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Prebiotic Adenine Synthesis from HCN—Evidence for a Newly Discovered Major Pathway

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Three new imidazole derivatives have been isolated and characterized from oligomerizing HCN solutions. On the basis of these results as well as the earlier identification of a new precursor to adenine, a new and major pathway leading to the formation of adenine is suggested. The route accounts for the synthesis of adenine-8-carboxamide from *cis*-diaminomaleonitrile, without requiring an isomerization to the *trans* configuration or reactions with formamidine. The formation of previously reported imidazoles is also explained.

INTRODUCTION

The formation of biologically important molecules via the oligomerization of HCN is well established. Oro (*1*) and Oro and Kimball (*2*) isolated and identified the purine adenine from aqueous ammonium cyanide solutions (1–15 *M*). This first result has been supported and explored further (*3, 4*). Subsequently, several different classes of compounds have been reported including purines, pyrimidines, and imidazoles as well as amino acids, urea, oxalic acid, and guanidine (*5–11*). In this context, the oligomerization of HCN is considered to have been a major process in chemical evolution on the primitive Earth (*12, 13*).

However, the pathways for the formation of these compounds in the HCN oligomerization are not understood entirely. Suggested mechanisms for the formation of adenine are summarized in Fig. 1 (*3, 11, 12*).

The pathway from the trimer, aminomalononitrile **1**, is now considered to be unlikely in comparison to reactions involving the tetramer diaminomaleonitrile **2** (*12*). High steady-state concentrations of formamidine are also thought not to be plausible except in solutions with a high concentration of ammonia. The route to adenine *via* **2** is dependent on a photochemical isomerization and ring-closure reaction, and further conversion to adenine has been modeled by means of reaction with formamidine.

We have reported previously the isolation and preliminary characterization of a new precursor of adenine in the HCN oligomerization prior to hydrolysis. This compound—adenine-8-carboxamide (**6**), Fig. 5—was identified spectroscopically and by synthesis ((*8*) and unpublished results). The isolation of this precursor suggested a new pathway to adenine which was proposed tentatively (*8*). We have now isolated and identified several new compounds which support this mechanism, which we propose as a major route to purines in chemical evolution.

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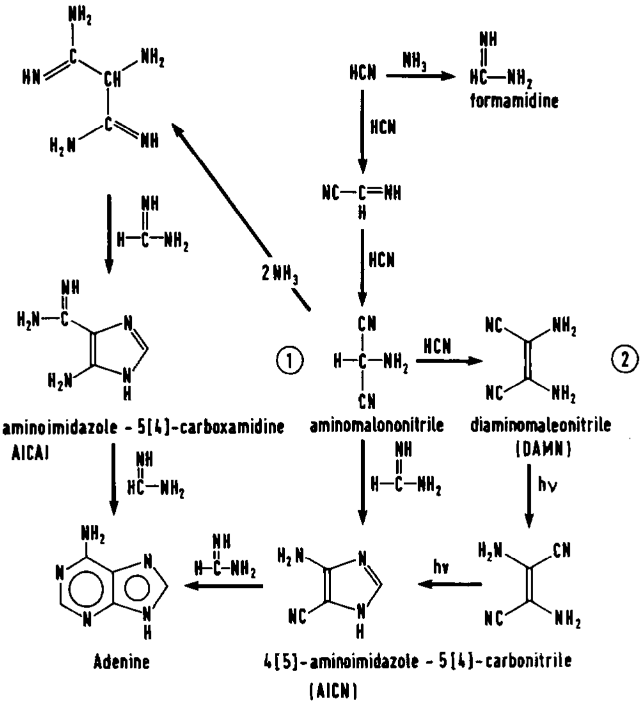


FIG. 1. Summary of earlier proposed pathways for the formation of adenine in aqueous ammonium cyanide solutions.

