Pyrazine Formation from Amino Acids and Reducing Sugars, a Pathway Other than Strecker Degradation

Chi-Kuen Shu

Bowman Gray Technical Center, R. J. Reynolds Tobacco Company, Winston-Salem, North Carolina 27105

Because the reaction of acyloins with ammonia generates pyrazines and acyloins are not involved with Strecker degradation, this study uses acetoin (a simple acyloin, from sugar degradation) to react with amino acids to determine whether ammonia is released, leading to the formation of tetramethylpyrazine. The results show that all reactions between α-amino acids and acetoin generate not only tetramethylpyrazine but also the corresponding Strecker aldehydes. However, the reactions between *β*-, *γ*-, or *ϵ*-amino acid and acetoin generate only tetramethylpyrazine. Quantitative analysis shows that among such reactions, both α- and *β*-amino acids generate significantly higher amounts of tetramethylpyrazine than *γ*- and *ϵ*-amino acids. On the basis of these data, it is proposed mechanistically that α-amino acids decarbonylate to generate the reactive intermediates, 1-hydroxyamines, deamination of which leads to the formation of the Strecker aldehydes. This study demonstrates that pyrazines and Strecker aldehydes are also formed from α-amino acids and reducing sugar via deamination in addition to their formation via the well-known Strecker degradation.

Keywords: *Strecker degradation; pyrazine formation; deamination; decarbonylation; acyloin; α-amino acid; β-amino acid; γ-amino acid; ϵ-amino acid; Strecker aldehyde*

INTRODUCTION

Pyrazines are generally considered as important flavor components in foods and have been identified as components of various food products (Maga, 1992). Several mechanisms have been proposed for the formation of pyrazines (Vernin and Parkanyi, 1982; Heath and Reineccius, 1986). The most accepted mechanism for pyrazine formation from α-amino acid and reducing sugar is based on the Maillard reaction and Strecker degradation. The reaction of α-amino acids and reducing sugars initially generates Amadori/Heyns compounds, rearrangement of which leads to the formation of reductones including α-dicarbonyls, and then Strecker degradation converts the α-dicarbonyls into α-aminocarbonyls, which in turn are condensed to pyrazines. Because sugar degradation also provides α-dicarbonyls, pyrazines can also be formed directly from the Strecker degradation alone.

In the literature, there are numerous studies using model systems to correlate alkylpyrazine formation with Strecker degradation. Among the current publications are those of Hwang et al. (1994) and Keyhani and Yaylayan (1996). Mechanistically, it is important to determine whether there is an alternate pathway to form pyrazines from α-amino acids and reducing sugars. Although pyrolysis of amino acids can generate ammonia (Lien and Nawar, 1974; Sohn and Ho, 1995), there is little information in the literature to demonstrate that, via a mechanism other than Strecker degradation, pyrazines are formed from the ammonia released from amino acids and the sugar degradation products. The objective of this study was to investigate this alternate pathway.

The main strategy of this study was to take advantage of two facts: (1) the reaction between acyloins and ammonia takes place readily to form pyrazines even at room temperature (Shu and Lawrence, 1995); (2) acyloins are not involved with Strecker degradation. Therefore, if the reaction of an acyloin with an amino acid generates pyrazine, it will prove that deamination occurs and pyrazine formation is not through Strecker degradation. During the course of this study, acetoin, a simple acyloin and a sugar degradation product, was chosen to react with the simple α-amino acids. In addition, a *β*-amino acid (*β*-aminobutyric acid), a *γ*-amino acid (*γ*-aminobutyric acid), and an *ϵ*-amino acid (*ϵ*-aminohexanoic acid) were also chosen to react separately with acetoin for comparison of the deamination reactivities.

EXPERIMENTAL PROCEDURES

Material. Leucine, isoleucine, valine, alanine, glycine, α-aminobutyric acid, *β*-aminobutyric acid, *γ*-aminobutyric acid, *ϵ*-aminohexanoic acid, 3-hydroxy-2-butanone (acetoin), and tetramethylpyrazine (TMP) were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Preparation of the Reaction Mixtures from Amino Acids and Acetoin. In an enclosed reaction vessel (Parr Instrument Co., Moline, IL), 2.5 mmol of acetoin and 2.5 mmol of each amino acid listed above were heated in an oven at 250 °C for 7 min, and then each reaction mixture obtained was cooled to room temperature prior to the analysis.

Headspace Solid-Phase Microextraction (SPME) Analysis. The reaction vessel containing the reaction mixture obtained above was immediately and tightly covered with aluminum foil. A fused silica fiber coated with poly(dimethylsiloxane) from Supelco Co. (Bellefonte, PA) was inserted through the foil into the reaction vessel; the fiber remained in the headspace for 3 min prior to GC/MS analysis.

