Mechanisms of Hydrogen Cyanide Formation from the Pyrolysis of Amino Acids and Related Compounds[1]

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Amino acids were pyrolyzed in a helium atmosphere at temperatures from 700 to 1000°. Under these conditions, HCN was a major pyrolysis product. For straight chain and branched acids, HCN production varied in the order $\gamma \gg \beta > \alpha$. The cyclic acids, proline and 4-hydroxyproline, gave the largest yields of all amino acids studied. Extensions of the study to pyrolysis intermediates such as pyrrolidine and 2-pyrrolidone led to observations of high yields of HCN. Unsaturation of the ring containing the nitrogen atom was shown to cause a decrease in yield, as did methyl substitution on nitrogen. Compounds which contain nitrogen as a part of an aliphatic ring which can produce methylenimine—or can furnish such rings as intermediates—were shown to give relatively high amounts of HCN. At 1000° maximum yield was obtained with five-membered-ring compounds. All of these observations support the general hypothesis that HCN formation is favored in those instances in which the pyrolyzed compound either is itself an aliphatic nitrogen containing ring or is formed into such a ring as a reaction intermediate. Under ideal circumstances almost 100% of amino nitrogen is converted to HCN.

Heyns and Pavel[2] showed that thermal treatment of amino acids produces small quantities of hydrogen cyanide. They exposed glycine, alanine, leucine, phenylalanine, tyrosine, and gelatin to temperatures between 310 and 340° and showed HCN to be formed from glycine, alanine, and gelatin.

Winter and Albro[3] pyrolyzed amino acids at 300° and gas chromatographed the resulting C_1 to C_5 amines as a means of characterizing the acid. Each amino acid was shown to give a unique amine profile. Merritt and Robertson[4] also pyrolyzed 17 amino acids under conditions which led to a characteristic pyrolysis product for each acid. Recently, Bryan and Olafsson[5] carried out differential enthalpic analysis of aromatic and heteroaromatic amino acids and obtained thermograms which are characteristic of specific amino acids. In all these investigations, the possible formation of HCN was not mentioned. Patterson, *et al.*,[6] pyrolyzed lysine, leucine, and tryptophan at 850°. HCN was mentioned as a pyrolysis product of lysine hydrochloride, but no quantitative data was given.

It was the purpose of this work to study the pyrolysis of amino acids under conditions such that the factors responsible for HCN formation could be studied. Experiments were chosen so that the molecular structural features favoring HCN formation could be determined and mechanisms for the conversions elucidated.

Experimental Section

Chemicals.—Amino acids were purchased from Nutritional Biochemicals Co. These acids which are homogeneous by paper chromatography, can be assumed to be better than 99.5% pure. Amines and other liquids were obtained from various sources and purified by distillation. 1,4-Diaminobutane dihydrochloride and other solids were used as received from various suppliers.

Pyrolysis.—A modification of an apparatus described by Honaker and Horton[7] was used. This consisted of a 9.53-mm-o.d. Vycor tube enclosed in a 89-mm-long oven. This tube was joined through a toggle valve to a stainless steel "T" which was connected to the injection port of a F & M Model 720 gas chromatograph. An in-line sintered Cambridge filter pad was used to protect the chromatographic column from high-molecular-weight materials. All connections to the injection port were wrapped with a heating tape which was maintained at 150°. Pyrolysis of solids was accomplished by magnetically pushing a porcelain boat containing the sample into the equilibrated hot zone and retracting the magnet. Temperatures, which were monitored at the wall of the pyrolysis tube by a pyrometer, dropped 10° on sample introduction. Liquid samples were pyrolyzed by direct injection into the pyrolysis zone. In all cases, 10^{-5} mol of compound was pyrolyzed. Helium was used as the carrier gas at a flow rate of 80 ml/min. After 1 min the pyrolysis unit was cut off from the chromatograph by closing the toggle valve. Chromatography was continued using helium furnished by a line which bypassed the pyrolysis apparatus and fed into the injection port *via* the "T" assembly.

