CHEMICAL STRUCTURE OF A PREBIOTIC ANALOG OF ADENOSINE

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Abstract. Upon heating a dry mixture of ribose and adenine, condensation products are formed. They were identified as isomers of N6-ribosyl-adenine (Fuller, Sanchez and Orgel, 1972). Due to the current interest in nucleotide analogs as potential constituents of primitive RNA catalysts, the products were further characterized by mass spectroscopy and proton NMR. Our results fully substantiate the previous proposals.

1. Introduction

Several lines of reasoning are leading to the idea that RNA was preceded in evolution by polymers containing analogs of nucleotides (Joyce *et al.*, 1987). Natural or modified RNAs may contain most of the chemically reactive groups that play a role in classical protein catalysis (Orgel, 1986; Benner *et al.*, 1987). Imidazole is a special case. While free adenine contains an imidazole ring, this functional group is no longer available in adenosine, where the N9 of the ring is linked to the C1′ of ribose or deoxyribose. However, if condensation of adenine and ribose is carried out non-enzymatically, the ribose is linked preferentially on the N6 of adenine (Fuller, Sanchez and Orgel, 1972) and the imidazole group of adenine remains free for catalysis (Maurel and Ninio, 1987).

Another isomer of adenosine, N3-ribosyl-adenine, attracted much attention recently. Hill *et al.* (1988) demonstrated the suitability of N3-ribosyl-adenosine 5′ phosphate as a monomer for non-enzymatic Poly(U)-directed oligomerization, and Wächtershäuser (1988) proposed a model of an all-purine ancestral DNA containing N3-ribosyl-adenine. In this compound, the imidazole ring is still available for catalysis.

The current emphasis on N3 rather than N6-ribosyl-adenines as the ancestors of adenosine (Wächtershäuser, 1988; Hill *et al.*, 1988), in addition to the plausibility of chemical rearrangements in the monomers after their synthesis (e.g., Groziak, Bhat and Leonard, 1988), prompted us to reinvestigate the structure of our product, with the help of physical techniques, now used in the study of biochemical compounds.
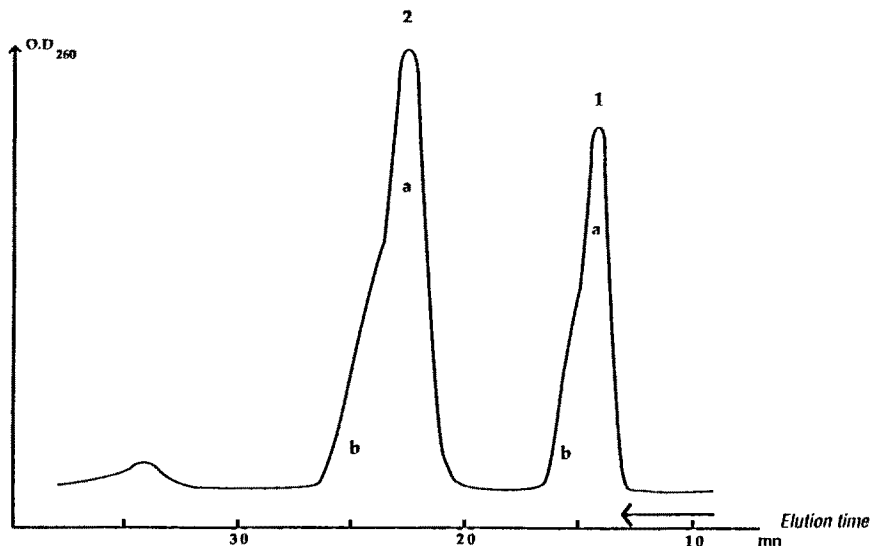
Fig. 1. HPLC fractionation of condensation products of ribose and adenine. In this system the compounds are mainly distinguished on the basis of the β (peak 1) or α (peak 2) mode of linkage of the base to the sugar.

2. Methods

Free adenine ad ribose were condensed according to Fuller *et al.* (1972) as described by Maurel and Ninio (1987). The products were purified by HPLC on a reverse phase C18 magnum 9 column (Partisil ODS3). Elution was performed in the isocratic mode with 20% methanol (flow rate of 2 ml mm^{-1} and pressure of 70 bars). A typical HPLC elution profile of the synthesized material is shown in Figure 1: two major peaks, 1a and 2a, are observed with lagging shoulders, 1b and 2b. Using the criteria of chromatographic and electrophoretic mobility of Fuller *et al.* (1972), the following structural assignments can be proposed: 1a, β ribopyranosyl adenine; 1b, β ribofuranosyl adenine; 2a, α ribopyranosyl adenine; 2b, α ribofuranosyl adenine (Figure 2). When the lagging shoulders 1b and 2b are chromatographed on HPLC just after their purification, they generate the initial profiles of peaks 1 or 2. This rapid isomerization precludes any attempt of separate mass spectrometry or NMR study for the shoulder materials.

Mass spectroscopy (Fast Atom Bombardment Mass Spectrometry): The spectra were performed on an R1010 Nermag mass spectrometer. A sample of 50 μg was dissolved in a glycerol matrix and deposited on a copper target. Argon was used as fast atom beam (acceleration energy of 8KV).

