Volume 5 Number 3 March 1978 Nucleic Acids Research

New observations concerning the chloroacetaldehyde reaction with some tRNA constituents. Stable intermediates, kinetics and selectivity of the reaction

J. Biernat, J. Ciesiołka, P Górnicki, R.W. Adamiak, W.J. Krzyżosiak and M. Wiewiórowski+

Department of Stereochemistry of Natural Products, Institute of Organic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland

Received 22 December 1977

ABSTRACT

The stable intermediates formed in the reaction of cytosine, cytidine and adenosine with chloroacetaldehyde were isolated. The $-CH_2CH/OH/-$ bridge between the exo and endo nitrogen atoms of the parent base was found in these compounds by means of PMR spectroscopy. Their acid-induced dehydration resulted in formation of appropriate ethenoderivatives. The rate constants of the intermediate formation and its dehydration were found to be $38x10^{-4}$ and $47x10^{-4}$ /min^{-1}/ for adenosine, and $33x10^{-4}$ and $10x10^{-4}$ /min^{-1}/ for cytidine. The pH range of 4.5 - 5.0 was found to be optimum for both adenosine and cytidine reactions. The quantitative modification of these two nucleosides in the presence of guanosine may be achieved with high selectivity only at a low pH of 3.0 - 4.0. N^6-methyladenosine and N^4-methylcytidine react quantitatively with chloroacetaldehyde and the reaction rate is higher than in the case of the parent nucleosides. The structure of the reaction products was assigned on the basis of PMR spectroscopy.

INTRODUCTION

A number of reactions between chloroacetaldehyde and nucleic acid constituents[1-5] and their analogs[3,6-9] have been reported since the discovery of a chloroacetaldehyde reaction with adenine and cytosine derivatives[10]. The fluorescent reaction products were the subject of many physicochemical studies[11-16] and have found wide application in biochemistry[17,18]. Because of the mildness of the reaction conditions[10] and the selectivity for specific bases[1,2] the chloroacetaldehyde reaction was used in the chemical modification of nucleic acids[19-22]. In each reported case the reaction with chloroacetaldehyde was found to be a very clear and simple process leading directly to one product with nearly quantitative yield.

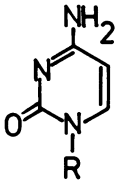
These results were very promising, so in starting our systematic study in the field of chemical modification of specific tRNAs[23], we decided that chloroacetaldehyde would be the first reagent we would use. At the very begining we examined the reaction once more on the monomer level; in order to reinvestigate the optimum reaction condi-

tions and to look for some intermediates which one should expect in such complex reactions. We had also decided to find out more information concerning the reactivity of common, modified and hypermodified tRNA constituents. It seemed that these studies on monomers would be very helpful in the interpretation of the data we could obtain on tRNA.

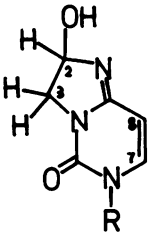
RESULTS AND DISCUSSION

Reaction intermediates

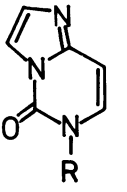
Up to now, the reaction of adenine and cytosine derivatives with chloroacetaldehyde has been described as a reaction leading directly from the substrate to the product without any intermediates[1,2]. At the same time it was suggested[3] that the reaction path goes through three steps : 1. alkylation of the endocyclic nitrogen atom, 2. cyclization by addition of exoamine group to the carbonyl bond, 3. double bond formation by dehydration. Precise tlc control of these reactions in proper solvent systems enabled us to observe the presence of intermediates /2/, /5/, /8/ and /11/ in the reaction mixtures.
Isolated compounds /2/, /5/ and /11/ were transformed quantitatively to the corresponding ethenoderivatives in the presence of catalytic



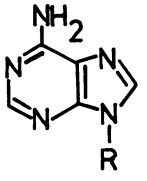
/1/ R = H
/4/ R = Ribose



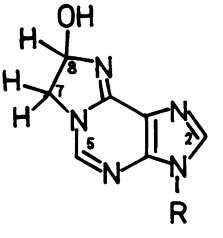
/2/ R = H
/5/ R = Ribose



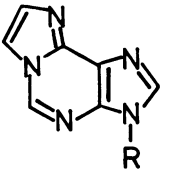
/3/ R = H
/6/ R = Ribose



/7/ R = H
/10/ R = Ribose



/8/ R = H
/11/ R = Ribose



/9/ R = H
/12/ R = Ribose

amounts of trifluoroacetic acid. Such transformation for /2/ followed by PMR is shown on Fig.1.

The PMR spectra of /5/ and /11/ are more complex because the signals of the $-CH_2CH/OH/-$ bridge protons and sugar protons are to some extent overlapped. Compounds /8/ and /11/ are less stable than /2/ and /5/. Isolated /11/ always contains ca. 20% of /12/.