Chromatography.—The separation of HCN was accomplished by using a 3.05 m × 6.35 mm stainless steel column packed with 80–100 mesh Porapak S. The flow rate of 80 ml/min was not changed. The column was kept at room temperature for 5 min and then temperature programmed to 130° at 20°/min. After 10 min at this temperature, programming was resumed at 5°/min. Programming was continued until 250° was reached. All connections between the pyrolysis zone and the injection port of the chromatography were maintained at 150° by using heating tapes. Under these conditions, HCN eluted sharply and cleanly at 18.5 min. The detector was operated at a temperature of 280° and a filament current of 140 mA. Calibration of recorder response with known samples of HCN assayed on a mass spectrometer permitted quantitative determination of HCN. Overall reproducibility in experiments by this procedure was ±5% for moderate quantities of HCN and ±10% for extremely high amounts. Acetonitrile was eluted at 28.0 min.

Ammonia was separated by the method of Burks.[8] Triethanolamine (5%) was coated onto 40 mesh firebrick. A 2 m × 6.35 mm stainless steel column was used at room temperature with helium flow of 80 ml/min. Ammonia was eluted in 1.5 min.

Results and Discussion

Pyrolysis of α-Amino Acids.—The results of the pyrolysis of some α-amino acids at 1000° are given in Table I. HCN yields can be seen to vary from 8 to 45%. If we consider these results in terms of glycine, we will note that the substitution of hydrogen with alkyl groups lowered HCN yields from 32 to 8%. However, substitution with hydroxymethyl or benzyl groups, raised yields from 32 to 45%.

Of the thermal reactions that glycine might be expected to undergo such as deamination, decarboxylation, and 2,5-piperazinedione formation, the first two reactions should be rather independent of alkyl sub-

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(2) K. Heyns and K. Pavel, *Z. Naturforsch., B*, **12**, 97 (1957).

(3) L. N. Winter and R. W. Albro, *J. Gas Chromatogr.*, **1**, 1 (1963).

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(5) A. M. Bryan and P. G. Olafsson, *Anal. Lett.*, **2**, 505 (1969).

(6) J. M. Patterson, M. L. Baedecker, R. Muscik, and W. T. Smith, Jr., *Tobacco*, **168**, 24 (1969).

(7) C. B. Honaker and A. D. Horton, *J. Gas Chromatogr.*, **3**, 396 (1965).

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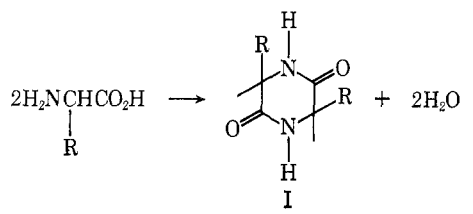
TABLE I

HCN YIELDS FROM α-AMINO ACIDS

Amino acid	Mol of HCN per mol of N (× 100), 1000°
Glycine	32
Alanine	12
Leucine	8
Isoleucine	8
Serine	45
Phenylalanine	43

stitution since functional groups are not thereby affected, while the last-mentioned reaction might be strongly affected if we can assume that bulky substituents might interfere with cyclization. If we can assume further



that the formation of these cyclic compounds represents the preferred route to HCN formation then two factors will be pertinent: (1) the tendency for ring formation to occur, and (2) the tendency, once formed, to give HCN. The observed order of HCN formation (glycine > alanine > leucine = isoleucine) would suggest that both factors are relevant. First, we suggest that, for this series, cyclization is easiest when R = H. Once formed, primary scission of I (R = H) will yield methylenimine (CH_2 = NH) or its diradical ($\dot{C}H_2\dot{N}H$). Dehydrogenation of either would afford HCN. In the cases where R = alkyl, similar cleavage of the ring would divert the reaction to alkyl cyanides which would require additional reaction in order that HCN be formed.

We were unable to put forth a reason for the high yields of HCN obtained from serine and phenylalanine. We feel, however, that these compounds deserve further study.

The suggestion that HCN formation was determined by the cyclic intermediates formed during the pyrolysis of the simple amino acids caused us to investigate various nitrogen heterocycles including the cyclic amino acids, proline and 4-hydroxyproline. Heterocycles were chosen so that structural requirements for HCN formation could be ascertained.

HCN Yields from Nitrogen Heterocycles.—The results of the pyrolysis of compounds containing ring nitrogen, including the amino acids proline and 4-hydroxyproline, at 700–1000° are given in Tables II and III. For all substances, HCN yields increased with increasing temperature. At all temperatures, pyrrolidine gave the highest yields of HCN, approaching 100% conversion at 1000°.

