STUDIES ON THE STRUCTURE OF HCN OLIGOMERS

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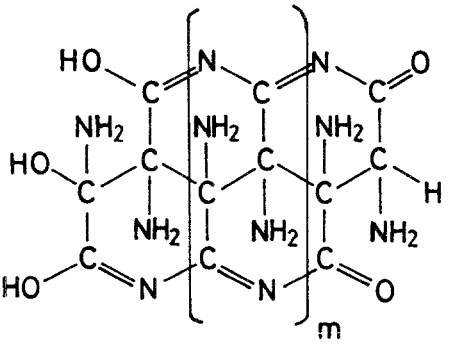
Abstract. The structure of the water-insoluble fraction of HCN oligomers (azulmic acid) was studied by IR, NMR, gel permeation chromatography, and chemical methods. The results show that nearly half of the nitrogen atoms contained in the oligomers are of the primary amino type and the other half are involved in —C = N— type bonding. It was found that the oligomers are easily and almost quantitatively acetylated, and the acetylated oligomers show characteristic acetyl amide IR absorption and NMR spectra. Owing to the greatly improved solubility due to acetylation, the molecular weight distribution was determined for the acetylated oligomers by gel permeation chromatography. Composite peaks were obtained ranging from 300 to 900 in molecular weight. Our results are essentially consistent with the structure proposed by Völker, but we point out there may be other possible structures also consistent with our experimental results.

Hydrogen cyanide has been considered as an important starting material in the prebiotic synthesis of proteins and/or nucleic acid bases. Many biologically important compounds such as amino acids and purine bases have been identified in the mixture of HCN oligomers or their hydrolysates. The water-soluble components of the oligomers or the hydrolysates have been well studied (Ferris and Hagan, 1984). However, water-insoluble or poorly soluble fractions of the HCN oligomers have been treated by only a few workers. Völker (1960) studied extensively the structure of the solid oligomers and proposed a structure I.



Structure I

Chemical and spectroscopic methods were applied by Ferris *et al.* (1981) to confirm the presence of various chemical groups in the oligomers. Labadie (1968) studied the solid HCN oligomers with special interest in their biological features. Matthews and

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coworkers (Matthews 1975; Matthews *et al.* 1977) continue their work on the structure(s) of the solid HCN oligomers, claiming that the oligomers contain precursors of polypeptides (polyamidine). Recently, a combined use of double-cross-polarization(DCP) MAS ^{15}N NMR and isotopic double-labeling has been applied by Matthews *et al.* to elucidate the structure of solid HCN oligomers and their hydrolysates (Matthews *et al.* 1984, McKay *et al.* 1984). The double-labeled oligomers were synthesized from an equimolar mixture of $H^{13}C^{14}N$ and $H^{12}C^{15}N$. According to their results, significant amount of ^{15}N absorption near 95 ppm (suggested as peptide nitrogen absorption) appears in the oligomers only after extensive hydrolysis. Before hydrolysis, the major fraction of the newly formed $^{13}C-^{15}N$ bonds seems to appear near 200 ppm, the region assigned as olefinic or aromatic nitrogen (McKay *et al.*, 1984).

1. Experimental

1.1. Synthesis of HCN oligomers and their acetylation

Oligomers were prepared by introducing HCN gas (0.25 mole) into 20 ml of concentrated aqueous ammonia and, after four days, collecting the precipitated solid oligomers which were then washed with water and dried over P_2O_5 in vacuo at 110° C. Acetylation was carried out by heating HCN oligomers with acetic anhydride in acetic acid under reflux for several hours, followed by evaporating the solvent and unreacted acetic anhydride under reduced pressure and finally drying the residue as described above. It was necessary to add a small amount of toluene before removing acetic acid completely from the oligomers.

1.2. Spectroscopic measurements

All IR spectra of the oligomers were measured as KBr pellets.

^1H NMR spectra of the oligomers in trifluoroacetic acid(TFA) were recorded at 90 MHz on a Hitachi R-42 spectrometer in continuous wave mode.

