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Structural Studies on HCN Oligomers*

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Summary. NMR spectral studies on the HCN oligomers suggest the presence of carboxamide and urea groupings. The release of CO_2, H_2O, HCN, CH_3CN, $HCONH_2$ and pyridine on pyrolysis is consistent with the presence of these groupings as well as carboxylic acid groups. No basic primary amine groupings could be detected with fluorescamine. Hydrazinolysis of the HCN oligomers releases 10% of the amino acids normally released by acid hydrolysis. The oligomers give a positive biuret test but this is not due to the presence of peptide bonds. There is no conclusive evidence for the presence of peptide bonds in the HCN oligomers. No diglycine was detected on partial hydrolysis of the HCN oligomers at pH 8.5 suggesting that HCN oligomers were not a source of prebiotic peptides.

Key words: HCN – HCN Oligomers – Peptide – Pyrolysis – Biuret test – Hydrazinolysis – ^{13}C-NMR – Chemical evolution – Primitive earth

Introduction

Dilute, aqueous solutions of HCN condense to give oligomers in mildly basic solution (Sanchez et al. 1967). Purines, pyrimidines and amino acids are released on hydrolysis of these oligomers, a finding which suggests that HCN may have been an important starting material for the synthesis of these three main classes of nitrogen-containing biomolecules on the primitive earth (Ferris et al. 1978 and the references therein). The structures of the HCN oligomers are of interest not only because they break down to biomolecules but also because the intact oligomers may have served as catalysts for prebiotic processes.

The oligomerization of HCN was first observed in the early 1800's (Proust 1806, 1807; Gay-Lussac 1815; Boullay 1830 a, b) but little progress has been made in elucidating the structural units present in these oligomers. In one postulate (Scheme 1, path A) it was suggested that the oligomers are formed by the polymerization of a HCN dimer (iminoacetonitrile, *1*) to a linear polymer (*2*) which then undergoes extensive crosslinking between the amino and nitrile groupings to give a ladder structure (*3*) (Völker 1960). A similar structure was proposed for thermalized polyacrylonitrile (Monahan 1966). Matthews and coworkers (Matthews and Moser 1967; Matthews 1979 and references therein) proposed (Scheme 1, path B) the oligomers are formed by the polymerization of azacylcopropenylideneimine (*4*) form of the HCN dimer to give heteropolyamidines (*5*) which hydrolyze to heteropolypeptides (*6*) in aqueous media. The present study was undertaken because there is little experimental data to support either structural postulate. The crosslinked structure (*3*) is supported mainly by the dark color of the HCN oligomers which is consistent with the presence of conjugated unsaturation. The heteropolypeptide structure (*6*) is supported by infrared absorption consistent with the presence of amide groups (Draganić et al. 1977), a positive biuret test (Draganić et al. 1976 a,b; 1977) and the release of amino acids on acid hydrolysis of the HCN oligomers.

There is also evidence which is not consistent with these structural postulates (Ferris 1979; Ferris et al. 1979). The dimer of hydrogen cyanide (*1* or less likely *4*) cannot be the monomer which condenses to the HCN

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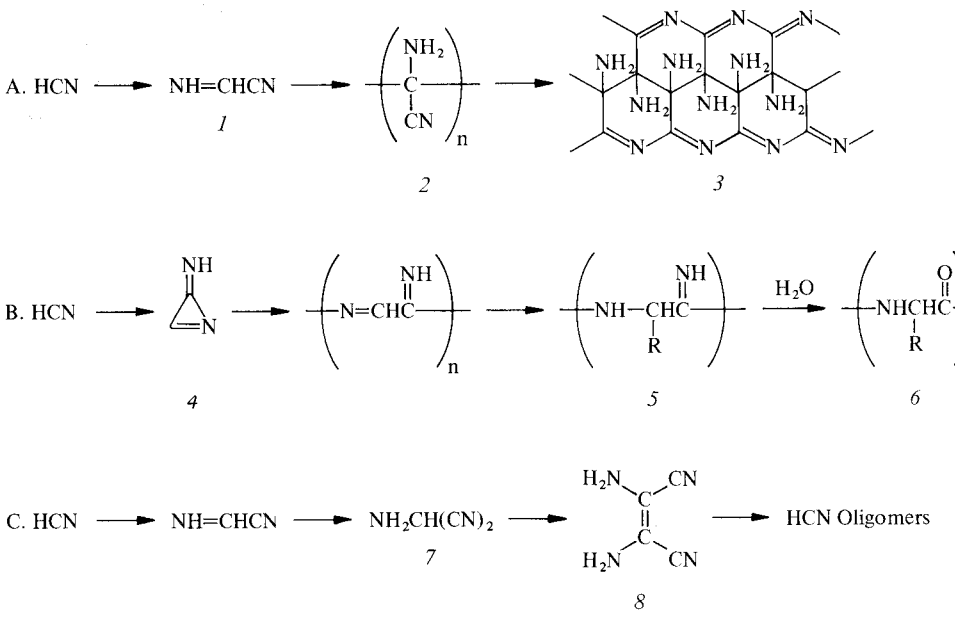


oligomers. It was shown that diaminomaleonitrile (DAMN) (*8*) is formed rapidly and irreversibly from HCN (Scheme 1, Path C) before the oligomers are observed (Ferris and Edelson 1978), hence DAMN (*8*) and not an HCN dimer must be the monomer which condenses to the HCN oligomers. The heteropolypeptide structure (*6*) is not consistent with the observation that the HCN oligomers are resistant to cleavage by proteolytic enzymes (Ferris et al. 1973; Labadie et al. 1967). Structure *3* is not consistent with the release of amino acids on hydrolysis of the HCN oligomers.

