Mechanistic Studies of 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline Formation under Low Temperature in 3-Hydroxy-2-butanone/Ammonium Acetate Model Systems

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Volatile compounds formed from the reaction of 3-hydroxy-2-butanone/ammonium acetate at 25, 55 and 85 °C were investigated. Six compounds were characterized by gas chromatography−mass spectrometry (EI and CI). Among the volatile compounds identified, an interesting intermediate compound, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline, was found. ^{15}N-Labeled ammonium acetate was used to confirm the structure of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline. The formation pathway of these volatile compounds was proposed. In these model systems, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline was formed at the reaction temperature below 25 °C. On the other hand, tetramethylpyrazine was the major component when the reaction temperature was higher than 85 °C. The amounts of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline and tetramethylpyrazine increased linearly with the increasing heating time at 55 °C. Protic solvents did not promote 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline formation but did favor the formation of tetramethylpyrazine. A kinetic study of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline formation was also performed, and the activation energy was found to be 16.5 kcal/mol.

Keywords: *2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline; GC−MS; kinetic study; activation energy*

INTRODUCTION

Oxazoles and oxazolines, which are oxygen- and nitrogen-containing heterocyclics, have been identified in many kinds of heated foods and have significant sensory contribution (Maga, 1981). 5-Acetyl-2-methyloxazole was first identified in coffee (Stoffelsma et al., 1968). Later, Vitzthum and Werkhoff (1974) reported 20 oxazoles in the aroma of coffee flavor. Interestingly, oxazoline, which has a structure similar to oxazole, was not found in coffee. As in the case of cocoa, 2,5-dimethyl-, 4,5-dimethyl-, 2,4,5-trimethyl-, and 5-methyl-2-propyloxazoles were identified, but oxazoline was not found in cocoa aroma (Vitzthum et al., 1975). Other vegetative foods, such as soy sauce (Nunomura et al., 1976) and wheat (Harding et al., 1978), have also been reported to contain only oxazoles.

The first oxazoline, 2,4,5-trimethyl-3-oxazoline, was reported by Chang et al. in the flavor of meat (1968). 2,4,5-Trimethyl-3-oxazoline isolated from the model reaction of ammonia, acetaldehyde, and 3-hydroxy-2-butanone has been reported to be a constituent of cooked meat at room temperature (Mussinan et al., 1976). 2,4,5-Trimethyl-3-oxazoline has been characterized as having woody, musty, and green aromas. Other oxazolines such as 2,5-dimethyl-3-oxazoline, 2,4-dimethyl-5-ethyl-3-oxazoline, and 2,5-dimethyl-4-ethyl-3-oxazoline were also identified, and their aromas were characterized as nutty, vegetable-like, and sweet (Mussinan et al., 1976). 2,4,5-Trimethyl-3-oxazoline was reported to be the major compound in boiled beef (Hirai, 1973). Peterson et al. (1975) also reported that the concentration of 2,4,5-trimethyl-3-oxazoline was higher than that of 2,4,5-trimethyloxazole in the sample of canned beef stew.

The oxazoline as discussed in these reports was formed in thermally generated aroma in food systems. However, no detailed study has been reported on the occurrence of oxazolines in heated foods. The purpose of this study was to isolate and identify the important flavor precursor, oxazoline, from the reaction of a 3-hydroxy-2-butanone/ammonium acetate model system at low temperature. The kinetic and formation pathways among the important volatile compounds, oxazole, oxazoline, and pyrazine, were also studied.

EXPERIMENTAL PROCEDURES

Materials. 3-Hydroxy-2-butanone and dimethylpyrazine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Ammonium acetate, methanol, ethanol, propanol, butanol, and the solvents for GC were chemical grade and obtained from Fisher Chemical Co. [^{15}N]Ammonium acetate was purchased from Isotec, Inc. (Miamisburg, OH).

Reaction Conditions. Reaction mixtures were composed of 0.0025 mol (0.625 M) of 3-hydroxy-2-butanone and 0.0075 mol of ammonium salts dissolved in 4 mL of deionized water or solvents. The vials were shaken regularly to assure that all the reactants were dissolved. The reactions were run in a water bath at the required constant temperatures.

