Table VI. Partition Coefficients of Acetates in Oil, at Different Temperatures, $K_{AO} \times 10^4$

acetates	25 °C	30 °C	35 °C	50 °C
		Coffee Oil		
butyl	3.11		5.30	10.68
pentyl	1.10		2.20	5.22
hexyl	0.39	0.59	0.89	
isopentyl	1.98	2.70	3.59	8.09
		Peanut Oil		
isopentyl	2.01		3.71	8.42
		Mineral Oil		
isopentyl	3.50		6.33	13.2

also kept refrigerated. Results are given in Table VI.

The activity coefficients of the acetates in coffee oil, assuming a molecular weight of 885.4 which corresponds to triolein, are about 0.7. This indicates a strong affinity between the host and solute molecules. Similar behavior was also found for aldehydes by Buttery et al. (1973). Mineral oil shows less affinity for the ester since it is a mixture of hydrocarbons.

Partition coefficients between oil and an aqueous phase for the acetates can be computed as $K_{OL} = K_{AL}/K_{AO}$.

LITERATURE CITED

Buttery, R. G., Ling, L. C., Guadagni, D. G., *J. Agric. Food Chem.* **17**, 385 (1969).
Buttery, R. G., Bomben, J. L., Guadagni, D. G., Ling, L. C., *J. Agric. Food Chem.* **19**, 1045 (1971).
Buttery, R. G., Guadagni, D. G., Ling, L. C., *J. Agric. Food Chem.* **21**, 198 (1973).
Chandrasekaran, S. K., King, C. J., *AIChE J.* **18**, 513 (1972).
Glasstone, S., Pound, A., *J. Chem. Soc.* **127**, 2660 (1925).
Kieckbusch, T. G., PhD Dissertation, University of California, Berkeley, 1978.
Kieckbusch, T. G., King, C. J., *J. Chromatogr. Sci.*, in press (1979).
Maier, H. G., *Angew. Chem. Int. Ed. Engl.* **9**, 917 (1970).
Massaldi, H. A., King, C. J., *J. Chem. Eng. Data* **18**, 393 (1973).
Massaldi, H. A. King, C. J., *J. Food Sci.* **39**, 434 (1974).
Nawar, W. W., *J. Agric. Food Chem.* **19**, 1057 (1971).
Radford, T., Kawashima, K., Friedel, P. K., Pope, L. E., Gianturco, M. A., *J. Agric. Food Chem.* **22**, 1066 (1974).
Schliephake, D., *Zucker* **16**, 523 (1963).

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Racemization of Amino Acids in Alkali-Treated Food Proteins

Patricia M. Masters and Mendel Friedman*

Casein, lactalbumin, Promine-D (soy protein), and wheat gluten exhibit significant racemization of aspartic and glutamic acids (or their amides), phenylalanine, and alanine when subjected to 0.1 N NaOH at 65 °C for 3 h. In order of lability to racemization, the proteins are Promine-D > casein > wheat gluten > lactalbumin. While racemization rates of individual amino acids vary among proteins, in any given protein, the relative rates are similar. The pH kinetics of aspartic acid racemization in casein indicate that k_{asp} is first order with respect to hydroxide concentration above pH 10. Racemization can impair nutritional values of food proteins by decreasing the amounts of the essential amino acid L enantiomers present, by decreasing digestibility, and as a result of specific toxicity of certain D enantiomers.

Commercial processing of food proteins often entails heating in alkaline solutions. Such treatments are intended to alter flavor and texture, destroy microorganisms, enzymes, toxins, or proteolytic inhibitors, and prepare protein concentrates. Undesirable changes also occur in the amino acid composition of proteins under such processing conditions. For instance, amino acid cross-linking (Provansal et al., 1975; Friedman, 1977, 1978; Hurrell and Carpenter, 1977), degradation (Asquith and Otterburn, 1977), and racemization (Provansal et al., 1975; Tannenbaum et al., 1970) have been reported.