Experimental[1]

General Procedures

Ultraviolet and visible spectra were recorded on Unicam Model SP 800A and Cary 219 spectrophotometers using one-cm path length quartz cells. Proton NMR spectra were recorded on either a Varian Model T-60 spectrometer (60 MHz), a Varian Model HA-100 spectrometer (100 MHz), or a Bruker Model WP-60 Fourier Transform spectrometer (60 MHz). A Varian Model C1024 time-averaging computer (CAT) was used in conjunction with the HA-100 for obtaining time-averaged spectra. Carbon-13 NMR spectra were obtained on either a Bruker Model WP-60 spectrometer (15.08 MHz) or a Varian Model XL-100 spectrometer (25.13 MHz). Infrared spectra were obtained on either a Perkin-Elmer Model 337 grating infrared spectrometer or a Perkin-Elmer Model 137B NaCl spectrophotometer. Mass spectra were obtained on a CEC Model 20–130 mass spectrometer. Fluorescence studies were performed using a G.K. Turner Model 430 spectrofluorometer. Amino acid analyses were performed on a Glenco Model MM-70 instrument equipped with a microbore column packed with a DC-4A cation exchange resin (Li^+). Paper chromatography was performed by the ascending method on Whatman 3MM paper. Electrophoresis was performed on 36" x 5" strips of Whatman 3MM paper at 3000 volts for 3.5 h on a Savant HV 5000 apparatus using a formic acid buffer prepared by adjusting 0.05 M formic acid to pH 2.7 with NH_4OH. HCN oligomers were fractionated by the ion exchange procedures described previously (Ferris et al. 1978). Freeze-dried HCN oligomers were prepared by freeze-drying the total oligomerization mixture. The dried product was heated at 70°C in vacuo for 10 days to remove urea and ammonium salts by sublimation. The term HCN oligomer is used synonymously with the acidic and amphoteric oligomer fraction (Ferris et al. 1978) unless designated as the freeze-dried oligomers.

Amino Acid Analyses

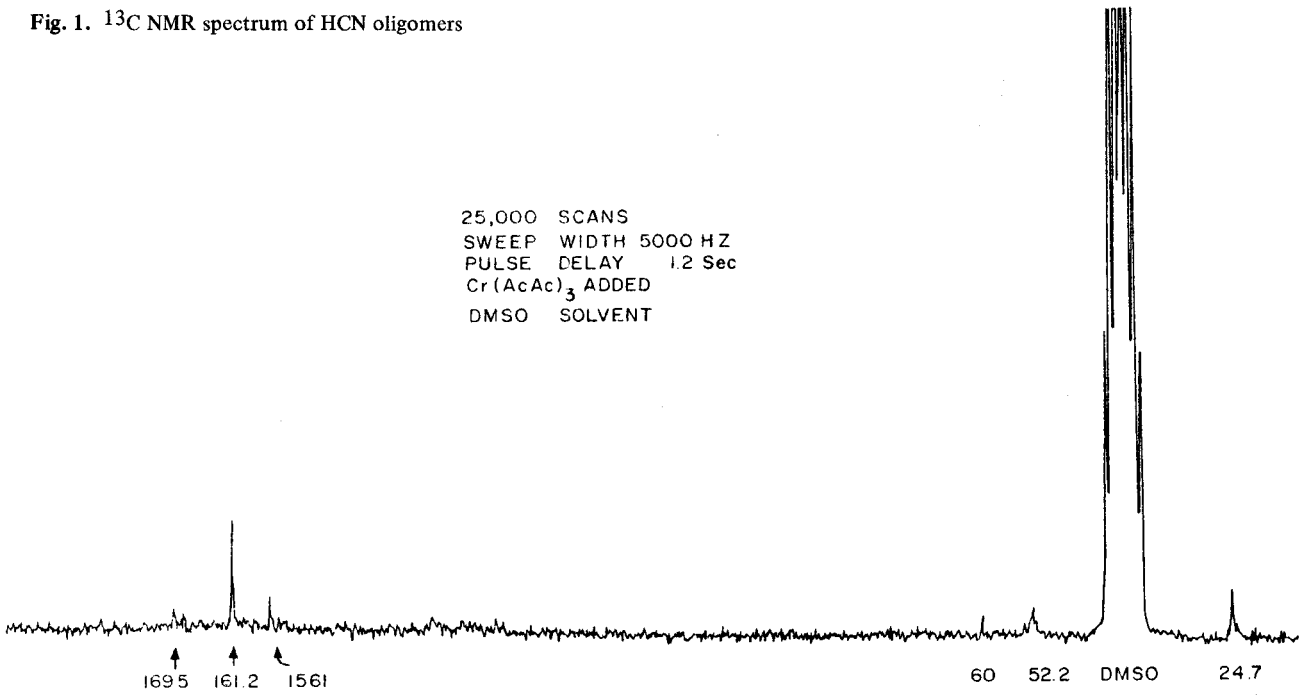
The basic and amphoteric fraction formed by the acid hydrolysis of various HCN oligomer preparations (Ferris et al. 1978) was analyzed. Qualitatively the same pattern of ninhydrin positive substances was obtained from the HCN oligomers prepared when oxygen was rigorously excluded (Ferris et al. 1978), when oxygen was not excluded, or if the freeze-dried oligomer was used (Ferris et al. 1973a; Edelson 1977). Some free amino acids were also detected in the HCN oligomers before acid hydrolysis. The amounts of these amino acids were much less in the freeze-dried oligomers. Presumably there is some degradation of the HCN oligomers by the acid treatment that is used to elute them from the ion exchange resin (Ferris et al. 1978). It was not possible to detect citrulline as one of the amino acids formed by hydrolysis using the amino acid analyzer. We did not investigate the presence of citrulline in the acid hydrolysate of the basic fraction of HCN oligomers. This fraction was reported to release the highest yield of citrulline (fraction F in Ferris et al. 1973).

