Two Novel 2-Hydroxy-3(2*H*)-thiophenones from the Reaction between Cystine and 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone

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From the reaction between cystine and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) at 160 °C for $^1/_2$ h at pH 2.4 in a closed system, 47 volatile components are identified including two novel compounds, 2,5-dimethyl-2,4-dihydroxy-3(2*H*)-thiophenone and 2,5-dimethyl-2-hydroxy-3(2*H*)-thiophenone. A comparison of the results of individual degradation products of cystine and DMHF under the same conditions revealed that the major reaction products were the two novel thiophenones, 2,5-dimethyl-4-hydroxy-3(2*H*)-thiophenone and 2,4-hexanedione. The formation mechanism of each of these products is proposed. The organoleptic properties of each novel thiophenone are also described.

INTRODUCTION

It is well-known that in food processing the Maillard reaction plays a very important role in the formation of flavor compounds (Hodge, 1967; Hurst, 1972; Kort, 1970; Jurch and Tatum, 1970). The α-dicarbonyl intermediates generated in the Maillard reaction have been shown to be precursors of many flavor compounds and undergo several reactions, namely, Strecker degradation (Schonberg and Moubacher, 1952), retroaldolization (Vernin and Parkanyi, 1982), cyclization and rearrangement (Hodge et al., 1972; Shaw et al., 1968), and recombination reactions (Mauron, 1981).

Cyclization and rearrangement reactions lead to a group of important flavor compounds, cyclic α-dicarbonyls, which typically possess a sweet, caramel, and fruity organoleptic character (Ohloff and Flament, 1979). These α-dicarbonyls may react further with amino acids and/or their degradation products to form various secondary flavor compounds (Kobayasi and Fujimaki, 1965; Rizzi, 1969; Kato et al., 1973; Takken et al., 1976; Ho and Hartman, 1982). DMHF or 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone is an α-dicarbonyl, which has been identified in many food sources (Rodin et al., 1965; Re et al., 1973; Tonsbeek et al., 1968; Takei and Yamanishi, 1974) and used extensively in many flavor areas (Hirvi et al., 1980; Re and Ohloff, 1974). Cystine and other sulfur-containing amino acids are considered important contributors to the formations of various food flavors especially to meat flavors (Hurrell, 1982; Ching, 1979; Fujimaki et al., 1969). The reaction between cystine and DMHF would, therefore, be expected to produce some interesting products chemically and organoleptically. In this study, two novel 2-hydroxy-3-(2*H*)-thiophenones along with other volatile components were generated from reaction at 160 °C for one half hour in a closed system. The results are compared with similar individual thermal degradations of cystine and DMHF previously reported by Shu et al. (1985a,b).

The literature relevant to the present study is very limited. The only available source was a study on the reaction between 5-methyl-4-hydroxy-3(2*H*)-furanone and hydrogen sulfide (van den Ouweland and Peer, 1975).

The heterocycle, 5-methyl-4-hydroxy-3(2*H*)-furanone, was discovered in beef broth (Tonsbeek et al., 1968) and described as a flavor associated with cooked beef. It has received great attention because of its applicability as a flavoring agent. Van den Ouweland and Peer (1975) investigated the possibility that this furanone could act as an intermediate to form additional meat flavors.

They prepared a model system consisting of 5-methyl-4-hydroxy-3(2*H*)-furanone and hydrogen sulfide in an aqueous medium heated at 100 °C for 4 h. The overall aroma generated from this reaction was described as resembling roasted meat.

Their study was really the reaction of 5-methyl-4-hydroxy-3(2*H*)-furanone with H_2S but not with cystine or cysteine. Although hydrogen sulfide can be generated from cystine and cysteine (Obata and Tanaka, 1965; Fujimaki et al., 1969), cystine and cysteine can also produce various additional primary and secondary breakdown products including sulfur and nitrogen compounds (Boelens et al., 1975) which, in turn, could react further with 5-methyl-4-hydroxy-3(2*H*)-furanone to lead a more complex volatile profile and a different aroma. Consequently, their reaction between 5-methyl-4-hydroxy-3(2*H*)-furanone and H_2S is not comparable to the reaction between this furanone and cystine or cysteine. The current paper is the first report pertaining to the study of the reaction between a 4-hydroxy-3(2*H*)-furanone and the sulfur-containing amino acid.