Characterization and Quantitation of Volatile Compound. *Gas Chromatography.* An HP 5890 gas chromatograph equipped with a fused silica gel column (60 m × 0.32 mm i.d., film thickness 0.25 μm, DB-1; J & W Scientific) and a flame ionization detector was used to analyze the volatile compounds. The operating conditions were as follows: injector and detector temperatures, 270 and 300 °C, respectively; helium carrier flow rate, 1.0 mL/min; temperature program, 40−260 °C at 2 °C/min and then isothermally held at 260 °C for 10 min. Dimethylpyrazine (150 mg/mL) was prepared and used as the internal standard; 10 μL of dimethylpyrazine was added after reaction. Then the mixture was saturated with sodium chloride and extracted by 1 mL of methylene chloride;

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S0021-8561(96)00734-0 CCC: $14.00

Table 1. Tentatively Identified Volatile Compounds in the 3-Hydroxy-2-butanone/Ammonium Acetate Model System at Low Temperature (25 °C)

peak no.	compound identified	MS *m/z* (relative intensity)
1	2,4,5-trimethyl-3-oxazoline	113(23), 98(30), 72(100), 71(24), 70(12), 69(21), 68(13), 60(8), 54(6)
2	2,4,5-trimethyloxazole	111(100), 96(1), 82(16), 70(3), 68(33), 55(50), 43(94), 42(74)
3	isomers, aldol condensation of 3-hydroxy-2-butanone	114(47), 113(8), 87(4), 72(100), 57(12)
4	2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline	157(1), 113(16), 112(100), 98(7), 72(10), 71(62)
5	tetramethylpyrazine	136(42), 121(1), 95(3), 80(2), 54(94), 53(23), 52(11), 51(7)
6	Schiff base	157(14), 131(7), 115(7), 114(5), 89(2), 72(100), 57(15)

Table 2. Quantitation of Tentatively Identified Volatile Compounds from the 3-Hydroxy-2-butanone/Ammonium Acetate Model System (Reaction Time: 4 h)

peak no.	compound identified	25 °C (mg)	55 °C (mg)	85 °C (mg)
1	2,4,5-trimethyl-3-oxazoline	0.078 (0.18%)[a]	0.068 (0.35%)	0.025 (0.04%)
2	2,4,5-trimethyloxazole	0.059 (0.11%)	0.056 (0.29%)	0.025 (0.04%)
3	isomers, aldol condensation of 3-hydroxy-2-butanone	0.027 (0.15%)	0.025 (0.10%)	
		0.042 (0.10%)	0.036 (0.19%)	
		0.009 (0.05%)	0.008 (0.04%)	
4	2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline	12.32 (97.9%)	14.28 (74.9%)	0.062 (0.11%)
5	tetramethylpyrazine	0.043 (0.10%)	4.84 (25.0%)	56.18 (99.6%)
6	Schiff base	0.008 (0.05%)	0.014 (0.07%)	0.136 (0.24%)

[a] (Amount of compound)/(total amounts of volatile compounds) × 100%.

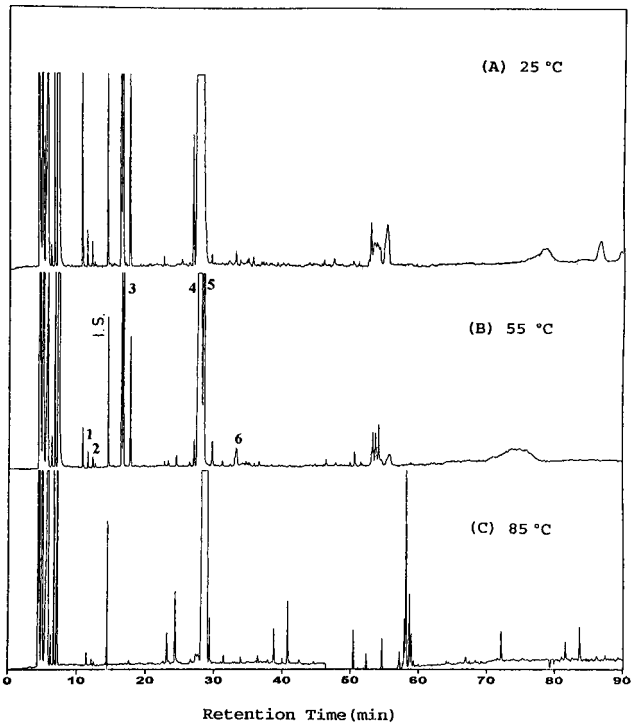

Figure 1. GC profile of volatile compounds formed at 25 °C (A), 55 °C (B), and 85 °C (C).