The ability of strong alkali to racemize amino acids has been demonstrated in early chemical studies on proteins (Dakin, 1912–1913; Levene and Bass, 1927, 1928, 1929). The reaction is thought to proceed by abstraction of the α proton from an amino acid or amino acid residue in a peptide or protein to give a negatively charged planar carbanion (Neuberger, 1948). A proton can then be added back to either side of this optically inactive intermediate, thus regenerating the L form or producing the D enantiomer. The reaction can be written as

$$\text{L-amino acid} \underset{k'}{\overset{k}{\rightleftharpoons}} \text{D-amino acid} \qquad (1)$$

where k and k' are the first-order rate constants for interconversion of the L and D enantiomers. For the amino acids discussed in this paper, $k = k'$. (Amino acids with two asymmetric centers exhibit k' slightly different from k.) Only L-amino acids are initially present in most proteins due to the stereospecificity of biosynthesis.

Provansal et al. (1975) have studied lysine racemization in sunflower protein under varying conditions of heat and hydroxide concentration. Enzymatic enantiomeric analysis disclosed measurable amounts of D-lysine in solutions in NaOH more concentrated than 0.2 N NaOH heated at 80 °C. Using microbiological assays, Tannenbaum et al. (1970) found methionine to be nearly completely racemized in fish protein concentrate heated 20 min at 95 °C in 0.2 N NaOH.

The present study uses quantitative chromatographic methods to measure the extent of racemization of seven amino acid residues from proteins subjected to relatively mild alkaline treatments. We have determined D/L en-

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Table I. Enantiomeric Ratios in Hydroxide-Treated and Untreated Proteins

treated proteins	time, h	D/L Asp	D/L Ala	D/L Val	D/L Leu	D/L Pro	D/L Glu	D/L Phe
casein	1	0.279	0.044	0.028	0.053	0.031	0.111	0.191
	3	0.432	0.154	0.065	0.075	0.056	0.210	0.286
	8	0.489	0.241	0.079	0.157	0.040	0.350	0.439
Promine-D	3	0.431	0.187	0.071	0.087	0.061	0.232	0.331
wheat gluten	3	0.409	0.156	0.040	0.059	0.033	0.349	0.304
lactalbumin	3	0.293	0.101	0.050	0.061	0.037	0.139	0.198
controls								
casein	0	0.022	0.023	0.021	0.023	0.033	0.018	0.029
Promine-D	0	0.023	0.021	0.027	0.034	0.033	0.018	0.023
wheat gluten	0	0.034	0.020	0.021	0.018	0.033	0.021	0.024
lactalbumin	0	0.032	0.022	0.030	0.028	0.032	0.030	0.023

antiomeric ratios for aspartic and glutamic acids (partly from their amides), alanine, valine, leucine, proline, and phenylalanine from casein, soy protein, wheat gluten, and lactalbumin.

MATERIALS AND METHODS

Commercial casein, lactalbumin, and wheat gluten were purchased from Nutritional Biochemicals Corporation, Cleveland, OH. Soy protein (Promine-D) was donated by Central Soya, Chicago, IL.

Alkali Treatment. A 1% solution of each protein in 0.1 N NaOH (pH ~ 12.5) was placed in a glass-stoppered Erlenmeyer flask and incubated at 65 °C in a water bath. The final pH did not significantly differ from the initial value. After 3 h, the sample was dialyzed against 0.01 N acetic acid for approximately 2 days and lyophilized. Control protein samples were dialyzed and lyophilized similarly (they were not heated).

Enantiomeric Analyses. Approximately 30 mg of protein was hydrolyzed in 12 mL of doubly distilled 6 M HCl for 24 h at 96 °C. The HCl solution was then brought to dryness at 50 °C under partial vacuum in a rotatory evaporator. The residue was redissolved in doubly distilled water and desalted on Dowex 50W-X8 (100–200 mesh) resin that had been cleaned with NaOH and protonated with doubly distilled HCl (Bada and Protsch, 1973). Amino acids were eluted from the column with 1.5 M NH_4OH prepared by bubbling NH_3 through doubly distilled water. The effluent was again evaporated to dryness. Aspartic acid was separated by chromatography on a calibrated column of Bio-Rad AG1-X8, 100–200 mesh, anion-exchange resin (Hirs et al., 1954). The resin was regenerated with four column volumes of 1 M sodium acetate, followed by two column volumes of 1 M acetic acid. Half of the sample was applied to the column; elution was carried out with 1 M acetic acid. The aspartic acid fraction was evaporated in a rotatory evaporator. The L-leucyl-DL-aspartic acid dipeptides were synthesized by the procedure of Manning and Moore (1968). The D/L aspartic acid ratio was determined with a Beckman Model 118 automatic amino acid analyzer.