Reaction kinetics

From the kinetical point of view, the reaction of adenine and cytosine derivatives with chloroacetaldehyde consists of two steps :

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

where k_1 is the rate constant of substrate disappearance /intermediate formation/ and k_2 is the rate constant of product formation /intermediate dehydration/. The reaction kinetics was studied for the /4/→/6/ and /10/→/12/ transformations in pH 3.5 and 4.5, respectively. These pH conditions were found to be optimum by Kochetkov[1] and confirmed by Leonard[2]. Intermediate /5/ shows higher stability in comparison with /11/ - Fig.2a,b. - and it is the reason why /11/ was difficult to iso-

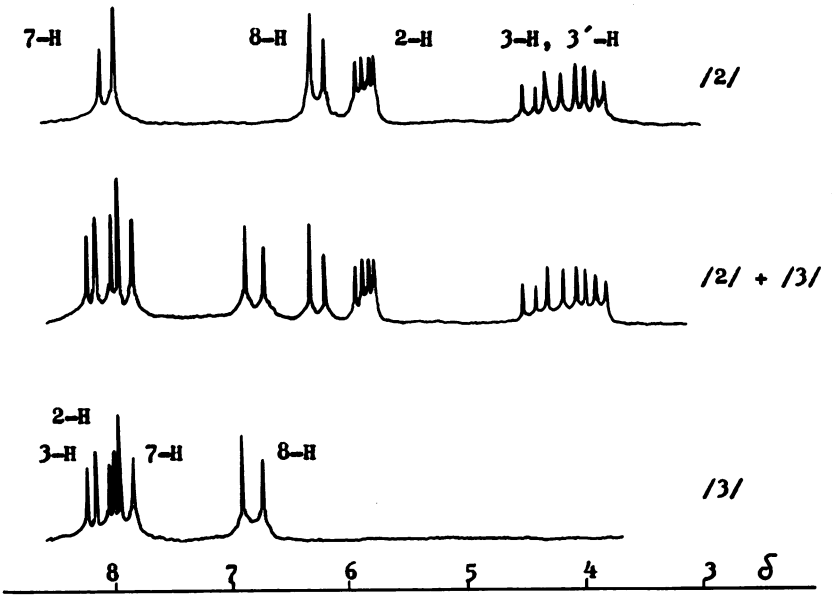


Fig.1. Three representative spectra of the /2/→/3/ transformation in DMSO-d_6.

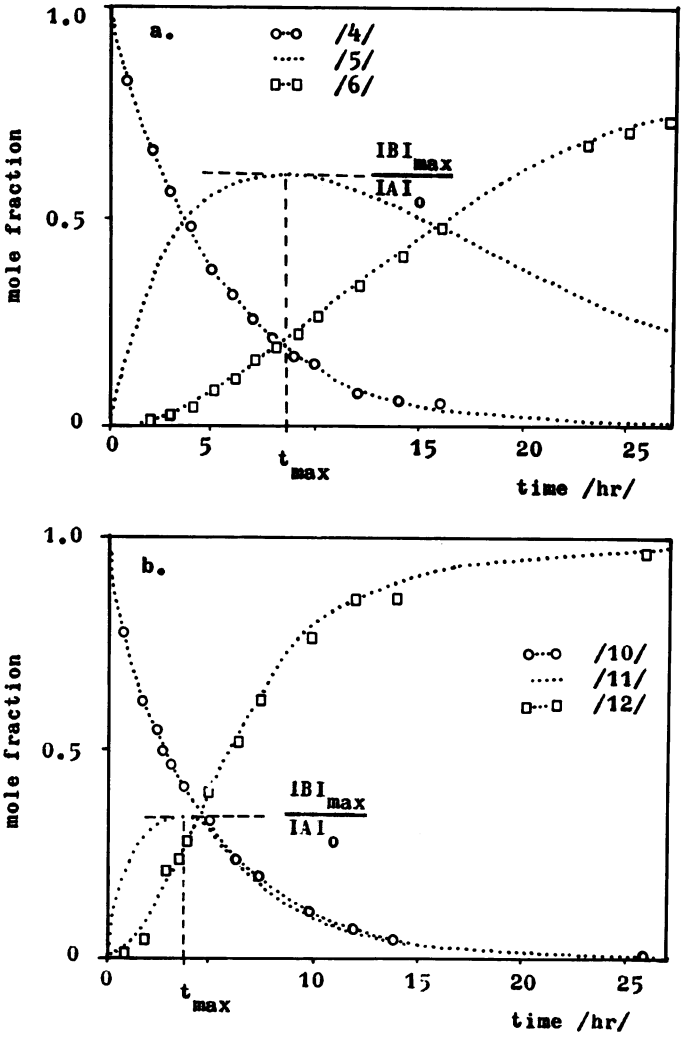


Fig.2a. Reaction of cytidine with chloroacetaldehyde, pH 3.5, 37°C.
2b. Reaction of adenosine with chloroacetaldehyde, pH 4.5, 37°C.

late in the pure state. The rate constants k_1 and k_2 for the /10/→/12/ transformation are comparable /Table 1/. In the case of the transformation /4/ ⟶ /6/ the formation of /5/ is three times faster than its dehydration. Thus, the second step /k_2/ determines the rate of the reaction and the complete disappearance of /4/ is not correlated with quantitative formation of /6/.

On the basis of the above results, we suppose that authors re-

Table 1. The rate constants and other kinetical parameters for reactions of adenosine and cytidine with chloroacetaldehyde.

