Amino Acid Catalyzed Condensation of Purines and Pyrimidines with 2-Deoxyribose†

Gary L. Nelsestuen*

ABSTRACT: Mild heating of aqueous mixtures containing 2-deoxyribose, amino compounds, and purines or pyrimidines produces derivatives of the purines and pyrimidines in high yield. Among the major products formed are 2,3-dideoxy-3-(1′-pyrimidino)pentose and 2,3-dideoxy-3-(9′-purino)pentose. The mechanism of the reaction includes amine-catalyzed dehydration of the α,β positions of the sugar followed by addition of the purine or pyrimidine to the double bond. Rapid addition of purines and pyrimidines to α,β-unsaturated carbonyl compounds (such as acrolein) is a general phenomenon which does not require an amine catalyst. While multiple derivatization of the purines will take place, the N-9 derivative is formed first.

Ideas about the genesis of living matter on the prebiotic earth often involve the view that nucleic acids were formed nonenzymatically and functioned in development of a reproducing system. In the course of investigations related to spontaneous nucleic acid synthesis, I have found that 2-deoxy sugars do condense with purines and pyrimidines. This reaction occurs readily, even in aqueous solution. The product is not a nucleoside in that the base is linked to C-3 of the sugar. The mechanism of the reaction involves amine-catalyzed dehydration of the deoxy sugar followed by addition of the base to the unsaturated carbonyl. This very facile addition reaction may be valuable in synthesis of biochemically active molecules.

Materials and Methods

Organic chemicals were purchased commercially and were of the highest grade available. 2-Deoxyglucose-*1*-^{14}C and 2-deoxyglucose-^{14}C (uniformly labeled) were purchased from New England Nuclear and were diluted with unlabeled 2-deoxyglucose before use.

Periodate oxidations were conducted in aqueous solutions of the appropriate compound (approximately 0.02 M) and periodic acid (0.1 M) in the dark at 22 °C for 18 h. Solid barium carbonate was added to neutralize the solution, and the solids were removed by centrifugation.

Sodium borohydride reductions were carried out at 22 °C for 6–16 h in solutions of approximately 0.02 M purine or pyrimidine reaction product and 0.2 M sodium borohydride. The solution was acidified with HCl and dried under reduced pressure. Methanol was added and evaporated three times. The residue was dissolved in water and used for chromatography.

Descending paper chromatography was conducted using Whatman no. 1 or 3-mm paper. Solvent system I (water-saturated butanol) was used most extensively. Other systems used were butanol–acetic acid–water (12:3:5) and ethanol–1 M ammonium acetate (pH 7.5) (7:3). Compounds were located on the chromatogram by their absorbance of ultraviolet light and were eluted with water.

Proton NMR spectra were obtained with a Bruker 270-MHz spectrometer operating in the Fourier transform mode. Spectra were obtained at 40 °C by using 0.03–0.06 M solutions in ^2H_2O. A total of 200 scans were obtained for each spectrum. Chemical shifts are expressed as parts per million downfield from the standard compound, sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$. Threefold lyophilization from ^2H_2O (99.8% ^2H) was performed to exchange the protons.

Ultraviolet absorption spectra were obtained using a Beckman Acta CIIA spectrophotometer. Spectra in acid solution contained 0.05 N HCl, and those in base contained 0.05 N NaOH. Molar extinction coefficients were estimated as follows. Products from the reaction of 2-deoxyglucose-^{14}C (known specific activity) with the purines or pyrimidines were purified by paper chromatography in solvent system I. The ultraviolet absorption spectrum and the ^{14}C associated with the product were determined. The extinction coefficient at the absorption maximum was calculated by assuming a 1:1 ratio (deoxyglucose–base) in the product.

