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Thermal Decomposition of Lysine

Dennis J. Breitbart[1] and Wassef W. Nawar*

The thermal decomposition products of lysine after heating for 1 h at 250 °C were examined by gas chromatography and mass spectrometry. The lysine pyrolyzate contained 14 pyridine compounds, three lactams, two piperidines, two pyrroles, three amides, a tertiary amine, hexamethylenimine, and cyclohexanone. Mechanisms for the formation of these compounds are proposed.

The thermal decomposition of many amino acids has been studied. These amino acids include simple amino acids such as glycine, alanine, and valine (Simmonds et al., 1972; Lien and Nawar, 1974; Ratcliff et al., 1974), sulfur-containing amino acids (Fujimaki et al., 1969; Kato et al., 1973), aromatic amino acids (Kato et al., 1971), and hydroxyamino acids (Kato et al., 1970; Wang and Odell, 1973). No major study dealing with the thermal decomposition of lysine has been reported. Lysine is of particular interest for two reasons. First, it is the limiting amino acid in many proteins, and secondly, the ϵ-amine of lysine has been shown to be highly reactive to food systems.

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This investigation is part of a study of the thermal interaction of proteins and lipids in foods. The thermal decomposition of lysine was examined. The thermal interaction of lysine and two simple triglycerides will be reported in a later communication.

EXPERIMENTAL SECTION

Materials. L-Lysine and DL-norleucine, free base, were purchased from Sigma Chemical Co. (St. Louis, MO). Purity of the amino acids was confirmed by paper chromatography, cold-finger distillation, and gas chromatography.

Whenever possible, reference compounds and reagents were purchased commercially at the highest purity available.

Heat Treatment. One-gram samples of the amino acid were sealed under vacuum (1 μm) inside a Pyrex ampule (8 in. long by 1 in. o.d.). The sealed ampules were heated at 250 ± 10 °C in a muffle furnace (Thermolyne Corp., Dubuque, IA) for 1 h.

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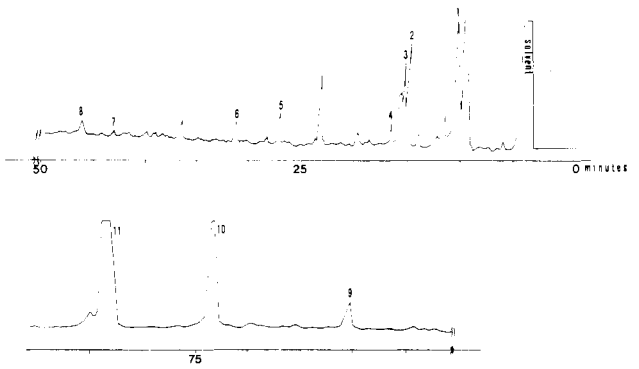


Figure 1. Gas chromatogram of heated lysine (ether extract), 500 ft × $^1/_{16}$ in. Carbowax 20 M capillary column, 70–170 °C at 4 °C/min.

Analytical Techniques. The heated amino acid samples were extracted with 20 mL of diethyl ether. The extract was concentrated at room temperature under a continuous stream of nitrogen gas prior to analysis. The concentrated ether extracts were separated by gas chromatography using an F & M Model 810 gas chromatograph equipped with temperature programming, sample splitter, and flame ionization detector. Two approaches were used to analyze the extracts. First, the compounds were trapped from a 6 ft × $^1/_8$ in. S.S. 28% Pennwalt 223 Amine + 4% KOH on 80/100 mesh Gas-Chrom R (Applied Science Laboratories, Inc., State College, PA) column and then rechromatographed on either a 6 ft × $^1/_8$ in. S.S. 10% Carbowax + 2% KOH on 80/100 mesh Chromosorb AW (Supelco, Inc., Bellefonte, PA) column or a 500 ft × $^1/_{16}$ in. S.S. Carbowax 20M capillary column.

