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Identification of 5-Hydroxy-3-mercapto-2-pentanone in the Maillard Reaction of Thiamine, Cysteine, and Xylose

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5-Hydroxy-3-mercapto-2-pentanone is claimed in the scientific literature as a key intermediate in the degradation of thiamine and the related generation of aroma compounds; however, there are no analytical NMR and MS data available. We have identified the compound in a thermally treated mixture of thiamine, cysteine, and xylose and characterized it by MS and NMR.

KEYWORDS: Maillard reaction; thiamine; cysteine; xylose; 5-hydroxy-3-mercapto-2-pentanone; NMR

INTRODUCTION

Thiamine is a key precursor for the generation of meat aroma. In beef bouillon it is responsible for the formation of 2-methyl-3-furanthiol, one of the impact compounds for the meaty odor note (*1*). As an important ingredient in the flavor industry it is used in meat-like thermal process flavorings (*2*−*5*). Van der Linde and co-workers (*6*) found 5-(2-hydroxyethyl)-4-methylthiazole, 4,5-dihydro-2-methyl-3-furanthiol, and 2-methyl-3-furanthiol to be important degradation products of thiamine, and they postulated 5-hydroxy-3-mercapto-2-pentanone as a key intermediate (**Figure 1**). The research group of Güntert was interested in thiamine degradation also and identified a range of new sulfur compounds in the steam-distillate of an autoclaved thiamine hydrochloride solution (*7*). They explain the formation of most of them via 5-hydroxy-3-mercapto-2-pentanone as the key intermediate, which they consider unstable and very reactive. They have not identified this compound, probably due to its instability or because it was not steam-distillable.

There is no scientific publication which reports on the spectroscopic properties of 5-hydroxy-3-mercapto-2-pentanone. Matsukawa and co-workers, in 1948, report on the synthesis of the acetylated title compound, 5-acetoxy-3-mercapto-2-pentanone, which is used as an intermediate in thiamine synthesis but not on the compound itself (*8*). The title compound was later synthesized from 5-hydroxy-2-pentanone, sulfur, and ammonia by Onural (*9*), but the author does not give any details on the procedure, yields, or analytical data. Finally a patent application (*4*) proposes 5-hydroxy-3-mercapto-2-pentanone as an ingredient for meat-like process flavors but does not mention how it was prepared or any analytical data.

To our knowledge the only reference in the scientific literature which mentions the identification of 5-hydroxy-3-mercapto-2-pentanone from degraded thiamine is the work of Matsukawa and co-workers (*10*). The authors report that they identified the compound in the diethyl ether extract of a refluxed aqueous solution of thiamine hydrochloride, based on comparison with a reference compound. However, the publication does not mention how the reference compound was synthesized, how it was compared with the compound, nor any MS or NMR data, techniques which were at their infancy in 1951, or any other analytical data for the compound.

In the present study 5-hydroxy-3-mercapto-2-pentanone was isolated from a model process flavor comprising thiamine, cysteine, and xylose and characterized by MS and NMR techniques.

MATERIALS AND METHODS

Materials. All reagents were of analytical grade. Dichloromethane, hydroxylamine hydrochloride, lithium hydroxide, pyridine, and sodium chloride were from Merck (Darmstadt, Germany). Ammonium chloride, ethyl acetate, and trifluoroacetic acid were from Acros (Morris Plains, NJ). Cysteine, hexamethyldisilazane, anhydrous sodium sulfate, sulfuric acid, thiamine hydrochloride, and xylose were from Fluka (Buchs, Switzerland). Diethyl ether and ethanol were from Carlo Erba (Val de Reuil, France), cyclohexane from Riedel de Haën (Seelze, Germany), and silica gel (32−63, 60 Å) from Brunschwig (Basel, Switzerland).