D/L enantiomeric ratios for alanine, valine, glutamic acid, leucine, proline, and phenylalanine in the remaining half of the desalted amino acid fraction were obtained by gas chromatography (Hoopes et al., 1978). The *N*-trifluoroacetyl-L-prolyl-DL-amino acid esters were synthesized and then separated on a Hewlett-Packard Model 5711A gas chromatograph equipped with a flame ionization detector and a 12-ft column of Chromasorb W-AW-DMCS solid support coated with 8% SP 2250.

Lysinoalanine content was determined by ion-exchange chromatography (Friedman, 1978).

RESULTS AND DISCUSSION

D/L enantiomeric ratios for seven amino acid residues are given in Table I. Extensive racemization of aspartic acid, phenylalanine, glutamic acid, and alanine is seen when the four proteins are treated with hydroxide. Valine, leucine, and proline are much less racemized.

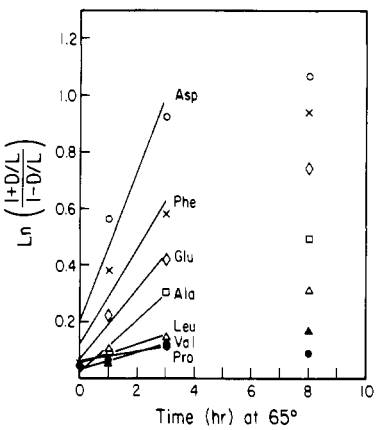

Figure 1. Time course of amino acid racemization reactions of casein in 0.1 N NaOH at 65 °C. The straight lines are constructed from the least-squares fit to the 0-, 1-, and 3-h points. (○) aspartic acid, (×) phenylalanine, (◇) glutamic acid, (□) alanine, (△) leucine, (▲) valine, (●) proline.

The percentage of D enantiomers relative to the total amount of the amino acid residue can be calculated by (D/D + L) × 100. D-Aspartic acid accounts for 30% of that residue (which is thus 60% racemized) in treated casein, Promine-D, and wheat gluten. In these three proteins, 22–25% of the phenylalanine (an essential amino acid) is the D enantiomer, and in wheat gluten, 26% of glutamic acid has been converted to the D form.

The small amounts of D enantiomers in the controls may be attributed to: (1) racemization occurring in the commercial preparation of the proteins, (2) acid-catalyzed racemization during the hydrolysis step of our analysis, or both factors.

It is apparent from Table I that racemization rates differ among these seven amino acids. In order to compare the results from the four proteins, rate constants were estimated from the data in Table I. For casein, the D/L ratios were measured at 0, 1, 3, and 8 h. Linear first-order kinetics were observed for all seven amino acids over the first 3 h of the reaction time as shown in Figure 1. First-order rate constants were calculated from the least-squares fit to the first three data points. On the assumption that the other proteins would also exhibit linear kinetics over the 3-h time course, apparent rate constants were calculated from the 0- and 3-hour points using the equation

$$\ln\left[\frac{1 + \text{D/L}}{1 - K'(\text{D/L})}\right]_t - \ln\left[\frac{1 + \text{D/L}}{1 - K'(\text{D/L})}\right]_{t=0} = (1 + K')kt \quad (2)$$

where $K' = 1/K_{eq}$ and K_{eq} is the D/L ratio at equilibrium

Table II. Relative Order of Apparent Racemization Rates[a] in Proteins Treated with 0.1 N NaOH at 65 °C

	k_{Leu}	k_{Val}	k_{Pro}	k_{Ala}	k_{Glu}	k_{Phe}	k_{Asp}
casein	1.0	0.9	0.5	2.6	3.7	4.9	7.8
Promine-D	1.0	0.8	0.5	3.1	4.1	5.9	8.1
wheat gluten	1.0	0.4	0	3.3	8.2	6.9	9.6
lactalbumin	1.0	0.6	0.2	2.5	3.4	5.4	8.2

[a] See text for derivation of rate constants.

