9
	7	285; 350	12,8; 28,5	260; 306	7,9; 5,0	7,15	1,15	1,38	1,70	1,70	4,10
	13	350	30,05	297	5,0	9,30	1,12	0,93	0,73	0,58	3,32
V	1	248; 350	16,8; 27,8	268	7,7	11,3	1,89	0,75	1,22	1,89	2,40
	7	248; 350	16,8; 27,8	268	7,7	11,3	1,89	0,62	1,16	1,89	2,40
	13	248; 350	16,8; 27,8	268	7,7	11,3	2,15	0,67	1,17	2,00	2,40
III	2	245; 345	34,0; 31,0	228; 290	28,5; 11,8	23,0	1,41	0,78	0,59	0,52	1,28
	6	245; 345	31,6; 33,8	226; 285	25,8; 11,2	21,4	1,44	0,77	0,55	0,52	1,47
	10	245; 345	34,0; 28,5	231; 295	31,8; 13,8	24,7	1,32	0,81	0,68	0,57	1,16
IX	1	250; 355	—	287	—	—	1,37	0,67	0,44	0,34	2,48
	7	253; 355	—	230; 295	—	—	1,13	0,81	0,69	0,43	2,05
	13	253; 355	—	230; 292	—	—	1,04	0,83	0,59	0,32	2,06
VI	1	253; 348	23,5; 23,5	231; 300	21,5; 9,6	22,4	1,07	0,83	0,62	0,49	1,05
	7	253; 350	23,2; 28,6	235; 305	22,0; 12,0	22,3	1,04	0,88	0,72	0,62	1,29
	13	350	31,5	311	13,2	20,8	1,06	0,91	0,77	0,70	1,51
VII	1	263	4,72	226	1,70	4,65	0,88	0,88	0,54	0,19	—
	8	250	5,0	233	3,4	4,20	1,07	0,70	0,38	0,13	—
	12	250	5,1	230	3,4	4,48	1,07	0,74	0,44	0,20	—
VIII	1	245; 350	—	265	—	—	1,94	0,97	1,02	1,06	3,25
	2	245; 350	—	265	—	—	1,85	1,00	1,32	1,77	13,2
	7	245; 350	—	265	—	—	1,68	0,94	1,34	1,74	10,3
	12	245; 350	—	265	—	—	1,87	1,00	1,43	1,94	10,4
IV	1	265; 350	—	247; 280	—	—	0,83	1,06	0,95	1,06	3,4
	7	245; 350	—	278	—	—	1,41	0,76	0,70	0,80	2,45
	13	245; 350	—	233; 275	—	—	2,6	0,71	0,71	0,84	3,40
XIII	1	245; 335	—	230; 300	—	—	1,29	0,92	0,62	0,56	0,64
	7	240; 290; 335	—	266; 310	—	—	1,26	0,96	0,86	0,81	1,03
	13	240; 285; 335	—	265; 308	—	—	1,18	0,90	1,03	1,05	1,05

Cytidine reacts with XIV to give 3-{β-[N-methyl-N-(4-formylphenyl)amino]ethyl}cytidine (II) in 2.4% yield after 3 days. In alkali, II is deaminated to 3-{β-[N-methyl-N-(4-formylphenyl)amino]ethyl}uridine (V). The rate constant for the deamination of II in 0.04 N alkali at 96° is $(2.64 \pm 0.11) \cdot 10^{-4}$ sec^{-1} and corresponds to that for 3-methylcytidine [7]. Compound II is not deaminated in 0.5 N ammonium hydroxide during chromatography on Dowex-50 (NH_4^+), which is in agreement with the mechanism of the deamination of cytidine [8]. Under the influence of hydrochloric acid and Dowex-50 (H^+), II undergoes 25% decomposition to form substances that do not absorb at 350 nm.