Model Reaction. Thiamine hydrochloride (25.00 g), cysteine (3.00 g), and xylose (11.00 g) were dissolved in potassium phosphate buffer (750 g, 0.5 mol/L, pH 5.0) and thermally reacted in a stirred pressure reactor (Glass, type 2, 1-L, Büchi, Uster, Switzerland) at 145 °C for 45 min. After cooling to room temperature, the product was saturated with sodium chloride and extracted twice with diethyl ether (300 mL + 200 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated to approximately 1.5 mL using a rotary evaporator (Büchi, Uster, Switzerland). The concentrate was analyzed by GC-MS and used for the isolation of 5-hydroxy-3-mercapto-2-pentanone.

Isolation of 5-Hydroxy-3-mercapto-2-pentanone. The concentrated solvent extract from the model reaction was submitted to flash chromatography on silica gel (20 × 2.5 cm) using cyclohexane/ethyl acetate/ethanol (8 + 1 + 1, v/v) as eluent. Fractions with the target compound, as revealed by GC-MS, were combined, and a spatula of silica gel was added, concentrated to dryness using a rotary evaporator, and again submitted to flash chromatography using the same conditions. The purest fraction after solvent evaporation yielded 20 mg and was analyzed by NMR and GC-MS.

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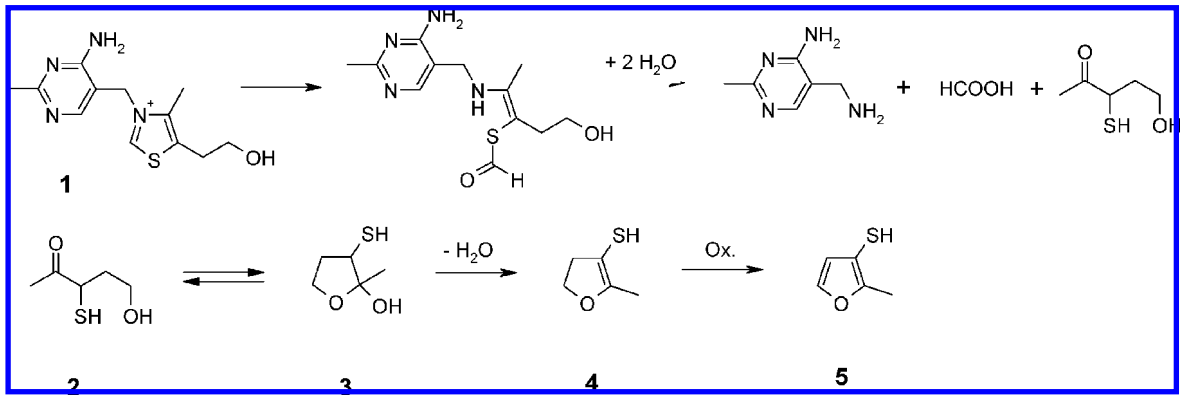

Figure 1. Proposed formation pathway of 2-methyl-3-furanthiol from thiamine via 5-hydroxy-3-mercapto-2-pentanone (6).

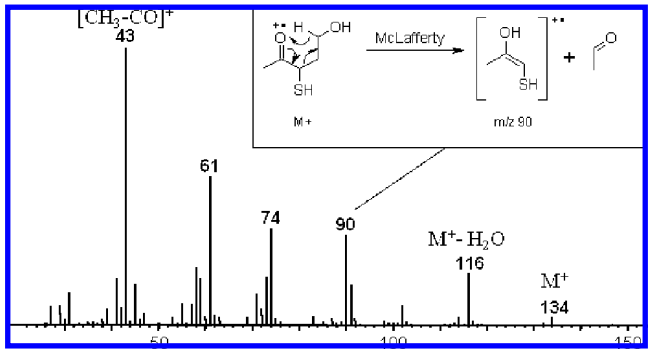

Figure 2. Mass spectrum (EI) of 5-hydroxy-3-mercapto-2-pentanone.

Derivatization of 5-Hydroxy-3-mercapto-2-pentanone. The deuterated solvent was evaporated under a stream of nitrogen from the NMR sample. Then a solution of hydroxylamine hydrochloride in pyridine (500 μL, 2.5% w/v) was added, and the closed vial was heated for 30 min at 70 °C. Hexamethyldisilazane (HMDS, 900 μL) and trifluoroacetc acid (100 μL) were added, and the closed vial was shaken and allowed to stand for 15 min. The derivatized sample was analyzed by GC-MS.