Substrate	k_1 /min^{-1}/	k_2 /min^{-1}/	$\frac{k_2}{k_1}$	$\frac{IBI_{max}}{IAI_o}$	t_{max} /hr/
/4/	$33x10^{-4}$	$10x10^{-4}$	0.31	0.59	8.7
/10/	$38x10^{-4}$	$47x10^{-4}$	1.23	0.33	3.9

porting the "lability" of oligonucleotides containing modified cytosine residues[21] observed the mixture of ethenoderivatives and intermediates.

Optimum pH

The reactions of /4/ and /10/ with chloroacetaldehyde were studied in order to find such a pH value, at which the selective modification of one of these compounds would be achievable. The pH range of 3.0 - 7.0 chosen for these studies was limited by the "lability" of the tRNA molecule on the one hand, and by instability of the chloroacetaldehyde solution, on the other. It was found that at a pH range of 4.5 - 5.0 is optimum for both /4/ ⟶/6/ and /10/⟶/12/ transformations - Fig.3a,b.

Furthermore we have found that in the pH range of 3.5 - 7.0, /4/ reacts faster than /10/ - Fig.5. and the greatest difference in the reaction rates is observed at pH 7.0. When the reactions are performed at that pH value 60% of /10/ and 90% of /4/ disappears after five hours of reaction.

Selectivity of reaction

Since we have been interested in the possibility of quantitative modification of both /4/ and /10/ in the presence of other tRNA constituents, the behaviour of guanosine /13/ was studied first[24]. Although it was reported[1] that /13/ was unreactive in the pH range of 2.0 - 5.0, we found considerable reactivity of /13/ at pH > 4.0 - -Fig.4a. In our studies the disappearance of /13/ was measured after the time necessary for the complete modification of /4/ and /10/. It was observed that in the pH range of 3.5 - 4.0 only about 5% of /13/ underwent the reaction, while at pH 6.0 at least 40%.

The reactivity of some accesible modified and hypermodified tRNA constituents was also investigated. When N^4-acetylcytidine /14/ was allowed to react with chloroacetaldehyde, /6/ was found to be the only

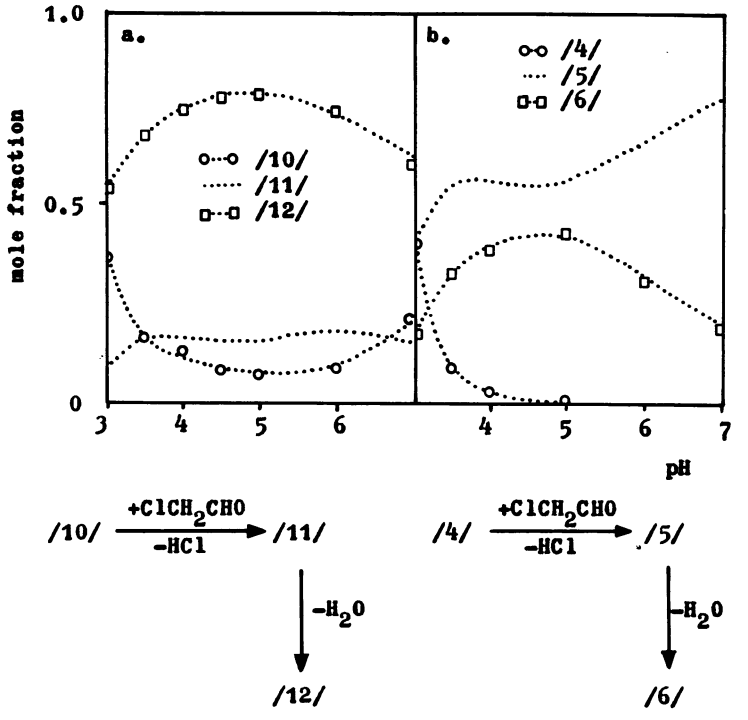


Fig.3. Dependence of a. adenosine and b. cytidine reactions on pH, measured after 10 and 12 hours, respectively.

product of the reaction. It is worth mentioning that this reaction requires more acidic conditions /pH 3.0 - 5.0/ because these conditions are necessary for initial acid catalyzed deacetylation of /14/ - Fig.4b.

N^6-methyladenosine /15/ and N^4-methylcytidine /17/ react with chloroacetaldehyde faster than their parent compounds /10/ and /4/, and the pH range of 5.0 - 5.5 was found to be optimum for these reactions - Fig.4c,d. It is proposed, on the basis of PMR spectroscopy that the quantitatively formed reaction products have the structures /16/ and /18/. The compounds /16/ and /18/ can be considered as stable alkylated analogs of intermediates /5/ and /11/.

The reactivity of N^6-/ Δ^2-isopentenyl/adenosine /19/ is similar to that of /10/ and the reaction rate is the highest at the pH range of 4.5 - 5.0. The structure /20/ was ascribed to the reaction product by Leonard[2] - Fig.4e.