[see Bada and Schroeder (1972) for derivation]. Theoretically, $K_{eq} = 1$ for amino acids having one asymmetric center, but the results plotted in Figure 1 indicate that $K_{eq} < 1$ for at least four of the residues. [A value of $K_{eq} < 1$ also has been reported for isoleucine in fossil mollusk shell protein; Masters and Bada (1977).] The rates within each protein were then standardized relative to that of leucine. The order of relative racemization rates is presented in Table II. Relative rates are very similar among the various proteins, with the exception of aspartic and glutamic acids in wheat gluten. This situation is discussed below.

The results in Table II can be compared with information on free amino acids. Bada (1972) has shown that for free amino acids at neutral pH, k(Ile):k(Val):k(Ala):k(Phe):k(Asp) = 1.0:0.8:2.4:4.4:8.6. Bada has pointed out that these rates agree with the order predicted from σ^* values of the respective R groups (Charton, 1964). The R groups that have the greater electron-withdrawing or resonance-stabilizing characteristics will induce faster racemization. Our results are compatible with the free amino acid data. (We have used k(Leu) rather than k(Ile) as the base of comparison, but the σ^* values for these R groups are similar.) This agreement indicates that the nature of the R group is the prime determinant of relative racemization rates in protein-bound as well as free amino acids.

It is interesting that even at pH ~12.5, the relative order of racemization rates in bound amino acids appear very similar to those of free amino acids at neutral pH. Since both the NH_2 and COOH groups are involved in peptide bonds, their ionic forms are no longer relevant in the reaction mechanism. The R substituents remain as the primary influence on relative racemization rates in polypeptides. At neutral and basic pH, the predominant ionic form of the β-carboxyl group of aspartic acid (and of the γ-carboxyl of glutamic acid) is COO^-. Although more electronegative than the undissociated carboxyl group, these ionized carboxyl groups still have greater electron-withdrawing capacity than do the alkyl groups of alanine, leucine, and valine. Phenylalanine, with an R group σ^* close to that of the β-carboxylate, may have a slower racemization rate relative to aspartic acid due to steric limitations on the formation of the planar carbanion intermediate. The protein-bound amino acid proline would be even more limited sterically.

The correlation between racemization rates in free amino acids and the σ^* values also supports the carbanion-intermediate mechanism of racemization (Bada, 1972). The R group can act to stabilize the negative charge on the α carbon so that the carbanion intermediate is more stable. Since the σ^* values also agree with the racemization rates observed in the present study, the same mechanism probably operates with protein-bound amino acids.

Where a relative rate differs widely from the observed pattern, as is the case of glutamic acid in wheat gluten, the apparent rate enhancement may be the result of the very high proportion of glutamine in the protein (Kasarda et al., 1976). The δ-amide group should increase the inductive character of the R substituent so that glutamine should racemize faster than glutamic acid. Because glutamine (and asparagine) are probably deamidated to some extent during the alkali treatment and completely during acid hydrolysis of the proteins, our D-glutamic acid values actually represent the sum of both D-amino acid enantiomers. This circumstance may explain the relative rate difference for wheat gluten if deamidation during hydroxide treatment is slower than racemization under our experimental conditions.

Deamidation is sequence dependent with half-times ranging from 18 to 507 days for asparaginyl peptides and 96 to 3409 days for glutaminyl residues at 37 °C, pH 7.4 (Robinson et al., 1973). The pH dependence of the deamidation rates was studied in phosphate buffer (Scotchler and Robinson, 1974). Extrapolation of the curve for one glutaminyl peptide to pH 12 (approximating the conditions used in our study) results in a $k_{deam} \simeq 2x$ that of the expected k_{rac} of glutamic acid. Since the majority of the 24 sequences studied (Robinson et al., 1973) deamidate more slowly than this peptide, it seems probable that most glutamines will remain intact during the 3-h time course of our treatments.