Synthesis of 5-Hydroxy-3-mercapto-2-pentanone. The 5-hydroxy-3-mercapto-2-pentanone was synthesized from 5-(triethylsilyloxy)-3-thioacetyl-2-pentanone, obtained from 3-chloro-5-hydroxy-2-pentanone by triethylsilylation of the hydroxyl group and substitution of the chloride group by a thioacetoxy group. The 5-(triethylsilyloxy)-3-thioacetyl-2-pentanone (26.5 mg) was dissolved in tetrahydrofuran (2.50 mL) and the solution added dropwise at 4 °C to an aqueous solution of lithium hydroxide (5%). After 60 min at 0−4 °C, the mixture was added to an aqueous solution of ammonium chloride (8.9%) at 0−4 °C. The temperature was allowed to climb to 20−25 °C. The mixture was saturated with sodium chloride and extracted with diethyl ether (5

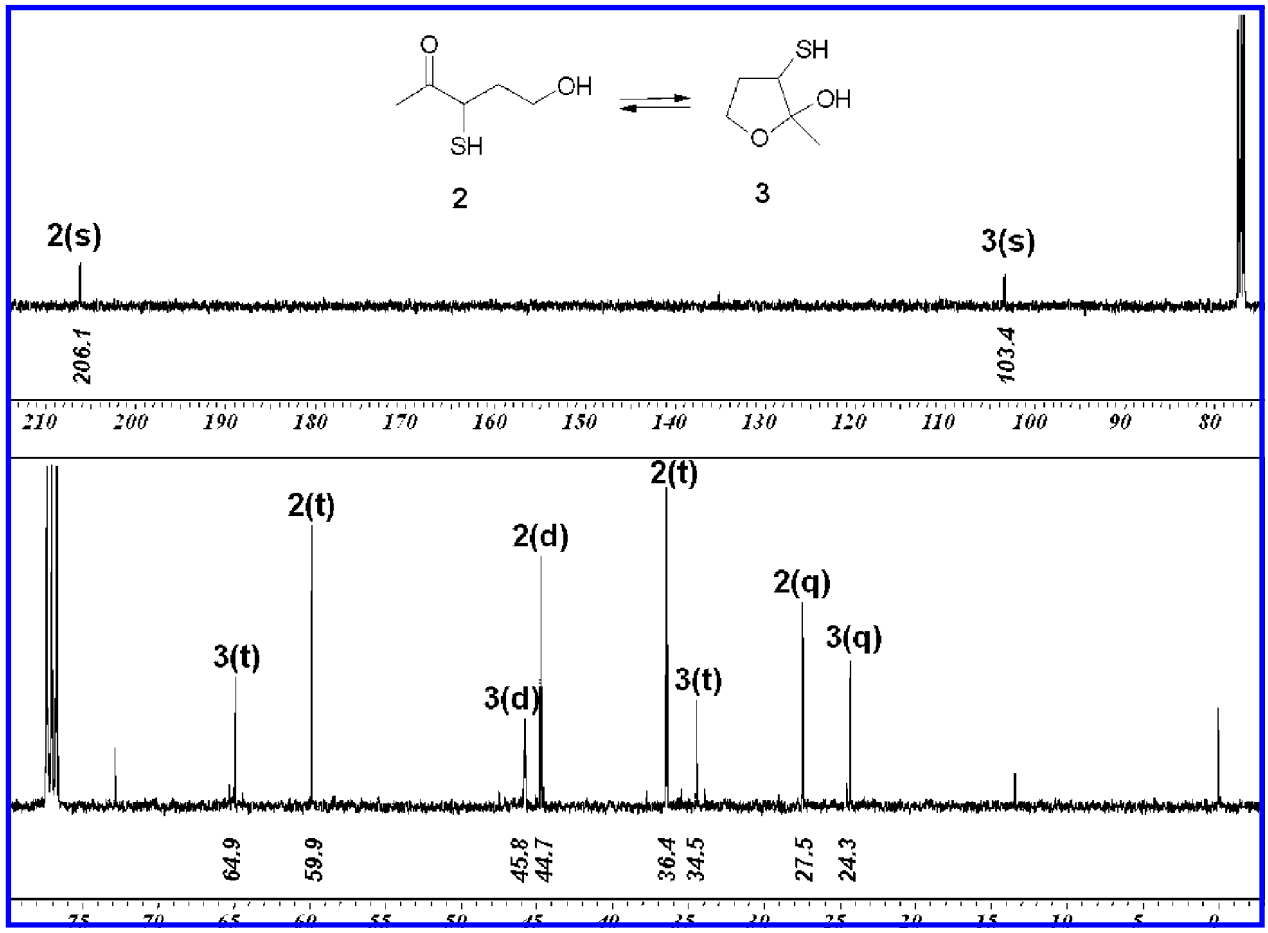

Figure 3. ^{13}C NMR spectrum of 5-hydroxy-3-mercapto-2-pentanone.