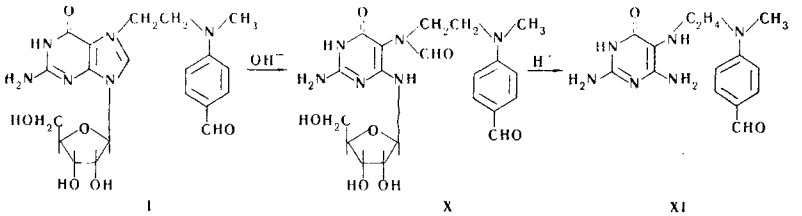
Under the above conditions, inosine undergoes 3.8% alkylation to give 1-{β-[N-methyl-N-(4-formylphenyl)amino]ethyl}inosine (III). Compound III is resistant to the action of 0.1 N alkali at room temperature, but undergoes hydrolytic cleavage of the pyrimidine ring after 30 min at 100° to give 52.5% imidazole-4-carboxamide (VI) and 47.5% imidazole-4-carboxylic acid (VII). Also found in the hydrolyzate in amounts equivalent to the VII content is 4-dialkylaminobenzaldehyde (VIII). The 4-(N-β-aminoethyl-N-methylamino)benzaldehyde structure [9] is proposed for this compound on the basis of the scheme of the transformation and the changes in the UV spectrum of VIII at pH 1 and 2 (Table 2).



The cleavage of the ring of III under these conditions is consequently accompanied by ~50% hydrolysis of the amide group in VI. The cleavage of the pyrimidine ring of 1-alkylinosines under these conditions is well-known [10, 11]. It unambiguously attests to the alkylation of inosine in the 1 position. This direction of the alkylation of inosine XIV does not contradict the data in [12], according to which the methylation of inosine derivatives by dimethyl sulfate leads to 67% of the 1-methyl derivative and 18% of the 7-methyl derivative. Ribose and 1-{β-[N-methyl-N-(4-formylphenyl)amino]ethyl}hypoxanthine (IX) are obtained in quantitative yield in the hydrolysis of III in 0.5 N HCl at 100°.

To estimate the reactivity of pseudouridine (Ψ), which constitutes up to 4% of t-RNA, we investigated the reaction of the accessible thymine with XIV. The alkylation of thymine gives 1.5% 1-{β-[N-methyl-N-(4-formylphenyl)amino]ethyl}thymine (IV) after 3 days. In analogy with this, it might be expected that the rate of alkylation of Ψ to form (probably) 1-substituted Ψ will be of the same order of magnitude as the rate for thymine [13].

The ratio of the rate constant for the alkylation of guanosine in 17% aqueous dioxane by XIV at pH 5-6 and the rate constant for the hydrolysis of XIV under the same conditions (k/a) is constant during the reaction at 10.5 ± 0.5 mole^{-1}. Only one substance – I – is formed during the alkylation of guanosine under these conditions. The k/a ratio for the alkylation of guanosine at pH 7-8 decreases with time [5]. To ascertain the reasons for the decrease in k/a, we measured the rate of hydrolytic cleavage of the imidazole ring of I to form pyrimidinone X under the alkylation conditions. The rate constant of this reaction at pH 7.5 and 50° is (8.0 ± 0.3) · 10^{-6} sec^{-1}. Thus the rate of hydrolytic cleavage of I proves to be greater than the rate of ionization of XIV – the step that limits the alkylation [6] [k = (3.58 ± 0.1) · 10^{-6} sec^{-1}] – and is consequently the reason for the decrease in k/a during alkylation in weakly alkaline media.



The resulting mixture of I and X cannot be analyzed, since X is eluted along with the starting materials during chromatography on Sephadex, and in addition to hydrolysis of the glycoside and formamidine bonds, acid hydrolysis of the reaction mixture and chromatography on Dowex-50 (H^+) lead to transformations with the participation of the aldehyde and NH_2 groups of the substances formed. Thus no more than 37% 5-alkylformamido-2,6-diaminopyrimidin-4-one (XI) – the product of the simple conversion of X in acid [5] – is determined during the action of hydrochloric and formic acids on X.