EXPERIMENTAL SECTION

A partially dissolved mixture was prepared from 0.05 mol (12 g) of cystine (Ex. Ajinomoto Co., Tokyo, Japan), 0.05 mol (6.4 g) of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Ex. International Flavors and Fragrances), and 482 g of distilled water. The pH of the mixture was measured as 2.4. The mixture was then placed in a 2-L Parr bomb (Parr Instrument Co., Moline, IL) and heated for $^1/_2$ h. The reaction mass was subjected to vacuum steam distillation, extraction with methylene chloride, and concentration in that order and the concentrate was analyzed by gas chromatography–mass spectrometry (GC–MS) on the fused silica columns as described previously (Shu et al., 1985a,b). The concentrate obtained was 392 mg.

In order to isolate and identify the unknown components, a portion of the concentrate (125 mg) was separated by column chromatography (10 g of silica gel, 5% deactivated, methylene chloride/ethyl acetate gradient elution), followed by isolation of the individual components with gas chromatographic trapping technique (glass capillary columns, 30 M × 0.32 mm, Carbowax 20M and OV-1). The isolates were then spectroscopically characterized by mass spectrum (MS), proton nuclear magnetic resonance (NMR, Varian XL-100, $CFCl_3$, relative to internal standard tetramethylsilane), and infrared (IR, Perkin Elmer 397, neat liquids).

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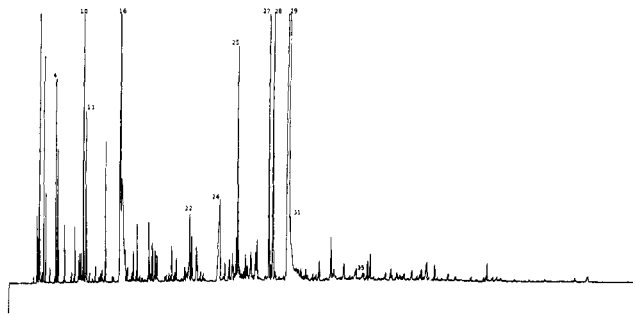

Figure 1. The GC profile of the volatiles from the reaction of cystine and DMHF in water, initial storage (OV-1 column). Column: OV-1 fused silica capillary (50M × 0.32 mm). Temperature: 50–225 °C programmed at 2 °C/min. Detector: flame ionization.

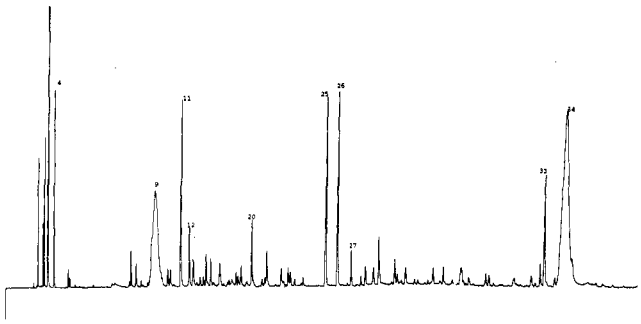

Figure 2. The GC profile of the volatiles from the reaction of cystine and DMHF in water, initial storage (CWX column). Column: CWX. Temperature: 50–225 °C programmed at 2 °C/min. Detector: flame ionization.

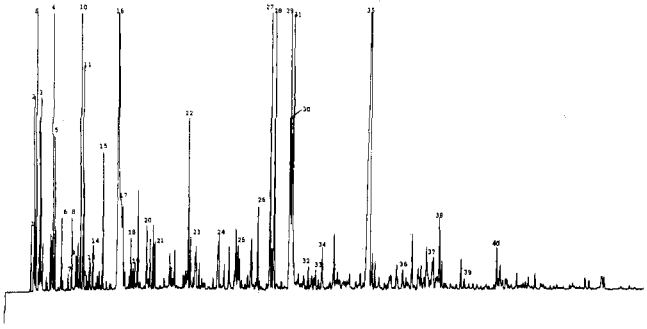

Figure 3. The GC profile of the volatiles from the reaction of cystine and DMHF in water, in storage of two weeks at refrigerated temperature (OV-1 column). Column: OV fused silica capillary (50 M × 0.32 mm). Temperature: 50–225 °C programmed at 2 °C/min. Detector: flame ionization.

