Mechanistic Studies on the Formation of Thiazolidine and Structurally Related Thiazines in a Cysteamine/2,3-Butanedione Model System

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Phosphate was found to dramatically enhance the formation of 2-methyl-2-acetylthiazolidine from a cysteamine/2,3-butanedione model system. In addition to the major component, 2-methyl-2-acetylthiazolidine, significant amounts of two structurally closely related compounds, 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine and 5-acetyl-2,3-dihydro-1,4-thiazine, were characterized by using GC/MS (CI and EI). There was an oxidative transformation of 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine to 5-acetyl-2,3-dihydro-1,4-thiazine in the presence of azodicarbonamide. A formation mechanism for 2-methyl-2-acetylthiazolidine and structurally related 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine and 5-acetyl-2,3-dihydro-1,4-thiazine is proposed.

Keywords: *2-Acetyl-2,3,5,6-tetrahydro-1,4-thiazine; cysteamine; 2,3-butanedione; popcorn-like compounds; oxidative transformation*

INTRODUCTION

Model systems composed of D-glucose and L-cysteine have long been used to study the thermal generation of nitrogen- and sulfur-containing flavor compounds (Scanlan et al., 1973; Mulders, 1973). Among the volatile compounds identified, thiazolidines and thiazines are of special interest.

Thiazolidines generally possess a characteristic popcorn-like flavor (Yeo and Shibamoto, 1991). 2-Acetyl-2-methylthiazolidine was first characterized by Umano et al. (1995) from the headspace of a heated D-glucose/L-cysteine model system. The yield of 2-acetyl-2-methylthiazolidine is <0.01% (GC peak area). Umano et al. (1995) hypothesized that the reaction between cysteamine, the decarboxylated cysteine, and 2,3-butanedione, a glucose degradation product, may lead to the formation of 2-acetyl-2-methylthiazolidine. However, no detailed formation mechanism was provided.

On the other hand, an intensely roasted, popcorn-like odorant, 5-acetyl-2,3-dihydro-1,4-thiazine, was identified in a D-ribose/L-cysteine model system by Hofmann et al. (1995a). It was proposed that a Schiff base was formed from the condensation between the amino group in cysteamine and the carbonyl group in 2,3-butanedione. Tautomerization and subsequent cyclization by a Michael-type nucleophilic attack of the thiol group at the activated methyl carbon atom yielded 5-(2-hydroxyethenyl)-2,3,6-trihydro-1,4-thiazine. Oxidation of this enaminol results in 5-acetyl-2,3-dihydro-1,4-thiazine, which, due to the electronegativity of the sulfur atom, tautomerizes into the more stable 5-acetyl-2,3-dihydro-1,4-thiazine (Hofmann et al., 1995a).

Interestingly, thiazolidine and thiazine formation were reported by different research groups, respectively, from the same model system, cysteamine and 2,3-butanedione. The objective of this research is to investigate in detail the formation of 5-acetyl-2,3-dihydro-1,4-thiazine and 2-acetyl-2-methylthiazolidine from the same model system.

EXPERIMENTAL PROCEDURES

Materials. Cysteamine, tributylamine, and 2,3-butanedione were purchased from Aldrich Chemical Co. (Milwaukee, WI). Sodium hydroxide, disodium hydrogen phosphate, sodium dihydrogenphosphate dihydrate, and azodicarbonamide were of chemical grade and obtained from Sigma Chemical Co. (St. Louis, MO).

Sample Preparation. Cysteamine (1 mM) and 2,3-butanedione (1 mM) were dissolved in either deionized water (100 mL) or a phosphate buffer (100 mL, pH 7.2, 0.2 M). Samples were put into a glass tube, capped tightly, and heated for 20 min in a laboratory autoclave at 121 °C. After cooling, the solution was extracted with dichloromethane. An internal standard, tributylamine (1 mM), was added to the extract.

Effect of Phosphate Buffer on 2-Acetyl-2-methylthiazolidine, 5-Acetyl-2,3-dihydro-1,4-thiazine, and 2-Acetyl-2,3,5,6-tetrahydro-1,4-thiazine Formation. Reaction mixtures without phosphate were adjusted to pH 7.2 with sodium hydroxide. To study the effect of the buffer system on the generation of volatile compounds in the model system, a phosphate buffer (0.2 M, pH 7.2) was utilized to replace the aqueous medium.