Although relative racemization rates of amino acids are usually similar, the overall lability of these four proteins to racemization by hydroxide differs considerably. In Table III, the proteins are ranked by the extent of racemization of each of the four most racemized amino acids. Promine-D is the most highly racemized protein for three of these amino acids. Lactalbumin has the lowest D/L ratios for all. These findings imply that there are protein-specific rates of racemization underlying the general uniformity of relative rates discussed above. Similar observations have been reported for diagenetic racemization rates in different fossil proteins (Masters and Bada, 1977; Miller and Hare, 1975; King and Neville, 1977). Variability among these food proteins in response to alkali treatment demonstrates that mild processing conditions for one protein (e.g., lactalbumin) constitute rather severe conditions for another (e.g., Promine-D).

The pH dependence of the racemization rate of aspartic acid in casein was also investigated. The results are plotted in Figure 2. Racemization rates are estimated from the log conversion of the D/L ratios (see caption to Figure 2). The pH of the NaOH buffer at 65 °C was calculated from the temperature variation of the pK_w of water (Robinson and Stokes, 1959). The pH values of the borate buffers at 65 °C were calculated using the temperature data of Bates (1964). The solid line represents rates which are first order with respect to hydroxide concentration. This line is a reasonable fit to the data points above pH 10. Further experiments are in progress with other proteins in order to identify the lowest OH^- concentrations that induce first-order racemization kinetics. If the critical base concentrations for racemization correspond with the different responses of the four proteins (see Table I and III), one may expect k(rac) for Promine-D to become first

Table III. Relative Order of the Proteins with Respect to Extent of Racemization of Each Amino Acid

Asp	Promine-D	=	casein	>	wheat gluten	>	lactalbumin
Ala	Promine-D	>	casein	=	wheat gluten	>	lactalbumin
Glu	wheat gluten	>	Promine-D	>	casein	>	lactalbumin
Phe	Promine-D	>	wheat gluten	>	casein	>	lactalbumin

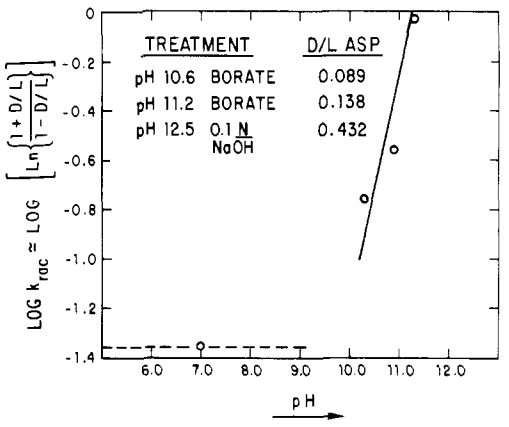

Figure 2. The pH dependence of aspartic acid racemization in casein. The initial pH values of the buffers are listed on the graph. The D/L ratios are plotted as a function of the actual pH values calculated by methods explained in the text. The expression log $k_{rac} \simeq$ log [ln (1 + D/L/1 - D/L)] is derived from eq 2. Under our experimental conditions, the $t = 0$ term, K', and t are all constants and eq 2 can be reduced to this one ln term when solving for k.

order at lower OH^- concentration than casein, while that of wheat gluten should be about the same as for casein. Lactalbumin may have the highest OH^- concentration tolerance.

Our findings imply that nutritional value may be reduced in food proteins processed under the conditions we have used here. A D-amino acid residue may be less readily metabolized than its L enantiomer (Berg, 1959; Kies et al., 1975). Of the three essential amino acids determined, significant racemization occurred in phenylalanine and trace amounts in leucine and valine. The L-phenylalanine content of wheat gluten, Promine-D, and casein is reduced 20–23% by racemization.

Another result that seems noteworthy in this work is that the control samples show 2–3% of the D form. Two or three percent conversion to D forms is not very important unless these products are specifically toxic.

While phenylalanine is not usually the limiting essential amino acid in foods, methionine frequently is in legume proteins (Bozzini and Silano, 1978). Because the inductive properties of methionine and phenylalanine are similar (Charton, 1964), the two amino acids should racemize at similar (fast) rates.