In this connection, it is interesting to compare the yield of I (obtained at pH 5-6, when a single substance is formed), determined on Sephadex A-25 (25.2%), with the yield of 7-alkylguanine (XII), obtained after hydrolysis of the reaction mixture in acid and chromatography on Dowex-50 (H^+) (23%). From the

TABLE 3. Identification of the Compounds from Their Differential UV Spectra with XIV

Compound	pH 1		pH 7		pH 13	
	λ_{max}, nm	λ_{min}, nm	λ_{max}, nm	λ_{min}, nm	λ_{max}, nm	λ_{min}, nm
II-XIV	279	245	280	—	267 b	—
3-Methylcytidine [24,7]	277,5	245	277,5	245	265	247
V-XIV	259 a	—	259	—	259 b	—
3-Methyluridine [25]	260	—	260	—	260	—
III-XIV	255; sh 270	230	253; Sh 270	225	253; Sh 270	
1-Methyl-2',3'-isopropylideneinosine [12]	—	—	250; Sh 270	—	250; Sh 270 b	—
IX-XIV	251	—	252,5	—	261,5	—
1-Methylhypoxanthine [23]	249	—	250	—	260	—
VI-XIV	265	250	266	242	266	242
1-β-D-Ribofuranosyl-5-amino-4-(N-β-carboxyethyl)imidazolecarboxamide [11]	267	255	268	—	268	—
IV-XIV	267	238	268	236	268	242
Thymidine [19, 20]	267	238	267	235	267	240,5
3-Methylthymine [15]	—	—	—	—	290 c	245c
1-β-D-(5'-phosphoryl)ribofuranozyl-5-aminoimidazole-4-carboxylic acid [10]	264 d	—	249 e	—	249 b	—

a) pH 2; b) pH 12; c) pH 11; d) 0.25 N H_2SO_4; e) pH 8.2.
Note: Sh – shoulder.

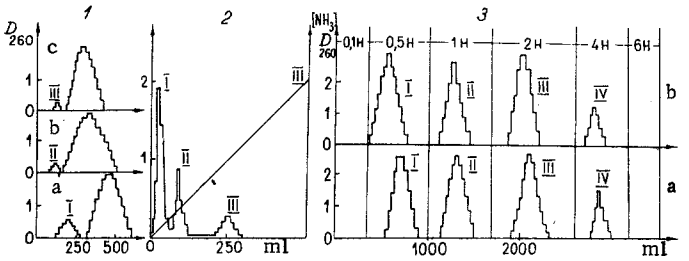


Fig. 1. Isolation of alkylnucleosides and alkylated bases: 1) on Sephadex A-25: a) 7-alkylguanosine (I), b) 1-alkylinosine (III); c) 1-alkylthymine (IV); 2) on Dowex-50 (NH_4^+): fraction I) cytidine and 4-(N-methyl-N-β-hydroxyethylamino)benzaldehyde; fraction II) XIV; fraction III) 3-alkylcytidine; 3) separation of the hydrolyzate of a mixture of alkylated guanosine (a) and inosine (b): fraction I) guanine (a), hypoxanthine (b); fraction II) 4-(N-methyl-N-β-hydroxyethylamino)benzaldehyde; fraction III) XIV; fraction IV) 7-alkylguanine (XII) (a) and 1-alkylhypoxanthine (IX) (b).

results of paper chromatography of the XII fraction, it is seen that hydrolysis is the reason for the reduced yield of XII, since it is accompanied by the side conversion (~6%) of XII to XIII with an absorption maximum at 335 nm. Lowering the temperature to 50° increases the hydrolysis time of I to 6 h [$k = (1.97 \pm 0.08) \cdot 10^{-4}$ sec^{-1} in 0.5 N HCl] and does not make it possible to eliminate the side reaction.

It follows from the above that the reactivities of nucleosides in the reaction with XIV in 36% aqueous dioxane at 50° (pH 5-6) decrease in the order guanosine > inosine > cytidine > thymine ≫ adenosine and uridine; and inosine, cytidine, and thymine are 36%, 21%, and 13%, respectively, as reactive as guanosine (k/a 10.5 $mole^{-1}$). The absence of the alkylation of adenosine is somewhat unusual, although it is known that some reagents react more slowly with adenosine contained in polynucleotides than with cytidine [14]. The results make it possible to assume that the rate of alkylation of adenosine by XIV, if this reaction does occur, is at least two orders of magnitude less than the rate of alkylation of guanosine. Thus a I concentration of 0.01 μmole/ml for a volume of 20 ml can be quite accurately (±0.5%) determined in the separation of the reaction mixture from the alkylation of guanosine by XIV on Sephadex A-25. Traces of alkylation products