NMR Spectra

a. ^{13}C NMR. A 445 mg sample of the oligomers prepared in the presence of air was dissolved in dimethylsulfoxide-d_6 with small amounts of tetramethylsilane and chromium(III) acetylacetonate added. 25,000 scans were accumulated using a 5,000 Hz sweep width, 1.2 s pulse delay and a 30 μs pulse width. The resulting ^{13}C NMR spectrum is shown in Fig. 1. The possible assignments of the observed peaks given in Table 1 were made by comparison with literature data (Levy and Nelson 1972; Stothers 1972).

[1] Detailed procedures are given in the Ph.D. Thesis of E. Edelson, Rensselaer Polytechnic Institute, 1977, and the M.S. Thesis of J. Auyeung, Rensselaer Polytechnic Institute, 1980

Fig. 1. ^{13}C NMR spectrum of HCN oligomers



b. ^1H NMR. The same sample of HCN oligomer used for ^{13}C NMR with the chromium(III) acetylacetonate removed was used to measure the ^1H NMR spectrum in dimethylsulfoxide-d_6. The chromium(III) acetylacetonate was removed by extraction with CH_2Cl_2. Three peaks were observed at 6.8, 7.3 and 7.8 ppm which decreased by 83% on addition of D_2O. There was also a small diffuse peak at 2.1 ppm which did not exchange with D_2O.

Table 1. ^{13}C NMR data for HCN oligomers

Chemical shift relative to TMS		Integral	Possible assignments
ppm	Hz		
169.5	4264.6	8	Acid, amide or ester carbonyl
161.2	4055.8	38	Urea carbonyl
156.1	3927.5	11	A carbonate, carbamate or N-substituted urea carbonyl
60.0	1509.8	5	Saturated carbon attached to oxygen, i.e., serine-type hydroxy or carbon α to a carboxyl group
52.2	1313.4	10	Saturated carbon attached to nitrogen, i.e., amine or imine or carbon α to a carboxyl group
24.7	621.5	12	Aliphatic carbon: methylene or methyl

Thermal Analysis of the HCN Oligomers

a. Differential Thermal Analysis. Differential thermal analysis of a 1 mg sample of the HCN oligomers purified by ion exchange chromatography was performed in a sealed pan. An endothermic transition was observed at 90°–110°C and at 195°C the pan burst. When the same analysis was performed in a open pan an 18% loss in weight was observed.

b. Pyrolysis GC/MS. A 2.6 mg sample of HCN oligomers was heated in a quartz boat in a Chemical Data Systems Model 190 Pyroprobe which was installed in the injection port of a Perkin Elmer 990 gas chromatograph. The injection port was maintained at 200° and the sample heated successively at 600°C, 850°C, and 1000°C for 20 s. The gaseous products evolved during each heating cycle were swept by the carrier gas onto a Porapak Q column and a Porapak R column (each 1/8" x 6') coupled in series. The products were initially identified by comparison of their retention times with those of authentic samples and their structures were established by mass spectral analysis of a sample that had been pyrolyzed only at 1000°C. The major reaction products were identified by GC/MS as CO_2, H_2O, HCN, CH_3CN, $HCONH_2$, and pyridine. Trace amounts of CH_4, $(CN)_2$ and $CH_3CH{=}CH_2$ were provisionally identified by GC analysis alone after heating to 600°C. Larger yields of $(CN)_2$, together with HCN and CH_3CN, were observed when the sample was further heated to 850°C and 1000°C while little or no pyridine and $HCONH_2$ were formed at these higher temperatures. A residue of 1.7 mg remained after heating the sample to 1000°C.

Attempted Reduction of HCN Oligomers with the Dimethylamine Borane Complex

A 10 mg sample of HCN oligomers was added to 5 ml of glacial acetic acid, the mixture was stirred at room temperature 30 min and then 10 mg $(CH_3)_2NH{\cdot}BH_3$ was added. The UV spectrum of this mixture did not change when monitored for 26 h and was identical to a control solution of the HCN oligomers in acetic acid. Initial studies indicated that 80% of the HCN oligomers are soluble in glacial acetic acid. The UV measurements were performed on the soluble portion. Aliquots of the reaction mixture and control solution were freeze-dried after 2 h. The samples were hydrolyzed with 6 N HCl at 110°C for 24 h and analyzed using an amino acid analyzer. There was essentially no difference in the pattern of amino eluted from the two samples indicating that $(CH_3)_2NH{\cdot}BH_3$ did not reduce the precursors to the amino acids.

Reaction of the HCN Oligomers with Hydroxylamine

a. Test for Aldehyde and Ketone Groups. Aliquots of aqueous solutions of HCN oligomers (10 mg/ml), formaldehyde (0.1 M) and acetone (0.1 M) were added to 1 ml of a 0.5% solution of hydroxylamine hydrochloride in 95% ethanol and the pH of the solution was determined using a pH meter (Shriner et al. 1964; Fritz et al. 1959). A decrease in pH indicates the presence of an aldehyde or ketone grouping. The results given in Table 2 are averages of duplicate determinations of the pH. A 0.1 mg HCN oligomer sample and 0.1 μmol of formaldehyde or acetone were just below the limit of detection for the test. These data indicate the presence of one carbonyl group per 1000 g of HCN oligomer.

