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Two Novel Thiophenes Identified from the Reaction between Cysteine and 2,5-Dimethyl-4-hydroxy-3(2H)-furanone

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From the reaction between cysteine and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) at 160 °C for 0.5 h at pH 2.2 in a closed system, 24 volatile components are identified including two novel compounds, 3-methyl-2-(2-oxopropyl)thiophene and 2-methyl-3-propionylthiophene. These compounds along with 2,4-hexanedione appear to be the major products from the reaction of cysteine and DMHF, based on a comparison of the results obtained from this system with those previously reported from the individual reactants under the similar conditions. The formation mechanism of these thiophenes is proposed.

INTRODUCTION

Investigations of the reactions of α -dicarbonyls with amino acids, especially sulfur-containing amino acids, have provided insight into the products and possible mechanisms operating during the heating of foods (Ho and Hartman, 1982; Hartman and Ho, 1984; Rizzi, 1969). Products formed from Strecker degradation involving sulfur-containing amino acids are believed to be extremely important to the production of food aroma (Vernin, 1982).

2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF) has been identified in many food sources (Ohloff and Flament, 1979) and used extensively in many flavor areas (Hirvi et al., 1980). The reaction between DMHF and sulfur-containing amino acids would be expected to produce some interesting products chemically and organoleptically and possibly mimic aspects of natural flavor production. In a previous paper (Shu et al., 1985c), the reaction between cysteine and DMHF was reported and two novel thiophenes were identified: 2,5-dimethyl-2,4-dihydroxy-3(2H)-thiophenone and 2,5-dimethyl-2-hydroxy-3(2H)-thiophenone. In the present study, we report the volatile components generated from the reaction between cysteine and DMHF at 160 °C for 0.5 h in a closed system. Background experiments on the thermal degradation of cysteine (Shu et al., 1985a) and thermal degradation of DMHF (Shu et al., 1985b) were previously reported.

EXPERIMENTAL SECTION

A 500-g mixture was prepared from 0.05 mol of cysteine-HCl-H₂O (Ex. Ajinomoto C., Tokyo, Japan), 0.05 mol

of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Ex. International Flavors and Fragrances), and distilled water. The pH of the mixture was measured as 2.2. The mixture was then placed in a 2-L Parr bomb (Parr Instrument Co., Moline, IL) and heated at 160 °C for 0.5 h. The reaction mass was subjected to vacuum steam distillation, extraction, and concentration in that order and the concentrate analyzed by gas chromatography-mass spectrometry (GC-MS) on fused silica columns (OV-1 and Carbowax 20 M) as described previously (Shu et al., 1985a).

In order to isolate and identify the unknown components, 125 mg of the concentrate was separated by column chromatography (10 g of silica gel, 5% H₂O deactivated, methylene chloride-ethyl acetate gradient elution), followed by isolation of the individual components from enriched fractions using gas chromatographic trapping technique (glass capillary columns, 30 m \times 0.62 mm, Carbowax 20M and OV-1). The isolates were then characterized by proton nuclear magnetic resonance (NMR, Varian XL-100, CCl₄, relative to internal standard Me₄Si) and infrared (IR, Perkin-Elmer 397) spectra.

RESULTS AND DISCUSSION

The yield of volatiles obtained from the 0.05-mol reaction of cysteine and DMHF was 180 mg. The odor of the mixture of volatiles was described as roasted, bread crust, and meaty. The GC chromatograms of the volatiles are shown in Figures 1 (OV-1 column) and 2 (CWX column). Table I lists the volatile components identified from this sample as compared to the components identified from the thermal degradations of cysteine in water and DMHF in water, respectively. Peak numbers correspond to those in Figures 1 and 2.

Of the components identified, our attention was drawn to peak 17 (OV-1), being the component present in largest concentration. The mass spectrum of peak 17 (Figure 3) suggested that the molecular weight was 154 with one sulfur atom [the (M + 2) peak was 4% of the M peak]. The strong loss of 43 (m/z 111) suggested the presence of acetyl group. The strong absorption of the IR spectrum

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Table I. Volatile Compounds Identified from the Reaction of Cysteine and 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF) at pH 2.2

