

As with peak 28, the presence of a P - 18 peak in the ms and an ir absorption band at  $3400\text{ cm}^{-1}$  confirmed peak 29 as an alcohol.

The absence of an nmr absorption in the region  $\delta$  3.5-4.0, which would correspond to the hydrogen on the hydroxyl-containing carbon, excludes the possibility of peak 29 being a nonallylic secondary alcohol. The splitting of the side-chain alkyl methyl group and the secondary splitting of the vinyl proton at  $\delta$  6.15, caused by the methine proton at the ring-side chain juncture, excludes the possibility of peak 29 being a tertiary alcohol. As in the case of peak 28, we are left with only two possible structures for peak 29, A and B, as shown previously. Structure B can be eliminated since we do not have an nmr absorption near  $\delta$  4.1-4.4, corresponding to an allylic proton on the hydroxyl-containing carbon. Structure B would also preclude the similarity in the mass spectra of peaks 28 and 29.

Obviously, the only difference between peaks 28 and 29 is the cis and trans relationship between the hydroxyl group and the side chain. This difference is responsible for the shifting of the hydrogen on the hydroxyl-containing carbon from its expected absorption at  $\delta \sim 4.4$  to a position farther downfield which was obscured by impurities. Cis and trans assignments have been made on the corresponding monoterpene alcohols (*cis*- and *trans*-yabunikeol) by Fujita *et al.* (1970) based on gc retention data and selected ir information. Accordingly, we would expect the trans isomer (peak 28) to elute before the cis isomer (peak 29) under our gc conditions. We feel this assignment is substantiated by the nmr since, in the case of peak 29, there is a downfield shift of  $\delta$  0.2 for one of the exocyclic methylene protons. This shift in  $\delta$  can be explained by the deshielding effect of the hydroxyl oxygen when it is brought into closer proximity to one of the methylene protons as is the case in the *cis* vs. *trans* isomers. To the best of our knowledge, peaks 29 and 28 are two novel compounds which we are giving the names *cis*- and *trans*- $\beta$ -sesquiphellandrol [5-(1,5-dimethyl-4-hexenyl)-2-methylene-3-cyclohexenol] from the parent hydrocarbon  $\beta$ -sesquiphellandrene.

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**Supplementary Material Available.** Figures showing ir and nmr spectra of peak 28, *trans*- $\beta$ -sesquiphellandrol, and the ir spectrum of peak 29, *cis*- $\beta$ -sesquiphellandrol, will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche ( $105 \times 148$  mm,  $24\times$  reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for \$3.00 for photocopy or \$2.00 for microfiche, referring to code number JAF-75-499.

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## Components Contributing to Beef Flavor. Volatile Compounds Produced by the Reaction of 4-Hydroxy-5-methyl-3(2H)-furanone and Its Thio Analog with Hydrogen Sulfide

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Reaction of 4-hydroxy-5-methyl-3(2H)-furanone or its thio analog with  $\text{H}_2\text{S}$  produces a complex mixture of compounds with an overall odor resembling that of roasted meat. The major components of the product mixtures have been isolated

by gas chromatography, and many of the components have been identified by infrared and mass spectrometry. The compounds identified included mercapto-substituted furan and thiophene derivatives.







The flavor of cooked meat is considered to be partly produced by sugar-amino acid reactions. Morton *et al.* (1960) patented reactions of cysteine and other amino acids with sugars producing a flavor with a basic meat

character. Since then much research effort has been spent on studying the volatile compounds that contribute to beef flavor; Herz and Chang (1970) reviewed the literature covering this topic. Reactions between amino acids and reducing sugars can account for many of the compounds reported.