Table 2. Hydroxylamine hydrochloride test for carbonyl groups

HCN Oligomers		HCHO		$(CH_3)_2CO$	
mg	pH	μmol	pH	μmol	pH
0	3.4	0	3.1	0	2.9
1.0	2.8	10	2.1	10	2.0
0.5	3.0	5	2.2	5	2.1
0.25	3.1	1	2.6	1	2.4
0.1	3.3	0.5	2.6	0.5	2.7
0.05	3.3	0.1	2.9	0.1	2.8

b. Test for Amide and/or Nitrile Groups. A 2 mg sample of the HCN oligomers was added to 0.5 ml of 1 M hydroxylamine hydrochloride in propylene glycol and 0.125 ml of 2 N KOH in propylene glycol was added. The mixture was heated to boiling for 2 min, cooled, and 0.2 ml of 5% $FeCl_3$ in ethanol was added. The color of the solution changed from yellow to red-brown on addition of $FeCl_3$. A blank was prepared to which the same substances were added but the solution was not heated (Shriner et al. 1964; Kasai et al. 1975). The blank did not change color on addition of $FeCl_3$. The visible spectrum of the test solution showed a maximum at 605 nm when the blank was used as a reference. Acetamide, diglycine and urea all gave positive tests using this procedure but the absorption maxima in the visible region were very concentration dependent in the range 500–700 nm so it was not possible to obtain quantitative data.

Reaction of HCN Oligomers with Hydrazine

A mixture of 25 mg HCN oligomers dissolved in 2 ml anhydrous hydrazine (distilled before use) was degassed by 3 freeze-pump-thaw cycles and then sealed in an ampule. The ampule was heated at 80°C for 24 h, and then cooled and the excess hydrazine was removed in a vacuum desiccator over Drierite, H_2SO_4 and P_2O_5. The residue was dissolved in water and the amino acids and the amino acid hydrazides were separated by ion exchange chromatography on 30 g of an IRC-50 cation exchange resin. The acidic and neutral amino acids were eluted with 500 ml water (Schroeder 1972). The eluate was evaporated to dryness, dissolved in 1 ml of pH 2.2 lithium citrate buffer and injected onto the column of an amino acid analyzer. HCN oligomers from the same batch were subjected to hydrolysis with 6 N HCl and the hydrolysate was analyzed on an amino acid analyzer. The results are given in Table 3.

Detection of Primary Amine Groupings with Fluorescamine

An aqueous solution of the compound to be tested was dissolved in 5 ml of 1 M pH 8.2 phosphate buffer and 0.1 ml of an acetone solution of fluorescamine (1 mg/ml) was added. The final volume of the solution was adjusted to 6 ml with water. The sample was excited at 390 nm and the emission was measured in the 420–520 nm range (Stein et al. 1973; Bohlman et al. 1973). Blanks with acetone or fluorescamine in acetone exhibited no fluorescence. The results are given in Table 4.

Biuret Tests for Peptide Bonds

a. Standard Test. To a 250 ml solution of 750 mg $CuSO_4 \cdot 5H_2O$ and 3 g sodium potassium tartrate were added 150 ml of 10% NaOH and 2 g KI and the mixture was diluted to 1 ℓ (Gornall et al. 1949). A solution of 5 mg bovine serum albumin (BSA) in 8 ml of this reagent gave an absorbance of 0.17 at 540 nm. No maximum was observed in the 500–600 nm region when either 10 mg of the freeze-dried oligomers or the acidic or amphoteric oligomers (Ferris et al. 1978) were dissolved in 4 ml of the reagent.

b. Modified Test. Stock solutions of 5 g $CuSO_4 \cdot 5H_2O$ in 100 ml distilled water and 175 g NaOH in 200 ml distilled water were prepared. The stock solution of $CuSO_4$ (1 ml) was mixed with 10 ml of the stock solution of NaOH and diluted to 110 ml before use. The blank which did not contain $CuSO_4$ was dis-

Table 3. Amino acids yields from hydrazinolysis and acid hydrolysis of HCN oligomers[a]

Amino acid[b]	Yield[c]		
	No hydrolysis	Hydrazinolysis	Acid hydrolysis
aspartic acid[d]	1.1[e]	0.2	4.1
d,l-diaminosuccinic acid	0.5	0.9	8.5
glycine	0.1	0.6	5.3
alanine	0	0.02	0.3
α-aminoisobutyric acid	0.01	0	0.9
Total	1.7 (0.8)[f]	1.7	19.1

[a]Freeze dried HCN oligomers were used

[b]The yields of those amino acids which have been conclusively identified are given (Ferris et al. 1974, 1978). Other unidentified amino acids were also observed. The pattern of amino acids released by hydrazinolysis differed from those released by acid hydrolysis

[c]mg/L of the original oligomerization mixture

[d]This substance may also be meso-diaminosuccinic acid (Ferris et al. 1974)

[e]There were many substances eluted in the aspartic acid region. The apparent high yield is probably due to overlap with some of these other substances

[f]Total assuming aspartic acid to be 0.2

Table 4. Fluorescence of HCN oligomers, glycine and urea when mixed with fluorescamine[a]

