Chemical Evolution. XIV. Oxidation of Diaminomaleonitrile and Its Possible Role in Hydrogen Cyanide Oligomerization[1]

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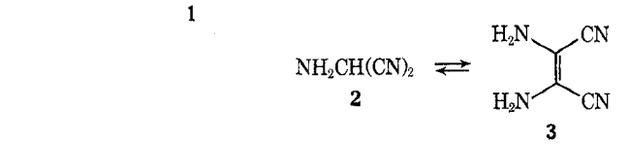
N,N'-Diisopropyldiaminomaleonitrile (**4**) is oxidized by oxygen to *N,N'*-diisopropyldiiminosuccinonitrile (**5**) in 47% yield in acetonitrile solution. The yield of **5** is much less in aqueous solution at pH 9.2 with monoisopropylurea as the other reaction product. Ammoniacal hydrolysis of **5** (0.1 mmol) gives urea (0.076 mmol) as the only reaction product, while hydrolysis in the presence of a 30-fold excess of cyanide gives an enhanced yield of urea (0.28 mmol). These results may be explained by the reduction of **5** to **4** by cyanide with the subsequent formation of cyanogen or cyanate which in turn reacts with ammonia to give urea. Oxygen oxidizes **4** to **5**, thus completing the cycle. Diaminomaleonitrile (**3**) is air oxidized in aqueous ammonia (pH 9.2) and urea was detected as a reaction product. Diiminosuccinonitrile (**6**) is the most likely initial oxidation product of **3**. A 50% yield of urea is obtained on hydrolysis of **6** in ammonical solution. However, this yield did not increase when the hydrolysis was performed in the presence of cyanide. A pH 9.2 solution of cyanide in the presence of oxygen decomposes more rapidly than one which has been degassed. The detection of urea and amino acids (after acid hydrolysis) from the degassed cyanide solution demonstrates that oxidation and reduction leading to urea and the amino acid precursors are taking place in the absence of oxygen, suggesting that these reactions were feasible on the primitive earth.

HCN condenses in aqueous alkaline solution to yield a variety of biomolecules, or their precursors, including purines,[2a] amino acids,[2b] and pyrimidines.[2c] These findings, together with the ease with which HCN is formed in primitive earth simulation experiments,[3,4] suggest that HCN condensation reactions may have been an important source of biomolecules on the primitive earth.

The condensation of HCN to diaminomaleonitrile (HCN tetramer, **3**) is well understood.[3,5] The reaction proceeds *via* the stepwise formation of dimer **1**, trimer **2**, and **3**. On further reaction the concentration of **3** gradually decreases and the so-called "HCN polymer" forms.[6,7] Amino acids have been identified among the acid hydrolysis products of this material.[2b] Substantial amounts of urea, together with oxalic acid, are also produced in the oligomerization mixture.[2b,7,8] These compounds must be formed by oxidation–reduction processes, because urea and oxalic acid are oxidation products of HCN while amino acids represent reduction products.



3 and to a lesser extent adenine are the highest molecular weight oligomers of HCN to be isolated and characterized from the oligomerization of dilute (0.1–1.0 *M*) aqueous cyanide.[2a,9] The apparent absence of higher oligomers suggests that the redox reactions leading to urea, oxalic acid, and the other substances found in the oligomerization mixture[8] involve compounds **1**, **2**, and **3**. The rapid conversion of **1** and **2** to **3** in the presence of cyanide suggested **3** to be the most likely candidate for oxidation and/or reduction.[3,5] The possible loss of **3** due to the facile oxidation or reduction of the low equilibrium concentrations of **1** or **2** cannot be excluded as a possibility.

Several lines of evidence suggested that cyanate was the direct precursor to urea in the oligomerization mixture.[7] First, much more urea is obtained when the oligomerization is performed in the presence of ammonium hydroxide than is formed at the same pH in the absence of ammonia hydroxide. Furthermore, *N*-methylurea is the product, instead of urea, when the oligomerization is performed in the presence of methylamine. Finally, carbamyl derivatives of amino acids have been detected as hydrolysis products of the "HCN polymer."[2c,7]

In the present work, the reactions of **3** and the dialkyl substituted derivatives of **3** were investigated with the aim of delineating which redox reactions are occurring during the HCN oligomerization.

Results

Reactions of *N,N'*-Diisopropyldiaminomaleonitrile and *N,N'*-Diisopropyldiiminosuccinonitrile.—A study of the solution chemistry of *N,N'*-diisopropyldiaminomaleonitrile[5] (**4**) was undertaken with the expectation that isopropyl groupings would facilitate product isolation and analysis. Compound **4** was found to be unstable in aqueous solution at pH 9.2. Its absorption maximum at 332 nm disappeared in 2–4 hr while the short-wavelength band at about 222 nm increased slightly. After standing for several days the 222-nm band disappeared and a band at 206 nm formed.

(1) For previous papers in this series see J. P. Ferris and D. E. Nicodem, *Nature (London)*, **238**, 268 (1972); J. P. Ferris, F. R. Antonucci, and R. W. Trimmer, *J. Amer. Chem. Soc.*, **95**, 919 (1973); and ref 7.

(2) (a) J. Oro and A. P. Kimball, *Arch. Biochem. Biophys.*, **94**, 217, 293 (1961); R. A. Sanchez, J. P. Ferris, and L. E. Orgel, *J. Mol. Biol.*, **38**, 121 (1968); (b) C. V. Lowe, M. W. Rees, and R. Markham, *Nature (London)*, **199**, 219 (1963); (c) J. P. Ferris and J. D. Wos, unpublished work.

