

Environmental Health, Mutagenesis Branch, Research Triangle Park, N.C., for the IR spectra of AME and AOH and for samples of the compounds and Hugo Hein, Jr., for assistance in the purification and separation of the compounds from pecan extracts.

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Received for review June 11, 1976. Accepted August 30, 1976.

Formation of Sulfur- and Nitrogen-Containing Compounds from the Reaction of Furfural with Hydrogen Sulfide and Ammonia

Seventeen compounds produced from the reaction of furfural, hydrogen sulfide, and ammonia in aqueous solution under cooking conditions were identified by a GC-MS technique. Major reaction products were cyclic methylene polysulfides and furan derivatives. The formation of oxazole and pyrazine derivatives indicated that furfural produced a smaller number of carbon units. The formation of thiophene and pyrrole derivatives indicated that the oxygen atom in the furan ring exchanged with the sulfur and nitrogen atoms. In addition to those compounds, two self-condensation products of furfural, difurylethylene and furil, were obtained in large amounts from this reaction.

The author has recently studied volatile chemicals generated in a D-glucose-hydrogen sulfide-ammonia heating system (Shibamoto and Russell, 1976), and identified many compounds which are associated with cooked meat flavors; they were thiols, sulfides, thiophenes, thiazoles, and furans. Most compounds identified in this system have also been found in cooked foods (Stoll et al., 1967; Kinlin et al., 1972). Quantitative analysis of these D-glucose-hydrogen sulfide-ammonia reaction products showed that this system produced a large amount of furfural (GC peak area % = 5.64, the third largest constituent following 2-methylthiophene and 2-acetylfuran). Furfural has been well known as a product from sugar caramelization (Hodge, 1967). Also, May (1960) obtained products which possess meat-like flavor from the reaction of cysteine or cystine with furan compounds including furfural.

It was noticed that there were some compounds which could be obtained by the reaction of furfural and hydrogen sulfide in this D-glucose-hydrogen sulfide-ammonia reaction mixture: methyl thiofuroate, 2-furylmethanethiol, and methyl furfuryl sulfide. Bruins (1929) obtained difurylethylene from the reaction of furfural and hydrogen sulfide in an aqueous solution with 18% yield. van den Ouweland and Peer (1975) reported ten thiophene derivatives produced from the reaction of 4-hydroxy-5-methyl-3(2H)-furanone, which had been identified in beef broth, and hydrogen sulfide. They suggested that an oxygen atom in a furanone ring was exchanged with a sulfur atom of hydrogen sulfide to produce thiophene derivatives. Rizzi (1974) reported the formation of N-alkyl-2-acetylpyrroles from the reaction of furfural and acetylfuran with α -amino acids. He proposed the exchange of an oxygen atom in the furan ring of acetylfurans and a nitrogen atom of amino acid to yield acylalkylpyrroles. Following from their studies, furfural was reacted with hydrogen sulfide and ammonia to investigate the formation

of sulfur- and nitrogen-containing compounds under the same conditions used for the D-glucose-hydrogen sulfide-ammonia heating system. If this system creates any of the same compounds which have been found in the sugar-hydrogen sulfide-ammonia model system or in some other food systems, furfural could be a flavor precursor as well as a sugar caramelization product or a flavor ingredient.

EXPERIMENTAL SECTION

Materials. Commercially obtained furfural was distilled at 95 °C (65 mmHg) before the reaction. All other chemicals and authentic samples were obtained commercially or were gifts from Ogawa & Co., Ltd., Tokyo, Japan, and were used without further treatment.

Sample Preparation. Furfural (9.6 g; 0.10 mol) was mixed with 100 ml of deionized water and hydrogen sulfide gas was bubbled through this solution at 0 °C for 10 min in a Kjeldahl flask (~0.02 mol of H₂S). Ammonium hydroxide solution (0.50 mol as NH₃) was then added to the above solution. The neck of the flask was flame-sealed and the flask placed in an oven at 100 °C for 2 h. Approximately 5 g of brown polymeric material was obtained from the reaction mixture which possessed sweet roast beef-like odor. After this polymeric material was removed by filtration, the reaction products were extracted from the filtrate with 200 ml of methylene chloride using a liquid-liquid continuous extractor for 16 h. The methylene chloride solution was dried over anhydrous MgSO₄ and concentrated to yield 0.5 g of a brown liquid. The identification of products was conducted following the GC-MS technique described previously (Shibamoto and Russell, 1976).