Results

Mixtures of purines or pyrimidines, 2-deoxyribose, and amino compounds react to form new ultraviolet-absorbing compounds. The proposed reactions and product structures for hypoxanthine are shown in Figure 1. The amine serves as a catalyst for the condensation, and most aliphatic amines (including ethanolamine, leucine, glycine, and glycinamide) will function. These derivatives are formed in substantial amounts; aqueous solutions of 2-deoxyribose (0.5 M), glycine (0.2 M), and a purine or pyrimidine (0.05 M) were heated at 80 °C for 4 h and streaked onto paper chromatograms, which were developed in solvent system I. The product yields (estimated from the total absorbance at 260 nm) from reactions containing hypoxanthine, uracil, adenine, and cytosine were 90, 20, 60, and 30%, respectively.

A number of methods were used to identify the reaction products. These include chromatographic properties, chemical reactivities, model compound and mechanism studies, the use of radioactive substrates, and the proton NMR and ultraviolet absorption spectra of the products. All results can be and are interpreted in light of the proposed reactions and structures (Figure 1). Since hypoxanthine gave the largest number of separable products (the four products shown in Figure 1 were obtained in approximately equal quantity), it was studied in the greatest detail.

The proton NMR spectra of one series of compounds are shown in Figure 2. Summaries of chromatographic data and NMR spectra are given in Table I. The peaks are complex due to multiple couplings and overlap of similar protons. Analysis of coupling constants was not attempted. Variable amounts of some minor peaks appear inconsistently in the spectra (e.g., the peaks at 1–2 ppm in Figure 2) and are

† From the Department of Biochemistry, College of Biological Sciences, University of Minnesota, St. Paul, Minnesota 55108. *Received November 2, 1978.* This work was supported in part by Grant No. HL 15728 from the National Institutes of Health.

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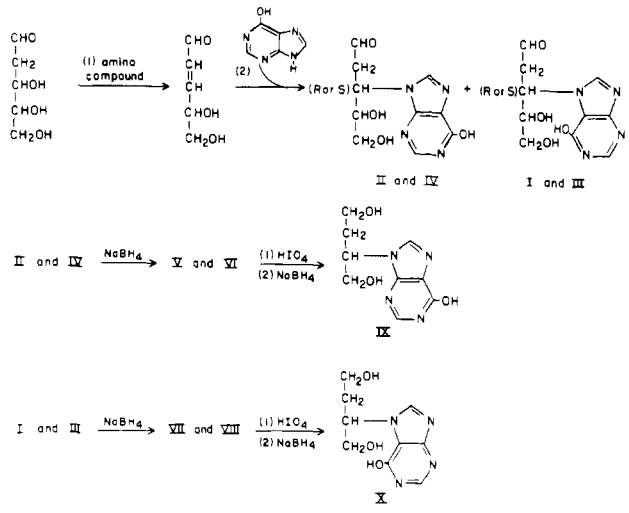

FIGURE 1: Reaction products formed from hypoxanthine and 2-deoxyribose. Compounds analogous to these products were obtained for both purines and pyrimidines (see text).

Table I: Selected NMR and Chromatography Data

compd[a]	base	sugar	R_{base}[b]	chemical shift[c] (ppm) H_1, H_5	H_2	H_3	H_4
I	Hyp	dRib	0.75				
II			0.70				
III			0.62				
IV			0.54				
V			0.52	3.48 3.65	2.31 2.43	4.92	4.24
VI			0.48	3.54 3.61	2.35	4.83	4.15
VII			0.70	3.48 3.64	2.26 2.44	5.19	4.24
VIII			0.62	3.52 3.67	2.40	5.15	4.26
IX (from V)			0.82	3.54 3.68	2.28	4.91	4.09
IX (from VI)			0.80	3.54 3.68	2.28	4.91	4.09
X (from VII)			0.95	3.54 3.65	2.30	5.09	4.03 4.12
X (from VIII)			0.98				
II and IV		dGlc	0.41				
I and III			0.49				
V and VI			0.27				
VII and VIII			0.47				
IX			0.80				
X			0.98				
II and IV	Ade	dRib	0.67				
V and VI			0.67				
II or IV		dGlc	0.57				
II or IV			0.49				
II and IV	Cyt	dRib	0.74				
V and VI			0.65	3.50	2.11	4.95	3.84
II and IV	Ura		0.62				
V and VI			0.52	3.60	2.11	HOD peak?	3.95
IX			0.87				
II and IV		dGlc	0.49				
V and VI			0.40				
IX			0.87				