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Table I. Compounds Formed in the Reaction of 4-Hydroxy-5-methyl-3(2H)-furanone with Hydrogen Sulfide

GLC peak	Structure	Name	Mass spectrometry;										Principal infrared bands, cm <sup>-1</sup>	Odor at GLC exhaust										
			<i>m/e</i> (upper), intensity %		(lower)																			
1 <sup>a</sup>		2-Methylthiophene	99	98	97	69	58	53	45	39	7	55	100	6	5	8	21	13	3075, 2955, 2925, 2860, 1780, 1443, 1240, 1167, 1080, 1045, 850, 690, 510	Onion-like, gasoline-like				
2		2-Methyl-2,3(or 2,5)-dihydrothiophene	102	100	85	65	59	45	41	39	4	81	98	28	100	45	24	46		Cabbage-like				
3 <sup>a</sup>		4-Mercapto-2-methylfuran	114	113	85	71	69	59	53	45	43	100	50	60	45	36	35	44	41	56	85	2950, 2920, 2850, 1585, 1518, 1510, 1440, 1387, 1225, 1195, 1123, 1088, 1018, 940, 888, 730	Green, meaty, herbaceous	
4 <sup>a</sup>		3-Mercapto-2-methyl-4,5-dihydrofuran	116	84	83	73	71	60	45	43	42	41	70	22	20	36	19	24	55	100	20	20	2960, 2920, 2890, 2860, 1740, 1663, 1635, 1480, 1435, 1400, 1380, 1365, 1220, 1060, 1030, 980, 960, 905, 680	Roasted meat
5 <sup>a</sup>		1-Methylthio-2-butanone	120	118	100	85	84	61	57	55	2	26	3	26	13	76	100	89					2970, 2935, 2915, 1705, 1455, 1434, 1420, 1408, 1374, 1346, 1320, 1210, 1190, 1105, 1050, 1030, 988, 970, 808, 685, 500, 402	Mushroom-like
6		3-Hydroxy-2-methyl-tetrahydrofuran	102	73	57	55	45	43	31	29	27	15	91	98	16	40	85	56	100	45				Fatty
7		4-Mercapto-3-oxo-tetrahydrofuran	120	118	85	61	57	43	41	39	4	39	32	95	100	66	47	25					2980, 2940, 2560, 1715, 1460, 1415, 1380, 1365, 1115	Green, meaty, maggi <sup>b</sup> -like
8 <sup>a</sup>		4-Mercapto-2-methyl-2,3-dihydrothiophene	132	117	99	97	85	84	59	58	45	41	100	95	58	37	36	38	28	100	42		2960, 2920, 2860, 2510, 1540, 1445, 1425, 1400, 1375, 1260, 1205, 1080, 930, 825, 790, 720, 680	Rubbery, meaty
9 <sup>a</sup>		4-Mercapto-5-methyl-3-oxotetrahydrofuran	132	117	100	99	85	59	58	45	43	41	20	100	12	25	40	14	30	12	27			Meaty, maggi <sup>b</sup> -like
10 <sup>a</sup>		3-Mercapto-2-methylthiophene	132	130	129	97	85	71	70	69	59	45	9	100	50	71	19	15	10	24	29	58	3100, 3070, 2950, 2910, 2850, 2540, 1520, 1432, 1375, 1340, 1180, 1153, 1085, 1015, 915, 870, 853, 700, 593, 470	Roasted meat
11		3-Mercapto-2-methyl-2,3-dihydrothiophene	133	130	129	117	97	85	60	59	43	41	62	60	55	47	65	55	67	94	35	52		Sweet, roasted meat

			(Starting material)				
12 <sup>a</sup>		4-Hydroxy-5-methyl-3(2H)-furanone					Rubbery
13 <sup>a</sup>		4-Mercapto-2-methylthiophene	132 130 129 97 90 85 61 45 41 8 100 52 79 65 26 30 64 95				Meaty, savoury
14		3-Mercapto-4-hydroxy-2-methyl-2,3-dihydrothiophene	150 149 148 116 114 85 59 43 41 10 10 80 10 100 30 30 40 90				Roasted meat
15 <sup>a</sup>		4-Mercapto-2-methyl-4,5-dihydrothiophene	132 131 103 97 71 67 59 45 43 41 48 41 31 58 45 35 35 85 100 53				Popcorn-like
16 <sup>a</sup>		4-Hydroxy-5-methyl-3(2H)-thiophenone	132 131 130 71 60 59 58 57 46 45 43 41 5 6 100 6 5 42 5 6 5 7 8 6				Meaty
17 <sup>a</sup>		3-Mercapto-2-methyl-4,5-dihydrothiophene	132 131 99 98 97 71 65 59 58 45 100 29 90 28 34 23 19 70 18 41				

<sup>a</sup> Structures of these compounds confirmed by comparison of their spectra with those of synthesized reference compounds. <sup>b</sup> Commercially available vegetable-meat extract named after inventor.