^{13}C solution NMR spectra of the oligomers were recorded on a Varian XL-300 spectrometer at 75.4 MHz, using a 5 mm switchable probe. The oligomers were dissolved in $H_3PO_4-D_2O$ mixture in 5 mm sample tubes. A 45° pulse was employed with 1.0 s pulse delay and the spectral width of 16.5 kHz. ^{13}C CP/MAS NMR was recorded on a Jeol FX-100 spectrometer at 100 MHz at Toray Research Center. The contact time was 2.0 msec and the spectral width was 10 kHz.

1.3. Determination of molecular weight distribution

The molecular weight distribution of the acetylated oligomers was determined by gel permeation chromatography with a TOYO SODA HLC-802UR through two G2000H8 columns connected in series. A saturated solution of acetylated oligomers in tetrahydrofuran (THF) was filtered and the filtrate was diluted ten times with THF, a 0.5 ml portion of which was injected. The columns were eluted with THF

rate of 1 ml min^{-1} at 40° C. The total elution volume was 30 ml and the eluent was monitored by a UV detector at 254 nm and also by a differential refractometer attached in parallel. The molecular weight was calculated from the calibration curve obtained from the elution time of standard polyethylene resins.

1.4. Hydrogenation of the Oligomers

About 0.05 g of PtO_2 suspended in 100 ml TFA was first equilibrated with H_2 in a conventional catalytic hydrogenation apparatus for one or two hours. Then the solid oligomer (0.07–0.10 g weighed out into a glass basket) was introduced into the TFA-Pt-black suspension. The temperature of the whole apparatus was kept within 19 ± 0.1° C in a temperature-regulated water-bath, because the result was sensitive to temperature due to the high vapor pressure of TFA. The total volume of hydrogen absorbed was measured using a gas buret.

2. Results and Discussion

Intact HCN oligomers have very poor solubilities in many solvents, and they are only partially soluble in acid media such as mineral acids (hydrochloric acid, sulfuric acid, and phosphoric acid). Up to now, the best solvent we have found for the oligomers is trifluoroacetic acid and various measurements have been carried out in this solvent. Also, acetylation improved the solubility characteristics of the HCN oligomers considerably.

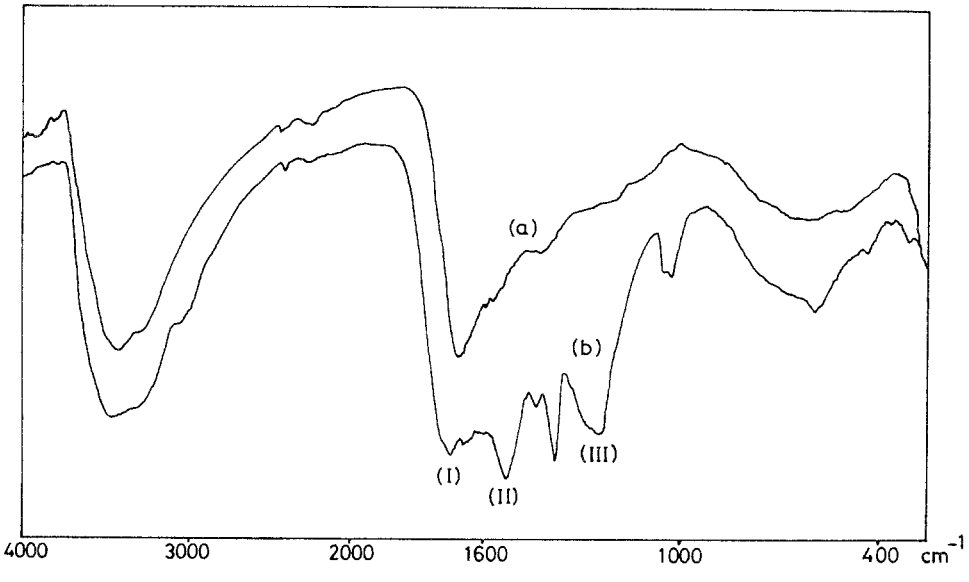


Fig. 1. IR spectra (KBr pellets). (a) HCN oligomers. (b) Acetylated oligomers.