Structural influences upon HCN formation can be defined if we interpret the data in terms of pyrrolidine. In light of this compound we might note effects on yield of ring size, ring unsaturation, substitution on the ring nitrogen, and substitution adjacent to the ring nitrogen.

The influence of ring size can be gleaned by noting that the yields from 2-pyrrolidone, 2,5-piperazinedione,

TABLE II

HCN YIELDS FROM NITROGEN HETEROCYCLES

Compd	Mol of HCN per mol of N (× 100) 700°	800°	1000°
Proline			86
4-Hydroxyproline			90
2-Pyrrolidone		67	90
Pyrrolidine	46	77	95
Piperidine	33	47	62
3-Pyrroline	...[a]	26	72
Pyrrole	...[a]	22	87
Piperazine	34	69	80
2,5-Piperazinedione	38	50	81
2-Oxohexamethylenimine			71
N-Methylpyrrole			40
N-Methylpyrrolidine			35

[a] Not measurable.

TABLE III

NITRILE YIELDS AT 800°

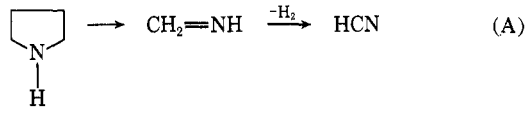
Compd	Mol of HCN per mol of N (× 100)	Relative yields of acetonitrile
Pyrrolidine	77	1
Succinimide	6	0.81
Succinamide	6	≪1
Piperidine	47	1
2,6-Dimethylpiperidine	18	8.1
Other compounds listed in Tables I–VII		None > 2

and 2-oxohexamethylenimine were 90, 81, and 71%, respectively. This evidence along with high yields obtained from pyrrolidine indicate that the five-membered ring is favored with respect to HCN formation.

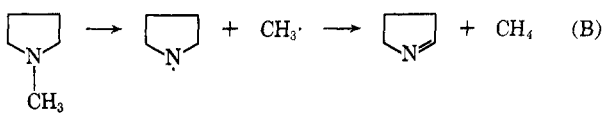
Further inspection of Table II gives an interesting picture of the role of ring stability in influencing the course of pyrolysis. Comparing pyrrolidine, 3-pyrroline, and pyrrole, it can be seen that unsaturation in the ring inhibits cleavage to HCN. Though this inhibition from the increased stability of the ring is somewhat overcome at 1000°, one can conclude that aromaticity of the ring containing nitrogen will be a factor which decreases HCN yields. Though no data are given here, this observation is in agreement with some we made previously concerning the pyrolysis of pyridine and chlorophyll, yields at 1000° being lower than those obtained at the same temperature in this study.

The substitution of methyl for the hydrogen attached to *nitrogen* is shown to greatly reduce HCN yield, cutting it by more than half of the value observed for the unsubstituted compounds (Table II).

This halving of the yields can be rationalized by considering the cleavage necessary to give HCN (eq A).



Applied to the *N*-methyl derivative, preferential cleavage of the NCH_3 bond would divert the reaction to other

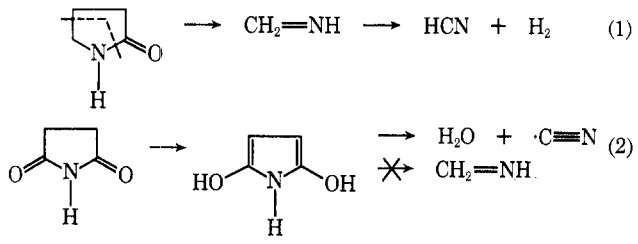


products (eq B). The relative strength of the NH bond would make cleavage according to eq B less likely.

Effects from substitution on carbon adjacent to the ring nitrogen are summarized in Table III.

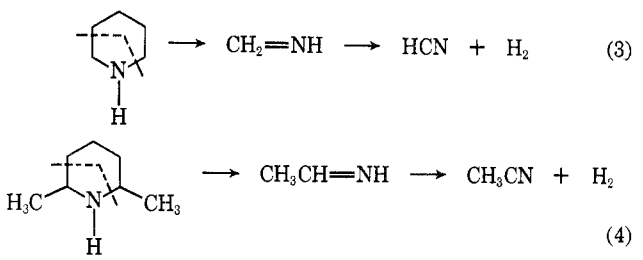
The presence of a single carbonyl group adjacent to the ring nitrogen seems to be of minor or no influence. Incidentally, the yields of HCN obtained from 2,5-piperazinedione are sufficiently high to support the contention that this species is responsible for HCN formation from glycine.