(3) R. A. Sanchez, J. P. Ferris, and L. E. Orgel, *J. Mol. Biol.*, **30**, 223 (1967).

(4) A. Bar-Nun and M. E. Tauber, *Space Life Sci.*, **3**, 254 (1972).

(5) J. P. Ferris, D. B. Donner, and W. Lotz, *Bioorg. Chem.*, **2**, 95 (1972); J. P. Ferris, D. B. Donner, and W. Lotz, *J. Amer. Chem. Soc.*, **94**, 6968 (1972).

(6) T. Volker, *Angew. Chem.*, **72**, 379 (1960).

(7) J. P. Ferris, D. B. Donner, and A. P. Lobo, *J. Mol. Biol.*, **74**, 499, 511 (1973).

(8) The term "oligomerization mixture" will be defined as in ref 7 to describe the complex mixture of reaction products formed from HCN. This term refers not only to the primary reaction products (**1–3**) which are true oligomers but also to the other products formed by oxidation, reduction and hydrolysis of these primary oligomeric species.[7]

(9) Adenine (HCN pentamer) has been isolated from more concentrated cyanide solutions.[2a]

Monoisopropylurea was isolated as a reaction product at the end of this time.

The rate of decomposition of **4** is independent of the concentration of added cyanide or isopropylamine but increased with increasing pH. The formation of monoisopropylurea is not inhibited by the addition of nucleophiles such as iodide or cyanide to the reaction solution. Monoisopropylurea was detected as a reaction product when the hydrolysis of *N,N'*-diisopropyldiaminomaleonitrile was performed in aqueous isopropylamine. It was not possible to ascertain if *N,N'*-diisopropylurea was a reaction product because we did not have a sensitive method for its detection. Thus, the loss of **4** apparently is not caused by the attack of a nucleophile, such as an amine or cyanide, on **4** or by the decomposition of a lower molecular weight oligomer in equilibrium with **4**. If nucleophilic attack were important the added nucleophiles should accelerate the decomposition of **4**, while the presence of cyanide would be expected to displace the equilibrium between a lower oligomer and **4** in the direction of **4**. This should slow the rate of decomposition of the lower molecular weight oligomer.

That the decomposition of **4** was due to atmospheric oxygen was demonstrated by comparing the rate of loss of **4** in degassed and nondegassed solutions. The uv maximum of **4** at 322 nm decreased only slightly in 8 hr in the degassed solution, while it disappeared in 40 min in the nondegassed solution.

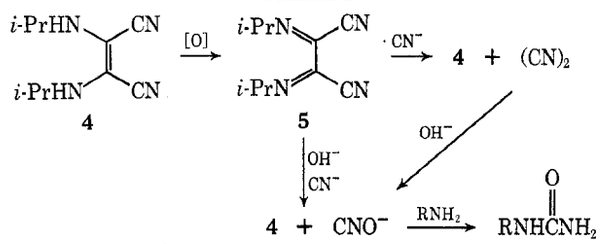
When the maleonitrile **4** was oxidized in acetonitrile, diisopropyldiiminosuccinonitrile (**5**) was isolated in 47% yield. The structure of **5** was assigned by comparison of its uv spectrum with that reported for *N,N*-di-*tert*-ocyldiiminosuccinonitrile.[10] The uv spectrum of **5** exhibits an intense maximum at 230 nm (ϵ 1.76 × 10^4) at about the same wavelength as the short-wavelength maximum of **4** [226.5 nm (ϵ 1.3 × 10^4)] as well as a weak maximum at 306 nm (ϵ 365). The decrease in the intensity of the uv maximum of **4** at 332 nm and the small variation in the spectrum at 225 nm is consistent with the oxidation of **4** to **5** in aqueous solution. It was also possible to isolate **5** as a reaction product when the oxidation was performed in aqueous medium.

Since we established **5** to be the oxidation product of **4** we then investigated the hydrolysis of **5** in ammoniacal solution at pH 9.2. Only urea could be detected as a reaction product and no isopropylurea was evident. The absence of isopropylurea was established by paper chromatography and by an independent color reaction which can be used to distinguish urea from monosubstituted ureas.[11] Therefore, urea formation from **5** must involve either (1) decomposition of **5** to a derivative which does not contain the isopropyl grouping, which in turn reacts with ammonia to give urea; (2) reaction of ammonia with diiminosuccinonitrile in such a way (*e.g.*, at the nitrile grouping) to give urea and no isopropylurea; (3) reaction of **5** with some other substance (*e.g.*, cyanide) to give a compound which yields urea on further reaction.

That alternate 3 above was the pathway for urea synthesis was established by performing the hydrolysis of **5** in the presence of cyanide. When the ammoniacal hydrolysis of 0.1 mmol of **5** was performed in the absence of cyanide 0.076 mmol of urea was produced. When the same reaction was performed in the presence of a 30-fold excess of cyanide, 0.28 mmol of urea was obtained.[12] Although urea is produced by the direct reaction of cyanide and ammonia, it will be shown later that its rate of synthesis is much slower than was observed in these experiments.

A plausible explanation for these data is given in Scheme I. In the presence of added cyanide ion **5**

SCHEME I



oxidizes the cyanide ion to cyanogen[13a] or cyanate.[13b] The cyanogen is cleaved by base to give cyanide and cyanate.[13a] The cyanate reacts with ammonia or amines to give the corresponding urea. More than 1 equiv of urea is obtained in this process because **4**, the reduction product of **5**, is readily air oxidized to **5** and the redox cycle can begin again. A small yield of monoisopropylurea is obtained in the absence of ammonia because the requisite cyanide and isopropylamine are formed in small amounts by the hydrolytic decomposition of **5**. In the presence of added ammonia (but no added cyanide) the isopropylamine is swamped out by the ammonia and urea is the reaction product.