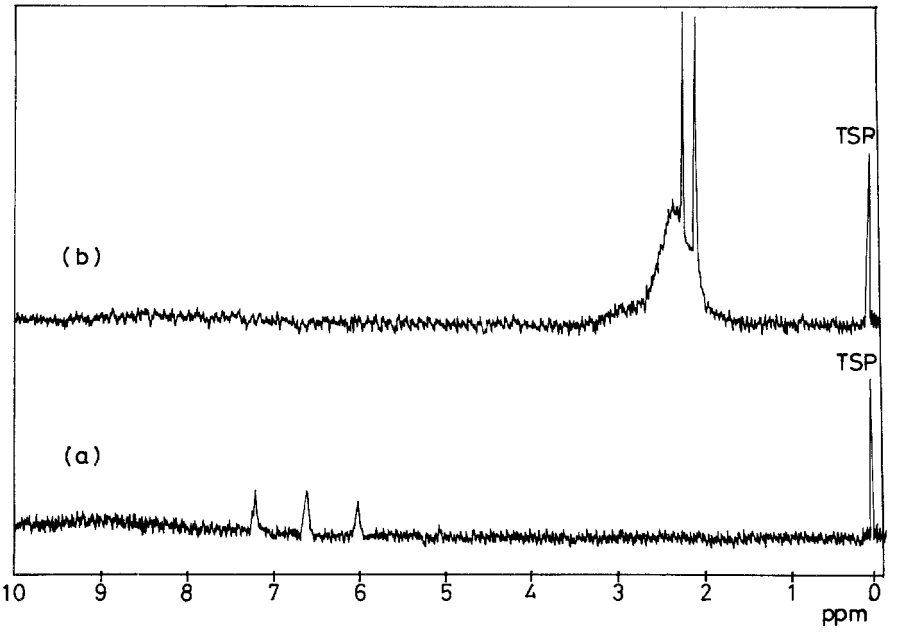


Fig. 2. ^{1}H NMR at 90MHz. (a) HCN oligomers in TFA solution. (b) Acelytated oligomers in TFA solution. Chemical shifts are measured from TSP(trimethylsilylpropionic acid sodium salt).

2.1. ACETYLATION OF THE PRIMARY AMINO GROUPS IN THE OLIGOMERS

We found that the oligomers are easily and nearly quantitatively acetylated by refluxing the oligomers with a mixture of acetic anhydride and acetic acid. The comparison of the IR spectra taken before and after acetylation is illustrated in Figure 1. The IR spectrum of the intact oligomers (Figure 1a) is nearly identical with that given by Völker (1960). It shows three rather broad absorption bands: The 3400 cm^{-1} band is due to N—H stretching, possibly overlapped with O—H stretching. A small amount of O—H group may be present in the oligomers but could not be confirmed. The possibility of overlapping of C—H stretching mode is unlikely from the consideration of the NMR results (see later). The 1660 cm^{-1} band is assigned to

$$\backslash$$
—C = H—, superposed with C = O stretching modes. The amount of the carbonyl
$$/$$

group should be comparatively small judging from the amount of oxygen found in the elemental analysis (see later). The 600 cm^{-1} band results from out-of-plane N—H wagging.

The IR spectrum of the acetylated oligomers (Figure 1b) clearly shows several new peaks in the range from 1600 to 1000 cm^{-1}. In reference to the standard secondary amide IR spectra, we assign the band near 1700 cm^{-1} to amide I, the one at 1530 cm^{-1} to amide II, the one at 1380 cm^{-1} to the bending mode of CH_3 in acetyl groups and the peak at 1260 cm^{-1} to amide III. Additional small bands appear near

