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RESTRICTED ROTATION OF THE AMINO GROUP IN CYTOSINE AND 5-ALKYLCYTOSINE DERIVATIVES CAUSED BY TFA COMPLEXATION

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Summary.

The restricted rotation of the amino group in analogs of dinucleotides 5RCyt/C3/5R'Cyt, where R,R'= H, CH_3, C_2H_5, as well as in free bases, cytosine and 5-alkylcytosine, is observed by proton magnetic resonance in TFA and DMSO-TFA solutions.
At low TFA concentration the complex bi-cytosine cations formation take place. The stability of these cations depends on basicity of cytosine derivatives and increases in the serie H < CH_3 < C_2H_5.

It is generally accepted, that cytosine exists predominantly in the amino form and the population of the imino tautomer is negligible under ordinary conditions [1]. The amino group of cytosine not paired with complementary base is reckoned to undergo relatively free rotation. However, the restricted rotation of the NH_2 group of 1-methylcytosine in DMF[+/] solution has been observed by 220 MHz proton magnetic resonance [2]. Previously, the rotational restriction of the amino group has been observed in N,N-dimethylcytosine derivatives [3,4] and in 5-substitu-

[+/] Abbreviations: DMF-dimethylformamide, DMSO-dimethylsulfoxide, TFA-trifluoroacetic acid.

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ted cytosine [5] , as well as in protonated cytosine derivatives [5,7] . Also the complementary base pair formation /cytosine-guanine/ results in restricted rotation of the amino group [6] .

We have studied the proton magnetic resonance spectra of the analogs of dinucleotides 5RCyt/C_3/5R'Cyt, where R,R'= H, CH_3, C_2H_5 /Fig. 1/ trifluoroacetic acid /TFA/ solution.

The aim of our investigation was to examine the influence of the alkyl substituents on properties of the cytosine residues in these model compounds. The results of the nmr study indicate that the cytosine residue is considerably more sensitive to the influence of substituents in 5 than 6 position of the pyrimidine moiety. The nmr spectra of the cytosine and 6-methylcytosine derivatives show only one slightly broadened singlet line at 8.20 δ or 7.95 δ respectively, due to the protons of NH_2 group. For 1,1'--trimethylenebis/5-alkyl/cytosine derivatives we have observed two two-proton singlet lines at 7.70 δ and 8.35 δ , which disappeared after D_2O addition. The 5-methylcytosine and 5-ethylcytosine nmr spectra show the same two singlet lines /Fig. 2/.

Small deviations of the chemical shifts of C^6-H protons for cytosine and 5-alkylcytosine /about +0.15 ppm/ show that these bases form the same solvation complex with TFA, and there is not observed any difference in their protonation /cytosine undergoes protonation at the 3-position of the pyrimidine ring [7,8] /. The nmr sharp narrow singlet line of the C^6-H protons for symmetrically substituted molecules 5RCyt/C_3/5RCyt /R = CH_3, C_2H_5/ proves that both 5-alkylcytosine residues are solvated by TFA by the same means. On the other hand we observe two nmr lines due to the protons of NH_2 groups for all symmetrically /R = R'/ and unsymmetri-

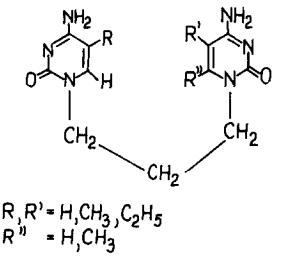

Fig. 1. 1,1'-Trimethylenebis/5- or 6-alkyl/cytosine derivatives as analogs of dinucleotides.

cally /R ≠ R'/ substituted analogs of dinucleotides, as well as for the free bases, 5-methyl- and 5-ethylcytosine. The presence of two amino signals may be interpreted as restricted rotation of the amino groups in 5-alkylcytosine residues.