Reactions of Diaminomaleonitrile (3) and Diiminosuccinonitrile (6).—The decomposition of **3** in aqueous pH 9.2 ammonia proceeds more rapidly in the presence of air and oxygen than when the solution is degassed (Table I). The formation of urea parallels the de-

TABLE I

OXIDATION AND HYDROLYSIS OF DIAMINOMALEONITRILE (**3**)

	1[a]		2[b]		3[c]		4[d]	
Time, days	**3**,[e] $M \times 10^4$	Urea,[f] $M \times 10^4$	**3**,[e] $M \times 10^4$	Urea,[f] $M \times 10^4$	**3**,[e] $M \times 10^4$	Urea,[f] $M \times 10^4$	**3**,[e] $M \times 10^4$	Urea,[f] $M \times 10^4$
0	93		93		93		93	
1	91							
2	89		40		22			
3	78	12						
4	61	21						
5	57		11		4		90	
6	46	27						
9			0	125	0	156		
13							82	
43							63	
76–82			0	110	0	99	46	0

[a] Open to the atmosphere. [b] Three freeze–pump–thaw cycles and opened to the atmosphere. [c] Three freeze–pump–thaw cycles and an oxygen atmosphere added. [d] Three freeze—pump–thaw cycles and sealed *in vacuo* in separate ampoules. One ampoule opened on each indicated day. [e] **3** measured from uv maxima at 295 nm.[3] [f] Determined by procedure of Ormsby.[11]

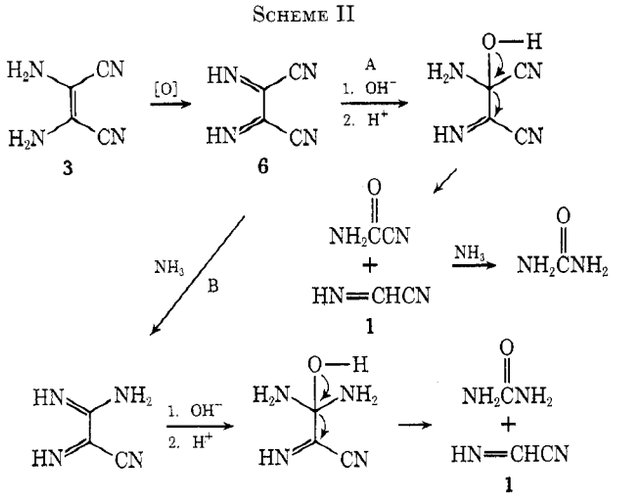
(10) L. deVries, *J. Org. Chem.*, **36**, 3442 (1971).
(11) A. Ormsby, *J. Biol. Chem.*, **146**, 595 (1942).
(12) The urea yields given in the Experimental Section were normalized here to those that would be obtained from 0.1 mmol of **5**.
(13) (a) K. Brotherton and J. W. Lynn, *Chem. Rev.*, **59**, 841 (1959); (b) W. M. Latimer, "Oxidation Potentials," Prentice-Hall, Englewood Cliffs, N. J., 1952, p 50.

composition of **3** (Table I) except in the degassed solution. There, no urea could be detected even after 76 days, when 50% of **3** had decomposed. The molar ratio of urea formed to the initial concentration of **3** was 1.35–1.7 after 9 days. This ratio was somewhat less after about 80 days, probably owing to the slow hydrolysis of the urea.

A 50% yield of urea is obtained on hydrolysis of **6** in a pH 9.3 aqueous ammoniacal solution, suggesting that **6** is the initial oxidation product of **3**. **6** has been obtained as the oxidation product of **3** in nonhydroxylic solvents.[14] Isopropylurea was obtained when the hydrolysis was carried out in the presence of isopropylamine. In contrast to the findings with *N,N*-diisopropyldiiminosuccinonitrile, the yield of urea did not increase when the hydrolysis of **6** was performed in the presence of a sixfold excess of cyanide. This difference between **6** and *N,N'*-diisopropyldiiminosuccinonitrile reflects the more rapid hydrolysis of **6**. It was impossible to detect **6** in a pH 9.2 reaction mixture after 13 hr while the disubstituted diiminosuccinonitrile (**5**) can be isolated from a 3-day air oxidation of **4** in aqueous solution. Apparently the reduction of **5** by cyanide is slower than the hydrolysis of **5**.

These experiments establish that urea must be formed directly from **6**. Two possible pathways are shown in Scheme II. Paths A and B differ only in

SCHEME II



the timing of the addition of hydroxide ion and ammonia to **6** respectively. These addition reactions are similar to those proposed for the addition of amines to **6** in nonpolar solvents.[14] The cleavage of **6** by base (path A) is similar to the cleavage of cyanogen to cyanate and cyanide by base[13a] and the hydrolysis of **3** to aminoacetonitrile.[3] Iminoacetonitrile (**1**) can serve as a leaving group because the negative charge is stabilized by the sp^2 carbon atom and the proximate cyano group.

The possibility that aminomalononitrile (**2**), in equilibrium with **4**, is the direct precurser to urea was eliminated by the following experiments. A fivefold excess of cyanide was added to an ammoniacal solution of **2** with the final pH 9.3. A 52% yield of **3** was observed after 10 min; however, no urea could be detected. A 19% yield of urea was detected after 2 days, a result consistent with the initial formation of **3** followed by the formation of urea.