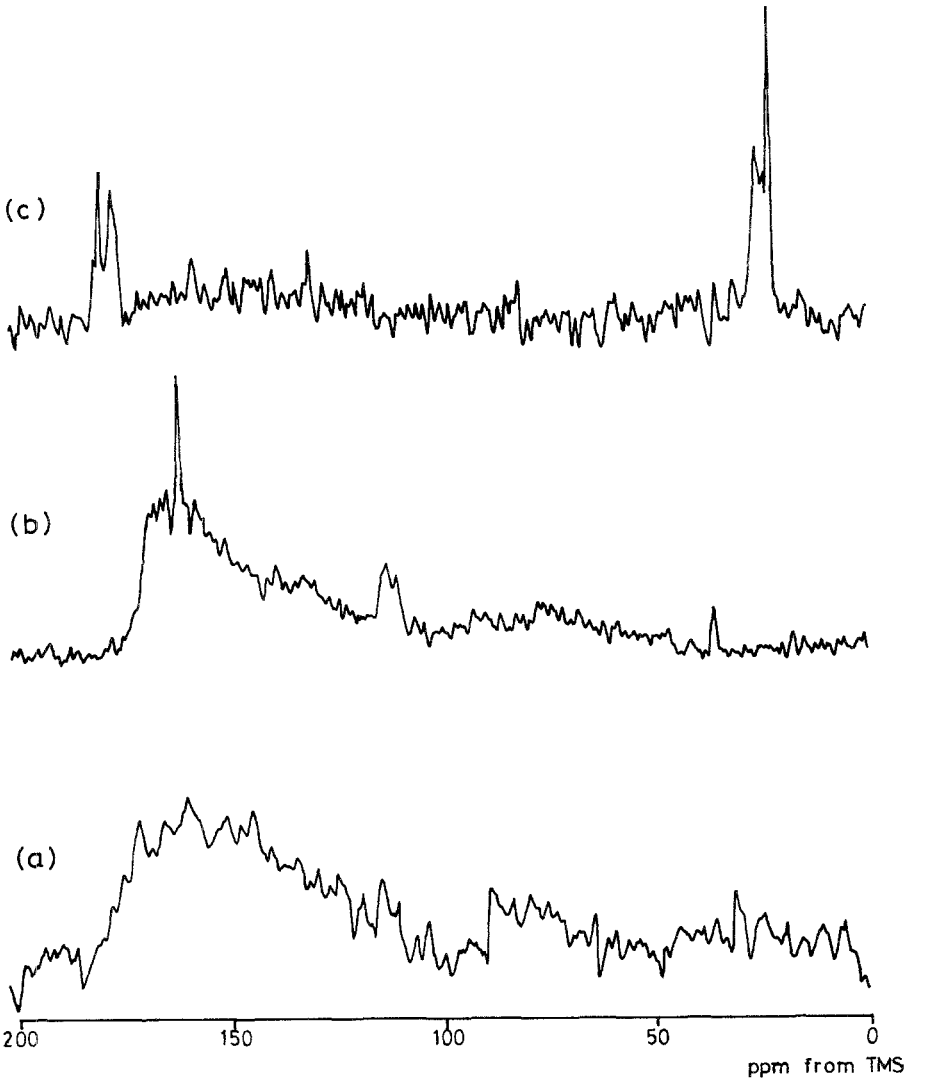


Fig. 3. ^{13}C NMR spectra of HCN oligomers and their derivatives. (a) CP/MAS NMR spectrum of the intact HCN oligomers at 25 MHz. 3000 scans. (b) Solution NMR spectrum of intact oligomers in H_3PO_4–D_2O mixture. 50000 scans without ^1H decoupling. (c) Solution NMR spectrum of acetylated oligomers in H_3PO_4–D_2O mixture. 6942 scans with ^1H decoupling. Chemical shifts of all ^{13}C NMR spectra are referred to TMS.

3000 cm^{-1} from the methyl group introduced by acetylation. Absorption at 1020 cm^{-1} is likely due to C—N stretching, but why it is missing intact oligomers is not clear.

Further quantitative results are obtained from the comparison of the elemental analysis of the acetylated oligomers with that of intact oligomers. As shown in Table 1, the elemental analysis of the intact oligomers gives atomic ratios (normalized to