Effect of Azodicarbonamide on 5-Acetyl-2,3-dihydro-1,4-thiazine Formation. To investigate the redox reaction in the model system, various concentrations of azodicarbonamide (0, 1, 2, and 3 mM) were added to the heated and then cooled solution and shaken in a water bath (35 °C) for 30 min. After shaking, the solution was extracted with dichloromethane. An internal standard, tributylamine (1 mM), was added to the extract.

Gas Chromatography (GC). An HP 5890 A gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a

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Table 1. MS Spectra of Thiazolidine and Thiazoline

no.	compd	RI[a]	MS
1	2-acetyl-2-methylthiazolidine	1134	M^+ = 145 (2), 102 (100), 61 (26), 60 (20), 59 (80), 42 (55)
2	2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine	1225	M^+ = 145 (2), 102 (100), 74 (39), 56 (32), 43 (24)
3	5-acetyl-2,3-dihydro-1,4-thiazine	1310	M^+ = 143 (100), 100 (25), 73 (15), 72 (24), 72 (24), 44 (27), 43 (84)

[a] RI, retention index.

fused silica column (60 m × 0.32 mm i.d.; film thickness, 0.25 μm; SPB-1, Supelco Co., Bellefonte, PA) and a flame ionization detector were used to analyze the dichloromethane extracts. The operational conditions were as follows: injector and detector temperatures, 220 and 240 °C, respectively; helium carrier flow rate, 1.0 mL/min; temperature program, 80−200 °C at 4 °C/min.

Gas Chromatography/Mass Spectrometry (GC/MS). GC/MS analysis was accomplished by using an HP 5890A gas chromatograph coupled to a 5972 mass selective detector. The mass spectra in the electron impact mode (MS-EI) were generated at 70 eV and in the chemical ionization mode (MS-CI) were obtained at 230 eV with methane as reagent gas. The filament emission current was 1 mA, and the spectra were recorded and analyzed with the HP 5989B MS Chemstation. The operating conditions were the same as those used in the GC analysis described above.

RESULTS AND DISCUSSION

Characterization of the Volatiles Generated in the Cysteamine/2,3-Butanedione Model System. Table 1 lists the major products characterized in a dichloromethane extract from a heated cysteamine/2,3-butanedione model system. At least four peaks appeared in the GC chromatogram as shown in Figure 1. Peak 1 was characterized as one of the reactants, 2,3-butanedione. The EI mass spectrum of the major peak (peak 2) was dominated by the base peak of *m/z* 102. A loss of a fragment of 43 indicates an acetyl group attached to a ring structure. The molecular weight of this compound was estimated to be 145 by MS-CI. This compound was elucidated as 2-acetyl-2-methylthiazolidine, which has been reported by Umano et al. (1995).

In addition to the major component, two other minor constituents were also characterized. Peak 4 with a retention time of 23.4 min in the gas chromatogram was characterized as 5-acetyl-2,3-dihydro-1,4-thiazine. The EI mass spectrum of this compound was dominated by a base peak of *m/z* 143. The CI mass spectrum revealed that this base peak is the molecular ion peak. The EI mass spectrum of this compound was in complete agreement with that of 5-acetyl-2,3-dihydro-1,4-thiazine (Hofmann et al., 1995a). Hofmann et al. (1995a) isolated and characterized 5-acetyl-2,3-dihydro-1,4-thiazine as the key odorant from a thermally treated solution of ribose and cysteine by aroma extract dilution technique.

Among the volatiles generated in the cysteamine/2,3-butanedione model system, peak 3 was particularly interesting. The molecular weight of this component was determined to be 145 by CI-MS (Figure 2A). Similar to that of 5-acetyl-2,3-dihydro-1,4-thiazine, the EI mass spectrum of this component (Figure 2B) showed a base peak at *m/z* 102 resulting from the loss of a fragment of 43 from the molecule ion, which strongly indicated that this compound also possesses an acetyl group. The loss of an ethylene moiety from the *m/z* 102 ion led to the formation of an *m/z* 74 ion (39%). The peak observed at *m/z* 56 (32%) represented a loss of the CH_2=S fragment from the *m/z* 102 ion as shown in Figure 3. This compound was tentatively characterized as 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine.