[a] The compounds are analogues of those shown in Figure 1 containing the indicated sugar and base. [b] The chromatographic mobility in solvent system I relative to the parent purine or pyrimidine. [c] The approximate center of the peak is given without consideration of splitting.

assumed to be due to contaminants, possibly derived from the paper chromatographs used in purification. The major resonance peaks are assigned to specific protons (Figure 2). In some cases the assignments are tentative and do not constitute an important part of the structural identification. The important specific assignments are the methylene protons, which are essential for interpretation of the deuterium exchange studies (see below), and the total integration of the peaks, which indicates a 1:1 ratio of deoxy sugar–base in the complex. The specific assignments given to the H_1, H_3, H_4, and H_5 protons are consistent with other data; they assist in tabulation of the protons for purposes of discussion and are therefore included in Figure 2. It should be noted that both the chemical shift and the area of each peak agree with the chemical nature and number of protons assigned to that peak. The methylene, hydroxymethyl, hydroxymethylene, and purine protons have chemical shifts which correlate with related protons of 2′-deoxyinosine (spectrum not shown).

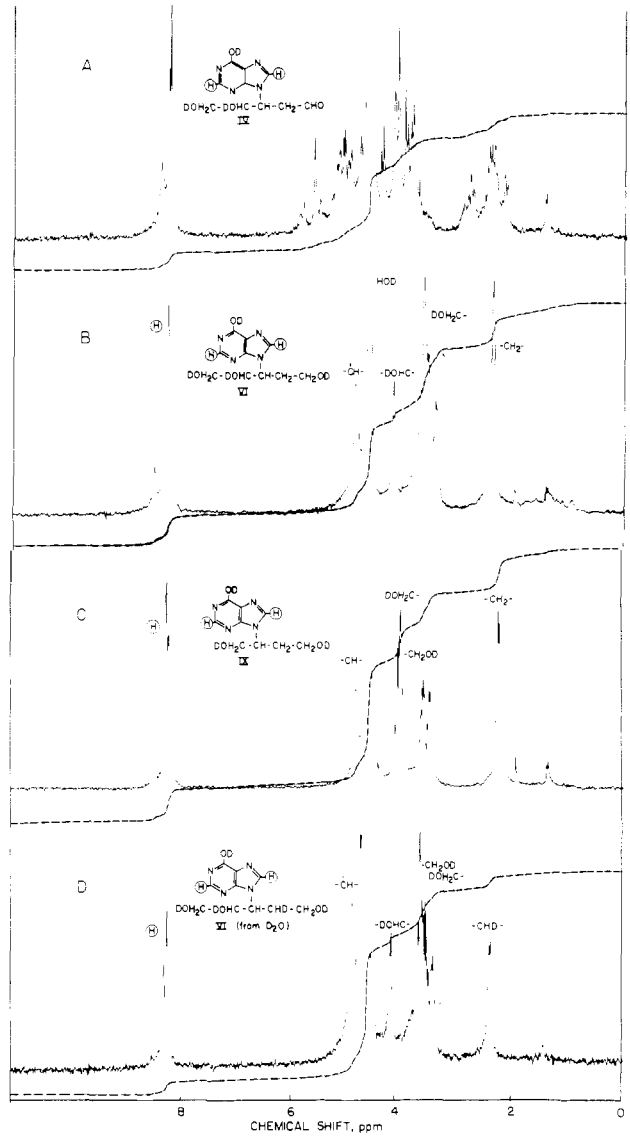

FIGURE 2: Selected proton NMR spectra of hypoxanthine derivatives. The identity of the compounds and tentative assignment of the resonance peaks are shown. The proton(s) assigned to a given peak appears above or to the side of that peak and can be further identified from the drawing of the entire structure. The HOD peak occurs at the same position in each spectrum. The peak assigned to $-CH_2OD$ in Figure 2B represents both the C_1 and C_5 hydroxymethyl groups. The dotted line shows the integration of the spectrum.