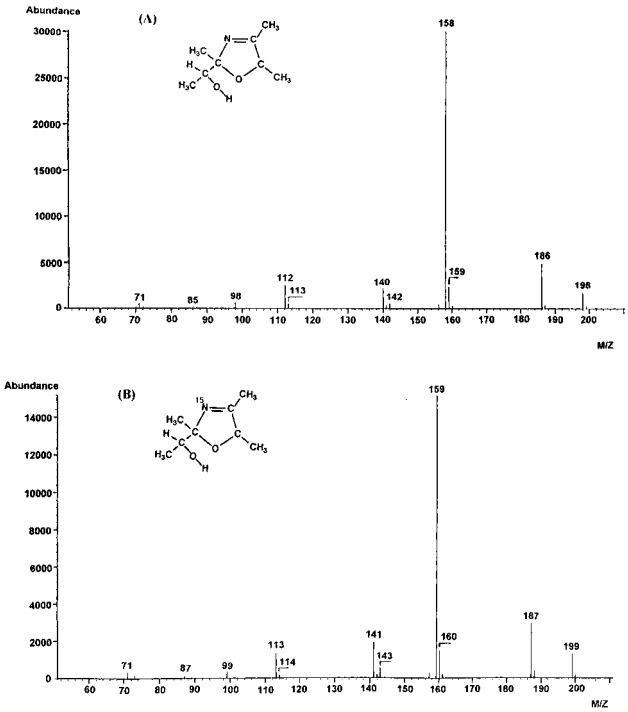

Figure 2. CI mass spectra in [^{15}N]- and [^{14}N]ammonium acetate/3-hydroxy-2-butanone model systems.

1 μL of extract was injected into the GC after drying by anhydrous sodium sulfate.

Gas Chromatography−Mass Spectrometry Analysis. GC−MS was accomplished by using a Hewlett-Packard 5890A gas chromatograph coupled to a Hewlett-Packard 5985B mass spectrometer equipped with a direct split interface. EI mass spectra were obtained using electron ionization at 70 eV and an ion source temperature of 250 °C. For CI-MS analyses, reactant gas (methane, CH_4) was utilized. The operating conditions were the same as those used in the GC analysis described above. An [^{15}N]ammonium acetate/3-hydroxy-2-butanone system was set up to confirm the structure of the 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline.

Kinetic Studies on 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline Formation at Low Temperature. The rate of oxazoline formation in 3-hydroxy-2-butanone/ammonium acetate in a water system at the three reaction temperatures (5, 15, 25 °C) was analyzed by GC in the reaction mixture. All kinetic studies were carried out in duplicate. The kinetics of formation of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline were determined using the basic equation for the rate of change of A with time: $dA = KA^n\,dt$, where A = concentration, t = time, K = rate constant, and n = reaction order. Slopes and intercepts were calculated by the linear least-squares method.

The temperature dependence of the reaction rate constant could be determined by the following Arrhenius equation:

$$K = K_o\, e^{-E_a/RT}$$

where K = rate constant, E_a = activation energy in kcal/mol, R = gas constant (1.987 cal/mol K), and T = temperature in K. Thus, the activation energy for formation of the 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline was calculated from the slope of the line generated by plotting the natural log value of the rate constant versus the reciprocal of the absolute temperature (Labuza, 1983).