EXPERIMENTAL

^{13}C nmr spectra were determined on a Bruker Model WM-250 (62.89 MHz) with DMSO as solvent and as internal standard. ^{1}H nmr spectra were recorded in DMSO on either a Bruker Model WH-90, WM-250, or WM-400 (TMS as internal standard). Mass spectral data were obtained with either a Varian MAT 711 double-focusing mass spectrometer with combined EI/FI/FD ion source or a Finnigan 3100 quadrupole GC/MS (solid probe method). IR spectra were determined on a Perkin–Elmer 457 spectrophotometer as potassium bromide micropellets. UV spectra were obtained on a Cary-15 spectrophotometer. HPLC was performed on Aminex A-25 resins as described previously (*14*). Sephadex G-15 (40–120 μm) was purchased from Pharmacia Fine Chemicals, and was used in column chromatography for fractionation of HCN oligomers (90 × 1.6 cm) as well as for desalting purposes (30 × 1.4 cm). In both cases H_2O was used as eluting solvent and the collected fractions were monitored continuously at 254 nm (LKB fraction collector connected to a Uvicord optical unit).

General procedure for the preparation of HCN solutions. HCN was freshly prepared by adding a saturated solution of NaCN to 75% aqueous H_2SO_4 at 70°C with continuous distillation of the formed HCN. For a 1 *M* solution, 40 ml of HCN was added to 900 ml of H_2O. The solution was then adjusted to pH 9.2 with

NH_4OH and made up to 1 liter with H_2O. Solutions were allowed to stand at least 7 months at room temperature.

4[5]-Amino-5[4]-carboxamide-2-cyano-imidazole (*3*). Ammonium cyanide (1 *M*, 350 ml, reaction conditions: 8 months, RT, and pH 9.2) was passed through a Millipore filter (0.45 μm) to separate insoluble azulmic acid. The filtrate was evaporated to dryness *in vacuo* below 30°C and the residue was extracted with ethyl acetate (3 × 100 ml). The residue was dissolved in 0.1 *M* HCl (20 ml) and the solution was extracted with EtOAc (3 × 100 ml). The combined, dried (Na_2SO_4) extracts were concentrated *in vacuo*. An aqueous solution of the residue was then applied to an Aminex A-25 HPLC column for fractionation. A peak with a retention time of 38.6 min was collected, desalted on Sephadex G-15, and evaporated *in vacuo* to yield 10–15 mg of product (0.09–0.14%). Mass: *m/e* 151(M^+), 134(100), 106, 81, 55, 54, and 53. IR: 3300, 1665 cm^{-1} (amide); 2215 cm^{-1} (C≡N). UV: 295, 240 nm (1 *M* HCl); 296, 238 nm (1 *M* NaOH); 298, 242 nm (H_2O). ^{1}H nmr (DMSO): δ = 8.73 and 8.42 ppm (see Results); 6.87 ppm (amino NH_2). ^{13}C nmr (DMSO): 90.57, 116.27, and 149.90 ppm (C=C and C≡N); 135.22 ppm (C–2); and 159.10 ppm (C–amide).

4[5]-aminoimidazole-2,5[4]-dicarboxamide (*4*). The residual 0.1 *M* HCl solution from the above extraction procedure was evaporated to dryness *in vacuo* and redissolved in H_2O (7 ml) and then applied to a Sephadex G-15 column. Thirteen fractions were collected (Fig. 2).

Fractions 7–9 were rechromatographed on an Aminex A-25 exclusion column; compound **4**, as the major peak in these fractions, was collected (A-25: 13.50 min) and was desalted by chromatography on Sephadex G-15. Mass: *m/e* 169(M^+), 152(100), 136, 124, 109, 107, 81, 55, and 54. IR: 3320 and 1670 cm^{-1} (amide); 1590 cm^{-1}. UV: 306, 251 nm (0.1 *M* HCl); 312, 253 nm (0.1 *M* NH_3); 305, 253 nm (H_2O). ^{1}H nmr: δ = 8.75 and 8.17 ppm (both asymmetric peaks); δ = 6.97 ppm (amino NH_2).

4[5]-N-(aminomethylidene)aminoimidazole-2,5[4]-dicarboxamide (*5*). The isolation of compound **5** was performed in the same way as described for compound

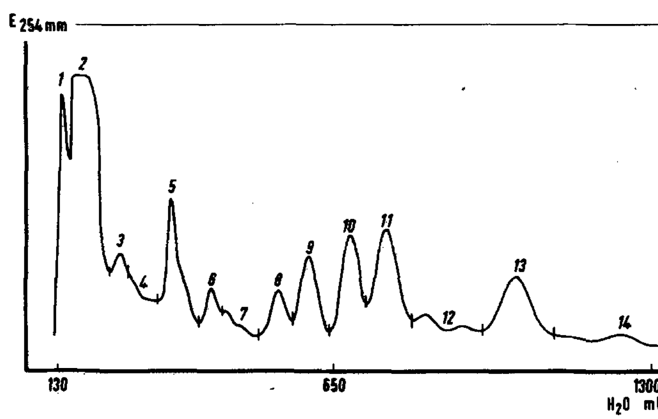


FIG. 2. Sephadex G-15 fractionation of the residual 0.1 *M* solution (see text).