However, two adjacent carbonyls have a profound effect. Thus succinimide was found to give less HCN than any cyclic compound studied (Table III). It gave a yield similar to that observed for succinamide which can be expected to cyclize to succinimide with loss of ammonia. In any event, prevention of facile formation of methylenimine as an intermediate apparently caused more than a tenfold drop in yield. Reac-



tion 2 would require the breaking of a double bond to give a cyanide radical, a process not favored compared to reaction 1.

Diversion of the pyrolysis to another nitrile is illustrated by methyl substitution in the 2,6 positions of piperidine (Table III). The substituted piperidine yielded eight times the amount of acetonitrile as did piperidine. Thermal rupture according to the equations below would rationalize these data. Reaction 4



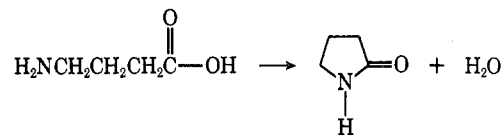
is consistent with the lower yields of HCN from alanine and leucine as opposed to glycine.

Pyrolysis of Isomeric Aminobutyric Acids.—Isomeric aminobutyric acids were pyrolyzed in order to ascertain the effects that amino group position might have an HCN formation and to determine whether this formation would follow the expected tendencies for cyclization to take place. The results are given in Table IV.

TABLE IV
HCN YIELDS FROM ISOMERIC AMINOBUTYRIC ACIDS

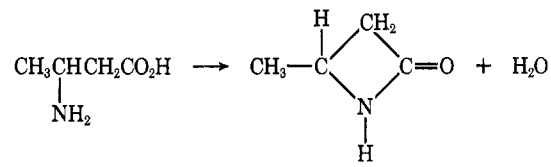
Amino acid	Mol of HCN per mol of N (× 100) 700°	800°	1000°
α-Aminobutyric acid	2	4	7
β-Aminobutyric acid	2	8	19
γ-Aminobutyric acid	30	67	77
α-Aminoisobutyric acid			7

The most striking features of these data are the high yields of HCN obtained from the γ acid. Notable, also, are the differences between the β and α acids. The unusually high yield from the γ acid can be attributed to the fact that a suitable pyrolysis intermediate (2-pyrrolidone) can be formed by intramolecular cycliza-



tion. The ability of the γ acid to cyclize by intramolecular reaction means that the necessary intermediate is generated easier than in the case of the α acid because the latter requires a bimolecular reaction. The competing reaction of deamination would then decrease the amount of nitrogen available for HCN formation.

The higher yield of the β as opposed to the α acid is suggestive of the idea that the β acid can react to some extent *via* a cyclic intermediate formed by an intramolecular reaction. Similarly, a 16% conversion to HCN



was obtained for β-alanine at 1000°, which is consistent with the 19% conversion obtained for β-aminobutyric acid (Table IV). To further check these points ammonia determinations were carried out. Table V gives

TABLE V
AMMONIA YIELDS FROM AMINOBUTYRIC ACIDS

Acid	Ammonia yields (peak heights), 1000°
α-Aminobutyric acid	1488
β-Aminobutyric acid	1280
γ-Aminobutyric acid	704

ammonia yields in terms of comparative peak heights. The order observed, namely $\alpha > \beta \gg \gamma$ is the inverse of that observed for HCN formation. The reactions proposed as being controlling in this study are consistent with the ammonia data.