The influence of TFA has been studied as well. We have obtained the nmr spectra of cytosine, 5-methylcytosine and 5-ethylcytosine in DMSO-d^6 solution at various TFA concentration.

The results are presented on fig. 3. In all cases the very mobile broad signal at low field is assigned to N^1-H and CF_3COOH protons.

For cytosine, as the TFA concentration is lowered the line of NH_2 group broadens and splits into two signals /Fig. 3/. The maximal splitting appears at the relation Cyt:TFA /mol: :mol/ about 1:10 and decreases for low TFA concentrations. The restricted rotation of the amino group is observed in ambient temperature. The higher field NH_2 signal may be assigned to H_A, e.g. the proton closest to N^3 and the one expected to participate in cytosine-TFA complex formation /Fig. 4/. This signal always shows the less height and the larger line width than the lower field one. Simultaneously, the C^6-H doublet is less clearly splitted than the C^5-H one. Such effect probably arises from a five-bond coupling of H_A

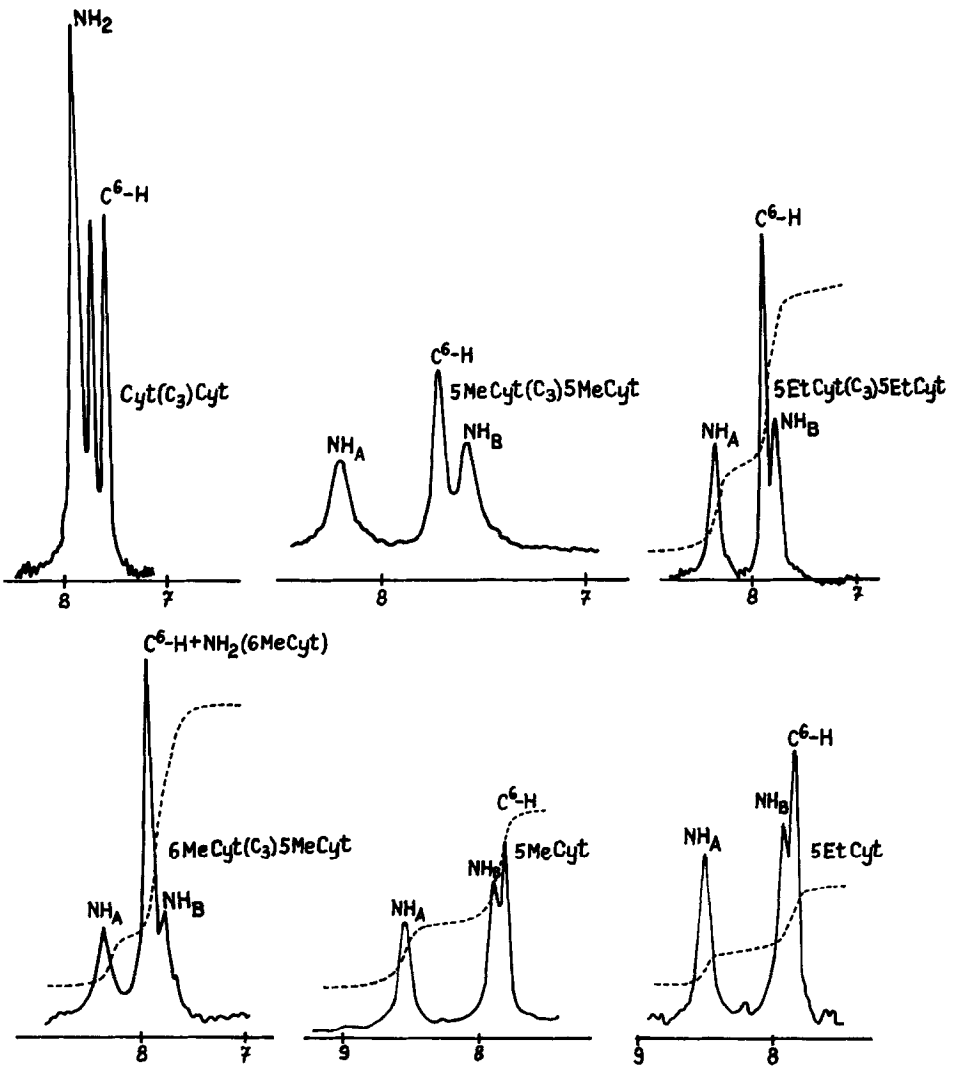

Fig. 2. The low field regions of the 60 MHz nmr spectra of analogs of dinucleotides and free bases in TFA solutions.

to the C^6-H proton, which are in a planar zig-zag configuration [2]. This observation makes additional support for the assignment of the higher field peak to H_A, and for a planar structure of the cytosine-TFA complex. 5-Methyl- and 5-ethylcytosine show the more complicated picture of changes /Fig. 3/. In 100% TFA we observe two lines due to the NH_2 group. But if the TFA concentration decreases, the lines