The second approach was direct analysis of the total concentrated ether extract using the 500 ft × $^1/_{16}$ in. S.S. Carbowax 20M capillary column (Figure 1).

A 6 × $^1/_8$ in. precolumn packed with 10% Carbowax + 2% KOH on 80/100 mesh Chromosorb AW (Supelco, Inc.) was used in conjunction with the capillary Carbowax column to detect the presence of acids in the sample.

Identification of the isolated thermal decomposition products was accomplished by combined gas chromatography–mass spectrometry (GC–MS). The mass spectrum and gas chromatographic (GC) retention time of the unknown compounds and those of authentic compounds were compared. Identifications were classified as tentative when based solely on the interpretation of the mass spectra or by matching them with those reported in the literature.

Two GC–MS systems were used: (1) a Varian Aerograph Model 1200 gas chromatograph coupled to a Perkin-Elmer Hitachi Model RMU-GA mass spectrometer via a heated line and a Bieman separator (both maintained at 200 °C) and (2) a Varian Mat 111 GC–MS system. In both cases, the ion source was operated at 80 eV.

RESULTS AND DISCUSSION

Table I lists the lysine thermal decomposition products identified and the amounts of these compounds present when quantitation was possible. When the gas chromatograms obtained using the capillary Carbowax column and the capillary Carbowax column in conjunction with the Carbowax + KOH subtractive loop were compared, no acids could be identified.

As shown in Table I, 13 compounds were positively identified, and 14 compounds were tentatively identified. The majority of the thermal decomposition products contained nitrogen. Quantitation of the compounds identified in the heated lysine samples was extremely difficult. When compounds were isolated by only trapping, no attempts were made to quantitate them. Only those compounds which were well separated from other compounds in the sample were quantitated.

Table I. Decomposition Products from Lysine Heated at 250 °C for 1 h under Vacuum

decomp. products	peak (Fig. 1)	μM/ 50 g of Lys	identification GC	identification MS
piperidine	1	*a*	*b*	*c*
tetrahydropyridine	2	*a*		*d*
pyridine	3	*a*	*b*	*c*
2-methylpyridine	4	*a*	*b*	*c*
pyrrole	5	149	*b*	*c*
2-methyl-6-ethylpyridine	6			*e*
N-acetylpyrrolidine	7	79		*d*
2-aminopyridine	8	140	*b*	*c*
2-pyrrolidone	9	941		*d*
2-piperidone	10	2020	*b*	*c*
2-oxohexamethylenimine	11	3097	*b*	*c*
2-methylpiperidine		*a*	*f*	*c*
hexamethylenimine		*a*	*f*	*c*
N-methyl-*N*-propyliso-propenylamine		*a*		*g*
cyclohexanone		*a*	*f*	*c*
2-methylpyrrole		*a*		*d*
2,5-dimethylpyridine		*a*	*f*	*c*
2,4-dimethylpyridine		*a*	*f*	*c*
N-ethylacetamide		*a*		*h*
2-propylpyridine		*a*		*i*
3-ethylpyridine		*a*	*b*	*i*
pyridine compound M_r 135		*a*		*g*
pyridine compound M_r 135		*a*		*g*
N-butylacetamide		*a*		*h*
pyridine compound M_r 135		*a*		*g*
pyridine compound M_r 149		*a*		*g*
pyridine compound M_r 135		*a*		*g*

[a] No attempt was made to quantitate this compound due to overlapping or because it could be isolated only by repeated trapping. [b] Retention agreed with that of authentic compound on two columns; Carbowax-KOH and Pennwalt. [c] Agreed with MS of authentic compound. [d] Porter and Baldas (1971). [e] Cornu and Massot (1966). [f] Retention agreed with that of authentic compound on Pennwalt. [g] MS interpretation only. [h] Mass Spectrometry Data Centre (1970). [i] Benyon et al. (1968).