RESULTS

The yield of the volatiles obtained from the 0.05 mol reaction mixture in aqueous medium was 392 mg. It was observed that during the storage at refrigerated temperature (4 °C) some components of the sample were unstable and changed considerably in the first two weeks. The GC profiles of the volatiles at the initial storage are shown in Figures 1 and 2 and those at two-week storage in Figures 3 and 4. Practically, it was very difficult to perform an in-depth analysis on this sample due to its instability during the initial storage; therefore, samples were analyzed after a two-week hold period.

Table I lists the volatile components identified from this sample. Peak numbers correspond to those in Figures 1–4. Most of the components including the major components, 3,5-dimethyl-1,2,4-trithiolane isomers, 3-hydroxy-2-pentanone, 2,4-hexanedione, and 2,5-dimethyl-4-hydroxy-3-(2*H*)-thiophenone were identified by GC–MS analysis.

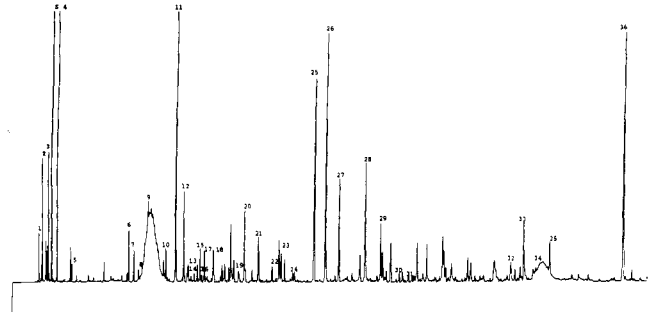

Figure 4. The GC profile of the volatiles from the reaction of cystine and DMHF in water, in storage of two weeks at refrigerated temperature (OV-1 column). Column: CWX. Temperature: 50–225 °C programmed at 2 °C/min. Detector: flame ionization.

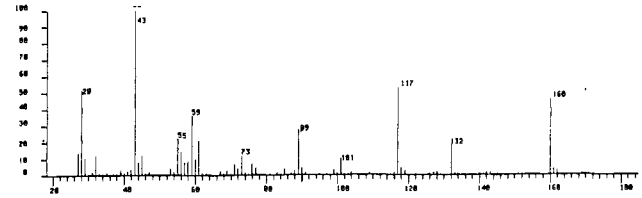

Figure 5. Mass spectrum of peak no. 35 from OV-1 column.

Comparison of the GC profiles between the initial storage and the two-week storage implied that during storage, 2,5-dimethyl-4-hydroxy-3(2*H*)-thiophenone [peak no. 29 on the OV-1 column (Figure 1)] decreased with an increase in peak no. 31 and 35 on the OV-1 column (Figure 3). Consequently, the structures of peak no. 31 and 35 were expected to be related to 2,5-dimethyl-4-hydroxy-3(2*H*)-thiophenone. Through column chromatography, these two unknown components (peaks no. 31 and 35) were enriched and trapped from the GC (OV-1 column).

The mass spectrum of peak no. 35 via a probe is shown in Figure 5, the highest peak suggested the molecular weight as 160. The structure contained one sulfur atom (M + 2 peak was 5% of the M peak) and a carbonyl group (M – 28). The strong absorption of the IR spectrum at 1670 cm^{-1} suggested a conjugated carbonyl group. The strong broad absorption in the region of 3000–3650 cm^{-1} indicated a hydroxy group; no thiol group was shown in the spectrum (2550–2600 cm^{-1}). From the proton NMR spectrum a singlet at δ 2.3 suggested a methyl group on a conjugated system. A sharp singlet at δ 1.72 indicated an additional methyl group. Two hydroxy groups were buried in the high field area. Therefore, the structure (A) in Figure 6 has been assigned to be peak no. 35 as 2,5-dimethyl-2,4-dihydroxy-3(2*H*)-thiophenone.