A second way in which racemization may lower the food value of alkali-treated proteins is by restricting the availability of essential amino acids by inhibiting digestion. The normal proteolytic enzymes are unable to break a peptide bond involving D-amino acid residues (Berg, 1959). Provansal et al. (1975) were able to show decreased pronase proteolysis of alkali-treated sunflower proteins. Though they did not measure D-amino acids other than lysine, they suggested that racemization of additional amino acids may have reduced the amount of essential amino acids released by proteolysis in vitro. Our results confirm that other amino acid residues are racemized and more rapidly than lysine. Thus, racemization of a residue next to an essential amino acid may inhibit its release as a free amino acid during digestion in vivo.

If one assumes that significant quantities of D enantiomers are released from treated proteins, their biological utilization depends on absorption and conversion to a metabolizable form. Uptake is slower for D-amino acids than L-amino acids in the intestine (Gibson and Wiseman, 1951) and kidney (Rosenhagen and Segal, 1974). If absorbed, D-amino acid oxidases present in the liver and kidney of most mammals can catalyze oxidative deamination to an α-keto acid (Meister, 1965). This may be followed by L-specific reamination. However, Wretlind (1952) has observed that simultaneously feeding several different D-amino acids may overload the oxidase system since D-essential amino acids are apparently not transaminated in sufficient quantities to support growth in rats. Consequently, too high D-enantiomeric content of nonessential amino acids is expected to inhibit interconversion of essential amino acids.

Other reactions such as cross-linking also occur during alkali treatment. Lysinoalanine formation, for example, can also impair digestibility. The occurrence of D-amino acids in alkali-treated proteins suggests that they may contribute to their biological action, now ascribed in part to lysinoalanine. Lysinoalanine contents of the four proteins of the present study treated with 0.1 N NaOH for 3 h at 65 °C are (in mol %): wheat gluten, 0.762; soy protein, 1.35; casein, 2.43; and lactalbumin, 2.62. Gould and MacGregor (1977) report that the rat kidney lesion induced by feeding alkali-treated soy protein is much more severe than observed after feeding alkali-treated lactalbumin, even though the lysinoalanine content of the latter is about twice that of soy protein. This puzzling observation may be due to the fact that amino acid residues in lactalbumin (including lysinoalanine) are racemized less readily than those in the soy protein treated under the same conditions. The greatest biological effect of alkali-treated soy protein compared to lactalbumin may therefore arise from a greater synergistic action with lysinoalanine of certain D-amino acid and/or dehydroalanine residues and/or from differences in diastereoisomeric composition of lysinoalanine in the two proteins. (Lysinoalanine has two optically active centers susceptible to racemization and serine and threonine can racemize via dehydroalanine and methyldehydroalanine intermediates; Friedman, 1977, 1978).

For those concerned with the nutritional value of food proteins processed with alkali and heat, a single measure of racemization would be useful. Even for the same treatment conditions, one cannot yet infer from one protein the susceptibility of another to racemization. Because of their often low concentrations and difficulty of analysis, reliable rate data for the essential amino acids relative to aspartic acid or alanine would be especially helpful. Then determination of the D/L aspartic acid or D/L alanine ratio would serve to indicate the amount of racemization of the essential amino acids. We are in the process of expanding this comparative rate study to the remaining essential amino acids.

The metabolic importance of racemization, aside from loss of essential amino acids, is emphasized by a recent report on the analgesic activity of D-phenylalanine (Chemical Week, 1978), which is suggested to act by inhibiting an enzyme responsible for destroying the natural opiate-like enkephalins in the brain. Since our study shows that phenylalanine is highly susceptible to racemization under alkaline conditions, the possible release of D-phenylalanine in vivo from racemized food proteins in sufficient amounts to exert a pain-killing effect is obviously of interest.

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LITERATURE CITED

Asquith, R. S., Otterburn, M. S., in "Protein Crosslinking: Nutritional and Medical Consequences", Friedman, M., Ed., Plenum Press, New York, 1977, pp 93–121.