N^6-/N-threonylcarbonyl/adenosine /21/ undergoes the reaction so

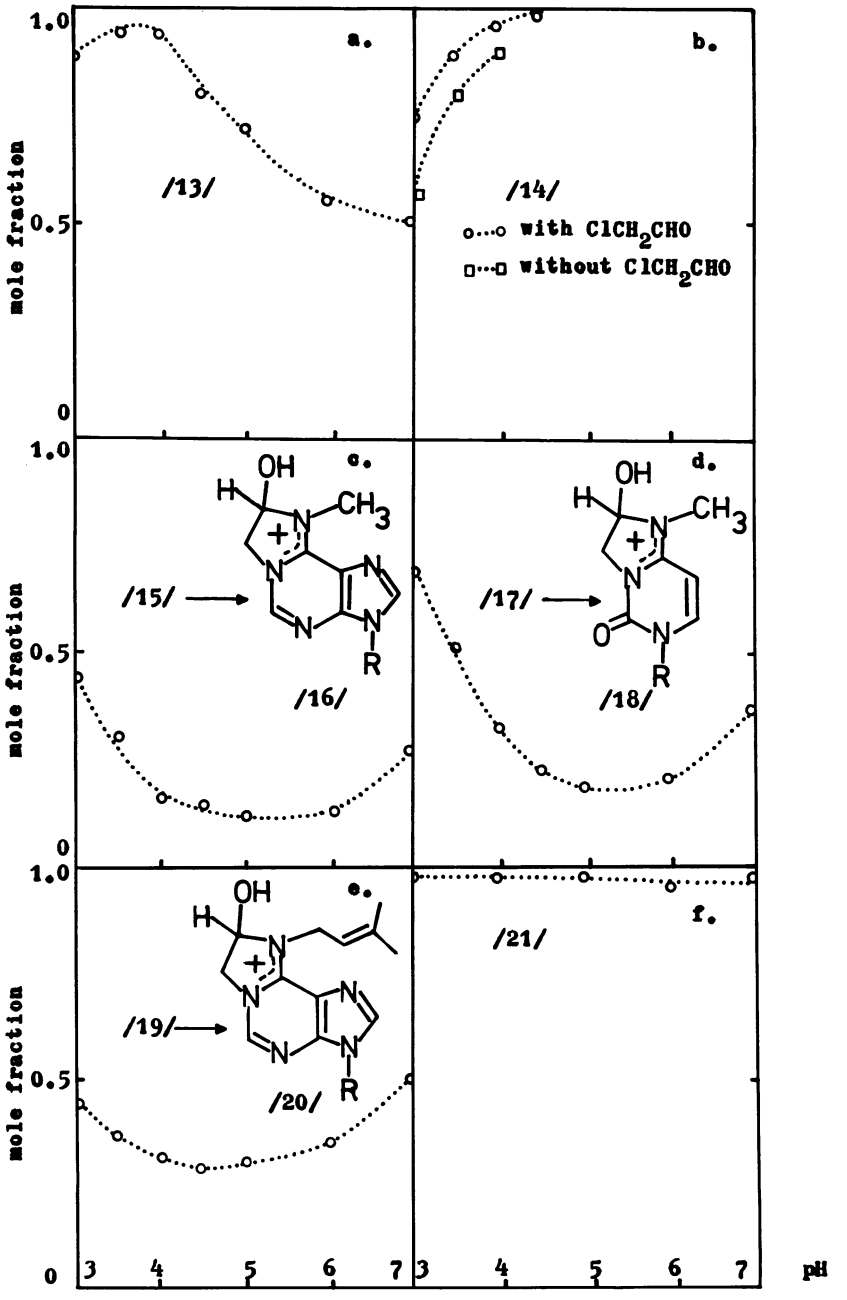


Fig.4. Dependence of substrate disappearance on pH in the reactions of a. guanosine /13/, b.N^4-acetylcytidine /14/, c. N^6-methyladenosine /15/, d. N^4-methylcytidine /17/, e. N^6-/Δ^2-isopentenyl/adenosine /19/ and N^6-/N-threonylcarbonyl/adenosine /21/ with chloroacetaldehyde, at 37°C, after 28, 28, 4, 2, 4,and 28 hours of reaction, respectively.

slowly that its reactivity can be neglected in our considerations - - Fig.4f.

The obtained results show that contrary to the alkylated on the exoamino group adenosine and cytidine derivatives the acyl compounds /including N^6-benzoyladenosine/ do not react with chloroacetaldehyde.

Final remarks

Our studies on reaction between chloroacetaldehyde and adenine and cytosine derivatives reveal that the reaction is not a direct one, but goes through stable intermediates. Nevertheless, the question how these intermediates are formed still remains open. Leonard, in one of his early papers[3] , suggested the alkylation of the endo nitrogen atom as an initial step in the reaction, which is followed by the condensation of the exoamino and carbonyl groups. A similar point of view was presented by Kochetkov[25].

We hope that our results, showing the possibility of an alternative and/or competitive mechanism will shed some new light on this problem.

It is shown on Fig.5. that in the case of adenosine, N^6-methyladenosine, N^6-/Δ^2-isopentenyl/adenosine and N^4-methylcytidine the reaction rates have a distinct maximum at a pH of about 5.0. In the case of cytidine the reaction rate also reaches the maximum value at a pH of about 5.0; however, it does not decrease at higher pH values.

The qualitatively different dependence of the reaction rates k_1 on pH, suggests the possibility of different mechanisms with different substrates. In the cases where the distinct maximum of k_1 appears, the addition of the exoamino group to the carbonyl bond is probably the initial step of the reaction.