	Amount (μg)	Fluorescence Maximum (nm)	Intensity[b]
1. glycine	2.2	485	9
2. HCN oligomers[c]	210	445	68
3. HCN oligomers	210	445	68
4. Mixture of HCN oligomers and glycine	210 and 30, respectively	465	95
5. Acid hydrolyzed HCN oligomers[a]	210	465	95
6. DAMN	200	–	–
7. Urea	200	–	–

[a]Basic and amphoteric compounds isolated when 210 μg of HCN oligomers subjected to hydrolysis (Ferris et al. 1978)
[b]Arbitrary units
[c]Blank without added fluorescamine

solved in a 1:11 dilution of the stock NaOH solution (Ellman 1962). Aliquots (50–250 μl) of a solution of the sample to be tested were added to each of two test tubes. Distilled water was added to bring the total volume in each tube to 300 μl. The diluted $CuSO_4$ solution was added to one tube and the diluted NaOH was added to the second tube. Reagent blanks were prepared in the same way using 300 μl of distilled water. The absorbance of each solution was measured after 30 min using the respective reagent blank as the reference. The difference in absorbance between the $CuSO_4$ and NaOH solutions of the samples at 263 nm was measured (Figs. 2 and 3). The reagents were cooled to 0–5°C prior to use and during the 30 min reaction period to minimize the loss of absorbance at 263 nm when HCN oligomers were tested. A linear plot of Δ absorbance vs increasing concentration of sample is considered to be a measure of the presence of peptide bonds (Ellman 1962). BSA (3 mg/ml) gave a slope of 6.4, tetraglycine (1 mg/ml) gave a slope of 10.3 while urea (1 mg/ml) did not give a complex. The freeze-dried HCN oligomers (2 mg/ml) and the acidic and amphoteric oligomers (2 mg/ml) (Ferris et al. 1978) gave slopes of 3.3 and 3.6, respectively. The basic solution of the HCN oligomer exhibit a very strong absorption in the 300–325 nm range (Fig. 2) which is not observed with the basic solutions of tetraglycine and BSA (Fig. 3). This absorption decreases when Cu(II) is present in the basic solution and a maximum at 263 nm is observed.

c. Rate of Change of the 263 nm Absorption. The biuret test was performed on aliquots of a 5 mg/ml solution of the HCN oligomers and a 2 mg/ml solution of diglycine. UV maxima were observed at 266 nm for diglycine and 263 nm for the HCN oligomers 30 min after mixing. The absorbance of a 0.5 ml aliquot of diglycine increased from 1.1 to 1.3 and the maximum shifted from 266 nm to 269 nm on standing at room temperature for 4 h. The absorbance at 263 nm of a 0.6 ml aliquot of the HCN oligomers decreased steadily from 0.44 to 0 at room temperature. The half-life for the loss was approximately 60 min.

d. Hydrolysis of the HCN Oligomers and Tetraglycine Monitored by the Modified Biuret Test. Solutions of 10–20 mg of tetraglycine and the freeze-dried HCN oligomers were prepared in 4.5 ml of H_2O, the pH was adjusted to 9 with concentrated NaOH and the mixtures were heated in sealed glass ampules for different time periods. The cooled solutions were then subjected to the modified biuret test (Ellman 1962). The HCN oligomers no longer gave a positive test (the slope of the Δ absorbance vs concentration plot = 0) when heated for 4 days at 110°C. Tetraglycine gave positive tests after 4 and 7 days at 110°C and a negative test after 4 days at 150°C.

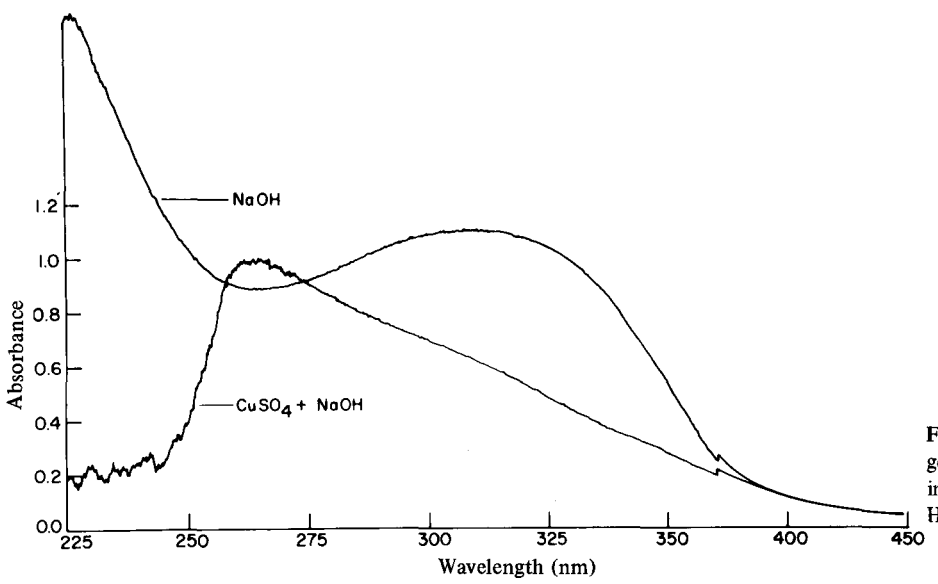


Fig. 2. Modified biuret test on HCN oligomers. NaOH curve – HCN oligomers in 2 M NaOH. $CuSO_4$ + NaOH curve – HCN oligomers in biuret reagent

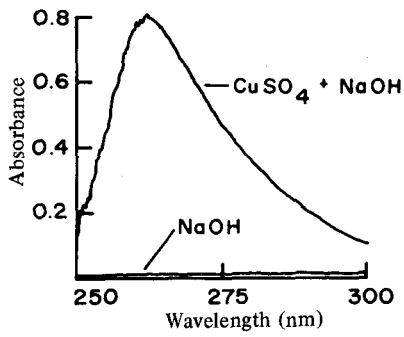


Fig. 3. Modified biuret test on diglycine. NaOH curve – diglycine in 2 M NaOH. $CuSO_4$ + NaOH curve – HCN oligomers in biuret reagent.