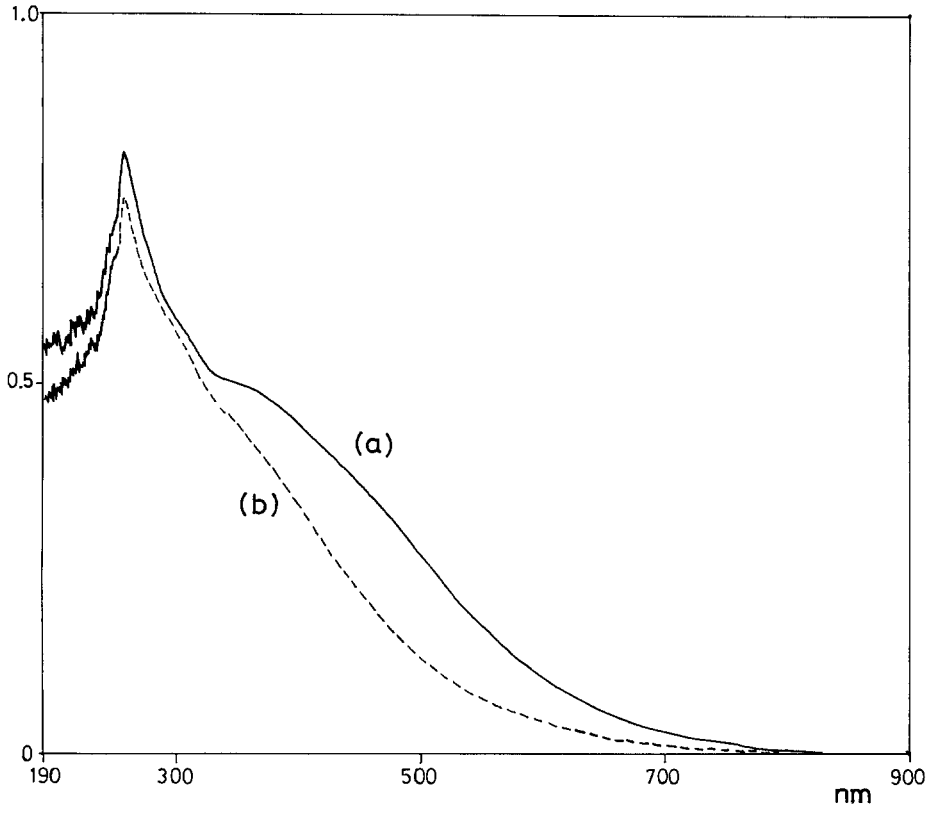


Fig. 4. UV/VIS absorption spectra of HCN oligomers in TFA solution. 89 mg of HCN oligomers was dissolved in 80 ml of TFA and hydrogenated. The UV/VIS spectra were taken by diluting the solution 50 times with TFA. (a) Before hydrogenation. (b) After hydrogenation.

the nitrogen content), which are in good agreement with the expected values for $(HCN)_x$, except for a small amount of oxygen present.

2.2. ^1H AND ^{13}C NMR SPECTRA

Figures 2(a) and 2(b) show the ^1H NMR spectra of the HCN oligomers and the acetylated oligomers dissolved in TFA. No distinct peak is observed in Figure 2a, except for a triplet due to ammonium ions and a broad N—H proton peak at 8-9 ppm. If the oligomers contained peptide bonds, we would expect to observe a C—H proton peak of —CH—NH—C(O)— type usually at 4–5 ppm. We actually observed such peptide C—H absorption in polypeptides of molecular weight ~ 100000, e.g. polylysine at 4.7 ppm and polyglutamic acid at 4.8 ppm, in TFA. Since no absorption could be observed for HCN oligomers in TFA in this region, the presence of peptide bonds can be ruled out in our HCN oligomers. The spectrum of the acetylated HCN oligomers, on the other hand, exhibits new peaks at 2–3 ppm due to the methyl protons of the acetyl group, indicating a successful acetylation of the primary amino

TABLE I

Elemental analysis of HCN oligomers and of their acetylation products[a]

	C	H	N	O
HCN Oligomers	39.6%	4.0%	46.1%	10.4%
	1.0	1.2	(1.0)	0.2
Acetylated Oligomers	47.7%	4.1%	27.0%	21.1%
	2.1	2.1	(1.0)	0.7
Calculated[b]	2.0	2.0	(1.0)	0.5

[a] Numbers in the first rows are the weight percent, followed by a row of the atomic ratios relative to the nitrogen content.

