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# NEW REACTION OF ADENINE AND CYTOSINE DERIVATIVES, POTENTIALLY USEFUL FOR NUCLEIC ACIDS MODIFICATION.

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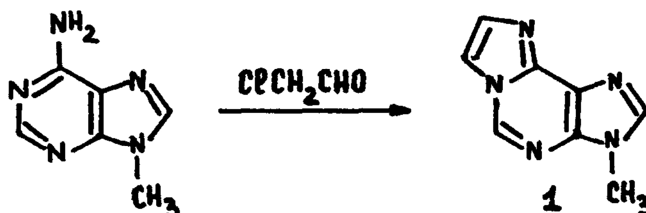
The chemical modification of the base residues in nucleic acids is of importance for structural and functional investigation of RNA and DNA /I/. A search of new specific chemical reactions for this purpose seems to be essential for the future development of this field.

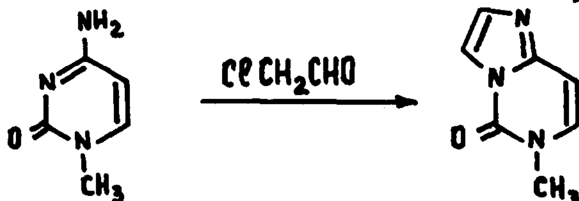
We wish to report our observations about interaction of chloroacetaldehyde with nucleic acid components. This reaction may form a basis for the new method of nucleic acid modification. We have found that chloroacetaldehyde reacts readily with 9-N-methyladenine and I-N-methylcytosine in weakly acidic aqueous solutions (in former case, addition of ethanol is necessary for homogeneity of the reaction mixture)to give 3-methylimidazo/2,1-i/purine(1) from 9-N-methyladenine.




The analytical sample of perchlorate of 1 has m.p. 295-6°(decomp.). Found % C 34.84, H 3.06, N 25.52, CI 12.93. Calculated for $C_8H_8N_5O_4CI$: C 35.11, H 2.95, N 25.60, CI 12.95. The NMR-spectrum of 1 corresponds to the structure assigned: δ(p.p.m., in DMSO - acetone) 8.08 and 8.38 (2H, pair of doublets, H-7 and H-8, $J_{7,8}$2cps), 8,60 (IH, s, H-5), 9.53 (IH, s, H-2), 12.05 (IH, broad s, NH). Conversion of 9-N-methyladenine into 1 leads to red shift in UV-spect-

rum. For 1 in 0.I N HCI: $\lambda_{max}$221, 276nm($\varepsilon$ 15500, 5620), $\lambda_{min}$246nm ($\varepsilon$ 2160); in 0.I N KOH: $\lambda_{max}$227, 270, 278nm ($\varepsilon$ 13500, 4480, 4310), $\lambda_{min}$248, 275nm ($\varepsilon$ 2890, 4000).

The interaction of I-N-methylcytosine with chloroacetaldehyde leads to 6-methyl-5-oxo-5,6-dihydroimidazo/1,2-c/pyrimidine (2).




The crystalline picrate of 2, m.p.169-70°, gives correct elemental analysis data. Found%: C 41.23, H 2.88, N 21.52. Calculated for $C_{13}H_{10}N_6O_8$:C 41.27, H 2.65, N 22.22. UV-spectrum of 2 in 0.I N HCI:$\lambda_{max}$213, 290 nm ($\varepsilon$ 10500, 9950), $\lambda_{min}$225nm ($\varepsilon$ 1650); in 0.I N KOH: $\lambda_{max}$214, 272nm ($\varepsilon$ 9050, 8260), $\lambda_{min}$ 228nm ($\varepsilon$ 1570). NMR-spectrum of 2: $\delta$(p.p.m., in $D_2O$): 3.44 (3H, s, $NCH_3$), 6.31 (IH, q, H-8), 7.06 (IH, d, H-7), 7.16 (IH, d, H-3), 7.35 (IH, q, H-2), $J_{2,3}$I.8cps, $J_{7,8}$7.7cps, $J_{2,8}$0.6cps.

The achieved modification of adenine and cytosine nuclei under mild conditions prompted us to study the interaction of chloroacetaldehyde with nucleoside.

Incubation of 0.0I M aqueous solutions of four common ribonucleosides with I M chloroacetaldehyde solution (pH 2.0 - 5.0, 50°) showed significant differences in their behavior, as shown of Fig.I. Rather fast hydrogen ion liberation occured in the adenosine solutions (curve I). The dependence of reaction rate on pH was bell-shaped curve with optimal value near pH 4.5. Analogous bell-shaped curve was observed for cytidine (curve 2), but pH optimum was near pH 3.5. As a consequence, the ratio of reaction velocities for adenosine and cytidine may be obtained as high as 2.44 (pH 4.5) or as low as 0.54 (pH 3.5). No reaction occured with uridine. Essentially the same situation exists for guanosine, although very slow hydrogen ion liberation was detected near pH 5,0.[x)]

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x) Decomposition and condensations resulting in self-acidification occur in alkali and neutral solutions of chloroacetaldehyde. At 50° these processes became detectable at pH 4.5 and their rates rapidly increases with the pH increasing (curve 3).