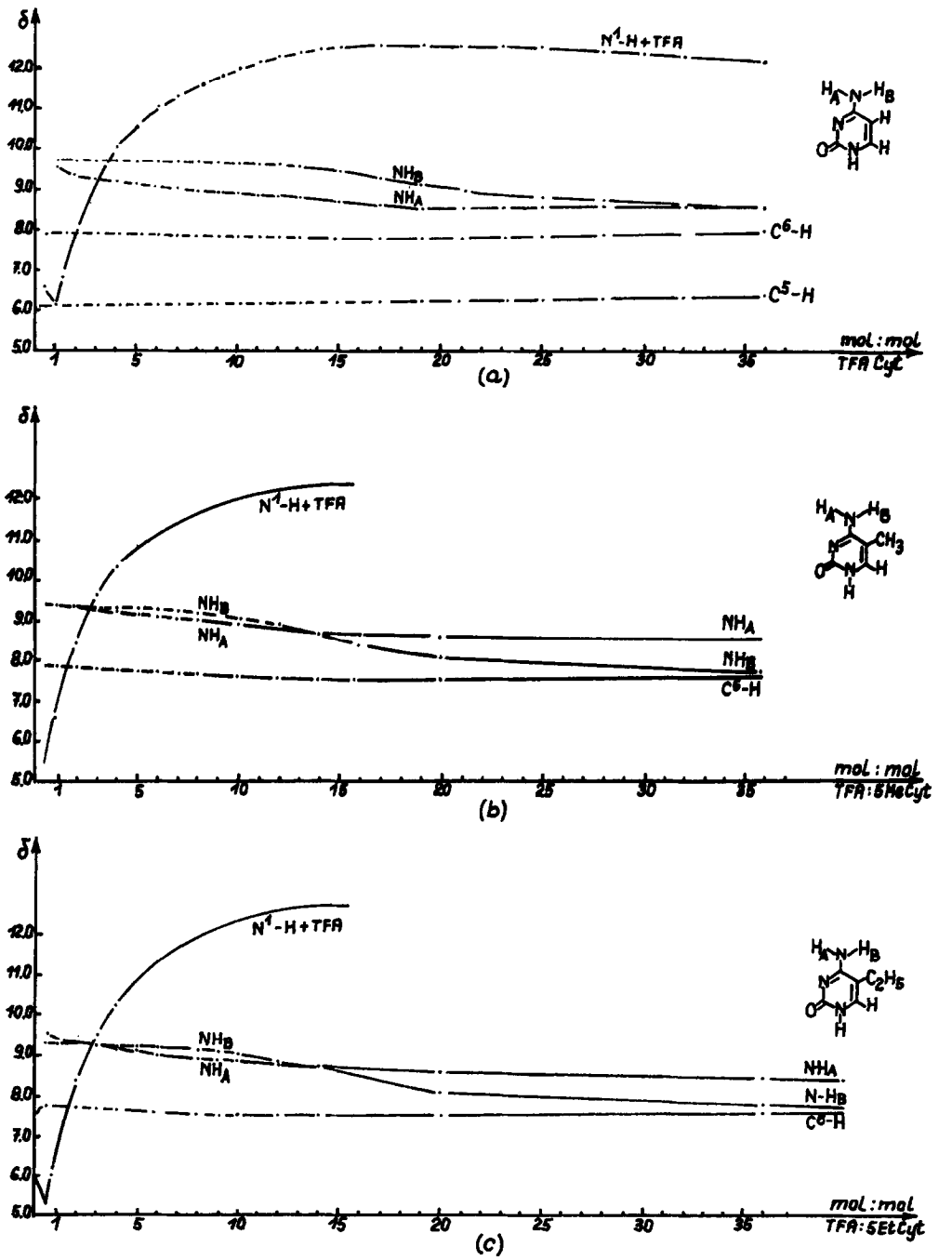

Fig. 3. The changes of the chemical shifts of N-H, C^6-H and C^5-H protons with increase of TFA concentration for cytosine /a/, 5-methylcytosine /b/, and 5-ethylcytosine /c/ in DMSO-d^6 solutions.

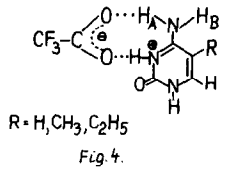

Fig. 4.

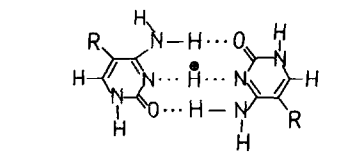

Fig. 5

Fig. 4. The structure of the 5-alkylcytosine - TFA complex.

Fig. 5. The bi-cytosine cation observed at low TFA concentration.

approach into one signal formation, subsequently, two lines appear again. Basing on the chemical shift changes and the line width analyses we assigned these signals to H_A and H_B protons of NH_2, group what is shown on Fig. 3. We notice, that the chemical shift changes of H_A with increase of the TFA concentration are identical for cytosine, 5-methyl- and 5-ethylcytosine. In all cases, the coupling of H_A to the C^6-H proton is observed. The H_B signal should be very sensitive to the influence of 5-alkyl substituents and solvent interactions, what is indeed observed in our experiment. The 5-alkyl substituents causes a diamagnetic shift of the H_B signal by about 0.5 ppm /Fig. 3/.