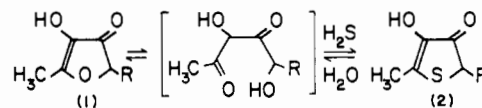


Figure 1. Initial stage in the reaction of 3(2H)-furanones with hydrogen sulfide (R = H, CH<sub>3</sub>).

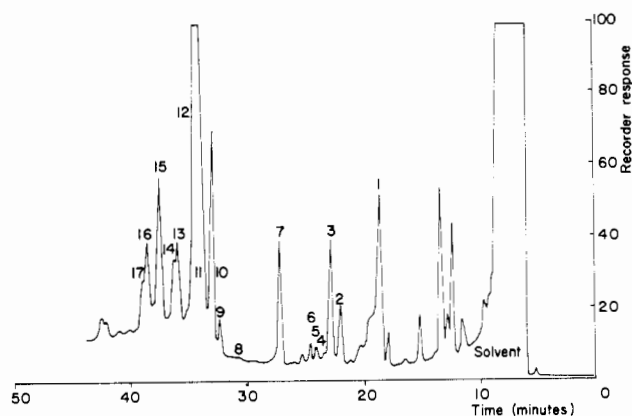


Figure 2. Gas chromatogram of the volatile compounds formed in the reaction of 4-hydroxy-5-methyl-3(2H)-furanone with hydrogen sulfide.

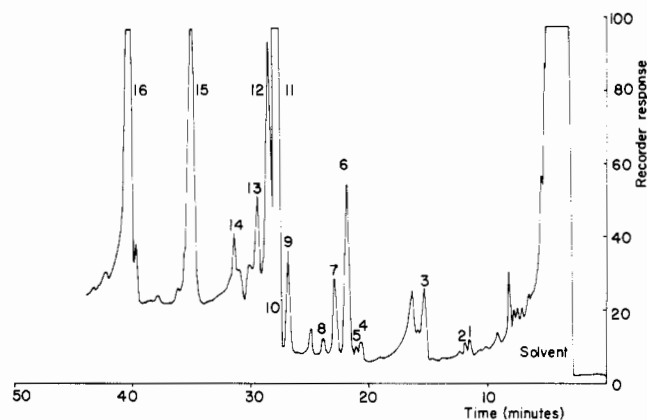


Figure 3. Gas chromatogram of the volatile compounds formed in the reaction of 4-hydroxy-5-methyl-3(2H)-thiophenone with hydrogen sulfide.

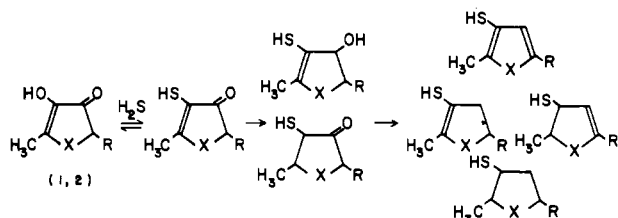
Tonsbeek et al. (1968) identified 4-hydroxy-5-methyl-3(2H)-furanone (1, R = H in Figure 1) and its 2,5-dimethyl homolog (1, R = CH<sub>3</sub> in Figure 1) in a natural beef broth and these compounds are also considered to be sugar degradation products. Both dihydrofuranone derivatives contribute to the flavor of beef broth, but in view of their relation to sugar degradation, it is interesting to know whether these compounds also act as intermediates in the formation of other meat flavor compounds.