In the case of cytidine, where the reaction rate reaches its plateau at a pH range of 5.0 - 7.0, it seems that alkylation of the endo nitrogen atom is the first step of the reaction. The rate of N-alkylation depends on the extent of protonation of the endo nitrogen atom only, and when this process is negligible, the reaction rate shows a constant maximum value.

In our mechanistic considerations, we assumed that the intramolecular cyclization, which follows initial addition or N-alkylation, is a very fast process and does not have any influence on the measured k_1 values.

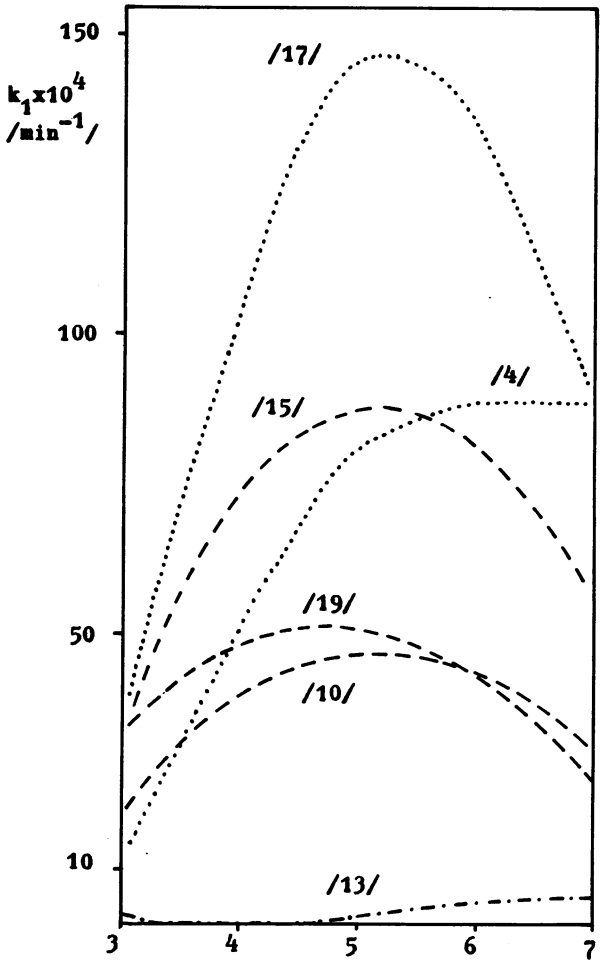


Fig.5. Dependence of the rate constants k_1 on pH for the reactions of cytidine /4/, N^4-methylcytidine /17/, adenosine /10/, N^6-methyladenosine /15/ N^6-/Δ^2-isopentenyl/-adenosine /19/ and guanosine /13/ with chloroacetaldehyde.

The results presented in this paper showing the complexity of the discussed reaction, do not change the fact that chloroacetaldehyde, if properly used, is one of the best reagents for chemical modification of nucleic acids. The pH range of 3.5 - 4.0 guarantees the smallest reactivity of guanosine but all adenosine and cytidine derivatives alkylated on the exoamino group react with a higher or similar rate. The reactivity of N^4-acetylcytidine is significant only at a low pH of 3.0 - 4.0, at which the hydrolysis of the amide function has already started.

EXPERIMENTAL SECTION

The chemicals came from commercial sources except for N^6-methyladenosine[26], N^4-methylcytidine[27], N^4-acetylcytidine[28] and N^6-/N-threo-

nylcarbonyl/adenosine[29] which were prepared according to known procedures. An aqueous solution of chloroacetaldehyde was obtained by following the Yasnitzky and Dolberg procedure[30]. The proton NMR spectra were obtained on a Varian EM 360 spectrometer /60 MHz/ with TMS as an internal standard. UV absorption measurements were performed on a VSU 2P spectrofotometer /C. Zeiss Jena/. Commercial Merck's silica gel and cellulose sheets were used for tlc.

Reaction intermediates

The chromatographic conditions listed in Table 2 enabled us to separate all compounds /substrate, intermediate and product/ occuring in every reaction mixture.

1. Isolation of 2,3-dihydro-2-hydroxy-imidazo[1,2-c]pyrimidin-5/6H/--one /2/.

352 mg /3.17 mmoles/ of cytosine were dissolved in 21 ml /31.5 mmoles/ of aqueous chloroacetaldehyde, pH 5.5, and incubated at room temperature for 13.5 hours. After that time when tlc /solvent system A/ revealed high concentration of /2/, the reaction was quenched by evaporation of chloroacetaldehyde and water. The remaining residue was dissolved in a small volume of water and separated by short column chromatography on silica gel HF_{254} using 10% water in isopropanol for elu-

Table 2. Rf values of the substrates, intermediates and products in different solvent systems.