At low TFA concentration the relation of the lower field N-H signal to the higher field one is 2:1. Simultaneously, the relation of the sum of both N-H signals to C^6-H signal is less than 2:1, and for 5EtCyt is precisely 1.5:1. Then, at low TFA concentration we observe a complex bi-cytosine cation formation /Fig. 5/. The nmr data show, that the stability of these cations increases in the serie H < CH_3 < C_2H_5, as well as the basicity of cytosine, 5--methyl- and 5-ethylcytosine /pK_a is 4.45 [9], 4.60 [10] and 4.81 [11], respectively/. This observation is confirmed by nmr studies of the crystalline 5-R-cytosine-TFA comple-

xes in DMSO solution /where R is H, CH_3, C_2H_5/ [14]. These results suggest that electronic factors and no steric hindrance are connected with the observed phenomenon.

Methods

All compounds were prepared in our Laboratory 5-alkylcytosine were synthesized by amination of corresponding 4-chloropyrimidine derivatives [12] . The analogs of dinucleotides 5RCyt/C_3/5R'Cyt were prepared by thiolation followed by amination of the analogs of uracil dinucleotides 5RUra/C_3/5R'Ura [12,13] . The chemical analysis and spectral data were given previously [12,13] . NMR spectra were recorded on Varian A-60, EM-360 and Tesla BS-487 /80 MHz/ spectrometers in CF_3COOH or DMSO-d^6 solutions using TMS as an internal reference.

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