Oligomerization of HCN.—Urea is produced in the HCN oligomerization mixture and the corresponding monosubstituted urea is formed when an organic amine is added to the reaction mixture.[7] In the present work we found that urea is formed slowly during the oligomerization of cyanide. No urea could be detected from 0.1 *M* cyanide after 20 days, and a 1.2% yield (based on initial cyanide) was detected after 83 days. A 5% yield (7% based on cyanide consumed) was obtained after 14 months. Urea is slowly hydrolyzing under the reaction conditions; so the actual amount formed is greater than 7%. Since **3** is readily oxidized, the effect of oxygen on the oligomerization of cyanide was then investigated. A portion of a stock solution of *ca.* 0.15 *M* cyanide was degassed by four freeze–pump–thaw cycles and then sealed under vacuum while the other portion was allowed to stand open to the atmosphere. Both solutions darkened at about the same rate. Analysis of the nondegassed solution after 1 month indicated that it was 0.135 *M* in cyanide.[15] After 7 months the degassed solution was 0.053 *M* in cyanide while the nondegassed was 0.024 *M*. The degassed solution was 1.9×10^{-3} *M* in urea after 7 months while the nondegassed solution was 3.7×10^{-3} *M* in urea. These data indicate that the same redox reactions are occurring in the presence and absence of oxygen. Further confirmation of this conclusion was obtained by the acid hydrolysis of the oligomerization mixture formed in the degassed and nondegassed cyanide solutions. Paper chromatography with ninhydrin spray indicated that the same amino acids were released in each instance.[16]

Conclusions

3 and the dialkyl-substituted derivatives of **3** are readily oxidized by molecular oxygen to the corresponding **6** derivatives.[9,10] Hydrolysis of **6** in the presence of ammonia yields urea. Since **3** is formed in significant amounts in the oligomerization of cyanide, one might expect that the presence of oxygen might exert a significant effect on this reaction. However, the oligomerization proceeds equally as well in the presence or absence of oxygen. The rate of cyanide loss is considerably less in the absence of oxygen; however, urea is produced in the oligomerization mixture on acid hydrolysis. Since redox steps must be involved in the formation of these compounds, either some other cyanide oligomers must be effecting these oxidation reactions or disproportionation reactions are occurring. The formation of amino acids and other biomolecules from HCN could have occurred on the primitive earth in the absence of oxygen.

The ease of oxidation of **3** suggests that it is being oxidized by other cyanide oligomers in the absence

(14) The reduction of diiminosuccinonitrile to diaminomaleonitrile in organic solvents by cyanide and the resulting formation of cyanogen has been reported: R. W. Begland, A. Cairncross, D. S. Donald, D. R. Hartter, W. A. Sheppard, and O. W. Webster, *J. Amer. Chem. Soc.*, **93**, 4953 (1971); O. W. Webster, D. R. Hartter, R. W. Begland, W. A. Sheppard, and A. Cairncross, *J. Org. Chem.*, **37**, 4133 (1972).

(15) A. A. Schilt, *Anal. Chem.*, **30**, 1409 (1958). This procedure was modified as described in ref 3.

(16) Similar results have been obtained in this laboratory by Drs. J. D. Wos and J. Wittmann.

of oxygen.[17] One pathway to the urea produced during the cyanide oligomerization is by the hydrolysis of **6**. The ease with which cyanide is oxidized to cyanate and cyanogen[13] in basic medium suggests that urea and oxalate may be formed by these routes as well.[7]

Experimental Section[18]

Materials.—Isopropylurea was prepared by the reaction of isopropylamine with KCNO, mp 148–152° (lit.[21] mp 154°), ir identical with that of a published spectrum.[22] Diisopropylurea was prepared by the reaction of phosgene with isopropylamine, mp 187–189° (lit.[23] mp 192°). Diiminosuccinonitrile was supplied by Dr. W. A. Sheppard of E. I. du Pont. **3**, *N,N'*-Diisopropyldiaminomaleonitrile,[5] and aminomaloninitrile *p*-toluenesulfonate[24] were prepared as described previously.

Quantitative Analysis for Urea.—The method of Ormsby[11] was modified in a few minor respects. The final volume of the solution after addition of potassium persulfate was made up to either 10 or 25 ml. The solution was then placed in a uv cell and the absorbance recorded with time. The maximum absorbance was used as the value for the determination. Standards were run simultaneously with the unknown sample.

Decomposition of *N,N'*-Diisopropyldiaminomaleonitrile in Aqueous Sodium Cyanide Solution.—A solution of sodium cyanide (117.3 mg, 2.4 mmol) and *N,N'*-diisopropyldiaminomaleonitrile[5] (8.6 mg, 0.05 mmol) in 10 ml of ethanol–water (1:1) was made up as a stock solution (solution A). A portion (2 ml) of solution A was diluted to 100 ml using ethanol–water (1:1) (solution B). A portion (2 ml) of solution A was diluted to 10 ml using ethanol–water (1:1) (solution C). The uv spectrum of the most dilute solution was followed initially and the more concentrated solutions were monitored in the later stages of the decomposition.