As indicated by borohydride reduction, all initial products (compounds I–IV) contain an aldehyde or ketone group. The initial compounds (e.g., Figure 2A) give very complex NMR spectra which are simplified by reduction (Figure 2B). The

complexity of the initial spectrum is probably due to the deoxyribose moiety, which can exist as the α or β anomer of the furanose or pyranose ring structures. Borohydride reduction also changes the chromatographic mobility of these compounds (Table I).

A second feature of the products is the presence of vicinal hydroxyl groups which are susceptible to periodate oxidation. The chromatographic mobility of each of these compounds was altered by this treatment (Table I). Oxidation followed by borohydride reduction should remove C-5, convert C-4 to a hydroxymethyl group, and destroy the chirality of C-4. Several lines of evidence indicate that these changes occur. Compounds V and VI are chromatographically separable and are proposed to be diastereomers (Figure 1, Table I). Removal of C-5 converts these compounds to enantiomers (compounds IX) which are not separable by simple chromatography (Table I). The NMR spectra of compounds V and VI also show differences, especially in the methylene protons, which give separated resonance peaks in compound V (Table I). Oxidation and reduction produce materials (compounds IX) which give indistinguishable NMR spectra (Table I; the patterns of peak splitting seen in Figure 2C were identical for compound IX from V or from VI). The areas of the hydroxymethyl and hydroxymethylene proton peaks undergo changes which can readily be interpreted in light of the proposed structure of compound IX (Figure 2C). As predicted from the proposed structures (Figure 1), periodate oxidation and borohydride reduction of the hypoxanthine–deoxyglucose adduct also produced compound IX. This is indicated by chromatographic evidence (Table I).

Compounds VII and VIII were subjected to the same treatments. The data (similar to those given for compounds V and VI, summarized in Table I) indicate that compounds VII and VIII are diastereomers which become enantiomers (compounds X) when C-5 is removed by oxidation.

The ultraviolet absorption spectra of purine and pyrimidine derivatives can be compared to standard spectra (Elion, 1962; Singer, 1975) and used to identify the site of derivatization. Comparison of spectra in acidic and basic solutions usually gives an unambiguous identification. Table II summarizes some of the data obtained. The spectra of compounds II and IV and their derivatives clearly indicate N-9 substituted hypoxanthine. The spectra are very similar to the nucleoside inosine (Table II). The spectra of compounds I and III are characteristic of N-7 derivatization [comparison with the data of Elion (1962)]. The NMR resonance peak assigned to H_3 also shows a difference in these two groups of compounds. This peak occurs at 4.9 ± 0.07 ppm for all compounds derived from II and IV and at 5.15 ± 0.06 ppm for compounds derived from I and III (Table I). This difference could arise from the chemical linkage involved in the two groups of compounds. The assigned H_3 proton peak for compound V shows considerable splitting which is diminshed when the methylene protons are decoupled by irradiation at 2.36 ppm (spectra not shown). This agrees with the adjacent position of H_3 and the methylene protons (Figure 1) and also supports the assignment of the H_3 proton resonance.