are not detected in the reaction of adenosine with XIV for 3 h, while 0.57 μmole/ml of I is formed in the same period of time. Except for those cases [15] in which the competition factors of two aliphatic β-chloroethylamines in the reaction with the major nucleotides were determined, there has been practically no quantitative comparison of the reactivities of nucleosides and nucleotides with respect to alkylating substances. The reactivities of the major bases constituting polynucleotides were qualitatively estimated for a number of reagents [14]. The reactivities of the minor components have not been compared. The order of activities of the major bases for the alkylation of polynucleotides by XIV at pH 7-8 is presented in [16]. The reactivities of the major nucleosides that we found generally agree with the known reactivities for polynucleotides, except for the behavior of adenosine.

EXPERIMENTAL

Descending chromatography on Leningrad medium paper in the following solvent systems was used in this study: isopropyl alcohol–ammonium hydroxide (sp. gr. 0.88)–water (7:1:2) (1), isopropyl alcohol–concentrated HCl–water (170:41:39) (2), isopropyl alcohol–water (6:4) (3), tert-butyl alcohol–water (6 : 4) (3), tert-butyl alcohol–methyl ethyl ketone–HCOOH–water (40 : 30 : 15 : 15) (4), ethanol–1 M ammonium acetate (pH 6.5) (5 : 2) (5). The alkylnucleosides were eluted from the paper with a stream of water, while alkylguanine was eluted with 0.1 N HCl. The solutions were concentrated at 30° (12 mm) with a rotary evaporator. The ribose content in the purine nucleosides was determined by the orcinol reaction [17], while the ribose content in the pyrimidine derivatives was determined by periodate oxidation [18]. The percentage of dialkylaminobenzaldehyde residues was determined from the absorption of the alkylation products at 350 nm, using the extinction of XIV [16] ($28.8 \cdot 10^3$). The known compounds were identified from the UV spectra [19, 20] and the R_f values. The percentages of the substances were determined from $D_{350\,nm}^{pH\,1}$ or $D_{260\,nm}^{pH\,1}$. The R_f values and the UV spectra of the new compounds are presented in Tables 1 and 2. The new compounds were identified from the differential UV spectra of these compounds with XIV (Table 3), from the ribose/dialkylaminobenzaldehyde residue ratio, and from transformations. Guanosine from the Reanal Company (Hungary) was recrystallized from water to give a product of 100% purity with mp 237° (dec.). Inosine from the Reanal Company was recrystallized from absolute ethanol to give a product of 97% purity with mp 218° (dec.). The thymine was obtained from the Th. Schuchardt Company (Federal Republic of Germany). Sephadex A-25 from the Farmacia Company (Sweden) was treated with 1 M NaCL [21]. Dowex-50W×4 (200-400 mesh) from the Serva Company (Federal Republic of Germany), which was converted to the H^+ form [22], was washed successively with 6 N and 0.1 N HCl; the Dowex was converted to the NH_4^+ form by washing with 2 N NH_4OH. Compound XIV was obtained by the method in [5] and crystallized from cyclohexane (mp 67.5-68.0°). The spectra were recorded with an SF-4 spectrophotometer, and the pH values were measured with an LPU-01 pH meter.

Alkylation of Nucleosides. A solution of 3.2 mmole of XIV in 36 ml of dioxane was added at 50° to a solution of 0.32 mmole of the nucleoside in 64 ml of water; the pH was brought up to 6, and the solution was held at 50° for 3 days while maintaining the pH at 5-6 by the periodic addition of 0.1 N KOH. The alkylation of guanosine at pH 7-8 was carried out as described above for 7 days.

Isolation of 7-{β-[N-Methyl-N-(4-formylphenyl)amino]ethyl}guanosine (I). At the end of the alkylation, the reaction mixture at pH 6 was cooled, and 10-ml samples of it were chromatographed on Sephadex A-25 (43 by 3.7 cm) with elution of 10-ml fractions by water (Figure 1, graph 1). Fractions I, which have absorption at 350 nm and D(350/260) = 1.9-2.0, were combined and dried lyophilically to give 11.4% of I after 3 days and 25% after 7 days.