Over a period of 3 hr, there was a decrease in the absorption at 332 nm and an increase in the absorption at 222 nm. After 1 hr, the absorbance at 222 nm began to decrease. After 6 days, the maximum of 222 nm disappeared, and a maximum of 206 nm appeared. There was a continuum of absorption to above 400 nm. The solutions were combined and the solvent was removed on a rotary evaporator to yield a brown-yellow solid residue. The residue was washed well with chloroform, the chloroform washings were combined, and the solvent was removed on a rotary evaporator. The presence of monoisopropylurea in the chloroform extract was established by paper chromatography (BAW) (R_f 0.83) followed by spraying with Ehrlich's reagent.

Decomposition of *N,N'*-Diisopropyldiaminomaleonitrile in Degassed and Nondegassed Aqueous Sodium Cyanide Solutions.—*N,N'*-Diisopropyldiaminomaleonitrile (2.4 mg) was dissolved in ethanol–water (1:1) (100 ml) to make a stock solution. The reaction vessel consisted of a Pyrex cell and a quartz cell at a 90° angle. These cells were part of one apparatus which could be outgassed on a vacuum line.

A.—Sodium cyanide (10.0 mg, solid) was placed in the quartz cell. A portion (*ca.* 3 ml) of the above solution was placed in the Pyrex cell and degassed by four freeze–pump–thaw cycles. The stopcock was then closed to maintain the system under vacuum.

B.—Sodium cyanide (29.8 mg, solid) was placed in the quartz cell. A portion (*ca.* 3 ml) of the stock solution was placed in the Pyrex cell. The solution was then carried through four freeze-thaw-cycles *without* degassing.

After degassing or simulated degassing the solutions in the Pyrex cells were then poured into the quartz cells containing the solid sodium cyanide and shaken to cause the sodium cyanide to dissolve. The uv spectra were recorded over a period of time. After 40 min, the absorbance at 332 nm (*N,N'*-diisopropyldiaminomaleonitrile) had disappeared for solution B (not degassed). This was a loss of 1.4 absorbance units. There was a loss of only 0.14 absorbance units in solution A during this time. The small loss in solution A may have been due to residual oxygen not removed in degassing. Consistent with this is the observation that after 8 hr, solution A had only lost 0.18 absorbance units. After 22.5 hr, solution A had lost 0.56 absorbance uuits (this loss on long standing may be due to slow leakage at the stopcock). The stopcock was then opened to allow air into the cell. Within 2 hr, all the absorbance at 332 nm has disappeared (loss of 0.94 absorbance units).

Decomposition of *N,N'*-Diisopropyldiaminomaleonitrile in the Presence of Nucleophiles Other Than Cyanide Ion.—A stock solution of *N,N'*-diisopropyldiaminomaleonitrile (57.6 mg, 0.3 mmol) in ethanol–water (10 ml, 1:1) was prepared. Two solutions were made up from this stock solution.

A.—A solution of *N,N'*-diisopropyldiaminomaleonitrile (11.3 mg, 2 ml of stock solution) and sodium iodide (345.8 mg, 2.32 mmol) was made up to 10 ml with ethanol–water (1:1).

B.—A solution of *N,N'*-diisopropyldiaminomaleonitrile (11.3 mg, 2 ml of stock solution) was made up to 10 ml with ethanol–water (1:1). The final pH of this solution was adjusted to 9.74 with 10% sodium hydroxide and 10% hydrochloric acid.

Analysis of the solutions after 6 days by paper chromatography (BAW, Ehrlich's reagent) indicated that there was monoisopropylurea in solution B not in solution A. After 25 days, solution B gave a weak positive test for cyanide ion.[15] Iodide ion interferes with this test, so that solution A could not be analyzed.

Conversion of *N,N'*-Diisopropyldiaminomaleonitrile to *N,N'*-Diisopropyldiminosuccinonitrile in Acetonitrile in the Presence of Anhydrous Sodium Carbonate.—*N,N'*-Diisopropyldiaminomaleonitrile (57.4 mg, 0.30 mmol) was dissolved in acetonitrile (10 ml). Anhydrous sodium carbonate (194.6 mg, 1.83 mmol) was added and the mixture was stirred at room temperature overnight. The sodium carbonate was removed by filtration. The acetonitrile was removed on a rotary evaporator to yield 53.4 mg of a white solid, mp 92–97°. After sublimation [40° (0.6 Torr)] a white solid, mp 98–99°, 27.1 mg (47%), was obtained. This was identified as *N,N'*-diisopropyldiminosuccinonitrile on the basis of its spectra: ir (KBr) 3012, 2933, 2230 (w, C≡N), 1618 (C=N), 1451, 1370, 1350, and 1163 cm^{-1}; uv max (CH_3CN) 306 nm (δ 365), 230 (1.76×10^4); nmr (CCl_4) δ 1.35 (d, J = 3 Hz, 6.8 H), 4.15 (septet, only five peaks resolved, 1 H); mass spectrum *m/e* (rel intensity) 190 (0.5), 175 (4), 133 (25), 96 (14), 95 (6), 76 (3), 55 (5), 54 (16), 43 (100), 42 (15), 41 (36), 40 (6), 39 (18), 27 (35).

Anal. Calcd for $C_{10}H_{14}N_4$: C, 63.16; H, 7.37. Found: C, 63.14; H, 7.46.