Compound	Rf values in the solvent system :				
	A	B	C	D	E
/1/	0.41	0.68			
/2/	0.20	0.37			
/3/	0.70	0.87			
/4/	0.46	0.74	0.51		
/5/	0.20	0.35	0.62		
/6/	0.67	0.84	0.87		
/7/				0.58	0.58
/8/				0.40	0.21
/9/				0.51	0.51
/10/				0.61	0.73
/11/				0.35	0.12
/12/				0.51	0.60

A - $iPrOH/H_2O$ 9 : 1
B - $iPrOH/H_2O$ 3 : 1
C - $iPrOH/NH_3$ conc./H_2O 3 : 1 : 1
D - $nBuOH/H_2O/AcOH$ 5 : 3 : 2
E - Dioxane/H_2O 9 : 1

Substances were visualized in UV light 254 nm.

tion. Fractions containing /2/ were collected and after evaporation of the solvent, 190 mg /38%/ of /2/ containing ca. 5% of ethenocytosine was obtained. PMR /DMSO-d_6/ δ 4.0/m, 2, 3-H and 3'-H/, 5.7/q, 1, 2-H/, 6.1/d, 1, J = 7 Hz, 8-H/, 8.0/d, 1, J = 7 Hz, 7-H/.

2. Isolation of 2,3-dihydro-2-hydroxy-imidazol1,2-c]pyrimidin-5/6H/--one-6-β-D-ribofuranosyl /5/.

150 mg /0.62 mmoles/ of cytidine were dissolved in 10 ml /15 mmoles/ of aqueous chloroacetaldehyde, pH 5.5, and incubated at room temperature. The reaction course was followed by tlc in the solvent system A. After 11 hours, when the concentration of /5/ was high, chloroacetaldehyde and water were evaporated. The obtained oil was dissolved in a small volume of 10% water in isopropanol, applied onto a silica gel short column and eluted using the same solvent system. Fractions containing /5/ were collected, and after evaporation of the solvent, 75 mg /26%/ of /5/ containing ca. 5% of ethenocytidine was obtained. PMR /DMSO-d_6/ δ 3.2 - 5.0/complex multiplet, about 11/, 5.55/q, 1, 2-H/, 5.75 - 5.95/two d, 2, 1'-H and 8-H/, 7.65/d, 1, 7-H/.

In the PMR spectrum of /2/ the doublet of doublets at 5.7 δ and the multiplet at 4.0 δ are characteristic for ABX protons - Fig.6.

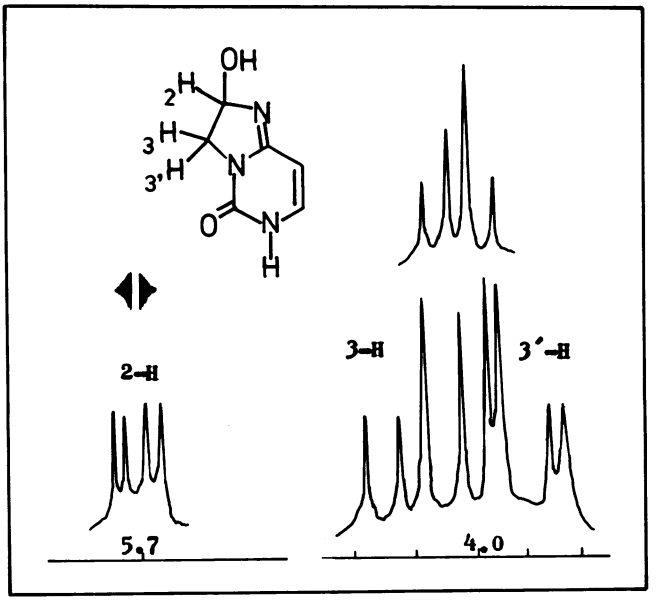


Fig.6. The spin decoupling of 2-H and 3-H, 3'-H protons in /2/, from the spectrum measured in DMSO-d_6 at 120 Hz sweep width.

Irradiation at the center of gravity of the doublet of doublets /2-H/ caused collapse of 3-H and 3'-H signals into two doublets. The presence of such a system of protons in a group attached to the cytosine ring determines univocally the structure of /2/. The coupling constants $J_{3,2} = 7.7$ Hz and $J_{3',2} = 2.6$ Hz were obtained by ABX analysis[31] of the spectrum measured at 120 Hz sweep width. $J_{3,3'} = -13$ Hz. Although PMR spectra of /5/ and /11/ are more complex, there are no doubts that the $-CH_2CH/OH/-$ bridge is attached to the base portion in these compounds.

Kinetical studies

An easily available and effective method for studies of such consecutive reactions was the combination of thin layer chromatography with UV measurements. The concentrations of the substrate IAI and product ICI were determined directly. The concentrations of the intermediate IBI were calculated from equation /1/ :

$$IBI = IAI_0 - /IAI + ICI/ \qquad /1/$$

where IAI_0 is the initial concentration of the substrate. Direct measurement of IBI was difficult, because the stability of the intermediate was not sufficient for that purpose. The first step of the reaction /intermediate formation/ is an example of pseudo first-order reaction, so the rate constants k_1 were calculated from equation /2/ :

$$k_1 = \frac{\ln \frac{IAI_0}{IAI_t}}{t} \qquad /2/$$

where IAI_t is the concentration of the substrate after time t of the reaction. The rate constants k_2, corresponding to the second step of the reaction /intermediate dehydration/, were calculated from equation /3/ :

$$IBI_{max} = IAI_0 \times / \frac{k_2}{k_1} / \frac{k_2/k_1}{1-k_2/k_1} \qquad /3/$$

where IBI_{max} is the maximum value of the intermediate concentration during the course of the reaction - Fig.2.