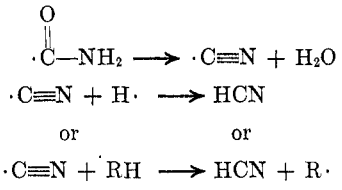
HCN Yields from Dicarboxylic Acids and Derivatives.—Further support of the idea of cyclization being a route favoring HCN production can be obtained from Table VI. The yield of HCN from aspartic

TABLE VI
HCN YIELDS FROM DICARBOXYLIC ACIDS AND DERIVATIVES

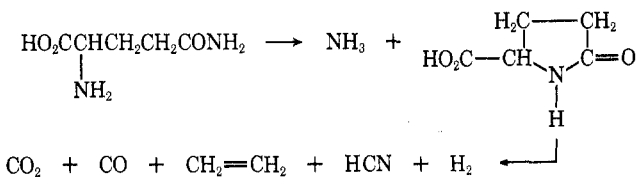
Amino acid	Mol of HCN per mol of N (× 100) 700°	1000°
Aspartic acid	0.7	35
Glutamic acid	14	71
Asparagine	2	21
Glutamine	10	46

acid at 1000° is closer to that observed for β-aminobutyric acid than it is to the value obtained from the α acid. Glutamic acid, on the other hand, pyrolyzes as if it were a γ acid. The substitution of a carboxyl group

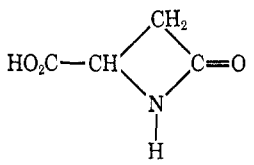
for a hydrogen on the amino bearing carbon had little effect on HCN formation. The substitution of an amide group for carboxyl in asparagine and glutamine is instructive also. If HCN were formed in appreciable quantities from



the yields obtained from these two amides should be similar. However, glutamine yields are significantly higher, 5:1 at 700°, and more than 2:1 at 1000°. The 46% conversion to HCN of glutamine at 1000° is indicative of a process in which more than 90% of the nitrogen in the pyrrolidone carboxylic acid intermediate is converted to HCN. Asparagine would require, of course,



a four-membered ring to give similar yields. Further-



more, considering yields from both asparagine and glutamine, it is clearly shown that HCN attributable to the amide group is produced in less quantities than that obtained from amino nitrogen. Once again, the overriding factor in HCN formation appears to be breakdown of a cyclic structure.

Pyrolysis of Amines.—The pyrolysis of C-4 amines at 800° proved to be instructive (Table VII). The order of HCN production observed for these amines was 1,4-diaminobutane > *n*-butylamine > *sec*-butylamine. The threefold increases in HCN yield of 1,4-diaminobutane over *n*-butylamine and its sixfold increase over *sec*-butylamine is consistent with the fact that the diamine, especially the dihydrochloride, can easily form pyrrolidine whereas the monoamines cannot. At 700°, 1,4-diaminobutane as the free base apparently did not cyclize with the efficiency of the hydrochloride (Table VII). The larger value observed for *n*-butylamine as opposed to the secondary amine is consistent with the expected greater ease of ammonia formation from the secondary amine. These observations are consistent with those made for amino acids.

TABLE VII
HCN YIELDS FROM AMINES

Amine	Mol of HCN per mol of N (× 100) 700°	800°
1,4-Diaminobutane dihydrochloride	20	46
1,4-Diaminobutane	10	42
n-Butylamine	10	14
sec-Butylamine	5	7

Registry No.—Glycine, 56-40-6; alanine, 56-41-7; leucine, 61-90-5; isoleucine, 73-32-5; serine, 56-45-1; phenylalanine, 63-91-2; proline, 147-85-3; 4-hydroxyproline, 51-35-4; 2-pyrrolidone, 616-45-5; pyrrolidine, 123-75-1; piperidine, 110-89-4; 3-pyrroline, 109-96-6; pyrrole, 109-97-7; piperazine, 110-85-0; 2,5-piperazinedione, 106-57-0; 2-oxohexamethylenimine, 105-60-2; *N*-methylpyrrole, 96-54-8; *N*-methylpyrrolidine, 120-94-5; succinimide, 123-56-8; succinamide, 110-14-5; 2,6-dimethylpiperidine, 504-03-0; α-aminobutyric acid, 80-60-4; β-aminobutyric acid, 541-48-0; γ-aminobutyric acid, 56-12-2; α-aminoisobutyric acid, 62-57-7; aspartic acid, 56-84-8; glutamic acid, 56-86-0; asparagine, 70-47-3; glutamine, 56-85-9; 1,4-diaminobutane dihydrochloride, 333-93-7; 1,4-diaminobutane, 110-60-1; *n*-butylamine, 109-73-9; *sec*-butylamine, 13952-84-6; HCN, 74-90-8.