[b] Atomic ratios calculated on the basis of the assumption that half of the nitrogen atoms in $(HCN)_x$ oligomers exist as amino groups which are completely acetylated.

groups. Sharp peaks at 2.2–2.4 ppm are most likely from the methyl groups of acetic acid or its derivatives produced by acid hydrolysis when acetylated oligomers were dissolved in TFA.

Figure 3a and 3b present ^{13}C CP/MAS and solution NMR spectra of the HCN oligomers. On the basis of similar spectra of thermally stabilized product of polyacrylonitrile (Yokota *et al.*, 1982), the broad peak in the 140–170 ppm region may be assigned to conjugated —C = N— bonds. Carbonyl carbon also has absorption in this region and it may be contained in this broad peak. Assuming the ladder type structure as proposed by Völker, we expect a ^{13}C NMR peak due to —C—NH_2 near 60 ppm. The peak intensity near 60 ppm, however, is weaker than expected for Völker's structure.

^{13}C NMR of aqueous phosphoric acid solution of HCN oligomers gives a very similar spectrum to that of CP/MAS solid state NMR with better resolution. A sharp peak at about 160 ppm is probably from the carbonyl carbon of urea or similar small molecules. The ^{13}C NMR of acetylated oligomers demonstrates additional peaks by the acetyl group.

2.3. Characterization of the Conjugated —C = N— Bond Structure

The dark-brownish color of the solid HCN oligomers suggests the presence of a conjugated double bond system. While many experimental and theoretical studies have been published on conjugated —C = C— systems, studies on linear conjugated —C = N— system are scarce (Aoki *et al.*, 1985).

In order to confirm the existence of unsaturated —C = N— double bonds in the HCN oligomers, we attempted Pt-black catalyzed hydrogenation. The amount of hydrogen absorbed by the oligomers corresponded to about one molecule of hydrogen to two HCN molecules. This is interpreted as being due to a unit in the oligomer which is formed from two molecules of HCN, e.g. —$C(NH_2)C$ = N—, and the unit absorbs one mole of hydrogen. During the hydrogenation, we visually

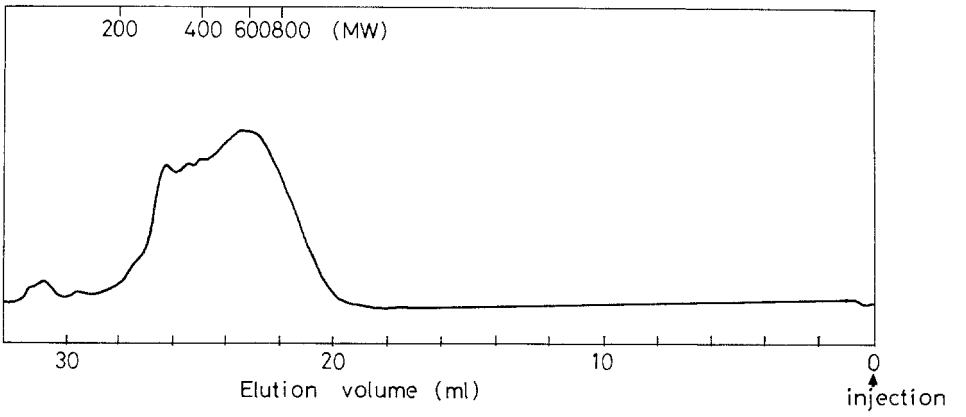


Fig. 5. Gel permeation chromatogram of acetylated oligomers.

observed, and also confirmed by UV/VIS spectra, a hypsochromic shift (Figure 4). This may be due to the saturation of —C = N— bonds and the breaking of conjugation in the oligomers. Since the hydrogenation reaction is quite slow and the gas volumetry is semi-quantitative, we continue to search for a hydrogenation catalyst more effective than Pt-black.