The collected decomposition products were examined for primary amines by GC–MS. None of these compounds were found. The more volatile products (e.g., NH_3, CO_2, etc.) were not collected under the experimental conditions used in this study. The formation of such compounds, however, from the heating of amino acids has been well documented (Ratcliff et al., 1974; Winter and Albro, 1964).

The thermal decomposition of lysine involves the same basic reactions as shown for other amino acids reported in the literature. These reactions include decarboxylation, decarbonylation, and deamination. Lysine, however, differs from these amino acids in that it has both an ε- and an α-amino group. Ratcliff et al. (1974) pyrolyzed a series of monoamino acids at 500 °C for 10 s in a helium atmosphere to determine the effect of the position of the amino group relative to the carboxylic acid group. Alanine, β-alanine, α-aminobutyric acid, β-aminobutyric acid, norvaline, 5-aminopentanoic acid, and 6-aminohexanoic acid were studied. It was concluded that decarboxylation was the major mode of thermal decomposition of α-amino acids, while deamination reactions predominated for β-amino acids. They also concluded that deamination of the amino acid became less important as the amino group moved farther away from the carboxylic acid group. This conclusion was based on the large amounts of carboxylic acids produced from the β-amino acids and significantly smaller amounts of carboxylic acids produced as the amino

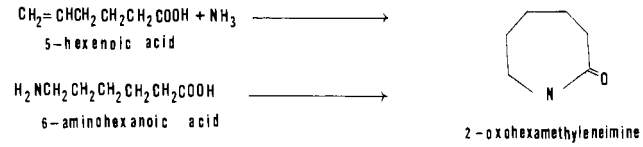
group was moved farther away from the carboxylic acid group. No carboxylic acids were produced by the pyrolysis of 6-aminohexanoic acid. α amino acids also do not produce carboxylic acids upon pyrolysis. In the case of 6-aminohexanoic acid, 2-oxohexamethylenimine was isolated along with a series of nitriles and amines. Ratcliff et al. (1974) predicted that the nitriles resulted via the lactam, 2-oxohexamethylenimine and that the amines occurred directly from the parent compound.

The lysine pyrolyzate in this study contained no detectable carboxylic acids or the 1,5-diaminopentane which was expected. The absence of these compounds suggest that the total deamination of lysine to form carboxylic acids and the decarboxylation of lysine to form 1,5-diaminopentane does not occur to any appreciable extent, or that if these compounds are formed they decompose or react readily with other compounds in the sample. Deamination of lysine to produce a series of amino acids cannot, however, be discounted. It is expected that deamination of the α-amine group occurs to form 6-aminohexanoic acid. In addition, deamination of the ϵ-amine group and cleavage of the hydrocarbon chain may also occur to form α-amino acids. From the data obtained, it is not possible to determine which amine group would be preferentially deaminated. Although α-deamination would seem most likely, the mass spectral fragmentation of lysine suggests the possible fragmentation of the hydrocarbon chain. According to Bieman and McCloskey (1962), the base peak in the mass spectrum of lysine is m/e 30, which corresponds to the cleavage of the C_4–C_5 bond to produce $^{+}NH_2{=}CH_2$. The lysine pyrolyzate was not analyzed for intermediate amino acids in this study. Several monoamino acids have been reported to produce amino acids upon pyrolysis; cystine produces alanine, isoleucine, and methionine; cysteine produces alanine, cystine, and isoleucine (Fujimaki et al., 1969); and phenylalanine produces glycine (Kato et al., 1971).

The thermal decomposition of the amino acid intermediates would be expected under the conditions used in this study. Furthermore, a closed system (the sealed ampule) allows recombination of all the possible intermediate amino acid decomposition products. The proposed intermediate amino acids produced by the thermal decomposition of lysine are glycine, alanine, 2-aminobutyric acid, norvaline, norleucine, and 6-aminohexanoic acid. Of these amino acids, only norleucine has not been previously studied. When norleucine was pyrolyzed under the same conditions as lysine, no lysine pyrolysis decomposition products were isolated. The thermal decomposition products produced by alanine, α-aminobutyric acid, norvaline, and 6-aminohexanoic acid were reported by Ratcliff et al. (1974), while Simmonds et al. (1972) reported the thermal decomposition products when alanine and glycine were pyrolyzed.

