# Formation of 2-(1-Hydroxyalkyl)-3-oxazolines from the Reaction of Acyloins and Ammonia Precursors under Mild Conditions

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Studies on the reaction between acetoin and an ammonia precursor under mild conditions revealed that two major products were formed, one of which was tetramethylpyrazine (TMP) and the other an unknown compound. By spectral elucidation (MS, IR, NMR), this unknown compound has been identified as 2,4,5-trimethyl-2-(1-hydroxyethyl)-3-oxazoline (OXZ). Two homologs of OXZ were also prepared. A 3-week storage study demonstrated that OXZ was readily formed, after which its formation declined. In contrast, TMP formation gradually increased before it leveled out in 15–20 days. At the end of this experiment, only TMP remained as the product, while OXZ was not found. Mechanistically, it is proposed that condensation of acetoin and ammonia generates α-hydroxyimine or α-amino ketone by tautomerism, which may lead to product formation via two pathways. One of the pathways is to form TMP via self-condensation of the α-amino ketone, dehydration, and dehydrogenation, which is well-known. The other pathway may be that the α-hydroxyimine condenses with a second molecule of acetoin and then is cyclized by Schiff base formation to OXZ. These storage results also indicate that the second pathway may be reversible, so that OXZ initially formed is able to be converted back to α-hydroxyimine, which, in turn, was tautomerized to α-amino ketone, so that the first pathway to form TMP could be followed.

**Keywords:** *Acyloins; ammonia precursor; 2-(1-hydroxyalkyl)-3-oxazolines; 2,4,5-trimethyl-2-(1-hydroxyethyl)-3-oxazoline; 2,4-dimethyl-2-(hydroxymethyl)-3-oxazoline; 2,4,5-triethyl-2-(1-hydroxypropyl)-3-oxazoline; acetoin; acetol; 4-hydroxy-3-hexanone; diammonium hydrogen phosphate*

## INTRODUCTION

Alkylpyrazines are very important flavor compounds for foods; their formation has been extensively studied. Basically, two pathways are involved in alkylpyrazine formation in food systems. One of them takes place at relatively high temperatures, such as in roasting or baking, in the presence of an α-amino acid and a reducing sugar (Hodge, 1967). The α-diketones derived from the Amadori rearrangement or sugar degradation form α-amino ketones (acyloins) via Strecker degradation. Self-condensation of the α-amino ketones followed by dehydrogenation generates the alkylpyrazines. A second pathway that takes place is at relatively low temperatures, such as in fermented foodstuffs, in the presence of ammonia and acyloins (Kosuge et al., 1971; Rizzi, 1988), which are biologically available. Alkylpyrazines have been found in several fermented products, including cheese (Liardon et al., 1982), fermented soy foods (Liardon and Ledermann, 1980), and fermented cacao (Barel et al., 1985). Recently, the formation of tetramethylpyrazine (TMP) from ammonia and 3-hydroxy-2-butanone (acetoin) under high hydrostatic pressure was investigated (Huang et al., 1994). Both Rizzi (1988) and Huang et al. (1994) demonstrated that reaction of acyloins and ammonium salts yielded alkylpyrazines; however, information on whether the other products were also formed in this reaction was lacking. The objectives of this study were to determine whether other major products would be formed in addition to alkylpyrazines under mild conditions and to propose the formation mechanism and the possible chemical relationship between the alkylpyrazines and the other major products.

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## EXPERIMENTAL PROCEDURES

**Material.** Tetramethylpyrazine (TMP), acetoin (ACT), acetol, 4-hydroxy-3-hexanone, diammonium hydrogen phosphate (DAP), ammonium acetate (AAc), diammonium hydrogen citrate (DAC), ammonium bicarbonate (ABC), and ammonium hydroxide were purchased from commercial suppliers and were used as supplied.

**Preparation of the Solutions from Different Ammonia Precursors.** Each mixture was prepared with 0.01 mol of ACT, 0.01 mol of each of the above ammonia precursors, and 4 mL of water, and all of the solutions were sealed and stored at room temperature. During the 4-week storage period, the physical changes of these solutions were examined, and at the end of the storage period, each solution was diluted with 15 mL of water and extracted with methylene chloride (20 mL × 3). The combined extracts were dried over anhydrous sodium sulfate and concentrated by a rotary evaporator to 20 mL, and the concentrate was analyzed by GC/MS.

**Preparation of the Solutions with DAP.** For the storage study, a series of solutions were prepared with 0.01 mol of ACT, 0.01 mol of DAP, and 4 mL of water and stored at room temperature. At different storage time intervals (1, 2, 3, 5, 10, 15, and 20 days) one of the solutions was extracted and analyzed as described above.