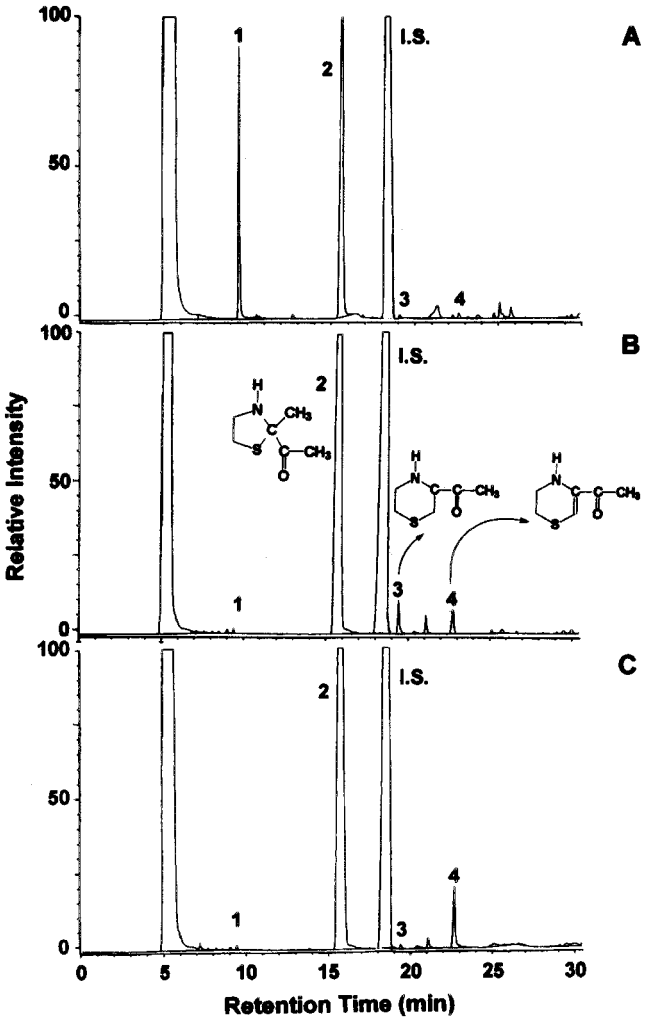

Figure 1. Gas chromatograms of the dichloromethane extract from the cysteamine/2,3-butanedione model system: (A) without phosphate buffer, pH 7.2; (B) with phosphate buffer, 0.2 M, pH 7.2; (C) with phosphate buffer, 0.2 M, pH 7.2, and 3 mM azodicarbonamide. Peaks: 1, 2,3-butanedione; 2, 2-acetyl-2-methylthiazolidine; 3, 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine; 4, 5-acetyl-2,3-dihydro-1,4-thiazine.

Effect of a Phosphate Buffer System on Thiazolidine Formation. Quantitative data (Table 2) obtained in this experiment revealed that phosphate is a very effective buffer system for the promotion of 2-methyl-2-acetylthiazolidine formation. The addition of a phosphate ion resulted in 16-, 12-, and 21-fold increases of 2-acetyl-2-methyl-thiazolidine, 5-acetyl-2,3-dihydro-1,4-thiazine, and 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine formation, respectively, as compared with an aqueous system at pH 7.2. The phosphate may act as both hydrogen acceptor and donor, which catalyzes Schiff base formation during the generation of a thiazolidine and thiazine.