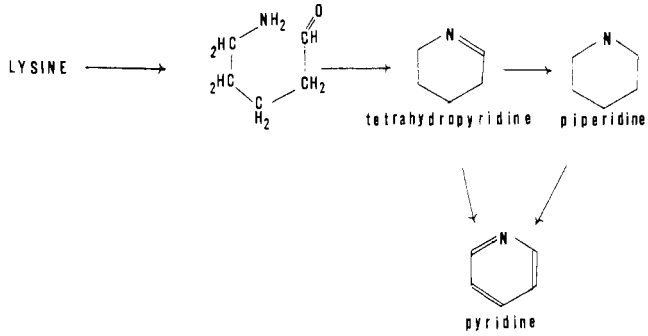
In addition to these potential intermediates, Johnson and Kang (1971) isolated hydrogen cyanide from lysine by pyrolyzing at 700 °C and Winter and Arbro (1964) produced ammonia, ethylamine, dimethylamine, tripropylamine, and tributylamine by pyrolyzing lysine at 300 °C.

Table I shows that the major thermal decomposition products produced from lysine are the lactams, 2-pyrrolidone, 2-piperidone, and 2-oxohexamethylenimine. The major component, 2-oxohexamethylenimine, has been previously isolated from the pyrolyzate of 6-aminohexanoic acid (Ratcliff et al., 1974). These authors, however, reported that 2-oxohexamethylenimine was not the major decomposition product. Therefore, another reaction pathway capable of producing 2-oxohexamethylenimine as well as the other lactams found must be present. One possible pathway would involve the reaction of unsaturated carboxylic acids with ammonia. The reaction of 3-butenoic acid, 4-pentenoic acid, and 5-hexenoic acid with ammonia should yield 2-pyrrolidone, 2-piperidone, and 2-oxohexamethylenimine, respectively. Alkenoic acids would

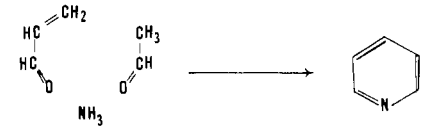


result from the deamination of lysine. As stated earlier, no carboxylic acids were isolated from the heated lysine sample. If acids were produced, these reactions may account for their absence.

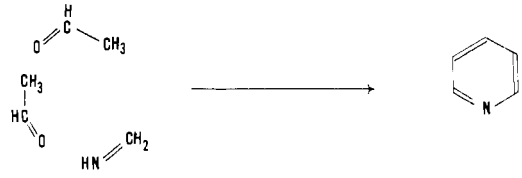
The reaction of aldehydes with ammonia and amines to form imines via dehydration has been reported by Sprung (1940) and Layer (1962). This reaction serves as the basis for the formation of many of the cyclic nitrogen compounds isolated. α deamination and decarbonylation of lysine would result in the formation of 5-aminopentanal. It can also be speculated that an intramolecular aldehyde–amine dehydration would produce tetrahydropyridine. Dehydrogenation may then occur to form pyridine and further hydrogenation would form piperidine.



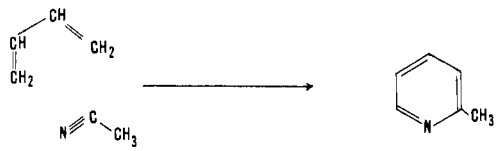
Intermolecular aldehyde–ammonia reactions of thermal decomposition products produced by the proposed intermediate amino acids can account for the presence of the cyclic nitrogen compounds identified. For example, the reaction of 2-propenal, acetaldehyde, and ammonia would yield pyridine (Chichibabin and Oparina, 1923).



It may be speculated that methylimine produced from lysine could also react as shown by the reaction of acetaldehyde and methylimine to form pyridine.