Extinction coefficients for ultraviolet absorption were estimated by using deoxyglucose-^{14}C (Table II). The coefficients obtained from uniformly labeled or deoxyglucose-*1*-^{14}C were approximately the same (Table II). This indicates that the carbon chain of the sugar is not fragmented during product formation. The estimated extinction coefficients are similar to those of known standards. This suggests that the ratio of base–deoxyglucose in the products is 1:1. These observations

Table II: UV Absorption Properties

	in acid		in base	
	λ_{max} (nm)	$\epsilon \times 10^{-3}$	λ_{max} (nm)	$\epsilon \times 10^{-3}$
hypoxanthine II and IV[a]	250[c]	10.2[d] 11.2[e]	254	1.06χ[f]
hypoxanthine I and III	256[c]	8.1[d] 8.9[e]	263	1.07χ[f]
inosine[b]	248[c]	12.2	253	13.1
adenine II and IV[a]	259	13.0[d] 12.3[e]	261	1.03χ[f]
adenosine[b]	257	14.6	260	14.9
uracil II and IV[a]	265	6.9[d] 7.4[e]	264	0.75χ[f]
uridine[b]	262	10.1	262	8.5
cytosine II and IV[a]	282	χ	272	0.68χ[f]
cytidine[b]	280	13.4	271	9.1

[a] These compounds are analogues of those shown in Figure 2. [b] Taken from the table "Properties of the Nucleic Acid Derivatives" compiled by Calbiochem, San Diego, CA. [c] These maxima were determined at neutral pH. [d] Extinction coefficient is estimated from the ratio of 2-deoxyglucose-^{14}C (uniformly labeled) to ultraviolet absorbance. A single determination is made. [e] The extinction coefficient is based on 2-deoxyglucose-*1*-^{14}C as outlined in *d*. [f] The extinction coefficient in base is expressed relative to the extinction coefficient in acid (i.e., extinction coefficient in acid = χ).

are in agreement with the NMR data.

The results of a number of experiments with other sugars and bases are summarized in Tables I and II, and a few of the principal observations will be outlined here. Only two products separated from the hypoxanthine–deoxyglucose reaction (Table I). The ultraviolet absorption spectra indicated that one product contained N-9 derivatized hypoxanthine and that the other contained N-7 derivatized hypoxanthine (Tables I and II). The diastereomers of the hypoxanthine-deoxyglucose adduct apparently do not separate in solvent system I. A single major product separated from adenine–deoxyribose reactions, while two products were obtained from adenine–deoxyglucose reactions. These latter products may be analogous to the diastereomers II and IV (Figure 1). Ultraviolet absorption spectra indicated that all adenine products contain N-9 derivatized adenine. The extinction coefficient (Table II) indicates a 1:1 ratio of adenine–deoxyglucose in the product. Reactions of adenine also contained small quantities of additional products which were not studied.

The pyrimidines each produced a single major product when reacted with either 2-deoxyglucose or 2-deoxyribose (Table I). The ultraviolet absorption spectra and extinction coefficents indicate N-1 derivatization (Table II) and a 1:1 ratio of pyrimidine–deoxy sugar. The NMR spectra showed resonance peaks closely related to those of the hypoxanthine derivatives. Both the chemical shifts (Table I) and the peak areas were similar.

Reaction Mechanism. The structures and reactions shown in Figure 1 obtain further support from mechanism and model compound studies. A 2-deoxy sugar is an aldol compound and therefore should be subject to the dehydration shown (rection 1, Figure 1). Proton-exchange studies suggest that this reaction occurs. When the condensation is conducted in ^2H_2O, the product shows a 50% reduction of the methylene proton peak (Figure 2D). This relative change was observed for all four of the hypoxanthine products. Exchange of this single proton is consistent with the dehydration shown (Figure 1). The methylene proton peak is reduced by no more than 50% (Figure 2D), and it follows that reactions 1 and 2 (Figure 1) are not in rapid equilibrium.

The amino compound would most likely function by forming a Schiff base with the aldehyde. This might labelize the α proton and thereby stimulate loss of the β-hydroxyl group. Labelization of an α proton would be similar to the first step of the Amadori rearrangement (Hodge & Fisher, 1963). The similarity of these reactions is supported by the failure of aromatic amines to function in this reaction; no derivatives of adenine or cytosine were obtained unless an aliphatic amine was added. A Schiff base containing an aromatic amine is not sufficiently basic to participate in labelization of the α proton (Hodge & Fisher, 1963).