TABLE 1

HIGH-RESOLUTION MASS SPECTROMETRY OF COMPOUND 3

Mass	C	H	N	O	
M^+151.0494	5	5	5	1	
134.0239	5	2	4	1	$M^+ - 17$ [-NH_3]
106.0282	4	2	4	0	[-C=O]

4, but with fractions 9 and 10 of the initial Sephadex G-15 separation (A-25: 24.50 min). Mass: *m/e* 196(M^+, 100), 179, 153, 151, 134, 124, 108, 81, 55, and 54. IR: 3400 and 1650 cm^{-1} (amide); 1600 cm^{-1}. UV: 307 nm (0.1 *M* HCl); 310 nm (0.1 *M* NH_3); 302 nm (H_2O).

RESULTS

The identification of compound **3** was based on the combined interpretation of the spectra (mass, nuclear magnetic resonance, and infrared spectra). Table 1 presents the molecular formulas for the molecular-ion peak and two successive fragment peaks as determined by high-resolution mass spectrometry.

Infrared spectra supported the structure proposed and showed a broad absorption peak at 3300 cm^{-1} and a sharp band at 1665 cm^{-1} which indicated an amide functional group. Another very important peak present was at 2215 cm^{-1} which suggested a nitrile group attached to a conjugated system. The ^1H nmr spectrum showed three main signals in the ratio of 1 : 1 : 2. No methine proton was present in

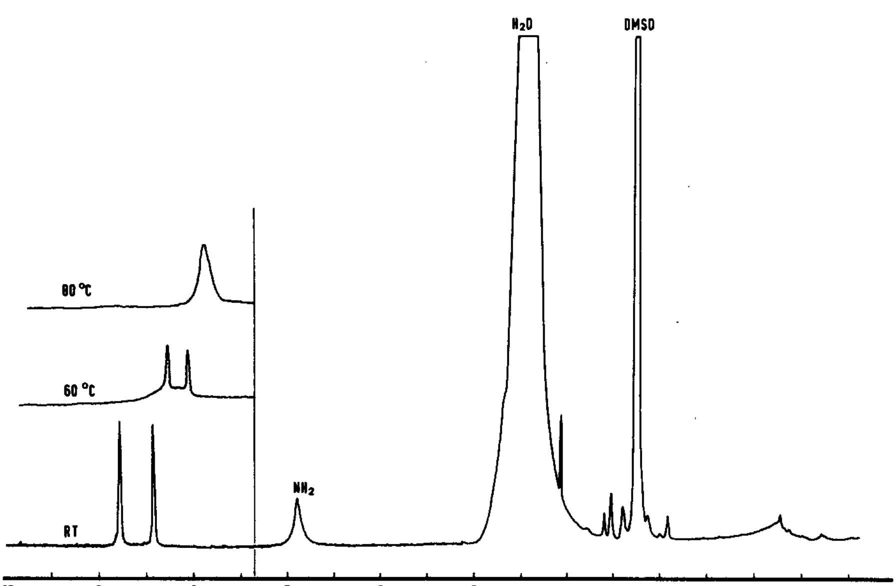


FIG. 3. ^1H nmr spectra of compound **3** at different temperatures.