Reaction of the dihydrofuranone derivatives with cysteine indeed resulted in the formation of an interesting roasted-meat flavor. Flavor analysis of these reaction mixtures showed that an important part of the meat flavor formed is due to trace constituents whose identification proved to be difficult. We could establish that the initial stage in the reaction between the dihydrofuranones and cysteine involves a partial but incomplete substitution of the ring oxygen by sulfur to give the thio analog 2. This reaction presumably proceeds via the intermediate 2,4-diketone which, upon reaction with H<sub>2</sub>S and subsequent ring closure, would give compound 2 as shown in Figure 1. Thus, in this reaction, cysteine acts as an H<sub>2</sub>S

Table II. Compounds Formed in the Reaction of 4-Hydroxy-5-methyl-3(2H)-thiophenone with Hydrogen Sulfide

GLC peak	Structure	Name	Mass spectrometry; <i>m/e</i> (upper), intensity % (lower)	Principal infrared bands, $\text{cm}^{-1}$	Odor at GLC exhaust
1 <sup>a</sup>		2-Methylthiophene	See peak 1 of Table I	See peak 1 of Table I	Paraffinic
2 <sup>a</sup>		2,3-Pentadione	100 57 43 42 10 35 100 7		Butter-like
3		2-Methylidihydrothiophene	See peak 2 of Table I	See peak 2 of Table I	Cabbage-like
4		2,3-Dimercaptobutane	124 122 89 88 61 3 25 20 10 100		
5		4-Mercapto-3-oxo-tetrahydrofuran	120 118 100 89 85 61 57 2 32 7 9 26 80 100		Maggi <sup>b</sup> -like
6 <sup>a</sup>		2-Methyl-3-oxo-tetrahydrothiophene	118 116 101 88 74 60 59 46 45 5 100 7 24 63 95 66 95 85	2980, 2930, 2870, 1735, 1448, 1400, 1370, 1270, 1198, 1150, 1115, 1040, 983, 827, 450, 415	Acetylenic
7		2-Butyl-5-methyl-furan	138 123 96 95 81 41 39 60 55 100 63 70 40 30		Green
8		2-(2'-Hydroxybutyl)-5-methylfuran	154 121 96 81 43 41 39 10 22 58 30 100 42 20		Green
9 <sup>a</sup>		4-Mercapto-2-methyl-tetrahydrothiophene	134 86 85 84 61 59 49 45 43 41 100 42 46 62 36 38 88 45 46 52	2958, 2920, 2860, 2540, 1450, 1435, 1375, 1268, 1205, 1182, 1030, 1000, 940, 735, 715, 700	Meat-like
10 <sup>a</sup>		4-Mercapto-5-methyl-3(2H)-furanone	132 131 130 129 98 97 96 85 59 52 51 50 10 14 100 46 10 51 10 14 12 39 36 27	2920, 2850, 2530, 1510, 1435, 1375, 1175, 1090, 890, 855, 715	Sweet, meat-like
11 <sup>a</sup>		3-Mercapto-2-methyl-thiophene	See peak 10 of Table I	See peak 10 of Table I	Roasted meat
12 <sup>a</sup>		3-Mercapto-4-hydroxy-2-methyl-2,3-dihydrothiophene	See peak 14 of Table I	See peak 14 of Table I	Phenolic
13 <sup>a</sup>		<i>cis</i> -3-Mercapto-2-methyl-tetrahydrothiophene	134 85 74 69 59 55 47 32 19 38 20 27 28 28	2950, 2920, 2860, 1455, 1440, 1375, 1312, 1260, 1200, 1170, 960	Meaty
14 <sup>a</sup>		<i>trans</i> -3-Mercapto-2-methyl-tetrahydrothiophene	134 74 69 67 59 55 47 45 41 39 82 70 54 35 26 48 26 36 100 45	2960, 2920, 2860, 1450, 1440, 1370, 1330, 1270, 1200	Meaty, savory
15 <sup>a</sup>		4-Hydroxy-5-methyl-3(2H)-thiophenone	See peak 16 of Table I	See peak 16 of Table I	Popcorn-like
16 <sup>a</sup>		4-Mercapto-5-methyl-3(2H)-thiophenone	148 147 146 145 132 130 129 114 113 100 99 98 97 11 10 100 20 15 40 15 28 35 16 18 13 43 85 71 69 61 60 59 58 57 55 53 47 46 45 40 25 10 12 25 95 30 10 20 15 10 12 63	3100, 2970, 2920, 2860, 2530, 1730, 1660, 1560, 1445, 1388, 1370, 1260, 1200, 1140, 1080, 860, 790, 730	Nutty

<sup>a</sup> Structures of these compounds confirmed by comparison of their spectra with those of synthesized reference compounds. <sup>b</sup> See footnote b to Table I.