**Preparation of the Reactions of Other Acyloins with Ammonium Hydroxide.** A solution of 0.01 mol of ammonium hydroxide, 0.01 mol of acetol, and 4 mL of water was prepared and kept at room temperature for 2 weeks prior to the extraction and analysis as described above. In the same manner, the reaction from 4-hydroxy-3-hexanone was also prepared except that no water was used.

**GC/MS Analysis.** Each concentrated extract was analyzed by GC/MS on a DB-Wax fused silica column (30 m × 0.32 mm i.d., 0.15 μm film thickness). The oven temperature was programmed from 50 to 190 °C at 6 °C/min, and a mass selective detector (EI, 70 eV) was used. Retention indices ($I_k$) were calculated (Majlat et al., 1974).

**CI/MS Analysis.** Some of the concentrated extracts were also analyzed under similar chromatographic conditions by MS

0021-8561/95/1443-2922$09.00/0 

detector in the chemical ionization mode, using methane as the reagent gas.

**Preparative GC.** The peaks of interest were collected from a preparative glass capillary column (Supelcowax 10, 30 m × 0.75 mm i.d., 1.0 μm film thickness), using a thermal conductivity detector under the same chromatographic conditions.

**IR Analysis.** The peaks of interest collected from GC were analyzed on a Mattson Polaris FT-IR microscope.

**NMR Analysis.** Proton NMR spectra were obtained on a Varian Unity 300 MHz spectrometer, using $CDCl_3$ or $CD_3OD$ as solvent. All chemical shifts were reported in parts per million downfield of internal TMS.

**Quantitative Analysis of TMP and the Unknown Compound.** The quantities of TMP and the unknown compound in the solutions prepared for storage study as described above were estimated by the external standard method, using TMP as purchased and the unknown compound prepared and isolated as follows:

To a three-neck reaction flask (100 mL), equipped with a thermometer, a condenser, an addition funnel, and a magnetic stirring bar and holding 20 mL of $NH_4OH$ (28% $NH_3$) was added dropwise acetoin (22 g) over 20 min, the temperature being kept below 35 °C by using an ice–water bath. The solution was then gradually brought up to room temperature and stirred for an additional 2 h. The product was extracted with methylene chloride (35 mL × 3), and the extracts were combined and washed with saturated salt water until the water layer became neutral. The extracts were then dried over anhydrous sodium sulfate. The methylene chloride was removed by a rotary evaporator to yield 12 g of a crude mixture, 5 g of which was subjected to column chromatography (silica gel), using methylene chloride and methanol for gradient elution. The fraction isolated by 5% of methanol in methylene chloride was found to contain 4.4 g of the unknown compound with purity of 98.5% after removal of the methylene chloride.

This quantitative estimate was performed on a longer fused silica column (DB-Wax, 60 m × 0.32 mm, 0.15 μm film thickness) under similar chromatographic conditions as described above, except that a flame ionization detector was used. The quantities (milligrams) of TMP and the unknown compound obtained were converted into yield (percent).

## RESULTS AND DISCUSSION

During the 4-week storage period of the solutions prepared with acetoin and different ammonia precursors, physical changes were observed. A brown color was found in all of the solutions during the entire storage period, except the one prepared with $NH_4OH$. In general, the pH values decreased in all of the solutions. It was interesting to note that precipitation took place first in the solution prepared with DAP and last in that prepared with ABC, but no precipitation was found in the solution prepared with $NH_4OH$. GC/MS analyses revealed that two major products were formed, one being TMP and the other an unknown, because no reference spectral data were available. In addition, the MS and the retention index confirmed that the precipitate was TMP. In general, TMP was readily generated in the solutions prepared with DAP, DAC, or AAc, and the unknown compound was readily generated in the solution prepared with $NH_4OH$, while the solution prepared with ABC generated both TMP and the unknown compound. Up to that stage, the results obtained clearly illustrated that TMP was not the only major product from such reaction during storage. Consequently, an attempt was made to identify the unknown compound.

In general, the unknown compound observed from the solutions prepared with acetoin and ammonia precursors showed two peaks very close to each other with the retention indices of 1609 and 1633. The mass spectra of these two peaks are very similar; both possess major fragments *m/z* 112 (76%), 72 (10%), 71 (71%), 45 (18%), 43 (100%), 42 (48%), and 41 (10%) suggesting isomers. To obtain the molecular ion, CI/MS was performed. The M + 1 fragment appeared to be *m/z* 158, implying a molecular weight of 157 and the presence of one or an odd number of nitrogen atoms.