1. Reaction of adenosine with chloroacetaldehyde.

1 ml of 1.14×10^{-2} M aqueous solution of adenosine and 1 ml of 1.5 M buffered solution of chloroacetaldehyde, pH 4.5, were heated to 37°C, mixed and then incubated at 37 ± 0.1°C. The composition of the reaction mixture was analysed at intervals of 0.5 or 1 hours. Aliquots

/50 µl/ were spotted on tlc sheets /5 x 6.5 cm/. Before and after chromatography in the solvent system D, the tlc sheets were dried for 10 minutes at room temperature. Spots originating from adenosine and ethenoadenosine were quantitatively transfered into centrifuge tubes, each time with an identical amount of silica gel. The nucleosides were extracted from the adsorbent with 3 ml of phosphate buffor, pH 6.8, at 25°C, under magnetic stirring for 25 minutes. The silica gel was removed by centrifugation, and absorbance was measured at 261 nm and 275 nm for adenosine and ethenoadenosine, respectively. Since the commercial silica gel HF_{254} contained a substance showing a weak absorption band in the region of measurements, it was necessary to compensate for its effect on the total absorbance by using a blank. Therefore, the reference solution was prepared for each analysis by extraction of the substance from the same quantities of silica gel as for analysed compounds.

2. Reaction of cytidine with chloroacetaldehyde.

The reaction of cytidine with chloroacetaldehyde at pH 3.5 was studied using the same method as described for adenosine. The initial concentration of cytidine solution was 1.36×10^{-2} M and the solvent system F was used for separation of the reaction mixture components. Absorbance was measured at 272 nm for both cytidine and ethenocytidine.

Optimum pH and selectivity of reaction

Studying the optimum pH conditions for reactions of adenosine and cytidine with chloroacetaldehyde and the reactivity of some modified and hypermodified nucleosides, all experiments were done under the standard conditions described above. There were no changes in the

Table 3. Some details concerning the method of kinetical measurements.

Compound	Time of analysis /hr/	tlc solvent system	Rf value	Wavelength for abs. measurements /nm/
/13/	28	D	0.56	252
/15/	4	F	0.45	267
/17/	2	D	0.50	277
/19/	4	D	0.74	270
/21/	28	D	0.47	270

F - nBuOH/H_2O 84 : 14

concentrations of the substrate and reagent, nor in the method of kinetical measurements.

The only exception was the reaction of N^4-acetylcytidine with chloroacetaldehyde. In this case a different method based on the direct absorbtion measurements was used to follow the reaction course. N^4-acetylcytidine was incubated in a buffered solution of pH 3.0 - 5.0 in the presence and absence of chloroacetaldehyde. After 28 hours, 50 μl of aliquots were withdrawn from each reaction mixture then diluted with 3 ml of phosphate buffer, pH 6.8, and the absorbance of the solution was measured at 300 nm. At that wavelength only N^4-acetylcytidine absorbs.

1. Synthesis of 7,8-dihydro-8-hydroxy-N^9-methyl-imidazoI2,1-iIpurin--3-β-D-ribofuranosyl chloride /16/.

141 mg /0.5 mmole/ of N^6-methyladenosine was dissolved in 17 ml /25.5 mmoles/ of aqueous chloroacetaldehyde, pH 5.5, and the reaction mixture was incubated at 37°C. After 30 hours, tlc /in solvent system F/ revealed the quantitative transformation of N^6-methyladenosine into /16/. The chloroacetaldehyde and water were evaporated off, leaving an oil. This compound has not been obtained in the crystalline state. PMR /D_2O/ δ 3.75/s, 3, CH_3/, 3.90 - 5.10/complex multiplet, about 7/, 6.10/q, 1, 8-H/, 6.32/d, 1, J = 5Hz, 1'-H/, 8.78 and 8.82/two s, 2, 2-H and 5-H/.

2. Synthesis of 2,3-dihydro-2-hydroxy-N^1-methyl-imidazoI1,2-cIpyrimidin-5/6H/-one-6-β-D-ribofuranosyl chloride /18/.

128 mg /0.5 mmole/ of N^4-methylcytidine was dissolved in 17 ml /25.5 mmoles/ of aqueous chloroacetaldehyde, pH 5.5, and the reaction mixture was incubated at 37°C. After 30 hours tlc /in solvent system : iPrOH/H_2O/NH_3 conc. 85 : 15 : 13/ revealed the quantitative transformation of N^4-methylcytidine into /18/. The chloroacetaldehyde and water were evaporated and the obtained oil was crystallized from ethanol - water mixture. Yield 156 mg /93%/. PMR /D_2O/ δ 3.32/s, 3, CH_3/, 3.5 - 4.4/complex multiplet, about 6/, 5.85 - 6.05/m, 2, 1'-H and 2-H/, 6.55/d, 1, J = 8Hz, 7-H/, 8.54/d, 1, J = 8Hz, 8-H/; /DMSO-d_6/ δ 3.23/s, 3, CH_3/, 3.6 - 4.6/complex multiplet, about 7/, 5.2 - 5.9/complex multiplet, 5, sugar OH, 2-H and 1'-H/, 6.70/d, 1, J = 8Hz, 8-H/, 8.06/d, 1, J = 8Hz, 2-OH/, 8.72/d, 1, J = 8Hz, 7-H/.