The mass spectrum of peak no. 31 (Figure 7) indicated that the molecular weight as 144 with one sulfur and a carbonyl group in the structure. The IR spectrum of the peak was very similar to that of 2,5-dimethyl-2,4-dihydroxy-3(2*H*)-thiophenone. It showed a conjugated carbonyl (1675 cm^{-1}) strong hydroxy absorption (3000–3600 cm^{-1}) and lacked a thiol band (2550–2650 cm^{-1}). The NMR spectrum suggested an allylic methyl group as a singlet at δ 2.37 and another methyl group as another singlet at δ 1.7. In addition, a vinyl proton appeared at δ 5.79. The hydroxy group was buried in the high field area. By combining the spectral information, the structure (B) in Figure 6 has been assigned to peak no. 31 as 2,5-dimethyl-2-hydroxy-3(2*H*)-thiophenone.

Apparently, during storage, 2,5-dimethyl-4-hydroxy-3-(2*H*)-thiophenone decreased with an increase of these two thiophenones. This phenomenon strongly suggests that these two thiophenones are derived from 2,5-dimethyl-4-

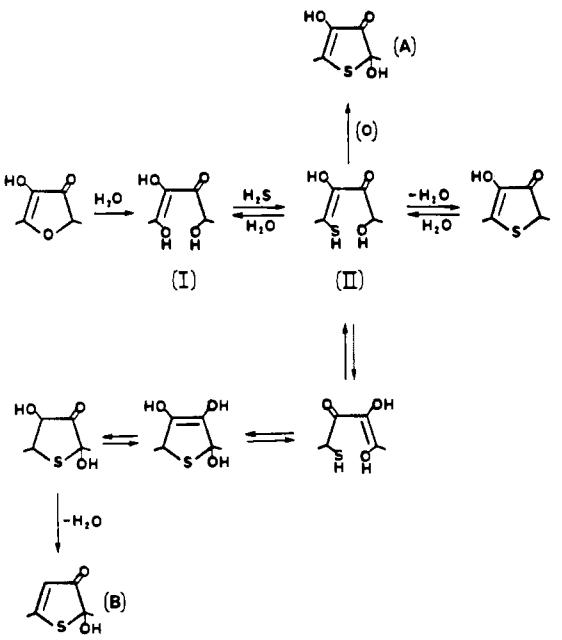

Figure 6. The postulated mechanism, relationship between the three thiophenones.

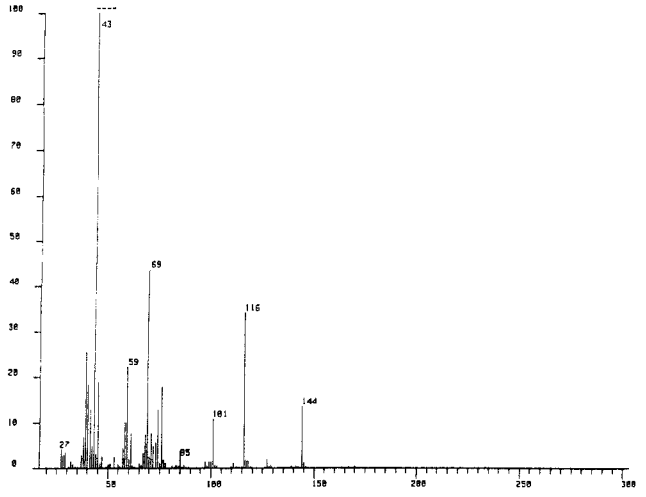

Figure 7. Mass spectrum of peak no. 31 from OV-1 column.

hydroxy-3(2*H*)-thiophenone.

These two structures and the relationship of the three thiophenones can be rationalized mechanistically as shown in Figure 7. Two intermediates (I and II) could be formed from the DMHF reaction with H_2S derived from cystine. Intermediate I was previously proposed by van den Ouweland and Peer (1975). Intermediate II could form 2,5-dimethyl-4-hydroxy-3(2*H*)-thiophenone by thioether formation or 2,5-dimethyl-2,4-dihydroxy-3(2*H*)-thiophenone by oxidation. Also intermediate II could rearrange to 2,5-dimethyl-2-hydroxy-3(2*H*)-thiophenone as described in Figure 6.