2.4. Nature of Oxygen in the Oligomers

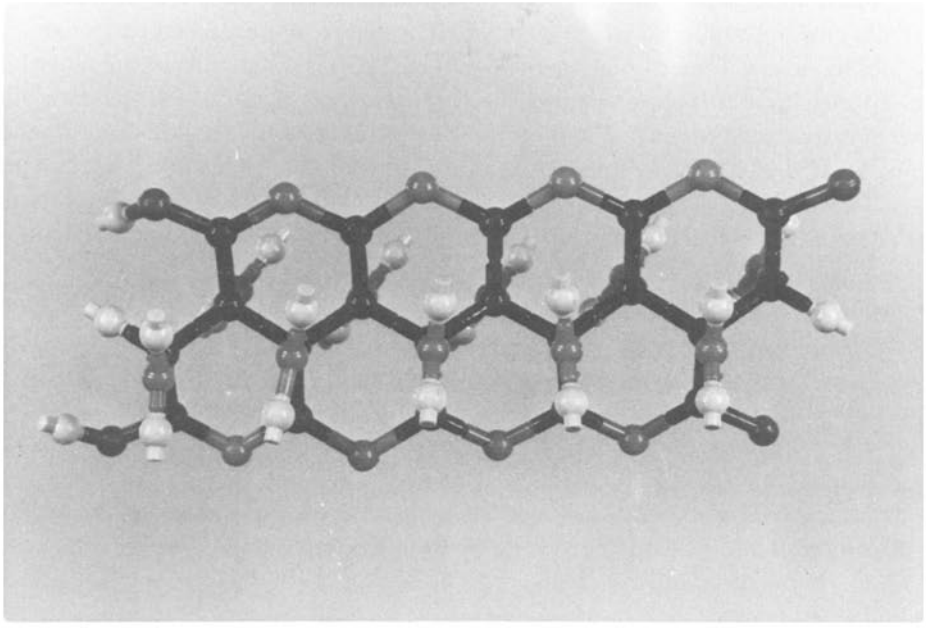
The elemental analysis (Table I) shows that the oligomers contain about 0.2 atom of oxygen per atom of nitrogen. The oxygen content depends on the polymerization conditions, and it is further known that anhydrous polymerization produces HCN oligoemrs which do not contain oxygen (Völker, 1960). It is therefore likely that oxygen is introduced into the oligomers from the solvent water through a hydrolytic processes:

$$\diagup\!\!\diagdown C{=}NH + H_2O \rightarrow \diagup\!\!\diagdown C{=}O + NH_3.$$

2.5. Molecular Weight Distribution of Acetylated Oligomers

Völker (1960) proposed the molecular formulas of HCN oligomers based on the elemental analysis and on the assumption that each oligomer molecule has either four or five oxygen atoms. However, as we described above, the oxygen content of the oligomers varies to some extent from preparation to preparation, and we consider Völker's molecular formulas quite tentative.

Previously, we reported the molecular weight distribution of the methanol soluble fraction of HCN oligomers, which were obtained by UV irradiation of HCN in the gas phase (Mizutani *et al.*, 1975). In that case, the molecular weight was found to be distributed from 200 to 1200. Generally, however, the poor solubility of solid HCN oligomers in most solvents makes it difficult to obtain well defined bands in