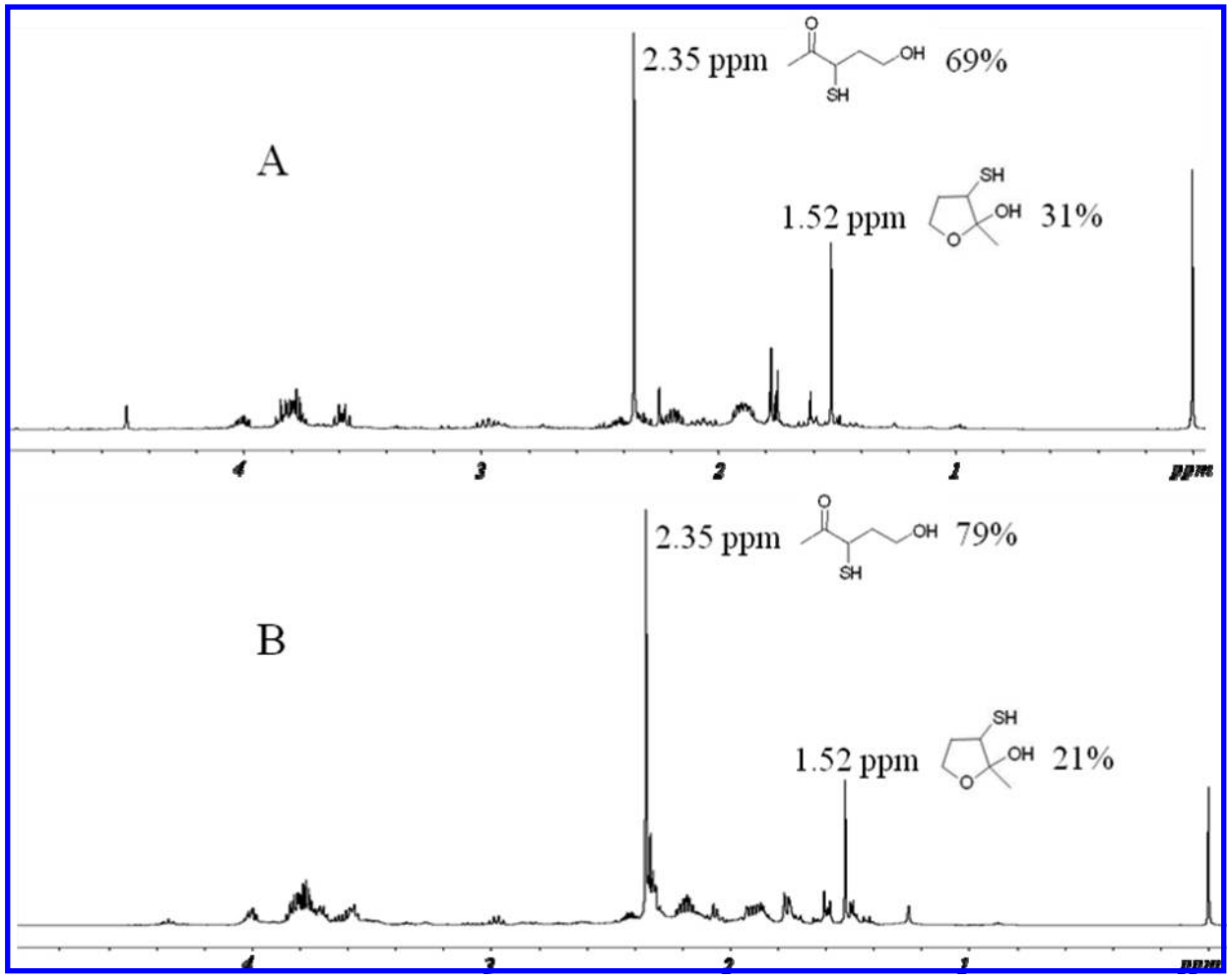

Figure 4. ^{13}C NMR spectrum of 5-hydroxy-3-mercapto-2-pentanone (A, isolated compound; B, synthesized compound).

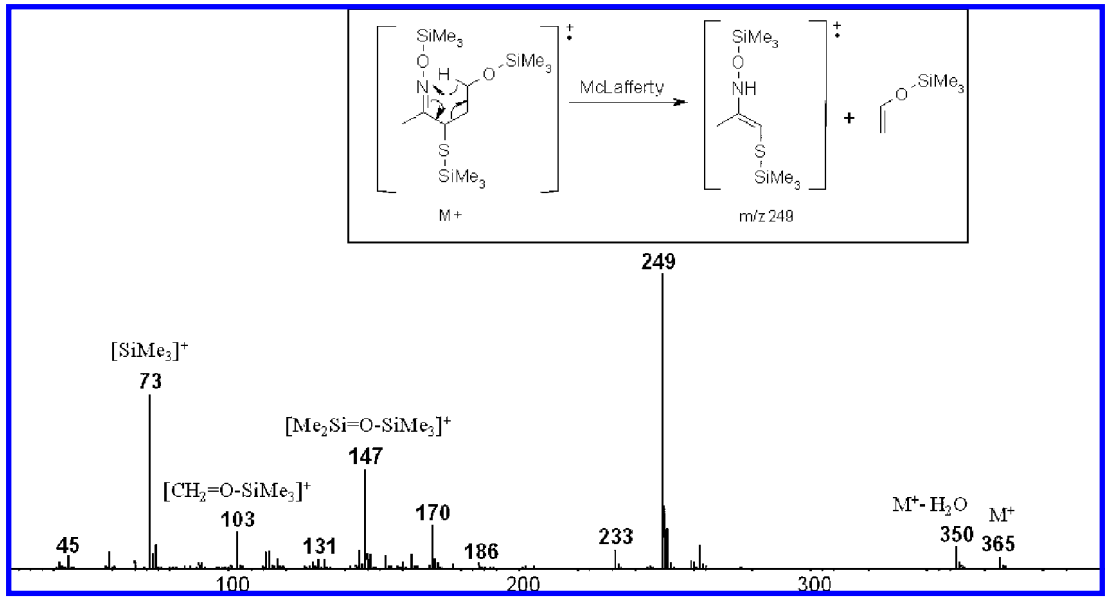

Figure 5. Mass spectrum (EI) of the trimethylsilylated oxime of 5-hydroxy-3-mercapto-2-pentanone.

× 0.50 mL). The aqueous phase was adjusted to pH 4−5 using sulfuric acid (0.5 mol/L) and then extracted with dichloromethane (6 × 2 mL). The organic phase was dried over anhydrous sodium sulfate, and then the solvent was evaporated.

Gas Chromatography. GC-MS analyses were performed on an Agilent GC 6890A coupled to an MSD 5973 using an HP-5MS capillary (30 m × 0.25 mm; film thickness 0.25 μm; Agilent, Palo Alto, CA) and an oven temperature program of 50 °C during 1 min, followed by a gradient of 10 °C/min to 250 °C. Samples (1 μL) were injected using a split injector (1:30). Mass spectra in the electron impact mode (EI) were generated at 70 eV and at a scan range from *m/z* 15−400.

Nuclear Magnetic Resonance Spectra. NMR spectra were recorded in deuterochloroform on a Bruker DPX 400 instrument (Bruker, Fällanden, Switzerland) with tetramethylsilane as internal standard.