Attempted Formation of Diglycine by Partial Hydrolysis of HCN Oligomers

a. Hydrolysis of Diglycine and Tetraglycine. Samples of diglycine and tetraglycine (1.75 mg) were separately dissolved in 5 ml of H_2O and the pH was adjusted to 8.5 with dilute NaOH. The solutions were heated at 150°C in sealed ampules for various time periods between 8 and 72 h. A 0.5 ml sample (final pH 8.2) was spotted on paper for analysis by electrophoresis in pH 2.7 formate. Three substances were detected using the Cl_2-toluidine spray for peptides (Scoffone and Fontana 1975) at 15.5 cm, di- and/or tetraglycine; 7.5 cm, glycine; 2.5 cm, the diketopiperazine of glycine. The hydrolysis of diglycine, tetraglycine and the diketopiperazine of glycine was complete in 48 to 72 h. Appreciable hydrolysis had already occurred at the end of 6 h as shown by the detection of glycine and its diketopiperazine in the hydrolysate of both diglycine and tetraglycine.

b. Hydrolysis of HCN Oligomers. Samples (15 mg) of HCN oligomers were dissolved in 5 ml of H_2O, the pH was adjusted to 8.5 and the solutions were heated at 150°C for time periods of 6, 18, and 36 h in sealed ampules. A 7.5 ml aliquot (final pH 7.5) was concentrated and spotted on paper for analysis by electrophoresis in pH 2.7 formate. No diglycine was detected using the Cl_2-toluidine reagent (Scaffone and Fontana 1975). The limit of detection of diglycine was found to be 5 μg using this reagent. Since a 3–5% yield of glycine is obtained by acid hydrolysis of the HCN oligomers a 225–375 μg yield would be obtained starting from 7.5 mg. Since the limit of detection of diglycine is 5 μg, a 1.3–2.2% yield of diglycine would have been detected. Diglycine was observed to absorb UV light after it was sprayed on the paper electrophoresis with 5% ClO^-. No diglycine was observed in the HCN oligomers hydrolysate using this detection method.

A substance which gives a positive color test with the Cl_2-toluidine reagent and an electrophoretic mobility identical with the diketopiperazine of glycine was also detected in the hydrolysate. It was shown not to be the diketopiperazine of glycine by a different R_f value on paper chromatography (n-amyl alcohol:pyridine:H_2O, 7:7:6) (Hais and Macek 1963) and by UV absorption maxima at 263 nm in acidic and neutral solution and 259 nm in basic solution. This substance was subsequently shown to be present in the HCN oligomers before hydrolysis.

Results

The heterogeneous nature of the HCN oligomers is suggested by the ill-defined ^1H- and ^{13}C-NMR spectra. The most intense ^{13}C-NMR resonance (Fig. 1) at 161.2 ppm is consistent with the presence of the urea carbonyl group which is reported to be at 161.2 ppm (Levy and Nelson 1972). This signal may also be due to urea present in the sample which did not sublime (Ferris et al. 1978). The signal at 169.5 ppm is assigned to the carbonyl group of carboxylic acid or its amide or ester derivative. This structural unit is consistent with the presence of the carboxyl groups of amino acids which have signals in the 168–172 ppm range (Wüthrich 1976) or less likely oxalic acid (166.3 ppm; Stothers 1972). The absence of a signal at about 120 ppm indicates few if any nitrile groups are present in the HCN oligomers (Levy and Nelson 1972). This is consistent with the IR spectrum which exhibits very weak absorption at 2250 cm^{-1} (Ferris et al. 1979). The carboxylic acid structural unit is consistent with the release of amino acids and oxalic acid on acid hydrolysis of the HCN oligomers (Ferris and Edelson 1978). Tentative assignment of the other ^{13}C-NMR signals is given in Table 1 based on literature tabulations of chemical shifts (Levy and Nelson 1972; Stothers 1972; Wüthrich 1976). The main signals in the ^1H-NMR spectrum at 6.80, 7.31 and 7.80 ppm are characteristic of protons bound to and spin decoupled with ^{14}N (Jackman 1959). These signals decrease in intensity when D_2O is added to the NMR tube indicating partial conversion of the N-H to N-D. This multiplet may be due either to the NH_2 of a substituted urea or to the presence of ammonium salts. Poorly defined signals at 2.1 ppm are consistent with the presence of aliphatic CH groupings.

Thermal treatment of the HCN oligomers resulted in a reaction at 90–110°C and release of gaseous products as the temperature was raised to 195°C. HCN, CO_2, CH_3CN, $HCONH_2$ and pyridine were identified by GC/MS after pyrolysis at 600°C. The pyrolytic formation of these products is consistent with the presence of carboxylic acid and amide groups in the HCN oligomers. Heterocyclic ring systems and structures which are thermalized to heterocycles are indicated by the formation of pyridine and HCN (Monahan 1966). HCN may also result from the pyrolysis of the amino acid precursors present in the HCN oligomers (Johnson and King 1971).