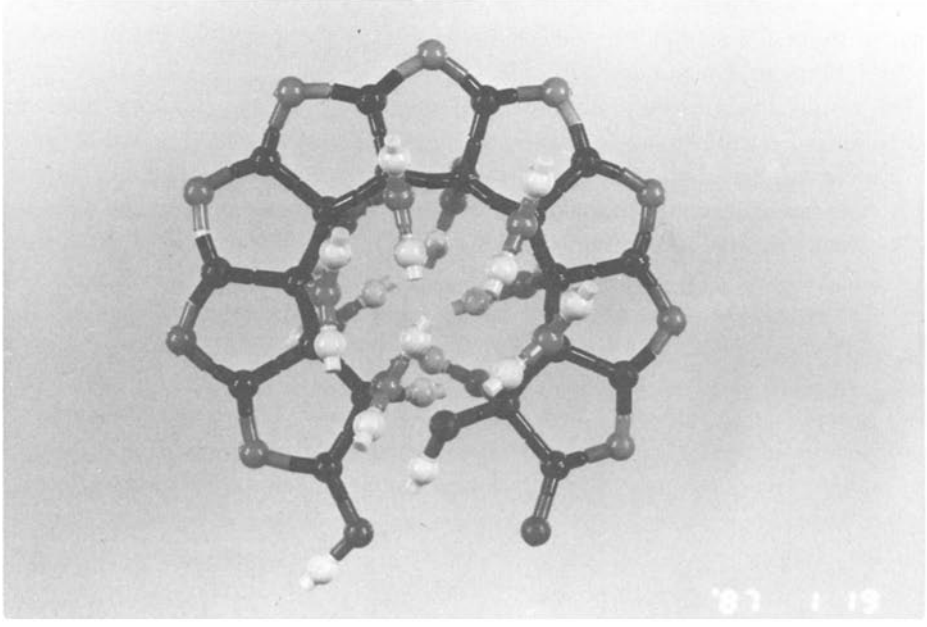


Fig. 6. HGS molecular models of the oligomer. (a) Double-ladder structure (I) proposed by Völker. (b) Single-ladder structure (II). (*black*: carbon, *white*: hydrogen, *gray*: nitrogen and *dark gray*: oxygen).

liquid chromatography. We have found that acetylation improves the solubility of the oligomers considerably. Using the THF solution of the acetylated oligomers (solubility: about 1 mg of oligomers in 1 ml of THF), we determined the molecular weight distribution. Figure 5 shows the chromatogram, from which the molecular weight is estimated to range from 300 to 900, the maximum of the dominant fraction being located at 600. If we assume one acetyl group has been introduced into a unit $-N=C-C(NH_2)-$ to give $-N=C-C(NHCOCH_3)-$, the group molecular weight of the acetylated unit is 96. This means that one oligomer molecule contains about six of such units, disregarding the contributions of terminal groups.

The molecular weight of the THF-insoluble fraction of the oligomers may be larger than the soluble fraction. Although details of the structural difference between THF soluble and insoluble fractions are not known, both fractions gave very similar IR spectra as that of the whole oligomers.

Considering these experimental results, the characteristics of HCN oligomers may be summarized as follows:

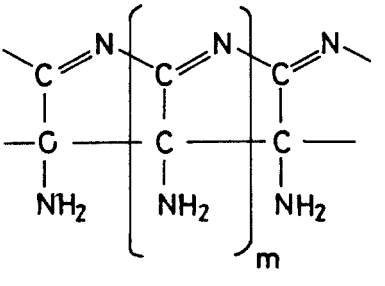
(1) Nearly half of the nitrogen in the oligomers is of the primary amino type.

(2) another half of the nitrogen is involved in the imino (—C = N—) type bonds.

(3) C—H bonds do not exist and almost all hydrogens in the oligomers are present in amino groups.

(4) —C = N— bonds are most likely conjugated judging from the color of the oligomers and the very weak absorption of nitrile groups in the IR spectrum. The details of the formation mechanism of the —C = N— conjugation are not certain, but we suggest a similar mechanism is involved as proposed in the pyrolysis of polyacrylonitrile (Glassie and McNeill, 1959).

Our results are essentially consistent with the double ladder structure proposed by Völker and we could see no indication of polypeptides, as far as the solid oligomers are concerned. Völker did not adopt a single ladder structure II, because of the steric repulsions between amino groups. We constructed both the double and the single ladder structures with HGS models.



Structure II

Since neither structure is completely free from steric strain, we consider that the single ladder structure may still be one of the candidates. Another possibility is a mixed ring structures composed of 5, 6, and 7-membered heterocyclic rings.

One difficulty with Völker's double ladder model I is that HCN polymerizes into a rod-like structure (Figure 6a) which can extend to any degree of polymerization. The molecular weight distribution observed for the acetylated oligomers (Figure 5) has a limited bandwidth in the low molecular weight region, and this is not well explained by Völker's model. The single ladder model II, on the other hand, forms a disk-like structure (Figure 6b), in which case the maximum degree of polymerization, n, for $(—N = C—C\ (NH_2))_n$ is 10–11. The observed molecular weight of the acetylated oligomers corresponds to the molecular weight of the intact oligomers of about 300, which is about two thirds of the maximum described above.

The structural problems of HCN oligomers have been studied for quite a long time, and we believe we are making a step forward in clearing up some of the problems. Further studies are underway.

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