Sensory evaluation of 2,5-dimethyl-2,4-dihydroxy-3-(2*H*)-thiophenone had a pot-roasted character in both aroma and taste; 2,5-dimethyl-2-hydroxy-3(2*H*)-thiophenone was described as roasted onion in aroma and taste.

It is interesting to note that 2,5-dimethyl-2,4-dihydroxy-3(2*H*)-thiophenone cannot be eluted from the Carbowax 20M fused silica column.

Previously we reported that degradation of cystine at pH 2.3 generated trithiolanes, thiazoles, thianes, and some carbonyls (Shu et al., 1985b) and degradation of DMHF at pH 2.2 generated carbonyls and furanones (Shu et al., 1985a). Comparison of those degradation data with the present results reveals that thiophenones and 2,4-hex-

Table I. Volatile Components Identified from the Reaction of Cystine and DMHF in Water

compounds identified	peak no.[a] OV-1	CWX	GC area, %
Aldehydes/Ketones			
acetaldehyde	1	1	T
acetone	2	2	T
methyl ethyl ketone	3	3	1.2
2-pentanone	4	4	1.5
3-hexanone	5	5	T
1-hydroxy-2-butanone	7		T
2,4-pentanedione	9		T
3-hydroxy-2-pentanone	10	11	4.2
2-hydroxy-3-pentanone	11	12	1.3
2,4-hexanedione	16	9	16.4
2-ethyl-5-methyl-2-cyclopenten-1-one		23	T
Esters			
acetol acetate	15	20	1.1
2-oxobutyl acetate	21		T
Thiazoles/Isothiazole–Thiazoline			
thiazole	6	7	T
3-methylisothiazole	12	8	T
2,5-dimethylthiazole	19		T
2-acetylthiazole	22	27	1.9
2-thiazolyl ethyl ketone	26	29	1.0
2-methylthiazole		6	T
2,4,5-trimethylthiazole		14	T
2-*n*-propylthiazole		15	T
2,4-dimethyl-3-thiazoline		16	T
2,5-dimethyl-4-ethylthiazole		18	T
2-methyl-5-ethylthiazole	23	17	T
Thiols			
1-mercapto-2-propanone	8		T
3-mercapto-2-pentanone	17	13	T
Pyrazine			
2,5-dimethylpyrazine	18	10	T
Furanones			
2,5-dimethyl-4,5-dihydro-3(2*H*)-furanone	13		T
2,5-dimethyl-4,5-dihydro-3(2*H*)-furanone	14		T
2,5-dimethyl-3(2H)-furanone	20	21	T
DMHF[b]	24	33	1.6
2,4,5-trimethyl-3(2*H*)-furanone		22	T
2,4-dimethyl-5-ethyl-3(2*H*)-furanone		24	T
Thiolanes/Thianes			
3,5-dimethyl-1,2,4-trithiolane	27	25	3.7
3,5-dimethyl-1,2,4-trithiolane	28	26	4.9
3-methyl-1,2,4-trithiane	34		T
4,6-dimethyl-1,2,3,5-tetrathiane	37		T
4,6-dimethyl-1,2,3,5-tetrathiane	38		T
3,6-dimethyl-1,2,4,5-tetrathiane	39	32	T
i-methyl-1,3-dithiolane		19	T
Thiophenes			
2-acetyl-5-methylthiophene	32		T
3-methyl-2-(2-oxopropyl)thiophene	33		T
4,5-dimethyl-2-acetylthiophene	36		T
2-acetylthiophene		30	T
3-methyl-2-thiophene carboxaldehyde		31	T
Thiophenones			
2,5-dimethyl-4-hydroxy-3(2*H*)-thiophene	29	34	22.5
2,5-dimethyl-2-hydroxy-3(2*H*)-thiophenone	31	36	
2,5-dimethyl-2,4-dihydroxy-3(2*H*)-thiophenone	35		
Unknowns			
unknown 1, M_r 128	25		T
unknown 2, M_r 157	30	28	2.3
unknown 3, M_r 183	40	35	T
methylene chloride (solvent)	S	S	

[a] T = trace, less than 1%. [b] Starting material.