Effect of Azodicarbonamide on 5-Acetyl-2,3-dihydro-1,4-thiazine Formation. Azodicarbonamide, a well-known hydrogen acceptor, was added to the reacted model system to study the influence of the oxido−redox reaction on the formation of 5-acetyl-2,3-dihydro-1,4-thiazine. The formation of 5-acetyl-2,3-dihydro-1,4-

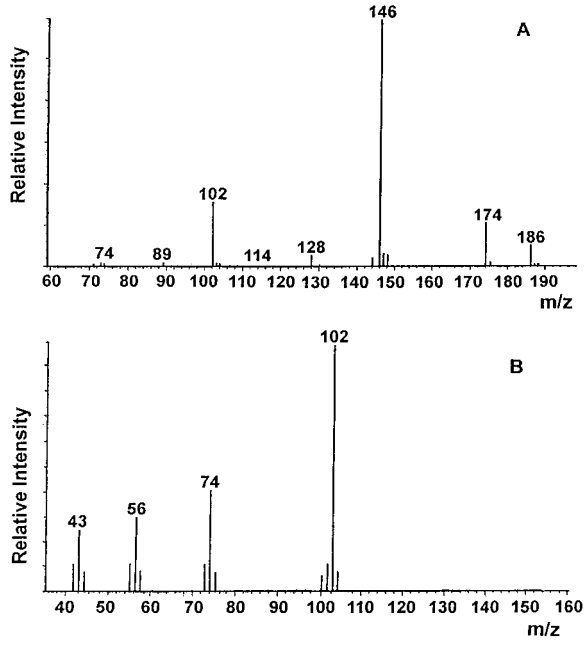

Figure 2. Mass spectra of 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine: (A) CI; (B) EI.

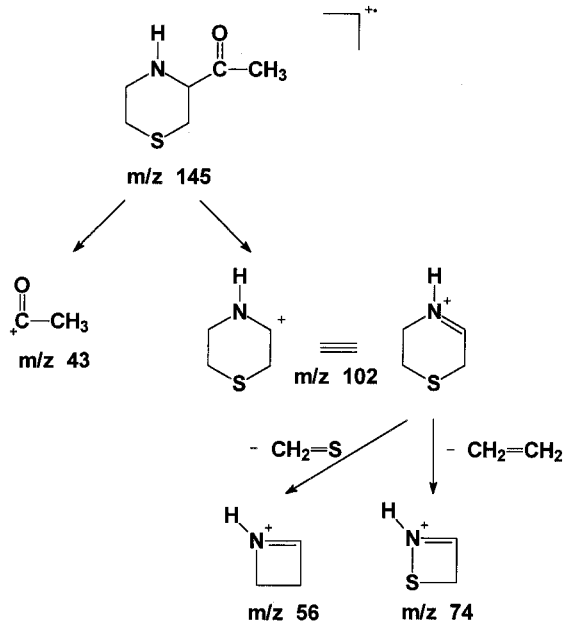

Figure 3. Fragmentation pattern of 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine.

Table 2. Effect of Phosphate Buffer on Volatile Generation in Cysteamine/ 2,3-Butanedione Model System at pH 7.2

	concn (mM)	
compd	phosphate	aqueous
2,3-butanedione	0.0017	0.0211
2-acetyl-2-methylthiazolidine	0.6596	0.0402
2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine	0.0231	0.0019
5-acetyl-2,3-dihydro-1,4-thiazine	0.1027	0.004

thiazine was found to increase linearly by increasing the concentration of azodicarbonamide in the range of 1−3 mM. The amount of 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine decreased with the increasing concentration of azodicarbonamide. The formation of 2-acetyl-2-methylthiazolidine was found to be independent of azodicarbonamide as shown in Table 3. The redox