RESULTS AND DISCUSSION

The compounds identified from the reaction of furfural with hydrogen sulfide and ammonia are listed in Table I and their gas chromatogram is shown in Figure 1. The

Table I. Compounds Identified from the Reaction of Furfural with Hydrogen Sulfide and Ammonia

Peak no.	Compounds	Odor description	Occurrence in foods	MS ref
1	2,4-Dimethyloxazole	Nutty, sweet		Porter and Bladas (1971)
2	2-Methylpyrazine	Nutty, roasted	Coffee, ^a popcorn ^b	Bondarovich et al. (1967)
3	α -Furyl cyanide	Sweet, pungent		IMSD (1969)
4	Furfural	Sweet, bread-like	Coffee, ^c popcorn ^b	Stoll et al. (1967)
5	2-Thiophenecarboxaldehyde	Sweet, coconut-like	Coffee, ^c filberts ^d	Stoll et al. (1967)
6	Furfuryl alcohol		Coffee, ^c popcorn, ^b peanuts ^e	Stoll et al. (1967)
7	Unknown			
8	2-Furylmethanethiol	Coffee-like	Coffee ^c	Stoll et al. (1967)
9	2-Acetylfuran	Tobacco-like	Coffee ^c	Stoll et al. (1967)
10	1,2,4-Trithiolane	Sulfurous		Morita and Kobayashi (1967)
11	Difurylethylene	Fatty		Stenhagen et al. (1974a)
12	2-Furfurylfuran	Caramelic	Coffee ^c	Stoll et al. (1967)
13	Unknown			
14	s-Trithiane	Pungent, sulfurous	Chicken ^f	Stenhagen et al. (1974b)
15	2-Pyrrolicarboxaldehyde	Corny, pungent	Popcorn, ^b cocoa beans ^g	Kinlin et al. (1972)
17	1,2,4,6-Tetrathiepane	Pungent, rubber-like	Mushroom ^h	Morita and Kobayashi (1967)
18	Unknown			
19	5-Methyl-2-pyrrolicarboxaldehyde	Pungent	Peanuts, ^e filberts ^d	Kinlin et al. (1972)
20	N-Furfuryl-2-aminofuran		(tentative)	
21	Difurfuryl sulfide	Toasted	Coffee ^c	Stoll et al. (1967)
22	Furyl furfuryl ketone		(tentative)	
23	Unknown			
24	Unknown (M ⁺ 268)			
25	Unknown (M ⁺ 268)			
26	Furil	Mild, sweet		Stenhagen et al. (1974c)
27	Unknown (M ⁺ 266)			
28	Unknown (M ⁺ 190)			

^a Bondarovich et al. (1967). ^b Walradt et al. (1970). ^c Stoll et al. (1967). ^d Kinlin et al. (1972). ^e Walradt et al. (1971). ^f Minor et al. (1965). ^g van Praag et al. (1968). ^h Morita and Kobayashi (1967).

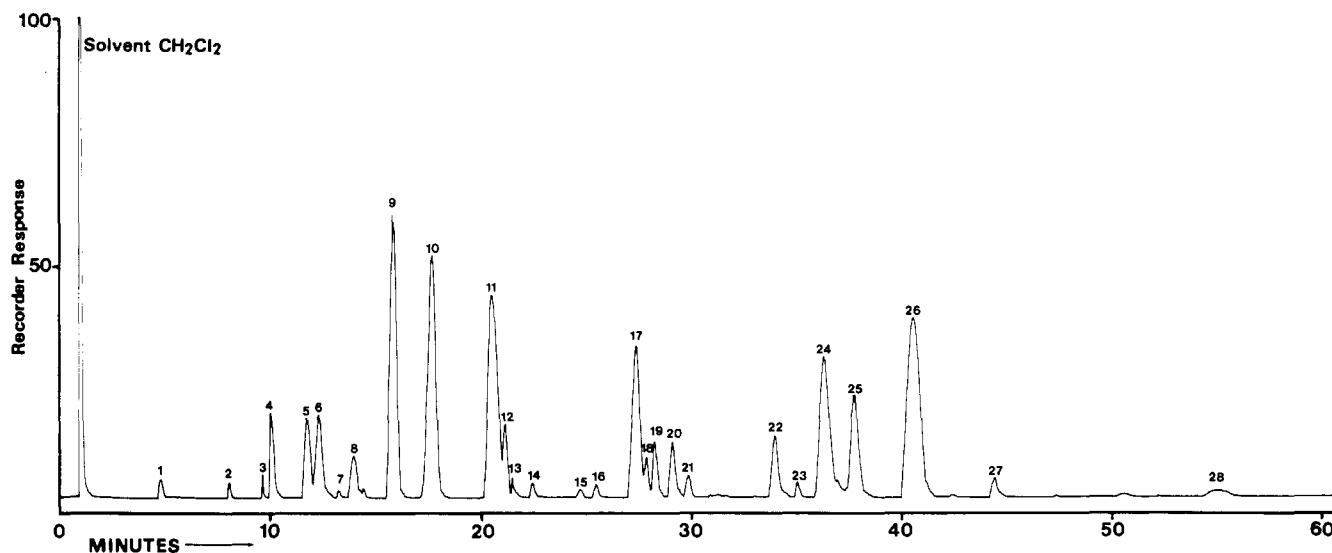


Figure 1. Gas chromatogram of compounds produced from the reaction of furfural with hydrogen sulfide and ammonia. A Varian Aerograph series 1200 gas chromatograph equipped with a flame ionization detector and 3 m \times 3 mm i.d. glass column packed with 5% OV-17 on 80-100 mesh Chromosorb W was used. The column temperature was programmed from 70 to 170 $^{\circ}$ C at 2 $^{\circ}$ C/min. Nitrogen carrier gas flow was 30 ml/min. The injector temperature was 200 $^{\circ}$ C and the detector temperature was 250 $^{\circ}$ C.