^{1}H NMR: The NMR samples were prepared by dissolving the corresponding product in DMSO, CD$_3$OD or D$_2$O. ^{1}H spectra were performed in the Fourier transform mode on Bruker WM 500 or Bruker WM 250 instruments. (Centre de Spectrochimie de l'Université Pierre et Marie Curie de Paris).

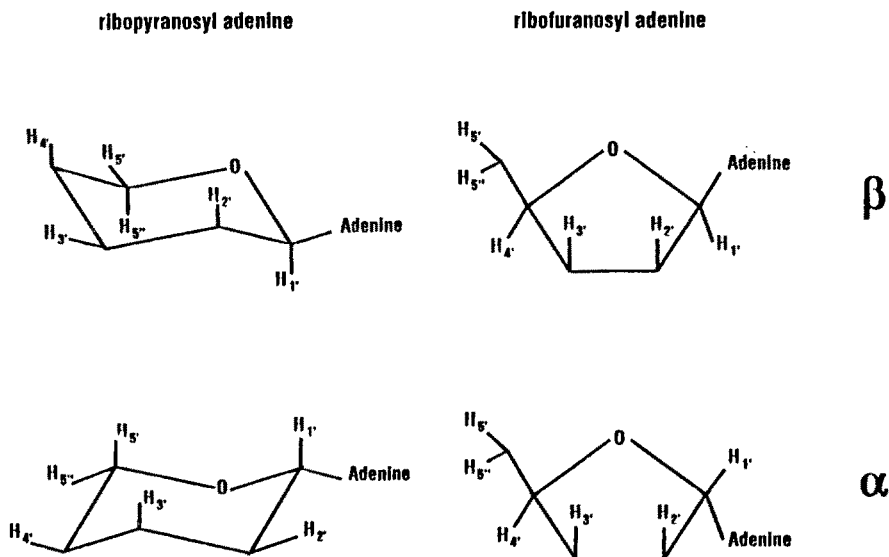**ribopyranosyl adenine**   **ribofuranosyl adenine**

Fig. 2. Structures for the four isomers of ribosyl adenine. The proximity relationship between pairs of protons in the sugar moiety are revealed by NMR analysis, thereby allowing the major isomeric form of the sugar to be identified.

3. Results and Discussion

MOLECULAR WEIGHT

Both samples 1 and 2 lead to an intense protonated MH^+ ion at m/z 268 (Mw=267), by using Fast atom Bombardment Mass Spectrometry.

POSITION OF THE GLYCOSIDIC BOND

The fixation site of the ribose on the adenine heterocycle may be determined by a simple comparison of the NMR spectra of samples 1 and 2 with that of adenosine
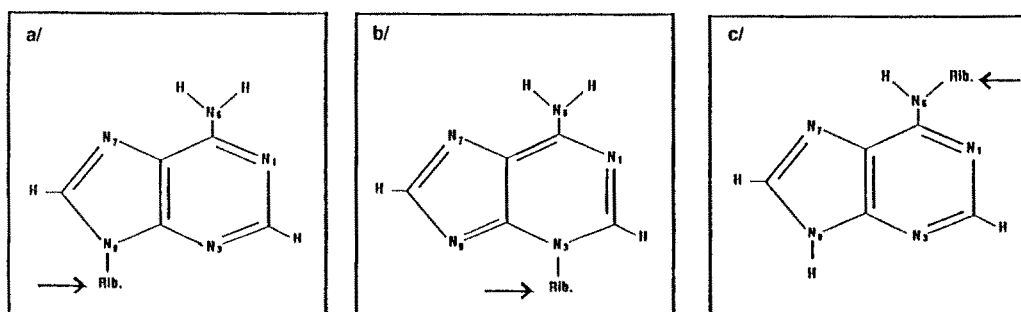