This work was supported by the Polish Academy of Sciences, project MR - I. 12. 1. 7. 11.

+ To whom all correspondence should be addressed.

REFERENCES

1. Kochetkov,N.J.,Shibaev,V.W.,Kost,A.A./1972/ Dokl.Akad.Nauk SSSR 205,100-103.
2. Barrio,J.R.,Secrist,J.A.III and Leonard,N.J./1972/ Biochem.Biophys. Res.Commun. 46,597-604.
3. Secrist,J.A.III,Barrio,J.R.,Leonard,N.J. and Weber,G./1972/ Biochemistry 11,3499-3506.
4. Sattsangi,P.D.,Leonard,N.J.,Frihart,C.R./1977/ J.Org.Chem. 42,3292.
5. Kochetkov,N.K.,,Shibaev,V.N.,Kost,A.A./1973/ Dokl.Akad.Nauk SSSR C2 13,1327-1330.
6. Secrist,J.A.III,Barrio,J.R.,Leonard,N.J.,Villar-Palasi,C. and Gilman,A.G./1972/ Science 177,279-280.
7. Jones,G.H.,Murthy,D.V.K.,Tegg,D.,Golling,R. and Moffat,J.G./1973/ Biochem.Biophys.Res.Commun. 53,1338-1343.
8. Shram,K.H.,Townsend,L.B./1974/ Tetrahedron Lett. 1345-1348.
9. Zhenodarova,S.M.,Sedelnikova,E.A.,Smeljaninova,O.A.,Shibaev,V.N, Kost,A.A./1975/ Bioorg.Khim. 9,1345-1351.
10. Kochetkov,N.K.,Shibaev,V.N.,Kost,A.A./1971/ Tetrahedron Lett. 1993-1996.
11. Tolman,G.L.,Barrio,J.R. and Leonard,N.J./1974/ Biochemistry 13, 4869-4878.
12. Spencer,R.D.,Weber,G.,Tolman,G.L.,Barrio,J.R. and Leonard,N.J. /1974/ Eur.J.Biochem. 45,425-429.
13. Lee,C.-Y. and Wetmur,J.G./1973/ Biochem.Biophys.Res.Commun. 50, 879-885.
14. Barrio,J.R.,Sattsangi,P.D.,Gruber,B.A.,Damman,L.G. and Leonard,N.J. /1976/ J.Amer.Chem.Soc. 98,7408-7414.
15. Wang,A.H.-J.,Barrio,J.R.,Paul,I.C./1976/ J.Amer.Chem.Soc. 98, 7401-7407.
16. Harvey,S.C.,Cheung,H.C./1976/ Biochem.Biophys.Res.Commun. 73,865.
17. Leonard,N.J.,Tolman,G.L./1975/ Ann.N.Y.Sci. 2255, 43-58.
18. Chladek,S.,Ringer,D.,Abraham,C.M./1976/ Nucleic Acids Res.3, 1215-1231.
19. Steiner,R.F.,Winner,W.,Lunasin,A. and Delac,J./1973/ Biochem. Biophys.Acta 294,24-37.
20. Paps,T.S.,Chirikjian,J.G.,Pry,T.W.,Massicot,J.G.,Irwin,R.D. and Chirigos,M.A./1974/ J.Virol. 14,1108-1114.
21. Shulman,H.,Pelka,H./1976/ Biochemistry 15, 5769
22. Greenfield,J.C.,Leonard,N.J.,Nystrom,R.F./1976/ J.Labelled Compd. Radiopharmaceut. 12,545-550.
23. M.Wiewiórowski/1976/ in "Synthesis,Structure and Chemistry of Transfer Ribonucleic Acids and Their Components",Proceedings of the International Conference,Poznań-Dymaczewo,Poland,pp.479-488.
24. In the lecture given by Prof.N.J.Leonard in our laboratory in July,1976,it was mentioned that during modification of the tRNA molecule by chloroacetaldehyde at pH 6.3,the reactivity of guanosine residues was found to be significant.
25. Kochetkov,N.K.,Kost,A.A.,Shibaev,V.N.,Sagitulin,R.S.,Kost,A.N., Zavjalov,Yu.V./1975/ Izv.Akad.Nauk SSSR,Ser.Khim.,2766-2770.
26. Jones,J.W.Robins,R.K./1963/ J.Amer.Chem.Soc. 85,193.

27.We thank Dr W.Markiewicz for supplying this compound.
28.Zorbach,W.W./1966/ in "Synthetic Procedures in Nucleic Acid Chemistry",Vol.1,p.285.
29.Adamiak,R.W. and Wiewiórowski,M./1975/ Bull.Acad.Pol.Sci.Chim. 23, 241-253.
30.Yasnitski,B.G.,Dolberg,E.B./1970/ Metody Poluch.Khim.Reaktiv.Prep. 21,5.
31.Becker,E.D./1969/ in "High Resolution NMR",p.149,Academic Press New York and London.