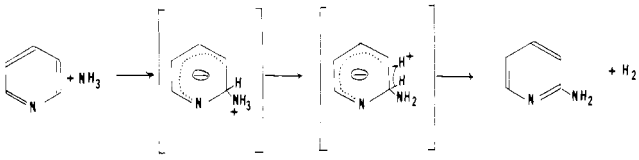


Another possible reaction pathway for the formation of cyclic nitrogen compounds would involve hydrogen cyanide or a cyanide compound and an alkene. Hawkins and Janz (1949) reported that low yields of pyridine were formed when 1,3-butadiene and hydrogen cyanide were heated at 400–600 °C without a catalyst. It was therefore speculated that the reaction of 1,3-butadiene and acetonitrile would form 2-methylpyridine. Similarly the reaction of 1,3-



butadiene and butyronitrile would produce 2-propylpyridine.

The formation of 2-aminopyridine differs from that of the alkylpyridines in that pyridine itself is one of the reactants. 2-Aminopyridine is formed by amination of pyridine in a Chichibabin-like reaction. Bergstrom (1937) predicted that ammonia or free aminopyridine could replace the monometal salt used in the Chichibabin reaction as an acceptor of the hydride ion which is liberated from the intermediate formed in the reaction. In the reaction proposed here, the proton liberated after addition of ammonia to the pyridine ring serves as the acceptor for the hydride ion in the final step of the reaction to form diatomic hydrogen and 2-aminopyridine.



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Formation of Monocarbonyl Compounds in Chicken Tissue

Kurt E. Moerck[1] and Hershell R. Ball, Jr.

Broiler adipose and thigh muscle tissue samples were aseptically obtained, ground, and then stored for 3 days at 22 °C or 7 days at 4 °C. Hexane-extracted carbonyl compounds were converted to their 2,4-dinitrophenylhydrazone derivatives and identified. Acetone was the only monocarbonyl present in fresh tissue samples. There was an increase in the concentration of all classes of aliphatic monocarbonyl compounds in stored tissue samples. More oxidation occurred in stored ground thigh muscle than in stored ground adipose tissue samples. 2-Pentanone was the only methyl ketone formed during storage. C_2–C_{10} alkanals were present in all stored samples. Hexanal and acetaldehyde were present in the highest concentrations. C_6–C_{11} 2-alkenals were isolated from ground thigh muscle samples and C_7–C_{10} 2-alkenals were identified in adipose tissue samples. 2-Nonenal was the predominant 2-alkenal in all samples. 2,4-Alkadienals were composed primarily of heptadienal, nonadienal, and decadienal, with decadienal being the predominant 2,4-alkadienal in all samples.

Lipid oxidation is a major cause of deterioration in the quality of stored poultry and poultry products. This oxidative process involves the reaction of unsaturated fatty acids with molecular oxygen to yield hydroperoxides, which in turn decompose to yield flavorable compounds (Holman, 1954; Lundberg and Jarvi, 1968; Labuza, 1971; Forss, 1972). Many of these flavor and odor compounds are aliphatic monocarbonyl compounds, namely methyl ketones, alkanals, 2-alkenals, and 2,4-alkadienals (Ellis et al., 1961; Gaddis et al., 1961; Badings, 1970). Hoffmann (1961), Smouse and Chang (1967), and others have demonstrated that flavor defects which occur because of autoxidative deterioration are many times due to monocarbonyl compounds which are formed from polyenoic fatty acids. These secondary products are responsible for a wide range of oxidized flavors and odors in chicken tissues (Dimick and MacNeil, 1970; MacNeil and Dimick, 1970; Dimick et al., 1972; Golovkin and Galkin, 1975). Aliphatic monocarbonyl compounds are important flavor and odor sources in poultry and other lipid-containing foods because of their low flavor threshold values (Forss et al., 1962; Meijboom, 1964; Badings, 1970; Siek et al., 1971).

The purpose of this investigation was to isolate and identify the aliphatic monocarbonyl compounds formed

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