compd ident	peak no.		GC area, %	compd ident in therm degradn	
	OV-1	CWX		Cys alone	DMHF alone
Acyclic Carbonyls					
acetaldehyde	1	1			+ ^c
acetone	2	2	13.3		+
methyl ethyl ketone	3	3	T ^b	+	
3-hydroxy-2-pentanone	4	7	1.1		+
2-hydroxy-3-pentanone	5	8	T		+
acetol acetate	6	10	T		+
2,4-hexanedione	7	6	4.7		
2,3-pentanedione		4	T		+
acetoin acetate		9	T		+
Cyclic Carbonyls					
2,5-dimethyl-3(2H)-furanone	8	11	1.4		+
2,4,5-trimethyl-3(2H)-furanone	10	12	T		+
DMHF ^a	11	24	5.8		
2,4-dimethyl-5-ethyl-3(2H)-furanone		14	T		
Sulfur Compounds					
3,5-dimethyl-1,2,4-trithiolane	13	15	3.1	+	
3,5-dimethyl-1,2,4-trithiolane	14	16			+
thienothiophene	16	22	T	+	
3-methyl-2-(2-oxopropyl)thiophene	17	21	23.0		
2-methyl-3-(propionyl)thiophene	18	20	2.0		
1,2,3-trithioa-5-cycloheptene	21	26	10.6	+	
2-methylthiazole		5	T	+	
tetrahydrothiophen-3-one		13	T		
2-acetylthiazole		17	T		
2,5-dimethyl-4-hydroxy-3(2H)-thiophenone	15	25	6.0		
2,5-dimethyl-2,4-dihydroxy-3(2H)-thiophenone	19		T		
2,5-dimethyl-2-hydroxy-3(2H)-thiophenone		27	T		
2-thiophenethiol	9		2.2		

^a Starting material. ^b T = trace. ^c + = identified in.

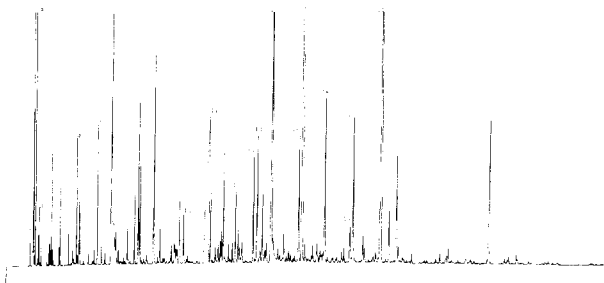


Figure 1. GC profile of the volatiles from the reaction of cysteine and DMHF at pH 2.2. Conditions: column, OV-1 fused silica capillary (50 m × 0.32 mm); temperature, 50–225 °C, programmed at 2 °C/min; detector, flame ionization.

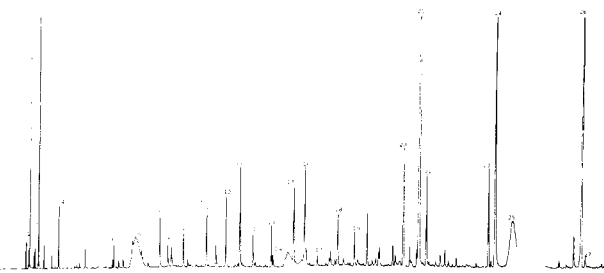


Figure 2. GC profile of the volatiles from the reaction of cysteine and DMHF at pH 2.2. Conditions: column, CWX 20M fused silica capillary (50 m × 0.32 mm); temperature, 50–225 °C, programmed at 2 °C/min; detector, flame ionization.

at 1708 cm⁻¹ suggested an unconjugated carbonyl group. From the proton NMR spectrum, two singlets at δ 2.15 and 2.04 were consistent with an acetyl methyl group and an additional methyl group on a conjugated system. The

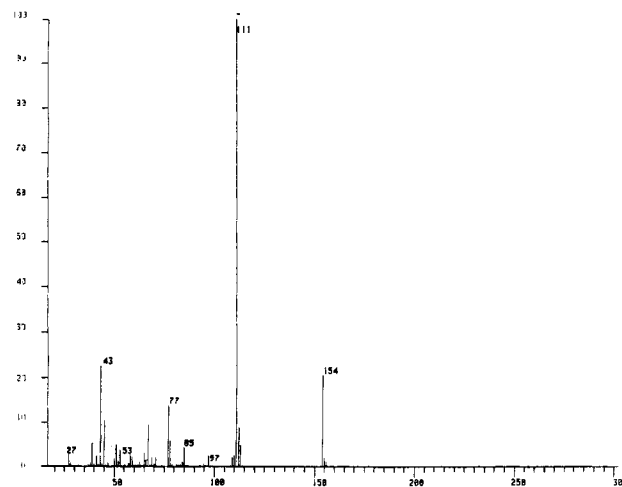


Figure 3. Mass spectrum of peak 17, OV-1 column.

two-proton singlet at δ 3.64 was consistent with a heavily functionalized methylene group, and two strongly coupled signals at δ 6.98 and 6.72 (d, *J* = 6 Hz) suggested two vicinal protons on an aromatic ring. Therefore, based on the combination of all of the spectral data, the structure of peak 17 has been proposed as 3-methyl-2-(2-oxopropyl)thiophene.