The formation of both oxalic acid and amino acids indicates that reduction and oxidation reactions must be involved in the oligomerization reaction (Ferris and Edelson 1978). Thre reduction of nitrile groups to imino functions was investigated by both the reaction of HCN with the dimethylamine borane complex and with hydroxylamine. Dimethylamine borane effects the reduction of imines to amines in acid solution (Billman and McDowell 1961, 1962). No reduction was detected as indicated by the absence of change in both the UV absorption spectrum of the HCN oligomers and in the yield and product distribution of the amino acids released from the HCN oligomers on acid hydrolysis. Low levels (1 mole per 1000 g of oligomers) of imine, aldehyde or ketone groups was further demonstrated by a hydroxyl-

amine test (Shriner et al. 1964; Fritz et al. 1959) for these functional groups.

Evidence for the presence of the amide, nitrile and urea groupings suggested by spectral studies was obtained using a modified hydroxylamine test (Shriner et al. 1964; Kasai et al. 1975). HCN oligomers, acetamide, urea and diglycine all gave a positive test with this reagent. It was not possible to use this test to make a quantitative assay of the number of groupings in the HCN oligomers which reacted with the reagent.

The possibility that the amide groups detected in the above studies might be peptides was then investigated. Chemical methods were utilized since proteolytic enzymes do not catalyze the hydrolysis of the HCN oligomers (Ferris et al. 1973; Labadie et al. 1967). Hydrazinolysis was used to determine the percentage of amino acids in the HCN oligomers with free carboxylic groups; this technique is used to determine the C-terminal amino acid in a polypeptide chain (Schroeder 1972). The C-terminal amino acid is released while the other amino acid residues are converted to their hydrazides. This C-terminal amino acid is then identified by its retention time on the amino acid analyzer.

An amino acid yield which is 5–10% of that produced by acid hydrolysis was obtained by hydrazinolysis of the HCN oligomers. Anhydrous hydrazine is as effective as 6 HCN in cleaving proteins and assuming hydrazine is as effective as aqueous acid in degrading the HCN oligomers these results indicate that 5–10% of the amino acid precursors bound in the HCN oligomers have free carboxyl groups (Table 3). These amino acid precursors are not at the C-terminus of a polypeptide chain since they are not cleaved by pronase or carboxypeptidase (Ferris et al. 1973).

The presence of basic primary amine groupings such as those found at the N-terminus of a peptide chain can be readily detected by the fluorescence of the adduct formed between fluorescamine and the sample (Bohlen et al. 1973; Stein et al. 1973). This is a sensitive technique as demonstrated by the ready detection of 2.2 μg of glycine by an emission band at 485 nm. This amount of glycine is equivalent to 10% of the total yield of amino acids obtained by acid hydrolysis of 210 μg of the HCN oligomers. We found that both the HCN oligomers and the acid hydrolysate of the HCN oligomers exhibited fluorescence at 445 nm in the absence of fluorescamine (Table 4). The intensity of this fluorescence did not increase when fluorescamine was added to the HCN oligomers but it did increase when the fluorescamine was added to the acid hydrolysate of the HCN oligomers and gave an emission maximum at 465 nm. We observed that the amount of amino acids mixed with the HCN oligomers may be determined approximately by the wavelength of the emission maximum (between 445 and 485 nm). Addition of 30 μg of glycine to 200 μg of HCN oligomers gives the same wavelength emission maximum as the acid hydrolysate resulting from 200 μg of HCN oligomers. This experiment confirms that a 10 ± 5% yield of amino acids is obtained by acid hydrolysis of the HCN oligomers and that the emission of the fluorescamine adducts are not quenched by the light absorbing groups in the HCN oligomers.

The above findings demonstrate the absence of basic primary amine groups in the HCN oligomers. The NH_2 groupings of amides, ureas or even that of diaminomaleonitrile may be present in the HCN oligomers since these groups do not form fluorescent adducts with fluorescamine (Table 4).

The observation of a positive biuret test has been cited as evidence for the presence of polypeptide groupings in the HCN oligomers (Draganić et al. 1976 a, b, 1977). A modification of the test was used (Ellman 1962) in which the UV absorption of the copper(II) complex of the oligomers was monitored at 263 nm because poorly defined spectra were observed at 560 nm, the standard wavelength for the assay (Gornal et al. 1949). We confirmed the report that the addition of Cu(II) to a strongly basic solution of the HCN oligomers gives a product with an absorption maximum at 263 nm and ill-defined absorption in the 560 nm region.

Three additional findings led us to conclude that the observed absorption at 263 nm is not due to the copper complex of peptide groupings in the HCN oligomers. First, a basic solution of the HCN oligomers exhibits a broad UV absorption band in the 300–235 nm region (Fig. 2) which is not observed with a basic solution of simple polypeptides (Fig. 3). This absorption may be due to the formation of the anion of the heterocyclic, phenolic or enolic groupings when the HCN oligomers are dissolved in strong base. The intensity of this absorption decreases and the UV maximum at 263 nm is produced when Cu(II) is added to the basic solution. The decrease observed on addition of Cu(II) indicates that a copper complex is formed with the functional group(s) responsible for the absorption at 325 nm in the HCN oligomers. These cannot be peptide groupings since peptides do not exhibit absorption in the 325 nm region in basic solution (Fig. 3). It is reported that the copper complex of imidazole exhibits a ligand band at 270 nm (Wilson et al. 1970), a finding consistent with the assignment of the 263 nm absorption band to the complex between Cu(II) and the anion of a nitrogen heterocycle. Second, the rate of hydrolysis of the substance giving the positive biuret test is at least twice as fast as the rate of hydrolysis of tetraglycine. Since it would be expected that any peptide links present in the HCN oligomers would be hydrolyzed at about the same rate as those in tetraglycine, we conclude that hydrolysis of peptide bonds is not responsible for the decrease in the absorbance at 263 nm. Third, the 263 nm absorption of the copper(II) complex of the HCN oligomers decreases with time ($t_{1/2} \sim 60$ min at room temperature) while that of diglycine increases slightly over a 4 h period and the maximum shifts from 266 nm to 269 nm.