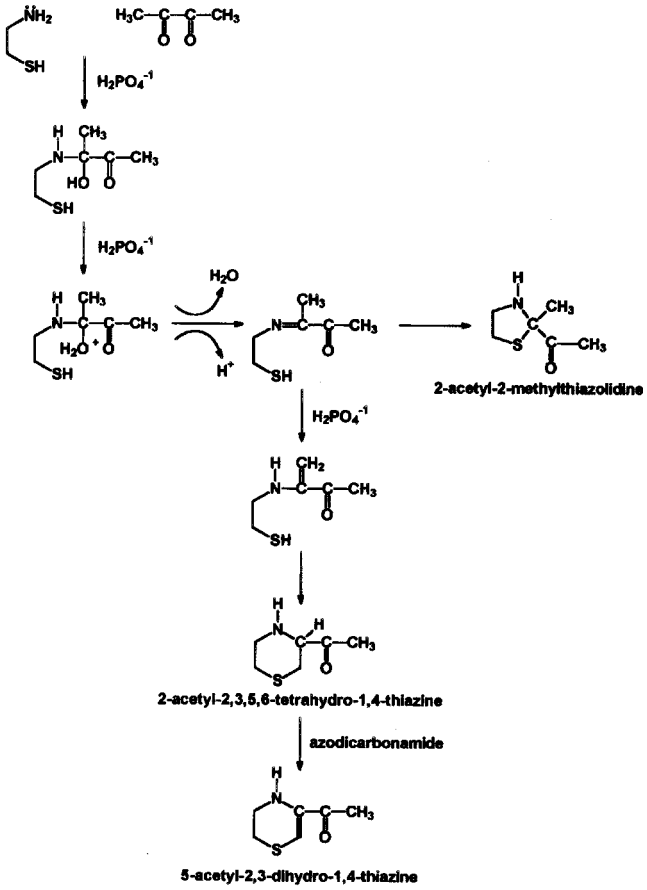

Figure 4. Proposed formation mechanism for 2-acetyl-2-methylthiazolidine, 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine, and 5-acetyl-2,3-dihydro-1,4-thiazine in the cysteamine/2,3-butanedione model system.

Table 3. Effect of Azodicarbonamide on Volatile Generation in Cysteamine/ 2,3-Butanedione Model System

	concn (mM)		
compd	1	2	3
2-acetyl-2-methylthiazolidine	0.6215	0.6674	0.6946
2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine	0.0135	0.0087	
5-acetyl-2,3-dihydro-1,4-thiazine	0.0159	0.0267	0.0349

reaction seems to lead to the conversion of 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine to 5-acetyl-2,3-dihydro-1,4-thiazine via a proton-transfer reaction similar to that in the conversion of tetramethyldihydropyrazine to tetramethylpyrazine (Huang et al., 1996). The redox reaction has been proposed by Huyghues-Despointes and Yaylayan (1996) in a Maillard model system composed of D-glucose and proline. They reported that an α-diketone/enediol redox couple participated in the generation of oxidation−reduction products in a Maillard system. Oxidation of thiazolidines in the presence of atmospheric oxygen may lead to the formation of the corresponding thiazoline (Sheldon and Shibamoto, 1987; Hofmann and Schieberle, 1996). Similarly, it was also observed in a cysteamine/methylglyoxal model system (Hofmann and Schieberle, 1995b). They attributed methylglyoxal as a proton acceptor in the formation of 2-acetyl-2-thiazoline from 2-acetylthiazolidine.

Proposed Mechanism for Thiazolidine Formation. The proposed mechanism for the formation of 2-acetyl-2-methylthiazolidine, 2-acetyl-2,3,5,6 -tetrahydro-1,4-thiazine, and 5-acetyl-2,3-dihydro-1,4-thiazine

is shown in Figure 4. In a reaction medium with a phosphate buffer, the nucleophilic amino group on a cysteamine molecule tends to attack the positively induced carbonyl carbon on the 2,3-butanedione molecule. The proton transfer facilitated by a phosphate ion not only makes the hydroxyl group a better leaving group, which is converted to a molecule of water, but also leads to the elimination of the hydrogen atom on the nitrogen atom (Huang et al., 1996). The dehydration and proton transfer gives a Schiff base. A Michael-type nucleophilic attack of the thiol group on the ethylene carbon leads to the formation of a 2-acetyl-2-methylthiazolidine following a pathway similar to that proposed for the thiazolidine formation in the aldehydes/cysteamine model system (Huang et al., 1998). In the presence of a phosphate ion, a delocalization of the unshared electron pair on nitrogen, the π-electron on a carbon−carbon double bond, and the π-electron on carbonyl double bond results in the formation of an activated methyl carbon similar to that proposed by Hofmann and Schieberle (1995a). Another Michael-type nucleophilic attack of the thiol group on this active carbon gives a 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine. A redox reaction catalyzed either by phosphate or by azodicarbonamide may facilitate the proton transfer from 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine to form 5-acetyl-2,3-dihydro-1,4-thiazine.

ABBREVIATIONS USED

bp, boiling point; i.d., internal diameter; GC/MS, gas chromatography/mass spectrometry; CI, chemical ionization; EI, electric ionization.

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