Decomposition of *N,N'*-Diisopropyldiaminomaleonitrile in Aqueous Isopropylamine.—Isopropylamine (4.1 ml, 48 mmol) was dissolved in water (10 ml) and the pH of the solution was adjusted to 9.2 using 10% HCl. The final volume was about 45 ml. A portion of this solution (20 ml, *ca.* 20 mmol) was placed in a 50-ml erlenmeyer flask and *N,N'*-diisopropyldiaminomaleonitrile (25.1 mg, 0.13 mmol) was added. Ethanol (95%, 5 ml) was added to bring the *N,N'*-diisopropyldiaminomaleonitrile into solution. After 3 days, the solution had turned yellow and fine white needles precipitated. The solution and needles were extracted with ether (2 × 25 ml). The ether extracts were combined and dried over anhydrous sodium sulfate. Tlc (silica gel–$CHCl_3$) gave two spots, one with an R_f corresponding

(17) Diaminosuccinic acid is one of the major amino acids produced by hydrolyses of the oligomerization mixture (J. D. Wos, unpublished). This result suggests that diaminomaleonitrile may also be serving as an oxidizing agent, although other explanations are also possible.

(18) The infrared spectral data were recorded on a Perkin-Elmer Model 137 sodium chloride spectrophotometer. The nmr spectra of solutions in deuteriochloroform, with TMS as an internal standard, were recorded on a Varian Model T-60 spectrometer. Ultraviolet spectra were recorded on a Unicam Model SP 800A spectrophotometer. Mass spectra were obtained using a Hitachi Perkin-Elmer RMU-6E mass spectrometer. pH measurements were made on a Radiometer Model 26 pH meter equipped with Corning 476050 electrode. Elemental analyses were carried out at Instranal Laboratory Inc., Rensselaer, N. Y. Melting points are uncorrected. Paper chromatography was by ascending development on Whatman 3MM at room temperature for *ca.* 15 hr. Abbreviations used: BAW, 1-butanol–acetic acid–water 5:2:3; BW, 1-butanol saturated with water; PA, 1-propanol–14.8 *M* ammonium hydroxide 3:1. Ehrlich's reagent[19] and Folin's reagent[20] were used as visualizing reagents. Compounds were visualized at 254 nm light source in all cases where a spray reagent was not used. Diaminomaleonitrile was estimated by its uv absorption at 295 nm (ϵ 1.35×10^4).[3]

(19) R. A. Heacock and N. E. Mahon, *J. Chromatogr.*, **17**, 338 (1965).

(20) I. M. Hais and K. Macek, "Paper Chromatography," Publishing House of Czechoslovak Academy of Sciences, Prague, 1963, p 809.

(21) Beilstein's "Handbuch der Organischen Chemie," 4th ed, Bd IV, J. Springer, Berlin, 1937, p 154.

(22) "Sadtler Standard Spectra," Sadtler Research Laboratories, Inc., Philadelphia, Pa., 1966, infrared spectrum no. 20089.

(23) T. Mukaryama and Y. Fujita, *Bull. Chem. Soc. Jap.*, **29**, 54 (1956).

(24) J. P. Ferris, R. A. Sanchez, and R. W. Mancuso, *Org. Syn.*, **48**, 1 (1968).

to N,N'-diisopropyldiiminosuccinonitrile. When the ether was stripped off and the residue was placed in a sublimator [room temperature (0.5 Torr)], a small amount of a white solid, mp 88–91°, sublimed. The infrared spectrum of this material was identical with that of N,N'-diisopropyldiiminosuccinonitrile, mp 98–99°.

Analysis of the water layer by paper chromatography using BAW gave a faint spot corresponding to isopropylurea when the paper was sprayed with Ehrlich's reagent.

Reaction of Isopropylamine with N,N'-Diisopropyldiaminomaleonitrile.—Isopropylamine (4.1 ml, *ca.* 48 mmol) was dissolved in 10 ml of water and the pH was adjusted to 9.16 with 25% hydrochloric acid. The final volume was about 35 ml. N,N'-Diisopropyldiaminomaleonitrile (23.0 mg, 0.12 mmol) was dissolved in 95% ethanol (7 ml) and added to the isopropylamine solution. The solution was allowed to stand at room temperature protected from room light. After 5 months, paper chromatography using BAW and Ehrlich's reagent showed the presence of monoisopropylurea. It was difficult to determine whether any N,N'-diisopropylurea had been formed. N,N'-diisopropylurea would be expected to be insoluble but no precipitate was observed. On paper chromatography using BAW and iodine vapors, it was difficult to get a good development of the spot for the authentic N,N'-diisopropylurea; so it was not certain if it was formed.

Decomposition of N,N'-Diisopropyldiiminosuccinonitrile in Aqueous Ammonia.—A portion (3.0 ml) of 14.8 *M* ammonium hydroxide solution was diluted to 10 ml with water. The solution contained about 45 mmol of ammonium hydroxide. The pH of the solution was adjusted to 9.2 with 10% hydrochloric acid. The final volume was about 35 ml. N,N'-Diisopropyldiiminosuccinonitrile (20.7 mg, 0.11 mmol) was added to this solution along with ethanol (15 ml). All the solid did not dissolve. The volume of the solution was made up to 50 ml with distilled water, and the solution was allowed to stand at room temperature protected from room light. After 43 days, analysis[11] indicated that it contained 5 mg (0.084 mmol) of urea. The presence of urea was confirmed by paper chromatography using BAW and Ehrlich's spray.

Decomposition of N,N'-Diisopropyldiiminosuccinonitrile in Aqueous Ammonia in the Presence of Cyanide.—A portion (3.0 ml) of 14.8 *M* ammonium hydroxide solution was diluted to 10 ml with water. The pH of the solution was adjusted to 9.2 with 10% hydrochloric acid. The final volume of the solution was about 35 ml. Potassium cyanide (355.9 mg, 5.5 mmol) was added to the solution and it dissolved completely. Diisopropyldiiminosuccinonitrile (35.4 mg, 0.19 mmol, mp 93–97°) was added to the solution along with ethanol (15 ml) in an attempt to solubilize the diisopropyldiiminosuccinonitrile. This was only partially successful and some solid remained undissolved. The volume of the solution was made up to 50 ml with distilled water and the solution was allowed to stand at room temperature, protected from room light. The solution contained 32 mg (0.53 mmol) of urea[11] and essentially no isopropylurea after 42 days.[11] We verified that both urea and isopropylurea can be distinguished by this procedure. Paper chromatography using BAW and spraying with Ehrlich's reagent confirmed the presence of urea.