1-{β-[N-Methyl-N-(4-formylphenyl)amino]ethyl}inosine (III) and 1-{β-[N-Methyl-4-(4-formylphenyl)-amino]ethyl}thymine (IV). These compounds were isolated in the same way as I (Fig. 1, graph 1) to give 3.8% of III, The ribose[dialkylaminobenzaldehyde residue was 1.00 : 1.03. The differential UV spectra of III with XIV correspond to the spectra of 1-methylinosine (Table 3). The yield of IV was 1.5%. The differential spectra of IV with XIV were similar to the spectra of thymidine and differed from those of 3-methylthymine. The alkylthymine[dialkylaminobenzaldehyde residue ratio was 1.00 : 1.02.

Isolation of 3-{β-(N-Methyl-N-(4-formylphenyl)amino]ethyl}cytidine (II). A) The reaction mass was concentrated to about a tenth of its original volume, and the precipitated XIV was removed. The solution was chromatographed on paper in system 1 to give 2.4% of II with R_f 0.71. The ribose/dialkylaminobenzaldehyde residue ratio was 1.0 : 1.1. The differential UV spectra with XIV corresponded to the UV spectra of 3-methylcytidine (Table 3).

B) A 5-ml sample of the reaction mass was separated on Dowex-50 (NH_4^+) (7 by 1.7 cm) in a linear gradient of ammonia concentrations from 0 to 1 N; the volumes of the mixing tank and reservoir were 0.25 liter each. The fractions were combined according to the chromatography profile (Fig. 1, graph 2), and 1 N HCl was added to pH 7. The percentage of II was determined from $D_{350\,nm}$ and $\varepsilon_{350\,nm}^{pH\,7} = 28.5 \cdot 10^3$. The yield of II was 0.6 μmole (2.4%). On Dowex-50 (H^+) in hydrochloric acid, II is eluted together with XIV by 2 N HCl and undergoes 25% decomposition. The site of elution and the stability in acid were judged from the results of chromatography of pure II on Dowex-50 (H^+). For this, 0.25 μmole of II in 6 ml of 0.1 N HCl was chromatographed on Dowex-50 (H^+). For this, 0.25 μmole of II in 6 ml of 0.1 N HCl was chromatographed on Dowex-50 (H^+) (12 by 0.9 cm) with successive elution by 100-ml portions of 0.1, 0.5, 1, 2, and 4 N HCl. The volume of the fractions was 4 ml, and the rate of elution was 24 ml/h. The yield of II was 0.18 μmole (75%).

Kinetics of the Alkylation of Guanosine by XIV. A solution of 0.5 mmole of XIV in 17 ml of dioxane was added to 0.37 mmole of guanosine in 83 ml of water, and the mixture was thermostatted at 50° while maintaining the pH at 5.5-6.0 by the periodic addition of 0.1 N KOH. After 4 and 8 h, 20- and 10-ml samples, respectively, were selected and applied to Sephadex A-25 (43 by 3.7 cm). The elution was carried out with water at 60 ml/h, and the volume of the fractions with D(350/260) = 1.9-2.0 were combined. The percentage of I [9] was determined from D_{350} and $\varepsilon_{358}^{pH\,7} = 24.8 \cdot 10^3$, while k/a was calculated as in [16].

Hydrolysis of I in Acid. A 5-μmole sample of I was allowed to stand in 20 ml of 0.5 N HCl for 30 min at 100°. The mixture was cooled, and the ribose and XII were separated on Dowex-50 (H^+) (Fig. 1, graph 3). The yield of XII was 83%. Fraction XII was chromatographed on paper in system 2, and XIII (6%) was eluted by 0.1 N HCl.

Kinetics of the Hydrolysis of I in Acid. A mixture of 0.102 μmole of I in 3 ml of 0.5 N HCl was held at 50°, and the isosbestic points at 255 and 311 nm and the spectral changes at 350 and 290 nm were recorded.