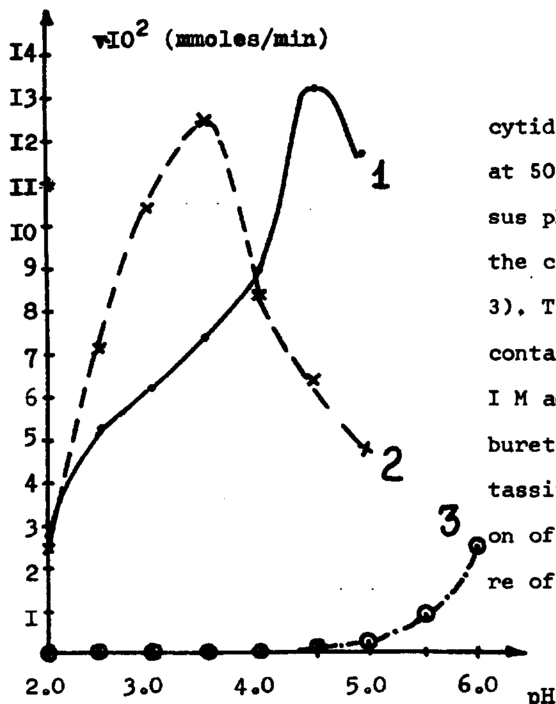


Figure I.

Interaction of adenosine (curve I) and cytidine (curve 2) with chloroacetaldehyde at 50°. The initial rate (v) is plotted versus pH. Initial rate data are corrected for the corresponding blank values (see curve 3). The vesel of Radiometer TTTI titrator contained 0.0I M solution of nucleosides in I M aqueous chloroacetaldehyde. Titration burette was filled with I M solution of potassium hydroxide. The slope of linear portion of pH-stat recording was taken as measure of initial velocity.

The observed specifity of chloroacetaldehyde reaction was confirmed by paper chromatographic study; its results are summarized in Table I. No UV-absorbing products were detected in uridine and guanosine solutions treated with chloroacetaldehyde. On the contrary, after incubation of adenosine solution with chloroacetaldehyde (pH 4.5) the starting nucleoside disappeared and a single product with characteristic UV-spectrum similar that of 1 could be identified. Analogous result was obtained on paper chromatogram of the reaction mixture containing cytidine and chloroacetaldehyde (pH 3.5). Its UV-spectrum is close to the UV-spectrum of 2. The reaction products obtained from adenosine and cytidine were detected on chromatogramm after spraying with sodium periodate - silver nitrate and alkali solutions, thus proving the intact α-diol grouping to be present in the reaction products.

Table I

Interaction of chloroacetaldehyde with nucleosides. Paper chromatography data.

| Nucleoside | Solvent systems ($^v/_v$) | | | | | |
|---|---|---|---|---|---|---|
| | BuOH - AcOH-$H_2O$ ( 5-3-2 ) | | i-PrOH - $H_2O$ - conc. $NH_4OH$ ( 85-15-1,3 ) | | i-PrOH - $H_2O$ - conc. HCl (170-250-41) | |
| | P.T[a)] | A.T.[b)] | P.T. | A.T. | P.T. | A.T. |
| Uridine | 0.41 | 0.41 | 0.48 | 0.48 | 0.82 | 0.82 |
| Guanosine | 0.30 | 0.30 | 0.17 | 0.17 | 0.61 | 0.61 |
| Adenosine | 0.41 | 0.35[c)] | 0.44 | 0.49[c)] | 0.76 | 0.69 |
| Cytidine | 0,28 | 0,52[d)] | 0.33 | 0.79[d)] | 0.78 | 0.84 |

[a)] P.T. = prior the treatment with chloroacetaldehyde.

[b)] A.T. = after the treatment with chloroacetaldehyde.

[c)] UV-spectrum of reaction product: in 0.I N HCl: $\lambda_{max}$274nm, $\lambda_{min}$247nm;
in 0.I N KOH: $\lambda_{max}$276nm, $\lambda_{min}$249nm.

[d)] UV-spectrum of reaction product: in 0.I N HCl: $\lambda_{max}$289nm, $\lambda_{min}$235nm;
in 0.I N KOH: $\lambda_{max}$272nm, $\lambda_{min}$239nm.

The experiments described show that chloroacetaldehyde reacts specifically with adenosine and cytidine. This reagent behaves unlike to glycidic aldehyde /2/ or glyoxal /3,4/, reacting preferentially with guanosine derivatives. The detailed mechanism of the reaction of chloroacetaldehyde and potentialities of this reagent as tool for nucleic acid modification are under investigation in this Laboratory.

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