GC/MS Analysis. Each fiber prepared above was injected into the GC injector to desorb the sample, which was analyzed by GC/MS on a DB-Wax fused silica column (60 m × 0.32 mm, 0.15 *μ*m film thickness) with a mass selective detector (EI; 70

S0021-8561(97)00999-0 CCC: $15.00
Published on Web 03/28/1998

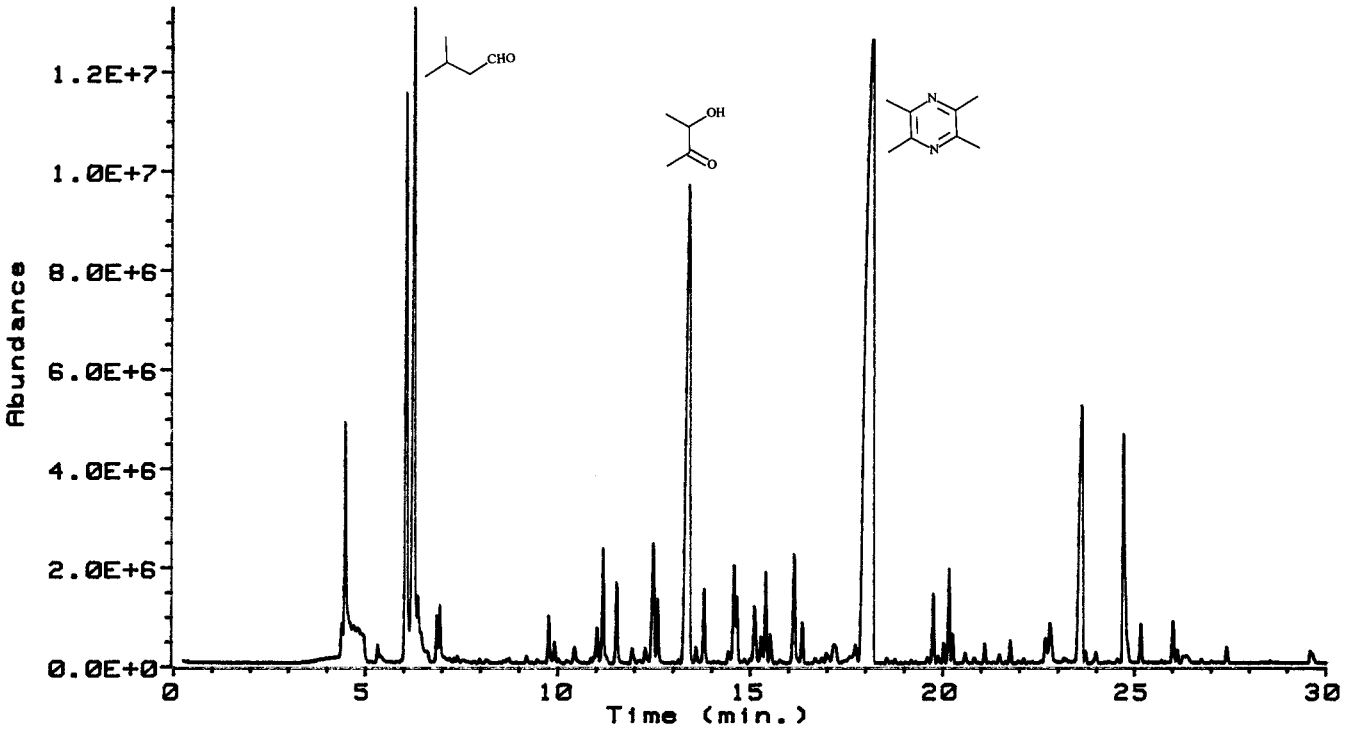

Figure 1. Total ion chromatogram of the headspace-SPME analysis obtained from the reaction between acetoin and leucine.

Table 1. Strecker Aldehydes Formed from the Reaction of Acetoin and Different Amino Acids

from the amino acid	Strecker aldehyde formed
alanine	acetaldehyde
valine	isobutyraldehyde
isoleucine	2-methylbutyraldehyde
leucine	isovaleraldehyde
glycine	formaldehyde, not found[a]
α-aminobutyric acid	propionaldehyde
β-aminobutyric acid	not formed
γ-aminobutyric acid	not formed
ϵ-aminohexanoic acid	not formed

[a] It is presumed that formaldehyde was not found due to its extremely high volatility and reactivity.

eV). The oven temperature was programmed from 50 to 200 °C at 6 °C/min.