Fig. 3. Adenosine isomers. (a) Adenosine (N9-ribosyl-adenine), (b) N3-ribosyl-adenine, (c) N6-ribosyl-adenine. The presence of a single proton linked to the N6 as well as its coupling constant to the ribose H1' proton make possible the unambiguous NMR identification of N6 as the site of linkage of ribose.
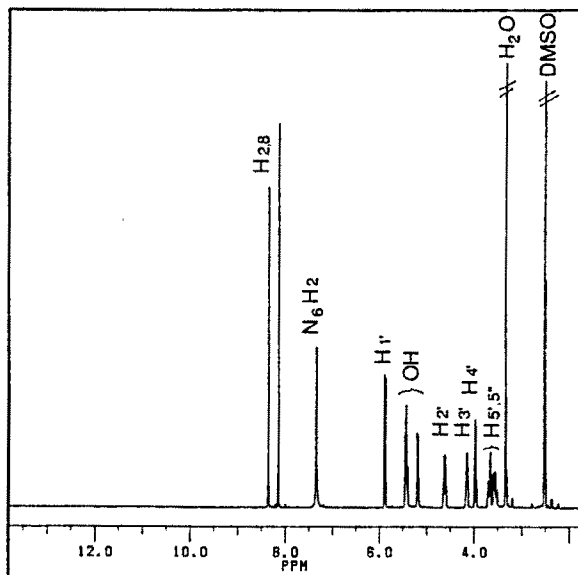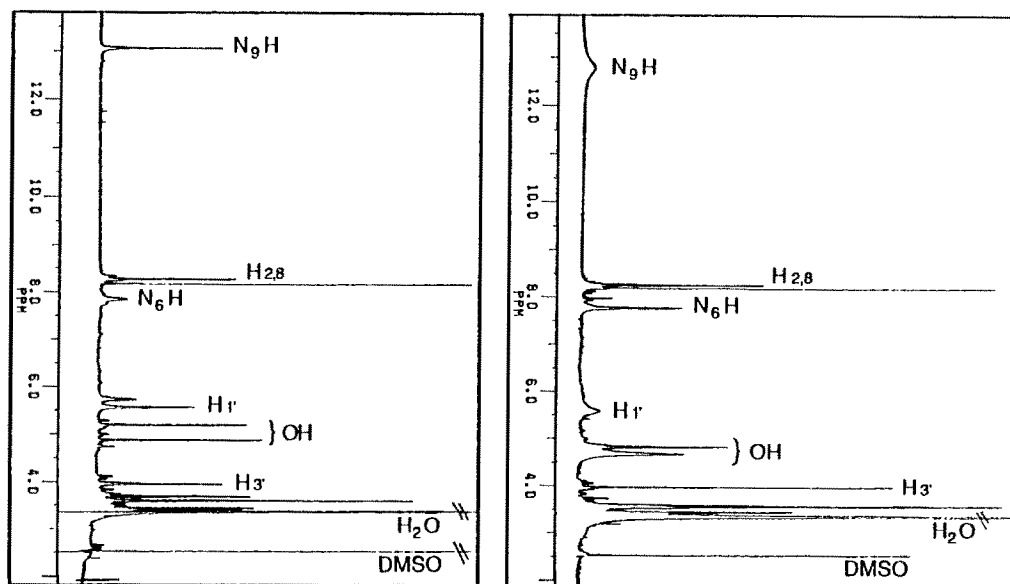
Fig. 4. NMR spectrum of adenosine in DMSO.

Fig. 5. NMR spectra of products 1 (right) and 2 (left) in DMSO.

(9βD-ribofuranosyl derivative of adenine) (Figure 3).

In fact, the NMR spectrum of adenosine in DMSO (Figure 4) presents a signal around 7.74 ppm corresponding to the two amino protons at position 6. In the samples 1 and 2 (Figure 5), the signal around 7.8 ppm corresponds to only one

proton: Its disappearance in the D_2O spectrum makes evident the amino nature of this proton. Moreover, in the spectra in DMSO, this signal is coupled to the H1′ sugar proton: in sample 1, it is a doublet (J=8.2 Hz), transformed in a singlet by irradiation of the H1′ proton at 5.56 ppm; in sample 2, it is a broad signal, the line width of which is reduced by about 6 Hz by irradiation of the H1′ proton at 5.54 ppm.

CONFORMATION OF THE SUGAR RING

Our analysis is based upon the extent of coupling constants and on the two well known assumptions (Altona and Sundaraligam, 1973; Jackman and Sternhell, 1969): firstly, in the pyranose ring, the vicinal coupling constant is larger for two protons in axial position than for protons in axial-equatorial or equatorial-equatorial positions; secondly in the furanose ring, the vicinal coupling constant is larger for protons in trans position than for those in cis position.

Concerning sample 1, the study of the NMR spectrum in CD_3OD provides two arguments in favour of a β pyranoside form of the sugar for the major product 1a. The first argument is the rather large value (9Hz) for the coupling constant $J_{1'2'}$. Moreover, a first order analysis of the system $H_{4'-5'5''}$ makes evident two very different values (10.4 and 5.3 Hz) for the coupling constants $J_{4'5'}$ and $J_{4'5''}$, in addition to the geminal coupling constant $J_{5'5''}$ of 10.9 Hz. These two features agree with a β pyranoside geometry for the sugar ring (Figure 2).

In contrast, from the NMR spectra of sample 2 in D_2O and CD_3OD it is possible to deduce rather small values for the three coupling constants $J_{1'2'}$, $J_{4'5'}$ and $J_{4'5''}$. This fact is consistent with an α pyranoside form for the sugar ring in the major component of sample 2.

These spectroscopic results fully substantiate the earlier proposals of Fuller *et al.* (1972): The ribose is linked to the N6 of adenine. Both α and β compounds are formed in comparable amounts. The ribose is predominantly present in the pyranose form. A minor form – possibly the more biologically usual furanose form – readily interconverts into the pyranose form. These conclusions strengthen the growing conviction that standard biological RNA molecules could have been much more difficult to synthesize in reasonable amounts under prebiotic conditions, than usually assumed in theoretical speculations. Adenosine would be exceptional both for its N9 sugar-base mode of linkage and its furanosyl sugar configuration. This argues in favour of early replicating molecules (Schwartz *et al.*, 1985) somewhat different from the biological nucleic acids.

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