Effect of Solvents on the Formation of the 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline and Tetramethylpyrazine. The reaction products of the intermediate and tetramethylpyrazine from 3-hydroxy-2-butanone/ammonium acetate in water, metha-

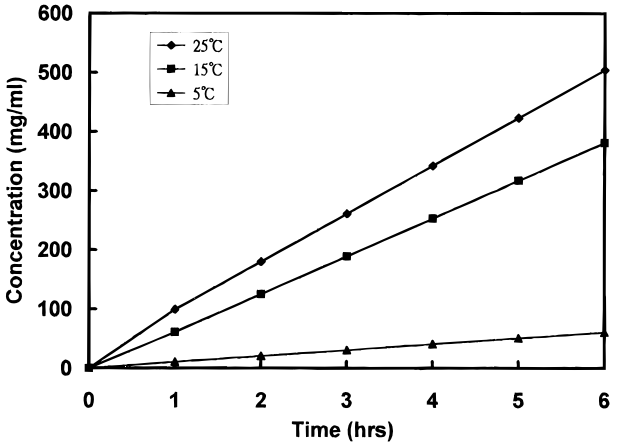

Figure 3. 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline formation at different temperatures.

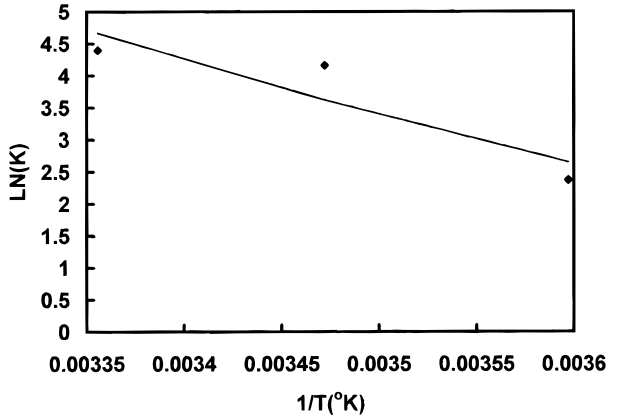

Figure 4. Arrhenius plot of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline formation.

nol, ethanol, propanol, and butanol at 25 and 55 °C for 4 h were quantified by gas chromatography. Dimethylpyrazine was used as an internal standard to quantify the amount of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline and tetramethylpyrazine formation in the model system.

RESULTS AND DISCUSSION

Volatile Compounds Identified in the 3-Hydroxy-2-butanone/Ammonium Acetate Model System. The gas chromatograms of volatile compounds formed at various temperatures (25, 55, 85 °C) are shown in Figure 1. Six compounds were tentatively identified by GC−MS(EI) to be 2,4,5-trimethyl-3-oxazoline (peak 1), 2,4,5-trimethyloxazole (2), isomers of aldol condensation product of acetoin (3), 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline (4), tetramethylpyrazine (5), and a Schiff base (6). Table 1 shows the major fragmentation data of these identified compounds. Among these compounds, we found an interesting oxazoline. Although oxazolines are usually formed at high temperature, we have found that 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline formed abundantly in our model system at low temperature. [^{15}N]Ammonium acetate was used to confirm the structure of this oxazoline compound. Figure 2A,B shows the results of GC−MS-(CI) in the [^{14}N]- and [^{15}N]ammonium acetate model systems, respectively. In the [^{14}N]ammonium acetate model system, m/z at 158 is interpreted as the M + 1 peak. Since the reactant gas was methane, the $CH_4^{\bullet+}$ ion may undergo fragmentation before it collides with a CH_4 molecule and produces CH_3^+. The fragment ion CH_3^+ may itself undergo an ion−molecule reaction and form $C_2H_5^+$ (Reg and Martin, 1991). Peak 186 which was shown on the CI spectrum is $(M + C_2H_5)^+$ ions which have a mass 29 units higher than the relative molecular mass of the molecular ion.

The peak at m/z 112 was due to the loss of CH_3-$CHOH^+$ from the parent ion (m/z 157). The peak at m/z 71 represented a loss of the CH_3CN fragment. One unit higher of mass to charge (m/z 159) was obtained from the [^{15}N]ammonium acetate model system. It showed that only one nitrogen atom was present in the molecular structure of this compound. No nitrogen was found in the fragment of m/z 71. This compound (peak 4) was therefore characterized as 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline. These results agree with those of Shu and Lawrence (1975).