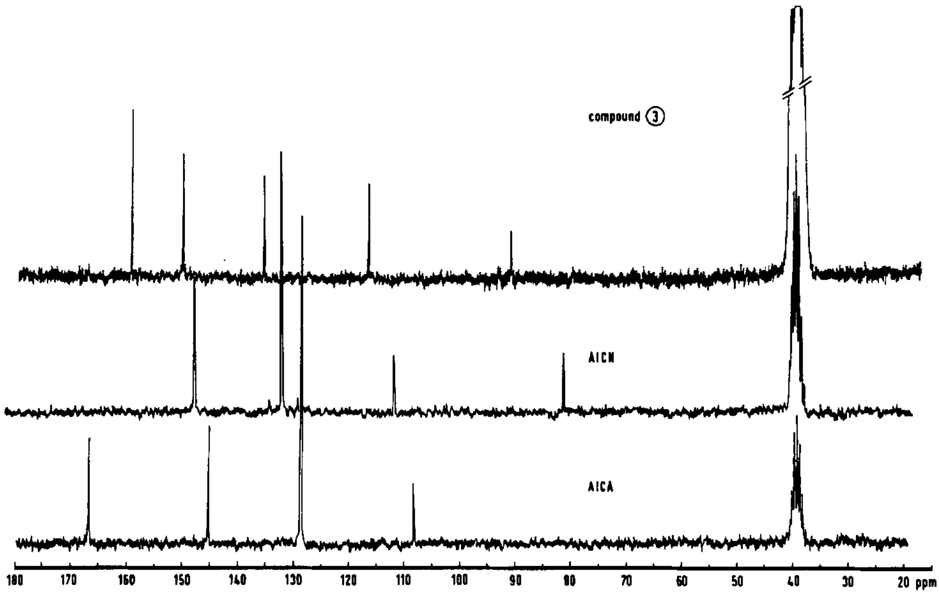


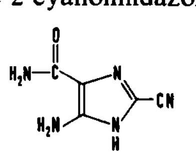
FIG. 4. ^{13}C nmr spectra of compound **3**, AICA, and AICN in DMSO.

the molecule, because of the facile proton exchange with D_2O indicating only NH or OH functions. When the spectrum was recorded at elevated temperatures (80°C), two of the three signals moved to higher field and showed overlapping, which is a strong indication of two protons belonging to the same functional group (Fig. 3).

Correlation of the ^{13}C nmr spectrum of isolated compound **3** with those of 4[5]-aminoimidazole-5[4]-carbonitrile (AICN) and 4[5]-aminoimidazole-5[4]-carboxamide (AICA) gave more information (Fig. 4).

The chemical shifts in AICA were 129.8 ppm for the C2 and 146.0 and 108.26 ppm, respectively, for the C=C. The amide absorption occurred at 165.3 ppm (Sadler ^{13}C nmr, No. 1631c, AICA · HCl; for the free AICA the amide absorption was at 161.3 ppm). AICN showed almost the same absorption pattern, but without the signal at 165.3 ppm and with new signals at 111 and 80.8 ppm for the nitrile function and the ring-carbon atom attached to the nitrile. The spectrum of compound **3** closely resembled the combined spectra of AICA and AICN. All the imidazole-ring absorption peaks were present as well as an amide function and a nitrile. The substitution of the C-2 position of the imidazole ring can be seen by the decrease in intensity of the absorption at 132 ppm compared to the corresponding peaks in AICN and AICA (absence of a Nuclear Overhauser Effect).

The spectral data suggested a structural formula for the isolated compound **3** of 4[5]-amino-5[4]-carboxamide-2-cyanoimidazole:



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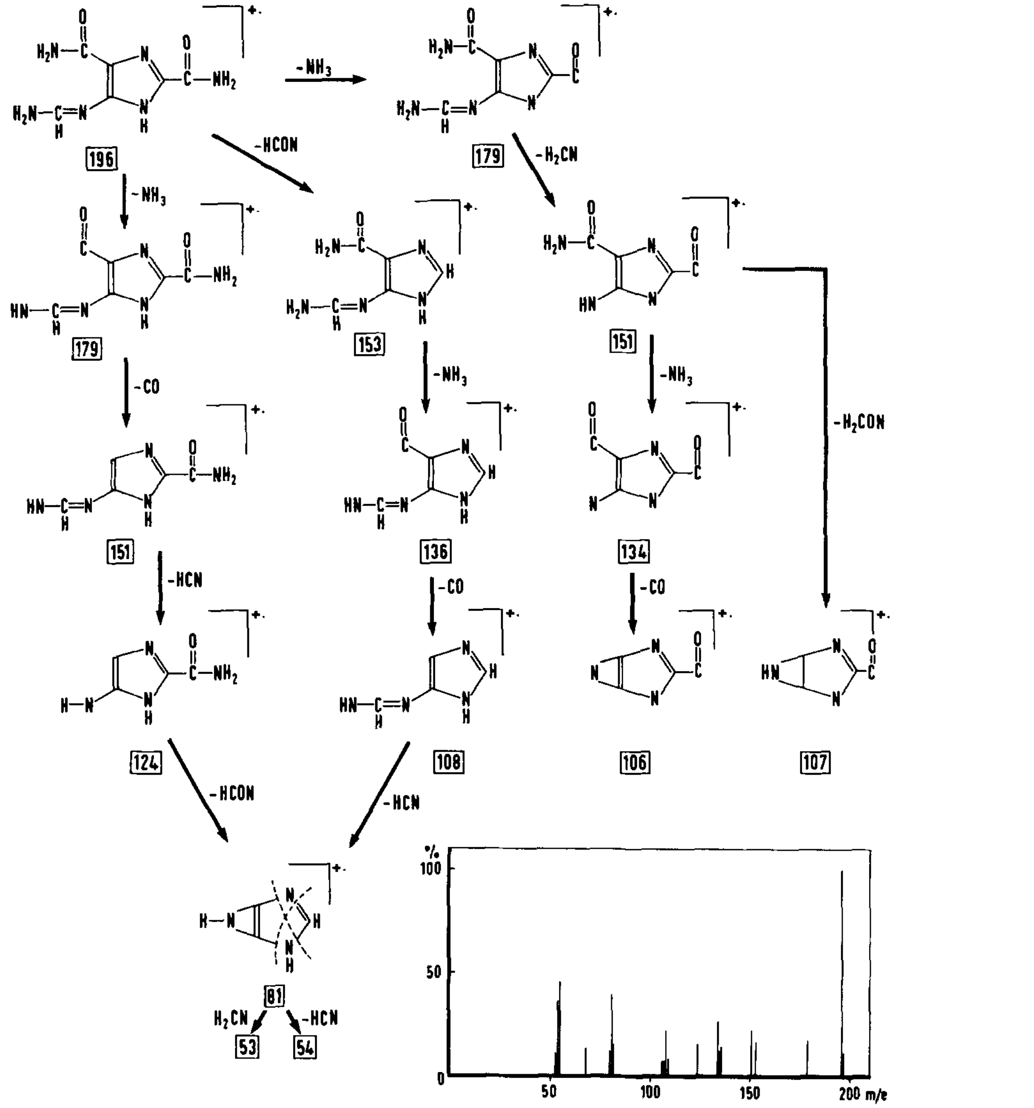
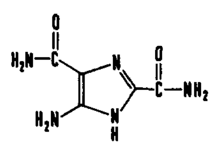


SCHEME 1

The nitrile function at the 4 or 5 position could be excluded by mass fragmentation data. No M^+-HCN fragment was produced as in the fragmentation of AICN. Another explanation of the positions of the functional groups in compound **3**, is the easy fragmentation of NH_3 by a proton donation of the amino to the amide function in a six-membered ring rearrangement (Mc Lafferty rearrangement).

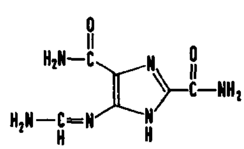
The mass spectrometry of compound **4** showed a *m/e* value of 169 as molecular-ion peak, which was established as $C_5H_7N_5O_2$ by high resolution (169.0590). The base peak (100%) was *m/e* 152 (152.0335, $C_5H_4N_4O_2$). The ^{1}H nmr spectrum of the isolated compound **4** resembled the spectrum of **3**. Once again two signals were present at room temperature at 8.75 and 8.17 ppm (NH_2 amide functional group(s)) and there was also a signal at 6.97 ppm from the amino group, but in the ratio 1 : 1 : 1. In the IR spectrum bands at 3220 and 1670 cm^{-1} suggested amino and amido functions whereas the band associated with a conjugated nitrile was absent. These combined spectral data, together with the knowledge that the molecule is a hydrolysis product of compound **3**, supported the structure **4**, viz., 4[5]-amino-imidazole-2,5[4]-dicarboxamide:



(4)

The third product from the HCN oligomerization was identified only on the basis of similarity to the compounds **3** and **4** and by mass spectral fragmentation (Scheme 1).