Quantitation of TMP Formed in the Reaction Mixture. Each reaction mixture obtained was dissolved in water (10 mL) and extracted with methylene chloride (5 mL × 4). The methylene chloride extract combined (20 mL) was analyzed under the same chromatographic conditions as described above except that a flame ionization detector was used. Ethyl decanoate was added to each extract as an internal standard (I.S.) for the quantitation of TMP formed. The calibration curve was established from four sets of mixtures containing a fixed amount of I.S. and different amounts of TMP. The yield of TMP is reported as yield percent, based on the stoichiometric calculation, from which 2 mol of acetoin and 2 mol of amino acid generate 1 mol of TMP.

RESULTS AND DISCUSSION

Figure 1 shows the typical total ion chromatogram of the headspace-SPME analysis obtained from the reaction between acetoin and the α-amino acid, using leucine as an example. From this particular reaction, it was revealed that TMP was formed, indicating that ammonia, which was released from leucine, reacted with acetoin. In addition, isovaleraldehyde, which is the Strecker aldehyde related to leucine, was also formed.

Table 2. Yields of TMP Formed from the Reactions between Acetoin and Different Amino Acids

from the amino acid	yield of TMP, %
glycine	11.40
alanine	14.16
valine	13.30
isoleucine	13.60
leucine	17.74
α-aminobutyric acid	17.38
β-aminobutyric acid	25.40
γ-aminobutyric acid	0.44
ϵ-aminohexanoic acid	0.44

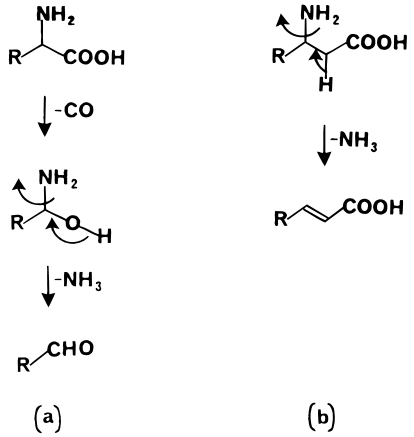

Figure 2. Proposed mechanism of deamination from α-amino acid (a) and β-amino acid (b).

Moreover, analyses of the other reaction mixtures prepared from acetoin and other α-amino acids including glycine, alanine, valine, and isoleucine also showed both TMP and the corresponding Strecker aldehydes (Table 1). However, analysis of the reaction mixtures prepared from β-aminobutyric acid, γ-aminobutyric acid, and ϵ-aminohexanoic acid produced only TMP without any Strecker aldehydes. For glycine, the corresponding