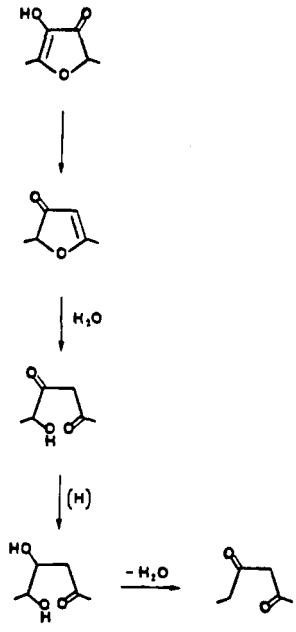

Figure 8. The possible formation of 2,4-hexanedione.

anedione are the major products from the thermal reaction of DMHF and cystine. The formation of 2,4-hexanedione is postulated as shown in Figure 8. The intermediate, 2,5-dimethyl-3(2*H*)-furanone, which was formed in degradation of DMHF (Shu et al., 1985a) as well as in the reaction of cystine and DMHF, leads to 2,4-hexanedione only when cystine is present. Therefore, cystine must play decisive role in the reduction to form 2,4-hexanedione.

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Registry No. DMHF, 3658-77-3; cystine, 56-89-3; acetaldehyde, 75-07-0; acetone, 67-64-1; methyl ethyl ketone, 78-93-3; 2-pentanone, 107-87-9; 3-hexanone, 589-38-8; 1-hydroxy-2-butanone, 5077-67-8; 2,4-pentanedione, 123-54-6; 3-hydroxy-2-pentanone, 3142-66-3; 2-hydroxy-3-pentanone, 5704-20-1; 2,4-hexanedione, 3002-24-2; 2-ethyl-5-methyl-2-cyclopenten-1-one, 78210-64-7; acetol acetate, 592-20-1; 2-oxobutyl acetate, 1575-57-1; thiazole, 288-47-1; 3-methylisothiazole, 693-92-5; 2,5-dimethylthiazole, 4175-66-0; 2-acetylthiazole, 24295-03-2; 2-thiazolyl ethyl ketone, 43039-98-1; 2-methylthiazole, 3581-87-1; 2,4,5-trimethylthiazole, 13623-11-5; 2-*n*-propylthiazole, 17626-75-4; 2,4-dimethyl-3-thiazoline, 60755-05-7; 2,5-dimethyl-4-ethylthiazole, 32272-57-4; 2-methyl-5-ethylthiazole, 19961-52-5; 1-mercapto-2-propanone, 24653-75-6; 3-mercapto-2-pentanone, 67633-97-0; 2,5-dimethylpyrazine, 123-32-0; 2,5-dimethyl-4,5-dihydro-3(2*H*)-furanone, 64026-45-5; 2,5-dimethyl-3(2*H*)-furanone, 14400-67-0; 2,4,5-trimethyl-3(2*H*)-furanone, 96504-23-3; 2,4-dimethyl-5-ethyl-3(2*H*)-furanone, 96504-24-4; 3,5-dimethyl-1,2,4-trithiolane, 23654-92-4; 3-methyl-1,2,4-trithiane, 43040-01-3; 4,6-dimethyl-1,2,3,5-tetrathiane, 96504-25-5; 3,6-dimethyl-1,2,4,5-tetrathiane, 67411-27-2; 2-methyl-1,3-dithiolane, 5616-51-3; 2-acetyl-5-methylthiophene, 13679-74-8; 3-methyl-2-(2-oxopropyl)thiophene, 96504-26-6; 4,5-dimethyl-2-acetylthiophene, 66587-69-7; 2-acetylthiophene, 88-15-3; 3-methyl-2-thiophenecarboxaldehyde, 5834-16-2; 2,5-dimethyl-4-hydroxy-3(2*H*)-thiophenone, 26494-10-0; 2,5-dimethyl-2-hydroxy-3(2*H*)-thiophenone, 96504-27-7; 2,5-dimethyl-2,4-dihydroxy-3(2*H*)-thiophenone, 96504-28-8.

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