RESULTS AND DISCUSSION

In a previous study (*11*) we have shown that thiamine **1** is an important precursor in the formation of the meat-like smelling aroma compound **5** in the reaction between thiamine, cysteine, and xylose. The analysis by HS-SPME-GC-MS revealed 3-mercapto-2-pentanone, 2-furfurylthiol, **4**, and **5** as main peaks in the chromatogram. In the present study thiamine, cysteine, and xylose were reacted using similar reaction conditions, and the resulting product was extracted with diethyl ether. GC-MS analysis of the solvent extract gave two major peaks, but neither of them corresponded to the volatiles mentioned before. One was identified as 5-(2-hydroxyethyl)-4-methylthiazol (sulfurol), a well-known thiamine degradation compound (*6, 10, 12*), and the mass spectrum of the other peak was previously unknown. Supposedly the sulfurol and the unknown compound are not volatile enough or too polar to be detected by SPME under the experimental conditions. Analysis of the spectrum (**Figure 2**) for the unknown analyte showed accordance with the structure of **2**. The signal at *m*/*z* 134 corresponds to the molecular ion M^+. The loss of water, as indicated by *m*/*z* 116, is characteristic for alcohols. Fragment *m*/*z* 43 results from the separation of the acetyl group. The peak at *m*/*z* 90 can be explained by a McLafferty rearrangement with neutral loss of acetaldehyde. The compound was isolated from the solvent extract by flash chromatography and analyzed by GC-MS and NMR. The isolated compound gave the same mass spectrum as the unknown compound in the solvent extract.

Since no literature NMR and MS data were available, **2** was synthesized starting from 5-(triethylsilyloxy)-3-thioacetyl-2-pentanone by cleaving the triethylsilyl and acetyl protection groups. The isolated compound and the synthesized reference compound showed the same mass spectra and the same H NMR and ^{13}C NMR spectra, thus corroborating the identification. The ^{13}C NMR data confirm the proposed chemical structure (**Figure 3**). The following ^{13}C NMR signals (δ/ppm) agree with the chemical structure of **2**: 206.1 (C, C-2); 59.9 (CH_2, C-5); 44.7 (CH, C-3); 36.4 (CH_2, C-4); 27.5 (CH_3, C-1). In addition, ca. 30% of ketal **3**, formed by cyclization of **2**, is present, as suggested by the following ^{13}C NMR signals (δ/ppm): 103.4 (C, C-2); 64.9 (CH_2, C-5); 45.8 (CH, C-3); 34.5 (CH_2, C-4); 24.3 (CH_3). **Figure 4** shows the ^1H NMR spectra of the compound which was isolated from the diethyl ether extract (A) as well as the ^1H NMR spectra of the synthesized reference compound (B). The singlet signals at 2.35 ppm and 1.52 ppm correspond to the methyl protons of the open chain molecule **2** (CH_3, C-1) and of the cyclic form **3** (CH_3), respectively. Integration of the peak areas indicates a share of 69% open chain form in the isolated compound and 79% for the synthesized reference. Because of the presence of one chiral carbon in **2** and two chiral carbons in **3**, in total six isomer compounds can be present. Information on the ratio of the optical isomers was not obtained from the ^1H NMR spectra.

In order to confirm the structure, the isolated compound was derivatized with hydroxylamine followed by trimethylsilylation and analyzed by GC-MS. The resulting mass spectrum (**Figure 5**) corresponds to the expected derivative: The signal at *m*/*z* 365 corresponds to the molecular peak (M^+) and *m*/*z* 350 indicates the loss of CH_3. The base peak at *m*/*z* 249 originates most likely from a McLafferty rearrangement with loss of the neutral enol fragment trimethylsilyloxyethene. The peaks at *m*/*z* 73, 103, and 147 are common fragments for trimethylsilylated compounds.

Hence, **2** could be unambiguously identified in a model process flavor containing thiamine, cysteine, and xylose. It was stable enough to be isolated and characterized. NMR data indicate the presence of both open chain and cyclic ketal form.

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