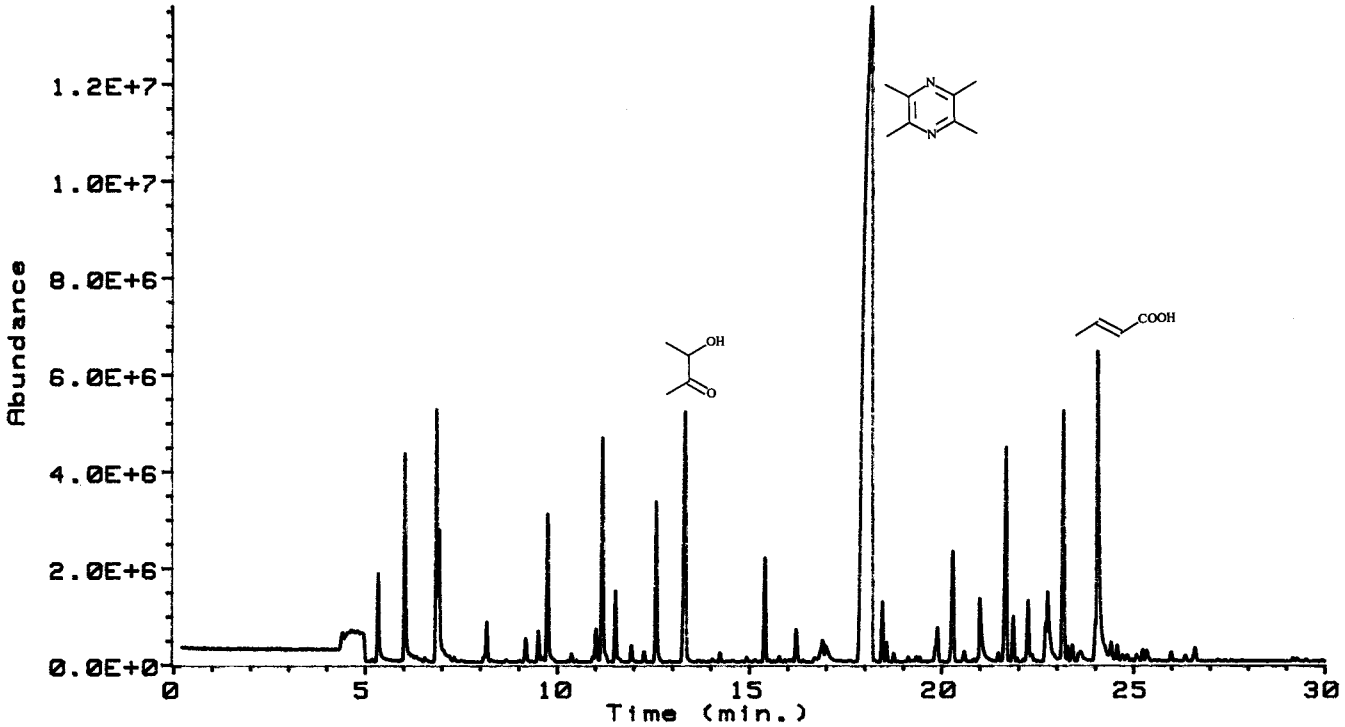

Figure 3. Total ion chromatogram of the headspace-SPME analysis obtained from the reaction between acetoin and β-aminobutyric acid.

Strecker aldehyde, formaldehyde, was not found. This is probably due to its extremely high volatility and reactivity. Overall, these results imply that all α-, β-, γ-, and ϵ-amino acids release ammonia during pyrolysis, as evidenced by the formation of TMP. However, when the quantitative data of TMP formed were considered (Table 2), it was found that the yields of TMP are different. The amounts of TMP formed from α- and β-amino acids were higher than those from γ- and ϵ-amino acids, indicating that α- and β-amino acids deaminated to a significantly greater degree than γ- and ϵ-amino acids. This indicates that the deamination mechanisms from these different amino acids may be different. As shown in Figure 2, it is proposed that the α-amino acid decarbonylates to generate the reactive intermediates, 1-hydroxyamines, deamination of which leads to the formation of the Strecker aldehydes. Also, it is proposed that deamination of β-amino acids (β-aminobutyric acid) takes place at the α- and β-positions to establish a conjugation with the acid group, which is relatively stable and readily formed. This proposed mechanism is supported by the identification of (*E*)-2-butenoic acid from the reaction of acetoin and β-aminobutyric acid (Figure 3). Taking this set of data into account, it is suggested that the reactivity of deamination from β-amino acids is slightly greater than that from α-amino acids. As for γ- and ϵ-amino acids, which generate very small amounts of TMP (0.22% yield, Table 2), it is assumed that for deamination both amino acids function as amines, which require a very high temperature.

This study demonstrates that in addition to the well-known Strecker degradation, pyrazines and Strecker aldehydes are also formed from α-amino acids and reducing sugars via deamination. Specifically, the ammonia released after decarbonylation of an α-amino acid reacts with acyloins, the sugar degradation products, to generate pyrazines. It is worth noting that when pyrazines and Strecker aldehydes exist in a reaction mixture, it is not always true that both products are derived solely from Strecker degradation.

LITERATURE CITED

Heath, H. B.; Reineccius, G. *Flavor Chemistry and Technology*; AVI Publishing: Westport, CT, 1986; pp 71−111.

Hwang, H.-I.; Hartman, T. G.; Rosen, R. T.; Lech, J.; Ho, C.-T. Formation of pyrazines from the Maillard reaction of glucose and lysine-α-amine-^{15}N. *J. Agric. Food Chem.* **1994**, *42*, 1000−1004.

Keyhani, A.; Yaylayan, V. A. Elucidation of the mechanism of pyrazinone formation in glycine model systems using labeled sugars and amino acids. *J. Agric. Food Chem.* **1996**, *44*, 2511−2516.

Lien, Y. C.; Nawar, W. W. Thermal decomposition of some amino acids. *J. Food Sci.* **1974**, *39*, 911−913.

Maga, J. A. Pyrazine update. *Food Rev. Int.* **1992**, *8* (4), 479−558.

Shu, C.-K.; Lawrence, B. M. Formation of 2-(1-hydroxyalkyl)-3-oxazolines from the reaction of acyloins and ammonia precursors under mild conditions. *J. Agric. Food Chem.* **1995**, *43*, 2922−2924.

Sohn, M.; Ho, C.-T. Ammonia generation during thermal degradation of amino acids. *J. Agric. Food Chem.* **1995**, *43*, 3001−3003.

Vernin, G.; Parkanyi, C. Occurrence and formation of heterocyclic compounds. In *The Chemistry of Heterocyclic Flavouring and Aroma Compounds*; Vernin, G., Ed.: Ellis Horwood: New York, 1982; pp 192−198.

Received for review November 24, 1997. Revised manuscript received February 18, 1998. Accepted February 18, 1998.

JF970999I