The IR spectrum showed the same kind of absorption bands as for compound **4**, 3400 and 1650 cm^{-1} for an amide function and 1600 cm^{-1} for the imidazole ring. High-resolution mass spectrometry gave for the molecular-ion peak (m/e = 196.0708) a formula of $C_6H_8N_6O_2$. These data suggest the probable structure of 4[5]-*N*-(aminomethylidene)aminoimidazole-2,5[4]-dicarboxamide (**5**):

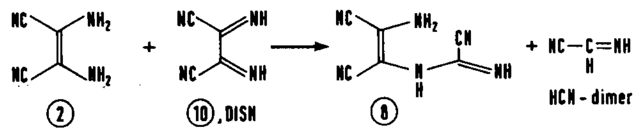


(5)

DISCUSSION

Prior to the present work, little was known concerning the intermediate compounds formed in the HCN oligomerization beyond the stage of the tetramer. Some attempts at structural analysis have been undertaken (*15*). However, in the very complex mixture of products formed by the HCN oligomerization it is very difficult—perhaps impossible—to indicate the total range of functional groups present. We previously reported the isolation of the first direct precursor of adenine in HCN-oligomerization mixtures—later confirmed to be adenine-8-carboxamide (**6**) ((*8*) and unpublished results)—and proposed a mechanism of formation. With the further isolation and identification of compounds **3, 4,** and **5** we now can refine and extend the proposed mechanism for the formation of adenine (Fig. 5).

An important step in this mechanism is the reaction of the HCN tetramer DAMN (**2**) with a cyanoimino derivative (**7**) leading to the formation of compound **8.** Diiminosuccinonitrile (DISN, compound **10**), an oxidation product of DAMN which can be formed in the presence or absence of oxygen (*16, 17*), could account for compound **7**:



However, attempts to find support for this reaction were unsuccessful. No higher yields of compound **6** were obtained after DISN was added prior to the oligomerization. A second possibility is the reaction of two molecules of DAMN, one of

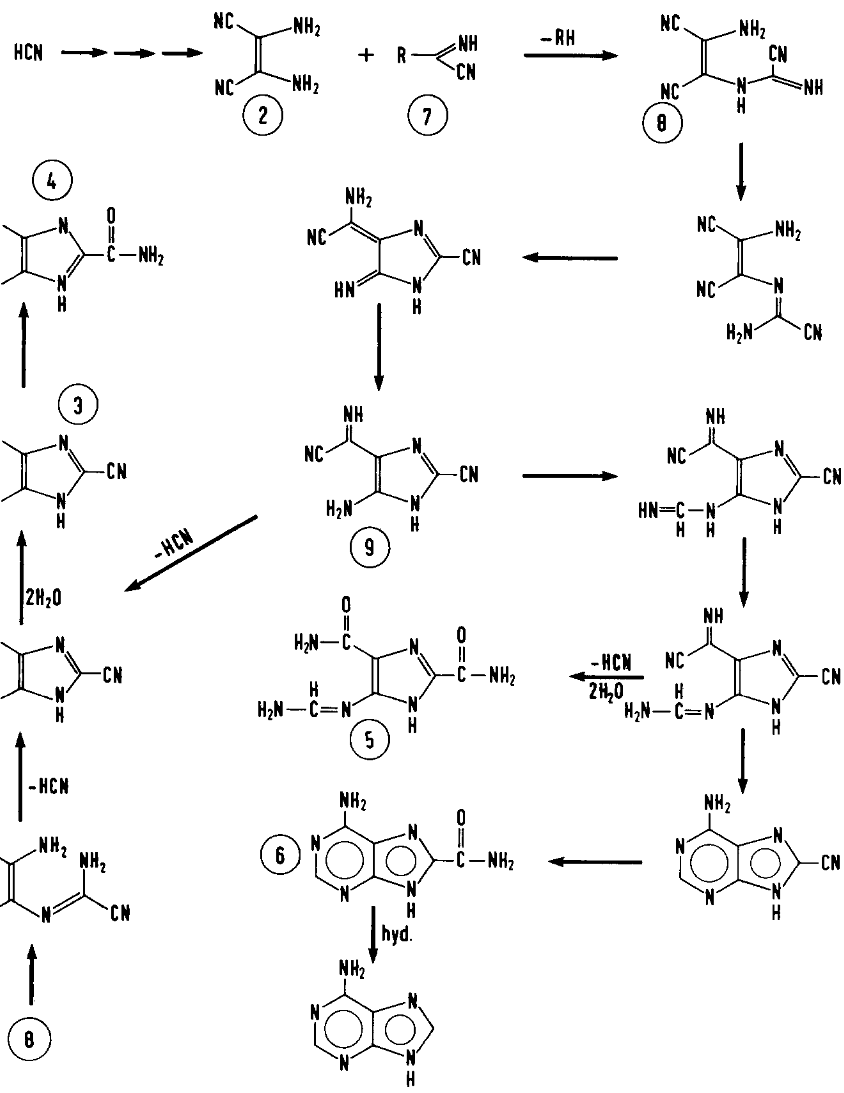
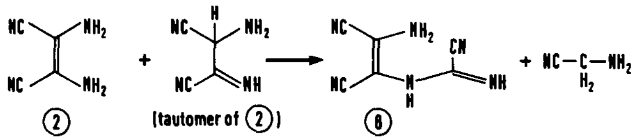