**Table 1. $^{13}C$ NMR Data ($CDCl_3$, TMS) for the Unknown Compound**

| chemical shifts, ppm | assignment |
|---|---|
| 171.72, 172.01, 172.10, 172.30 | −**C**=N− |
| 110.99, 111.05, 111.09, 111.34 | −O−**C**−N− |
| 82.40, 82.61, 83.47, 83.94 | −O−**C**−C=N− |
| 72.23, 72.37, 72.41, 73.02 | −**C**−OH |
| 20.94, 21.20, 22.93, 23.59 | −**C**$H_3$ |
| 18.89, 19.21, 19.98, 20.17 | −**C**$H_3$ |
| 17.17, 17.23, 17.26, 17.32 | −**C**$H_3$ |
| 15.25, 15.30 | **C**$H_3$−C=N− |

Preparative GC left the two peaks of the unknown compound unresolved. The IR spectrum of this GC trap showed a broad band in the 3000–3600 $cm^{-1}$ region, indicating a hydroxy group, while a strong absorption at 1664 $cm^{-1}$ suggested a C=C double or a C=N group. Combination of the IR information of the hydroxy moiety and the mass spectral fragments of *m/z* 112 (M − 45) and *m/e* 45 (a secondary alcohol) implied a −CH(OH)$CH_3$ moiety.

The proton NMR spectrum of this GC trap showed six signals. The signal at 3.66–3.78 ppm (m, 1H) refers to a methine adjacent to the alcoholic group, and the signal at 4.67–4.79 ppm (m, 1H) refers to a methine between two functional groups. Interestingly, both multiplets imply two or more quartets, indicating a mixture of isomers. The signal at 2.01/2.02 ppm (2s, 3H) appears to be due to an allylic methyl of two isomers at least. The signal at 1.41/1.42 ppm (2s, 3H) may be due to a methyl of two isomers at least. The signals at 1.33–1.37 ppm (m, 3H) and at 1.19–1.26 ppm (m, 3H) are two methyls, each of which shows four doublets, indicating four isomers. This proton NMR spectrum shows that probably this unknown compound is a mixture of four isomers.

The $^{13}C$ NMR shows eight carbon signals, of which each signal is composed of four chemical shifts strongly suggesting four isomers (Table 1).

All of the spectral data above lead to the identification of the unknown compound as a mixture of four isomers of 2,4,5-trimethyl-2-(1-hydroxyethyl)-3-oxazoline. As the stereochemistry is considered, there are three chiral centers at C-2, C-5, and the carbon adjacent to the alcoholic group. Theoretically, eight isomers (four pairs of enantiomers) are possible, but the spectra give evidence of only four isomers. The flavor characteristics of this compound have been described as mild aroma, yeasty, nutty, and bread-crust-like.

To study this compound further, two homologous compounds were prepared, one of which was 2,4-dimethyl-2-(hydroxymethyl)-3-oxazoline from acetol and ammonium hydroxide as a single peak ($I_k$ = 1732) on GC and the other 2,4,5-triethyl-2-(1-hydroxypropyl)-3-oxazoline from 4-hydroxy-3-hexanone and ammonium hydroxide as two peaks ($I_k$ = 1813 and 1846) on GC. The mass spectrum of 2,4-dimethyl-2-(hydroxymethyl)-3-oxazoline shows *m/z* 129 ($M^+$, 0%), 98 (93%), 70 (26%), 58 (16%), 57 (39%), 43 (25%), 42 (100%), 41, (12%), 39 (11%), 31 (24%), 29 (40%), and 27 (19%) and the IR bands as broad 3400 $cm^{-1}$ (hydroxy) and 1666 $cm^{-1}$ (C=N). Its proton NMR spectrum ($CD_3OD$, TMS) shows 2.07 ppm (s, 3H) as the allylic methyl group, 4.59 ppm (s, 2H) as the two protons between the double bond and the oxygen, 1.31 ppm (s, 3H) as the methyl group attached to C-2, and 3.53 and 3.58 ppm (2d, distorted,

**Table 2. Yields (Percent) of Products Obtained from the Mixture of Acetoin and DAP during Storage at Room Temperature**

| day | tetramethylpyrazine | 2,4,5-trimethyl-2-(1-hydroxyethyl)-3-oxazoline |
|---|---|---|
| 1 | 5.6 | 51.0 |
| 2 | 11.2 | 28.0 |
| 3 | 16.0 | 23.8 |
| 5 | 23.8 | 17.1 |
| 10 | 36.6 | 5.0 |
| 15 | 42.0 | 1.0 |
| 20 | 42.6 | not found |

2H, $J$ = 11.7 Hz each) as $H_A$ and $H_B$ of methylene adjacent to the alcoholic group due to the chiral center at C-2. Its $^{13}C$ NMR ($CD_3OD$, TMS) clearly shows the chemical shifts at 173.11 (C-4), 111.81 (C-2), 78.27 (C-5), 68.16 (C-OH), 22.54 ($CH_3$ at C-2), and 15.48 ppm ($CH_3$ at C-4).