Formation of 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline and Tetramethylpyrazine from the 3-Hydroxy-2-butanone/Ammonium Acetate Model System at Different Temperatures. Three temperatures (25, 55, 85 °C) were investigated in this model system. Quantitation of volatile compounds is summarized in Table 2. The data showed 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline formed predominately below 25 °C, whereas tetramethylpyrazine was the major product at reaction temperatures higher than 85 °C. At the temperature of 25 °C, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline (97.9% peak area of the total volatiles) was the major volatile compound formed in the model system. Kinetic analysis for the formation mechanism of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline was conducted at three temperatures (5, 15, 25 °C). The amount of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline increased linearly in all three temperatures. The rates of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline formation at each temperature followed pseudo-zero-order reaction kinetics as shown by the linear plot of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline concentration versus time (Figure 3). Rate constants were calculated from the slope using the least-squares fit method. The natural log of the rate constant as a function of the reciprocal of temperature is shown in the Arrhenius plot (Figure 4). Linear regression data summarizing the effects of temperature on 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline formation are shown in Table 4. The activation energy was found to be 16.5 kcal/mol.

When the reaction temperature was elevated to 55 °C, tetramethylpyrazine (25.0%) started to form along with 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline (74.9%) in the same system. Both major compounds, tetramethylpyrazine and 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline, increased linearly with increasing times. 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline showed a higher formation rate than tetramethylpyrazine. At a reaction temperature higher than 85 °C, tetramethylpyrazine was the major compound (99.6%) generated in the model system. It was found that 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline and tetramethylpyrazine were well separated by using GC analysis. The formation of tetramethylpyrazine was evidenced as the major component above 85 °C in this experiment.

Rizzi (1988) showed that tetramethylpyrazine could be derived from acetoin and ammonium acetate at temperatures as low as 22 °C. An HPLC method for tetramethylpyrazine determined developed by Rizzi was utilized in our previous study (Huang et al., 1996). In that study, it was determined that the activation energy of tetramethylpyrazine was to be 18.84 kcal/mol. The

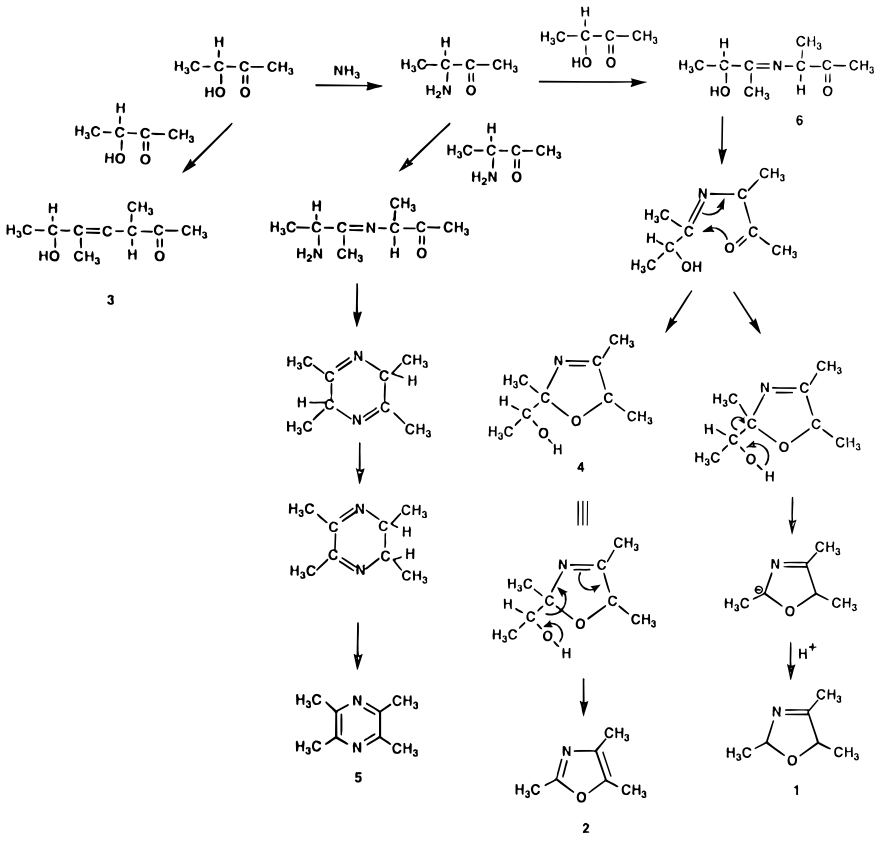

Figure 5. Overview of reaction pathways of 3-hydroxy-2-butanone with ammonium acetate.