Decomposition of Diaminomaleonitrile in Aqueous Ammonia.—A solution of ammonium hydroxide (296 mmol) was neutralized to pH 9.2 with 50% HCl and diluted to 200 ml in a volumetric flask. Diaminomaleonitrile (200 mg, 1.85 mmol) was added to this stock solution. A portion of this stock solution was left open to the atmosphere. A portion was carried through three freeze–pump–thaw cycles and then opened to the atmosphere. A 25-ml portion was degassed by three freeze–pump–thaw cycles and then sealed in the presence of 1 atm of pure oxygen. Portions (5 ml) were placed in bulbs, degassed by three freeze–pump–thaw cycles, and sealed under vacuum. The solutions were analyzed for diaminomaleonitrile and urea and the results are given in Table I.

Quantitative Analysis of the Amount of Urea and Cyanide Formed from Diiminosuccinonitrile in Aqueous Ammonia.—Ammonium hydroxide (3 ml of 14.8 *M* solution) was diluted to 10 ml with distilled water and the pH was then adjusted to 9.26 with 10% hydrochloric acid solution. The final volume was about 35 ml. Diiminosuccinonitrile (125.2 mg, 1.18 mmol) was dissolved in this solution and allowed to stand at room temperature for 1.5 months protected from light. Analysis by paper chromatography using BAW and Ehrlich's reagent indicated that there was urea present in the solution. The solution was filtered, diluted to 50 ml in a volumetric flask, and then analyzed for cyanide[15] and for urea.[11] These analyses indicated that the solution contained 1.34 mmol of cyanide and 0.53 mmol of urea.

Decomposition of Diiminosuccinonitrile in Aqueous Ammonia in the Presence of Excess Cyanide Ion.—A 35-ml solution containing 45 mmol of NH_4OH and 5.4 mmol of KCN was neutralized to pH 9.2 with 10% HCl and 0.9 mmol of diiminosuccinonitrile was added to it. After 16 days, a brown precipitate was removed by filtration and the filtrate was made up to 50 ml with distilled water. Analysis of the solution indicated that it contained 28 mg (0.46 mmol) of urea.[11] After 82 days, analysis indicated 31.1 mg (0.52 mmol) of urea. The presence of urea was confirmed by paper chromatography using BAW and Ehrlich's reagent.

Decomposition of Diiminosuccinonitrile in Aqueous Isopropylamine Solution.—Isopropylamine (4.1 ml, *ca.* 48 mmol) was dissolved in 10 ml of water and the pH of the solution was adjusted to 9.2 with 10% hydrochloric acid solution. The final volume was about 45 ml. Diiminosuccinonitrile (45.2 mg, 0.42 mmol) was dissolved in 20 ml of this solution. The solution was allowed to stand at room temperature for 3 days, at which time the solution was red brown in color and a black precipitate had formed. Analysis by tlc (silica gel–ethyl acetate) showed no starting material. Tlc [silica gel, chloroform–ethanol (1:1)] showed a spot corresponding to isopropylurea (R_f 0.85) when the plate was sprayed with Ehrlich's reagent. Paper chromatography (using BAW and spraying with Ehrlich's reagent) confirmed the presence of isopropylurea. There was no evidence for the presence of urea on tlc or paper chromatography.

Reaction of Aminomalononitrile with Cyanide Ion.—A portion (3.0 ml) of 14.8 *M* ammonium hydroxide was diluted with distilled water, and the pH was adjusted to 9.3 with 10% hydrochloric acid, giving a final volume of 35 ml. Aminomalononitrile *p*-toluenesulfonate (100 mg, 0.39 mmol) and KCN (137 mg, 2 mmol) were added and the solution turned yellow. The solution was made up to 50 ml in a volumetric flask using distilled water. The pH was 9.34. A uv spectrum measured 10 min after the start of the reaction indicated that the solution contained diaminomaleonitrile (4.00×10^{-3} *M*, 52%). No urea could be detected when a 1 ml aliquot of the stock solution was analyzed for urea.[11] A concentration of 0.05 mg/ml could have been detected. Urea could be detected after 1 month by paper chromatography using BAW and Ehrlich's spray.

In a repeat of this experiment, the ammonium hydroxide solution was made up as above and the pH was adjusted to 9.23. Aminomalononitrile (100.4 mg, 0.39 mmol) and potassium cyanide (132.7 mg, 1.9 mmol) were dissolved in the ammonium hydroxide solution. After 2 hr, a 59% yield of diaminomaleonitrile was obtained. After 2 days, the reaction solution contained 4.5 mg (0.075 mmol) (19%) of urea.[11] After 1 month the presence of urea was confirmed by paper chromatography using BAW and Ehrlich's reagent.

Analysis of HCN Oligomerization Solutions for Cyanide and Urea. A.—A solution of 0.103 *M* HCN in water was adjusted to 9.2 with ammonium hydroxide. After 14 months, the solution was analyzed for urea[11] and cyanide ion.[15] These analyses indicated that the solution was 0.03 *N* in cyanide ion (about 70% of cyanide gone) and 0.0053 *M* in urea (about 7% based on cyanide lost).