The mass spectrum of 2,4,5-triethyl-2-(1-hydroxypropyl)-3-oxazoline shows *m/z* 213 ($M^+$, 0%), 154 (54%), 126 (11%), 99 (22%), 57 (100%), 56 (15%), 41 (14%), 31 (11%), and 29 (30%) and the IR bands as broad 3450 $cm^{-1}$ (hydroxy) and 1660 $cm^{-1}$ (C=N). The proton NMR spectrum shows three multiplets, one between 4.51 and 4.59 ppm (1H) as the ring proton at C-5, one between 3.37 and 3.52 ppm (1H) as the methine proton adjacent to the alcoholic group, and another between 2.22 and 2.40 ppm (2H) as the allylic methylene protons at C-4; in addition, the upper field (below 2.00 ppm) shows overlapped multiplets as methyl and methylene protons of the side chains. This NMR spectrum strongly indicates that this compound is also a mixture of four isomers due to the three chiral centers.

The NMR spectra obtained from this study agree with literature information for the basic 3-oxazoline structure (Hua et al., 1992; Weber et al., 1992).

After this series of 3-oxazolines was identified, a stability study on a mixture of acetoin and DAP was initiated. During a 3-week storage study, both tetramethylpyrazine and 2,4,5-trimethyl-2-(1-hydroxyethyl)-3-oxazoline were monitored, quantitative data (yields) being obtained as shown in Table 2.

These data clearly demonstrate that during the 3-week storage, 2,4,5-trimethyl-2-(1-hydroxyethyl)-3-oxazoline (OXZ) was readily formed during the early stage of the reaction, after which its formation declined. In contrast, tetramethylpyrazine (TMP) formation increased more gradually before it leveled out in 15–20 days. At the end of this experiment, TMP remained as the only product, while OXZ was not found. This phenomenon strongly implies that a chemical pathway existed between these two products during the storage period. Figure 1 is the mechanism proposed for the formation of these two products from acetoin and ammonia derived from DAP.

Basically, condensation of acetoin and ammonia generates an α-hydroxyimine and an α-amino ketone by tautomerism, each of which leads to a product. The latter forms TMP via self-condensation, dehydration, and dehydrogenation, as is well-known. The former is proposed to condense with a second molecule of acetoin and then cyclize by Schiff base formation to OXZ. The data obtained from this storage study indicate that at the early stage of this reaction, OXZ was readily formed due to its lower $\Delta G_a$ (free energy of activation), so that the formation of OXZ may be considered to be kinetically controlled. However, as the reaction progressed, the formation of TMP began to increase due to the greater $\Delta G$ (free energy), so that the formation of TMP may be considered to be thermodynamically controlled. The data also suggest that the formation of OXZ is reversible. As a result, the OXZ initially formed was able to be converted back to α-hydroxyimine, which, in turn was tautomerized to α-amino ketone, which, then, followed the first pathway to form TMP.

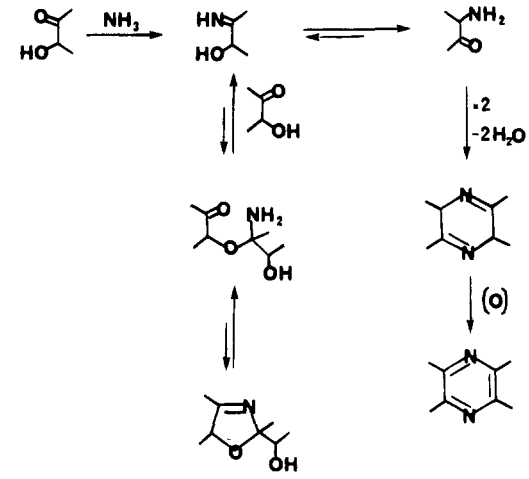


**Figure 1.** Formation mechanism of TMP and OXZ.

ACKNOWLEDGMENT

We thank Dr. Henry L. Chung, Mr. Sterling White, and Ms. Grayland Dobson for assistance with CI/MS, IR, and NMR spectra, respectively.

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Received for review April 10, 1995. Revised manuscript received September 5, 1995. Accepted September 18, 1995.[⊗]

JF9502562

[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1995.