1-{β-(N-Methyl-N-(4-formylphenyl)amino]ethyl}hypoxanthine (IX). This compound was obtained by the hydrolysis of III in acid as in the hydrolysis of I (Fig. 1, graph 3) to give 90% of IX. The differential UV spectra of IX with XIV correspond to the UV spectra of 1-methylhypoxanthine (Table 3). The IX/VIII residue ratio was 1.0 : 1.1. The percentage of IX was determined from the differential spectrum of IX with XIV from D_{260}^{pH1} and $\varepsilon_{260}^{pH\,1}$ of 1-methylhypoxanthine [27].*

Kinetics of the Hydrolytic Cleavage of I. A mixture of 2.04 μmole of I and 60 ml of 17% aqueous dioxane was held at 50° while maintaining the pH at 7.5 ± 0.2 by the periodic addition of 0.1 N KOH. Samples (3 ml) were selected, and the conversion of I to 5-{N-β-[N-methyl-N-(4-formylphenyl)amino]ethyl}-formamido-6-ribofuranosyl-2,6-diaminopyrimidin-4-one (X) was recorded from the change in absorption at 250, 272, 290, 300, and 350 nm. The isosbestic points were observed at 262 and 283 nm.

Hydrolytic Cleavage of the Pyrimidine Ring of III. A mixture of 0.9 μmole of III and 3 ml of 0.1 N KOH was held at 100° for 30 min. The solution was chromatographed on paper in system 5 to give VI (R_f 0.66), VII (R_f 0.45), and VIII (R_f 0.84). 1-Ribofuranosyl-4-{N-B-[N-methyl-N-(4-formylphenyl)amino]-ethyl}-5-aminoimidazole-4-carboxamide (VI) was obtained in a yield of 0.6 μmole (52.5%). The ribose/VIII residue ratio was 1.0 : 1.0. The differential UV spectra of VI with XIV were similar to the spectra of 1-ribofuranosyl-5-aminoimidazole-4-carboxylic acid (VII) was 0.51 μmole (47.5%). The VII/ribose ratio was 1.00 : 0.91. According to the UV spectra, VIII is probably 4-(N-β-aminoethyl-N-methylamino)benzaldehyde. The yield of VIII was 0.51 mmole (47.5%) with respect to $\varepsilon_{350}^{pH\,1}$ (taken as $14.1 \cdot 10^3$), similar to $\varepsilon_{350}^{pH\,1}$ of 4-(N-β-hydroxyethyl-N-methylamino)benzaldehyde [9].

Hydrolysis of X in Formic Acid. A mixture of 0.64 μmole of X and 0.3 ml of HCOOH was held at 25° for 4 days. The solution was lyophilized, and the residue was dissolved in 2 ml of 0.1 N HCl. The solution was chromatographed on Dowex-50 (H^+) (12 by 0.9 cm). The elution was carried out successively with 100-ml portions of 0.1, 0.5, 1, and 2 N HCl, followed by 4 N HCl (170 ml) and 6 N HCl (60 ml). The volume of the fractions was 4 ml, and the rate of elution was 24 ml/h. The fractions contained 0.6 μmole (94%) of ribose, according to the orcinol reaction, and 0.24 μmole (37%) of 5-N-{β-[N-methyl-N-(4-formylphenyl)-amino]ethyl}formamido-2,6-diaminopyrimidin-4-one (XI) [5].

Kinetics of the Deamination of II. A mixture of 0.6 μmole of II and 20 ml of 0.04 N KOH was held at 96°. Samples (3 ml) were selected, 1 N HCl was added to bring the pH to 2, and the conversion of II to V

*There is no [27] in Literature Cited of Russian Original – Consultants Bureau.

was recorded from the spectral changes at 248 and 285 nm. The isobestic points were observed at 265 and 310 nm.

3-[β-[N-Methyl-N-(4-formylphenyl)amino]ethyl]uridine (V). This compound was obtained by the deamination of 0.09 μmole of II for 6 h, as in the preceding case. The solution was chromatographed on paper in system 1, and V with R_f 0.65 was eluted quantitatively with water. The ribose/R residue ratio was 1.00 : 0.87. The differential UV spectra of V with XIV correspond to the UV spectra of 3-methyluridine [24] (Table 3).

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