Reaction 2 of Figure 1 is the formation of a Michael adduct (Bergmann et al., 1959) between the unsaturated carbonyl and the purine or pyrimidine. This reaction was studied with model compounds. A solution of methyl vinyl ketone (1.0 M) and adenine (0.1 M) was heated at 75 °C for 10 min. Essentially quantitative derivatization of the adenine occurred (the product chromatographed at R_{Ade} 1.25 in solvent system I). Amino compounds did not appear to facilitate this reaction. A solution of uracil (0.1 M) and methyl vinyl ketone (1 M) was heated for 2 h at 75 °C. Again, a nearly quantitative yield of a uracil derivative was obtained (R_{Ura} 1.2 in solvent system I). The ultraviolet absorption spectra of these compounds indicated that adenine was derivatized at the N-9 position and uracil was derivatized at the N-1 position. Acrolein was much more reactive with high yields of an adenine derivative formed after 10 min at 45 °C (0.05 M adenine, 0.15 M acrolein). The acrolein product showed the anticipated reactivity toward amino groups and borohydride reduction. This product was mixed with ethanolamine-^{14}C (0.1 M) and reduced with sodium borohydride. The resulting compound (R_{Ade} 0.1 in solvent system I) showed ultraviolet absorption properties of the parent compound and contained covalently bound ethanolamine-^{14}C. This is consistent with borohydride reduction of the Schiff base. These studies support the sequence of reactions shown in Figure 1 and indicate that reaction 2 is a very facile process which is not facilitated by an amine. An important feature of these condensations is the reactivity of the N-9 position of the purines; previous studies have shown that nucleophilic attack by N-3 predominates in other reactions (Leonard & Fujii, 1963; Leonard & Laursen, 1965).

Reaction of adenine with higher concentrations of acrolein or methyl vinyl ketone resulted in multiple derivatizations (indicated by both chromatographic mobility and ultraviolet absorption spectra). It was also found that acrolein and methyl vinyl ketone will derivatize adenosine, guanosine, and cytidine. These further additions may be valuable for derivatization of nucleic acids in general.

Discussion

These studies have demonstrated amine-catalyzed condensation of purines and pyrimidines with 2-deoxy sugars. The several lines of evidence outlined above provide considerable evidence that the reactions and derivations are as shown in Figure 1. These reactions are of potential importance. Specific amine-catalyzed dehydration of deoxy sugars may be a concern for storage of deoxy sugars or laboratory experiments involving deoxy sugars in the presence of amino compounds. Addition of purines and pyrimidines to unsaturated carbonyl compounds may be valuable for the synthesis of biologically active molecules which might function as nucleoside analogues and enzyme inhibitors. Furthermore, α,β-unsaturated carbonyl compounds may be valuable for derivatization of nucleosides and polynucleotides in general.

The initial goal of these studies was to demonstrate nucleic acid synthesis under prebiotic conditions. The reactions observed would rapidly deplete the components of nucleic acids and thereby present a major problem for prebiotic nucleic acid assembly. Other spontaneous reactions such as the Amadori rearrangement would also deplete the essential components of proteins and nucleic acids. The prebiotic synthesis of nucleic acids therefore appears to be much more difficult than is often appreciated. The relevance of nucleic acids under prebiotic conditions seems very questionable.

The products of the condensations observed here contain N-9 derivatized purines and N-1 derivatized pyrimidines. These are the sites of derivatization which allow base pairing by polynucleotides. Perhaps appropriate polymers of the spontaneously formed molecules are capable of acting as a template and may have functioned for early life forms. The structures of RNA and DNA may have arisen during evolution and displaced the earlier genetic material. Further investigations may identify other spontaneous condensations which may be relevant to the genesis of biological systems and useful to modern biology.

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