Bada, J. L., *J. Am. Chem. Soc.* **95**, 1371–1373 (1972).
Bada, J. L., Protsh, R., *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1331–1334 (1973).
Bada, J. L., Schroeder, R. A., *Earth Planet. Sci. Lett.* **15**, 1–11 (1972).
Bates, R. G., "Determination of pH: Theory and Practice", Wiley, New York, 1964, p 76.
Berg, C. P., in "Protein and Amino Acid Nutrition", Albanese, A. A., Ed., Academic Press, New York, 1959, pp 57–96.
Bozzini, A., Silano, V., in "Nutritional Improvement of Food and Feed Proteins", Friedman, M., Ed., Plenum Press, New York, 1978, pp 249–274.
Charton, M., *J. Org. Chem.* **29**, 1222–1227 (1964).
Chem. Week 47–48 (Oct 4, 1978).
Dakin, H. D., *J. Biol. Chem.* **13**, 357–362 (1912–1913).
Friedman, M., in "Protein Crosslinking: Nutritional and Medical Consequences", Friedman, M., Ed., Plenum Press, New York, 1977, pp 1–27.
Friedman, M., in "Nutritional Improvement of Food and Feed Proteins", Friedman, M., Ed., Plenum Press, New York, 1978, pp 613–648.
Gibson, Q. H., Wiseman, G., *Biochem. J.* **48**, 426–429 (1951).
Gould, D. H., MacGregor, J. T., in "Protein Crosslinking: Nutritional and Medical Consequences", Friedman, M., Ed., Plenum Press, New York, 1977, pp 29–48.
Hirs, C. H. W., Moore, S., Stein, W. H., *J. Biol. Chem.* **76**(4), 6063–6065 (1954).
Hoopes, E. A., Peltzer, E. T., Bada, J. L., *J. Chromatogr. Sci.* **16**, 556–560 (1978).
Hurrell, F. R., Carpenter, K. J., in "Protein Crosslinking: Nutritional and Medical Consequences", Friedman, M., Ed., Plenum Press, New York, 1977, pp 225–238.
Kasarda, D. D., Bernardin, J. S., Nimmo, C. C., *Adv. Cereal Sci. Technol.* 158–236 (1976).
Kies, C., Fox, H., Aprahamian, S., *J. Nutr.* **105**, 809–814 (1975).
King, K., Jr., Neville, C., *Science* **195**, 1333–1335 (1977).
Levene, P. A., Bass, L. W., *J. Biol. Chem.* **74**, 715–525 (1927).
Levene, P. A., Bass, L. W., *J. Biol. Chem.* **78**, 145–157 (1928).
Levene, P. A., Bass, L. W., *J. Biol. Chem.* **82**, 171–190 (1929).
Manning, J. M., Moore, S., *J. Biol. Chem.* **243**, 5591–5597 (1968).
Masters, P. M., Bada, J. L., *Earth Planet. Sci. Lett.* **37**, 173–183 (1977).
Meister, A., "Biochemistry of the Amino Acids", Vol. I, Academic Press, New York, 1965, pp 338–369.
Miller, G. H., Hare, P. E., *Carnegie Inst. Washington Yearbook* **74**, 612–617 (1975).
Neuberger, A., *Adv. Protein Chem.* **4**, 298–383 (1948).
Provansal, M. M. P., Cuq, J. -L. A., Cheftel, J-C., *J. Agric. Food Chem.* **23**, 938–943 (1975).
Robinson, A. B., Scotchler, J. W., McKerrow, J. H., *J. Am. Chem. Soc.* **95**, 8156–8159 (1973).
Robinson, R. A., Stokes, R. H., "Electrolyte Solutions", Butterworths, London, 1959, pp 336–369.
Rosenhagen, M., Segal, S., *Am. J. Physiol.* **227**, 843–847 (1974)
Scotchler, J. W., Robinson, A. B., *Anal. Biochem.* **59**, 319–322 (1974).
Tannenbaum, S. R., Ahern, M., Bates, R. P., *Food Technol.* **24**, 96–99 (1970).
Wretlind, K. A. J., *Acta Physiol. Scand.* **25**, 267–275 (1952).

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Thermal Decomposition of Lysine

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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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