FIG. 5. Suggested pathways for the formation of adenine and other identified products of the HCN oligomerization.

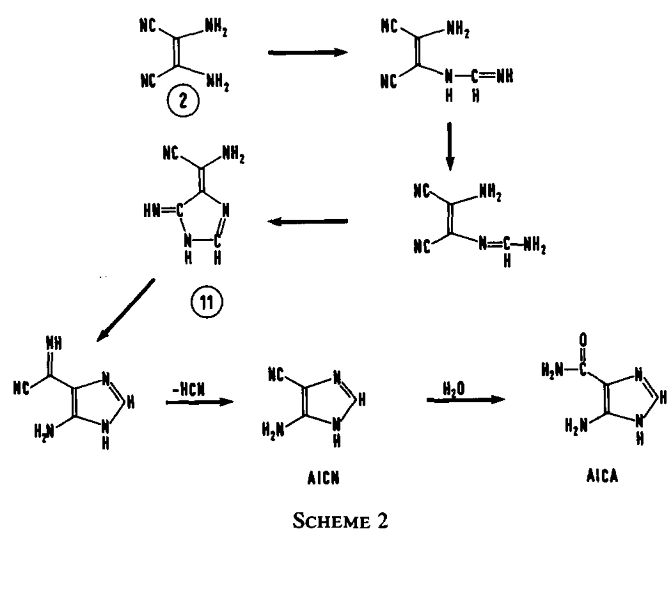
which is in the tautomeric, cyanoimino form. The elimination product—which would be synthesized together with compound **8**—is aminoacetonitrile, a precursor of glycine:



Other reactions involving DAMN or higher oligomers, or rearrangements of these, are also plausible.

It is important to note that the production of adenine, as well as the imidazoles which have been reported previously (*11*), can be accounted for without recourse to an isomerization of *cis*-DAMN (the major product) to the *trans* configuration. Thus AICN and AICA could be synthesized as shown in Scheme 2. The reaction from the tetramer **2** to compound **11** has been suggested previously for nonaqueous solutions (*18*). An analogous reaction of DAMN (**2**) with diiminosuccinonitrile (**10**) provides an alternative route to compound **9**.

Adenine is liberated *via* decarboxamidation (acid hydrolysis) of compound **6** (*8*). Earlier analyses for adenine after acid hydrolysis of the products of HCN oligomerization (0.1 *M* solutions at pH 9.2) have utilized 6 *M* HCl at 110°C for 24 hr. (*6*). The yields reported were 0.03 to 0.04%. We have found that these conditions produce extensive destruction of adenine and that maximal yields are obtained after 1.5 hr. Our analyses gave average yields for adenine of 0.1% based on the starting HCN. Hydrolysis experiments have shown that compound **6** is quantatively converted to adenine under these conditions and accounts for 42% of the total yield of adenine produced. Compound **6** therefore represents a major inter-



SCHEME 2

mediate in the route to adenine and is, in fact, the only direct precursor of this purine yet to be identified in dilute, oligomerizing solutions of HCN.

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