Table 3. Effect of Solvents on 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline and Tetramethylpyrazine Formation at 25 and 55 °C[a]

	quantification (mg)				
		solvent			
compound	water	methanol	ethanol	propanol	butanol
	25 °C				
2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline	12.32	12.99	9.92	12.00	15.42
tetramethylpyrazine	0.04	2.64	2.34	2.64	2.90
	55 °C				
2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline	14.28	7.54	7.80	7.30	6.56
tetramethylpyrazine	4.84	8.16	8.28	8.22	7.88

[a] Reaction time: 4 h.

Table 4. Rate Constant (*K*) and Corresponding Regression Coefficient of 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline Formation from the 3-Hydroxy-2-butanone/Ammonium Acetate System

T (°C)	*K* (mg/mL h)	R^2
5	10.54	0.99
15	66.09	0.98
25	81.64	0.99

amounts of tetramethylpyrazine were overestimated due to the close elution of tetramethylpyrazine and 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline by HPLC analysis. Low activation energy for tetramethylpyrazine formation at a level similar to that of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline could be expected.

Effect of Protic Solvents on the Formation of 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline. Table 3 shows the effect of protic solvents, methanol, ethanol, propanol, and butanol, on the formation of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline and tetramethylpyrazine. Water systems seem to favor the formation of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline which needed two Schiff base reactions. On the other hand, four Schiff base formation reactions are involved in one molecule of tetramethylpyrazine formation. More water needs to be removed for one molecule of tetramethylpyrazine formation than for 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline formation. It was proposed that protic solvents could promote Schiff base formation in our previous study (Huang et al., 1996). These data indicated that protic solvents did not promote 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline formation but increased the amount of tetramethylpyrazine at both 25 and 55 °C. These results are in good agreement with our previous studies. Therefore, protic solvent was more effective in tetramethylpyrazine formation than in 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline formation.

Proposed Mechanism of Volatile Compounds Formed in the 3-Hydroxy-2-butanone/Ammonium Acetate Model System at Low Temperature. Figure 5 summarizes the reaction scheme for the six major compounds. Two molecules of 3-amino-2-butanone, which was derived from the reaction of ammonia and 3-hydroxy-2-butanone, were condensed to form a Schiff base that can then be rearranged to form tetramethylpyrazine (peak 5). Three isomers (peak 3) were formed from the aldol condensation of 3-hydroxy-2-

butanone. The reaction also led to the formation of the Schiff base (peak 6). The major pathway involved the reaction of 3-amino-2-butanone with 3-hydroxy-2-butanone. The reaction formed a Schiff base which was rearranged to form 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline (peak 4) and then 2,4,5-trimethyloxazole (peak 2). 2,4,5-Trimethyl-3-oxazoline (peak 1) was also formed as another rearrangement product. A similar 2,4,5-trimethyl-3-oxazoline formation mechanism has been proposed by Piloty and Baltes (1979). They found that oxazoles, pyrazines, pyrroles, and pyridines were formed in the heated model system of amino acids and diacetyl (2,3-butanedione). We concluded that 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline (peak 4) was formed in the greatest amount indicating that this was the major pathway below 25 °C. Only small amounts of 2,4,5-trimethyl-3-oxazoline (peak 1), 2,4,5-trimethyloxazole (2), tetramethylpyrazine (5) and the compound from peak 6 were produced at low temperatures in this model system. However, tetramethylpyrazine was formed at temperatures higher than 85 °C.

ACKNOWLEDGMENT

This work was supported by a grant from the National Science Foundation, Taiwan, R.O.C. (CNS 85-2321-B 127-001).

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Received for review September 25, 1996. Revised manuscript received January 28, 1997. Accepted February 11, 1997.[⊗]

JF960734O

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1997.