B.—A 50 ml solution containing 3 ml of 14.5 *M* NH_4OH and 361 mg (5.6 mmol) of KCN was adjusted to pH 9.0 with 10% HCl. After 20 days, no urea could be detected in the solution.[11] After 83 days, analysis indicated that the solution contained 4.00 mg (0.067 mmol, 1.2%) of urea based on starting cyanide.

C.—HCN (2 ml) was dissolved in about 300 ml of distilled water, and NH_4OH (*ca.* 3 *N*) was added to adjust the pH to 9.2. The solution was then made up to 500 ml in a volumetric flask using distilled water (*ca.* 0.15 *M* in HCN). The final pH was 9.3. A portion (*ca.* 20 ml) of this cloudy solution was placed in a glass bulb, degassed by four freeze–thaw cycles, and sealed under vacuum (0.3 Torr). The remainder of the solutions was left open to the atmosphere. Both solutions turned pale yellow after about 4 days. After 1 month, the nondegassed solution was found to be 0.135 *N* in cyanide.[15]

After 7 months, analysis of 1 ml of the nondegassed solution for urea[11] indicated that it contained 0.22 mg urea/ml (3.7×10^{-3} *M*) and that it was 0.024 *M* in cyanide ion.[15] Examination of the uv spectrum of this solution did not reveal any absorp-

tion at 296 nm due to diaminomaleonitrile (1 ml to 10 ml dilution). The presence of urea in this solution was confirmed by paper chromatography using BAW and Ehrlich's reagent.

The degassed solution was opened after 7 months and the pH was found to be 9.36. A uv spectrum (1 ml to 10 ml dilution) had a broad continuum from 200 to 450 nm. The absorption at 296 nm was 0.97 absorbance units but it was impossible from this to say whether diaminomaleonitrile was present. Analysis of the solution for diaminomaleonitrile by paper chromatography using BAW and Folin's reagent did not give a spot corresponding to authentic diaminomaleonitrile. This solution was 1.9×10^{-3} *M* in urea[11] and 0.053 *M* in cyanide.[15]

Portions (15 ml) of the degassed and nondegassed solutions were placed in round-bottom flasks and evaporated to dryness on the rotary evaporator. The two different samples (degassed and nondegassed) were worked up in the same way so as to compare them. The residue in each flask was taken up in 6 *N* hydrochloric acid and the solutions were sealed in vials and heated overnight at 110°. The solutions were concentrated to dryness, and the residues were taken up in 1 ml of water and analyzed for amino acids by paper chromatography using both BAW and PA and using ninhydrin for detection. Both solutions (degassed and nondegassed) gave spots corresponding to authentic glycine, which had also been spotted on the paper. Other ninhydrin-positive materials were also detected. The presence of urea in the degassed solution was confirmed by paper chromatography using BAW and Ehrlich's reagent.

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Pyridazines. LVIII. Oxidative Transformations of Pyridazinyl Sulfides

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Different oxidizing agents have been employed for conversion of methylthiopyridazines or their *N*-oxides into the corresponding methylsulfinyl or methylsulfonyl derivatives. In some cases, besides S-oxidation, N-oxidation also took place.

It is well known that organic sulfides can be transformed into sulfoxides or sulfones by a variety of oxidizing agents.[1] In the pyridazine series oxidation can, *a priori*, occur at either the sulfur containing side chain(s) or ring nitrogens to give the corresponding *S*-oxides or/and N_1- or N_2-oxides. The reported results on oxidation experiments with some pyridazinyl sulfides are either conflicting with regard to structure assignment or there has been no assignment at all. Pyridazinyl sulfides have been converted into the corresponding sulfones with potassium permanganate or hydrogen peroxide,[2-6] chlorine,[4,7] or sulfur dioxide.[4] For the synthesis of sulfoxides hydrogen peroxide[2,8] or *m*-chloroperoxybenzoic acid[9] was used, but, depending on the quantity of the oxidizing agent and reaction conditions, sometimes a mixture of the corresponding sulfoxides and sulfones resulted.[2,10] Pyridazinyl sulfoxides were transformed into sulfones with potassium permanganate.[11]

An extensive study of oxidative transformations of alkylthiopyridazines with various oxidizing agents was reported by Takahayashi,[12] but the obtained products were mostly designated as monoxides, dioxides, or trioxides. Moreover, he also assumed that in some cases, in addition to the formation of *S*-oxides, N-oxidation took place.[12,13] Moreover, halogens or alkoxy groups bound on the pyridazine ring can suffer hydrolysis and the corresponding pyridazinone derivatives were obtained.[14,15]

We have studied oxidations of pyridazinyl sulfides under differing conditions with different oxidizing agents. We used 70% hydrogen peroxide alone or in admixture with various solvents or in the presence of sodium tungstate, as well as dichloromonoperoxymaleic acid, bromine, potassium permanganate, chromium trioxide, potassium metaperiodate, and ceric ammonium nitrate.

The structures of some products were proved through chemical transformations. In addition, it is possible to distinguish between different oxidation products by nmr and/or ir spectra as well as on hand of color tests.[16-18] Thus, in an analogous series, when observing chemical shifts for a methylthio, methylsulfinyl, and methylsulfonyl group we observed a distinct deshielding effect of approximately 1 τ unit. This is comparable to that observed in 3- or 4-methylthiopyridazines and their oxidation products.[19,20] Infrared spectra are also of diagnostic value since one can

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