**Figure 4.** Postulated pathways to components with a meat flavor from the reaction of 3(2*H*)-furanone ( $X = O$ ) or -thiophenone ( $X = S$ ) derivatives ( $R = H, CH_3$ ) with hydrogen sulfide.

donor (formation of H<sub>2</sub>S from meat during cooking is well known).

In an attempt to identify the odorous components formed during the reaction between the dihydrofuranones and cysteine, we decided to investigate the reactions of the compounds 1 ( $R = H$ ) and 2 ( $R = H$ ) with an excess of H<sub>2</sub>S. We used a much higher concentration of H<sub>2</sub>S than is normally obtained during the preparation of a natural beef broth in order to complete reactions and to obtain as much material as possible.

#### EXPERIMENTAL SECTION

4-Hydroxy-5-methyl-3(2*H*)-furanone (1,  $R = H$ ) (0.5 g) was dissolved in water (30 ml) and allowed to react with H<sub>2</sub>S (15 g) for 4 hr at 95–100° in a glass-lined autoclave. At the end of the reaction period the mixture was cooled, poured into ice-water (100 ml), and extracted five times with dichloromethane. The combined extracts (125 ml) were concentrated at atmospheric pressure to 10 ml and analyzed by GLC on a 600 × 0.4 cm glass column with Diatoport S as support. The stationary phase was 10% Aziepon L–1% Carbowax 20M. The carrier gas flow rate was 40 ml of nitrogen/min at 60°. The flame ionization detector temperature was 200° and column temperature was programmed from 60 to 220° at 5°/min. The gas chromatograph was fitted with an effluent splitter which directed 10% to the flame ionization detector and the remainder to the exhaust. From this exhaust the various microgram samples could be trapped and their mass and ir spectra were obtained according to the method described by Copier and van de Maas (1967).

4-Hydroxy-5-methyl-3(2*H*)-thiophenone (2,  $R = H$ ) was treated with H<sub>2</sub>S, worked up, and analyzed in exactly the same way as described for 1 ( $R = H$ ), except that the column temperature was programmed from 60 to 220° at 4°/min.

#### RESULTS AND DISCUSSION

Figures 2 and 3 represent the GLC patterns of the volatile compounds formed from the reaction of H<sub>2</sub>S with compound 1 ( $R = H$ ) and 2 ( $R = H$ ), respectively. The numbered peaks in the chromatograms correspond to the identified compounds, which are listed in Tables I and II. The odors detected upon elution of the components at the column exhaust are also given in these tables. From these data it may be postulated that formation of the odorous components proceeds according to the pathways illustrated in Figure 4.

The complex reactions occurring upon reactions of a vicinal diketone with H<sub>2</sub>S, e.g. thiocarbonyl formation, desulfurization, dehydration, and reduction, are also described by Mayer (1970).

The GLC retention times and odor assessments of various components isolated from reaction mixtures described above can also be ascribed to trace components of a natural beef broth. It may therefore be assumed that the furanone derivative 1 ( $X = O$ ), besides contributing as such to the general background of cooked meat flavor, is also involved in the formation of trace components responsible for meaty and other flavor notes.

From the many components isolated so far from cooked meat, no single compound has been identified with an aroma typical of meat. In the present paper evidence is given that from the reaction of the furanone derivative 1 ( $X = O$ ) with H<sub>2</sub>S a number of compounds are formed with a meaty odor. Because the dihydrofuranone derivative 1 ( $X = O$ ) is formed from ribose 5-phosphate via a dephosphorylation–dehydration reaction it is interesting to remark that the compounds which do have a meaty aroma are probably *not* formed via a Maillard-type reaction. However, none of the compounds with a meaty odor reported here have as yet been identified in meat, and their discovery remains a challenge to the flavor chemist with modern analytical tools.

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