This marked difference in stability of the copper complexes indicates that absorption at 263 nm is not due to a copper complex with peptide groups but rather with non-peptide groups in the HCN oligomers.

Hydrolytic studies demonstrate that diglycine is not released in detectable amounts from the HCN oligomers upon hydrolysis at pH 8.5. Since over 50% of the yield of amino acids formed by acid hydrolysis of the HCN oligomers is glycine (Edelson 1977) a high proportion of any peptide links would be expected to be between glycine units. Hydrolysis of the HCN oligomers, at time intervals which spanned the half-life for diglycine hydrolysis under the same reaction conditions did not result in the release of detectable amounts of diglycine or its diketopiperazine (Long et al. 1971). If $\sim$ 1% of the glycine released on acid hydrolysis were released as diglycine it would have been detected. It can be concluded from these experiments that either there are no diglycine units present in the HCN oligomers or that these units are present but the peptide bonds are hydrolyzed faster than the diglycine units are cleaved from the HCN oligomers. If there are no diglycine units present in the HCN oligomers then it is unlikely that other peptide links are present since glycine is the most abundant amino acid in its acid hydrolysate. If diglycine units are present but they are not released by hydrolysis at pH 8–9 then it appears unlikely that the postulated peptide links present in the HCN oligomers would have been effectively utilized on the primitive earth.

Discussion

In previous studies (Ferris and Edelson 1978) we established that a tetramer of HCN, DAMN (*8*), is formed irreversibly from HCN and concluded from this that HCN dimers cannot be the precursor to HCN oligomers. The irreversible formation of *8* was inferred from the lack of exchange of its cyano groups with $H^{13}CN$ at pH 9.2. This experiment was criticized because we did not observe the presence of ^{13}C-labeled *8* formed by the condensation of $H^{13}CN$ present (Matthews 1979). No ^{13}C-DAMN would be expected to be formed from the $H^{13}CN$ used in this experiment because the concentration of $H^{13}CN$ ($\sim$ 0.002 M) was less than the 0.01 M concentration required for HCN oligomerization to occur (Sanchez et al. 1967). Consequently, there is no reason to change our conclusion that *8* is formed rapidly and irreversibly from HCN and that the HCN oligomers are formed by the condensation reactions of *8*.

Functional group analysis of the HCN oligomers indicates the presence of ureas and carboxylic acid, carboxamide and other groupings with the same oxidation state as carboxylic acids. Nitrile groupings may be present as indicated by a weak infrared band at 2250 cm^{-1}. Preliminary ^{13}C-NMR data suggests that some of these carboxylic groups may be present as oxalic acid derivatives. No aldehyde, ketone, imine or basic primary amine groups were detected. Non-basic primary amine groups of the type found in ureas or *8* would not be detected by the analytical method used and may therefore be present in the HCN oligomers.

The release of amino acids by the acid hydrolysis of the HCN oligomers had led to the postulate that polypeptide units were present. However, the yield of amino acids is only 10 ± 5% (Edelson 1977) which places an upper limit of 10 ± 5% on the number of peptide bonds present. None of the findings of this research or in our previous studies (Ferris et al. 1973) support the presence of peptide links. A positive biuret test was believed to indicate the presence of peptide links but the present study showed that this test is misleading when applied to the HCN oligomers and careful analysis of the data indicates that peptide groups are not present. The amide absorption bands observed in the infrared spectra of HCN oligomers are consistent with the presence of simple amides as well as peptides (Draganić et al. 1977). We feel that this IR absorption is due to amide groups because of the chemical data observed in the present study which is consistent with the presence of simple amides.

It can be argued that if sufficiently sensitive methods of detection were used it might be possible to detect peptide links in the HCN oligomers. However, these peptide derivatives would be of significance to prebiotic chemistry only if the intact peptides were released on hydrolysis of the HCN oligomers under primitive earth conditions. No diglycine can be detected on partial hydrolysis of the HCN oligomers at pH 8.5, a finding which indicates that the HCN oligomers were not a source of peptides on the primitive earth.

The present study established the absence of peptide bonds in the HCN oligomers but it did not establish the structures of these oligomers. Although structure *6* has been eliminated by this research some elements of structure *3* may be present. The extensive UV-visible absorption observed for the HCN oligomers is consistent with the conjugated double bonds postulated in *3*. The hydrolytic release of heterocycles from the HCN oligomers is also consistent with the presence of cyclic structural units similar to those present in *3*. Although *3* is consistent with some of the chemical data it is much too simple a formulation to explain all the observed chemical transformations of the HCN oligomers. In addition, the HCN oligomers cannot be formed by pathway A in Scheme 1. Additional studies will have to be performed on fractionated samples of the HCN oligomers to obtain new insight into their structure.

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