The mass spectrum of peak 18 (OV-1) (Figure 4) also suggested a molecular ion of 154 with one sulfur atom, structurally related to the compound of peak 17. Fragment ion *m/z* 125 (*M* - 29) indicated a loss of C₂H₅ and fragment ion *m/z* 97 (*M* - 57) a loss of -COC₂H₅. In the proton NMR spectrum, a quartet was centered at δ 2.8 and a triplet at δ 1.19 in a 2:3 ratio indicated that the ethyl group was in a conjugated system. The singlet (3 H) at δ 2.54

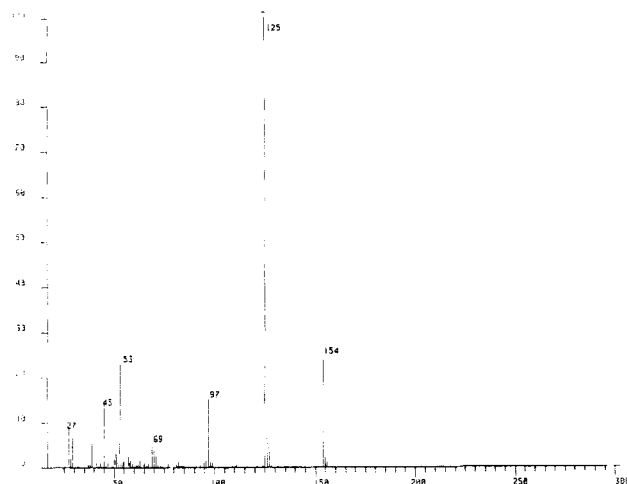


Figure 4. Mass spectrum of peak 18, OV-1 column.

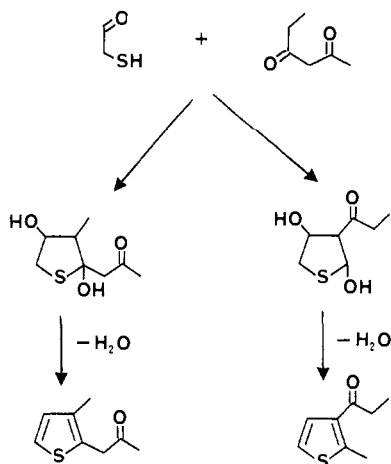


Figure 5. Postulated mechanism of the formations of these two novel thiophenes.

was consistent with aromatic methyl group. The two doublets at δ 6.84 and 7.21 (d, $J = 5$ Hz, 1 H each) are consistent with two protons in a conjugated system. Combining all the information, peak 18 was identified as 2-methyl-3-propionylthiophene.

These two structures can be related mechanistically via the condensation reaction between 2,4-hexanedione and β -mercaptoacetaldehyde as shown in Figure 5. The former was detected in this mixture and could have been derived from DMHF degradation (Shu et al., 1985c); the latter could have been derived from Strecker degradation of cysteine. Previously we reported that degradation of

cysteine at pH 2.3 generated 1,2,3-trithia-5-cycloheptene, trithiolanes, thianes, thiophenethiols, thiazoles, and some carbonyls (Shu et al., 1985a) and degradation of DMHF at pH 2.2 generated carbonyls and furanones (Shu et al., 1985b). Comparison of these degradation products with the products from this experiment reveal that the two novel thiophenes and 2,4-hexanedione are the major reaction products. Two trace components [2,5-dimethyl-2,4-dihydroxy-3(2*H*)-thiophenone and 2,5-dimethyl-2-hydroxy-3(2*H*)-thiophenone] identified in this study were also found as the major products of the reaction between cysteine and DMHF (Shu et al., 1985c).

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Registry No. DMHF, 3658-77-3; AcH, 75-07-0; AcMe, 67-64-1; AcEt, 78-93-3; AcCH(OH)Et, 3142-66-3; MeCH(OH)COEt, 5704-20-1; AcCH₂COEt, 3002-24-2; Me(CO)₂Et, 600-14-6; L-cysteine, 52-90-4; acetol acetate, 592-20-1; acetoin acetate, 4906-24-5; 2,5-dimethyl-3(2*H*)-furanone, 14400-67-0; 2,4,5-trimethyl-3(2*H*)-furanone, 64880-73-5; 2,4-dimethyl-5-ethyl-3(2*H*)-furanone, 74902-72-0; 3,5-dimethyl-1,2,4-trithiolane, 23654-92-4; 3-methyl-2-(2-oxopropyl)thiophene, 96504-26-6; 2-methyl-3-propionylthiophene, 100207-46-3; 1,2,3-trithia-5-cycloheptene, 13005-82-8; 2-methylthiazole, 3581-87-1; tetrahydrothiophen-3-one, 1003-04-9; 2-acetylthiazole, 24295-03-2; 2,5-dimethyl-4-hydroxy-3(2*H*)-thiophenone, 26494-10-0; 2,5-dimethyl-2,4-dihydroxy-3(2*H*)-thiophenone, 96504-28-8; 2,5-dimethyl-2-hydroxy-3(2*H*)-thiophenone, 96504-27-7; 2-thiophenethiol, 7774-74-5.

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