formation of 2-thiophenecarboxaldehyde and 2-pyrrolicarboxaldehyde indicated that the oxygen atom of the furan ring exchanged with either a sulfur atom of hydrogen sulfide or a nitrogen atom of ammonia. The formation of 2,4-dimethyloxazole, 2-methylpyrazine, and cyclic methylene polysulfides indicated that furfural fragmented into a small number of carbon units and reacted with hydrogen sulfide or ammonia. Degradation of furfural may produce formaldehyde which reacts subsequently with hydrogen sulfide to form 1,2,4-trithiolane, s-trithiane, and 1,2,4,6-tetrathiepane (Yasumoto et al., 1971; Minor et al., 1965). The other compounds obtained from this reaction were furan derivatives, the major portions of which were

products of reaction at the aldehyde group of furfural (e.g., difurfurylethylene, furil). From these results one may conclude that furfural can act as a precursor of heterocyclic flavor compounds obtained from food and model systems.

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Received for review June 14, 1976. Accepted October 7, 1976.

Effect of pH on the Hydrolysis of Chlorothalonil

Aqueous solutions of chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) were prepared at 0.5 ppm, buffered at pH 5 to 9, and stored in the dark. No hydrolysis was observed at pH 7 or lower. At pH 9, chlorothalonil hydrolyzed to 4-hydroxy-2,5,6-trichloroisophthalonitrile and 3-cyano-2,4,5,6-tetrachlorobenzamide. The rate of decline of chlorothalonil followed first-order kinetics and was determined to be 1.8% per day using gas chromatographic and radiotracer techniques.

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) is the active ingredient in the fungicide Bravo commercially marketed in the United States by the Diamond Shamrock Corporation. It was first registered in 1969 for the effective control of pathogens affecting potatoes and later for use on turf, ornamentals, tree crops, and vegetables. Vincent and Sisler (1968), Turner and Battershell (1970), and Tillman et al. (1973) found the diverse fungicidal activity of chlorothalonil to be attributed to its action as an alkylating agent for cellular thiols. The action and fate of chlorothalonil in biological systems were investigated by Long and Siegel (1975).

Ballee et al. (1976) developed analytical procedures to determine residues of chlorothalonil in crops and soil. Upon analysis of field samples, it was shown that chlorothalonil does not accumulate in soil. Its rate of decline was affected by the presence of moisture. In this study the conditions under which the hydrolysis of chlorothalonil occurs are investigated as well as the nature of the products of hydrolysis.

MATERIALS AND METHODS

[¹⁴C]Chlorothalonil, uniformly labeled in the ring, was synthesized with a specific activity of 0.026 mCi/mmol. Radiochemical purity, determined by thin-layer chromatography, was 99.3%. Separate benzene solutions of [¹⁴C]chlorothalonil and nonradioactive chlorothalonil were prepared at concentration levels of 100 and 50 µg/ml, respectively.

Clark and Lubs pH 5.0, 7.0, and 9.0 buffer mixtures were prepared as described in Lange's Handbook of Chemistry (1949).

To each of three 500-ml glass-stoppered Erlenmeyer flasks, 3 ml of the stock nonradioactive chlorothalonil solution was added and evaporated free of benzene under a stream of dry nitrogen. To the sample to be stored at a basic pH level, the equivalent of 5.5 µg of radiolabeled chlorothalonil was added. A 6-ml volume of the appro-

priate buffer was added to each of the three flasks. To each flask was then added 294 ml of sterile distilled water resulting in a final volume of 300 ml. The approximate final concentrations of the samples were 0.50 ppm for the pH 5 and 7 samples and 0.52 ppm for the pH 9 sample. The flasks were sealed and covered with aluminum foil to prevent any exposure to light, shaken 2 h, and stored at room temperature.

All samples were shaken on an automatic shaker for 30 min prior to any sampling. Periodic analyses were conducted by acidifying 10-ml portions of each aqueous solution with 10 drops of 1:1 sulfuric acid and partitioning with 20 ml of isopropyl ether. A 10-ml portion of the ether extract was evaporated to dryness, diluted to a known volume with benzene, and analyzed using a gas-liquid chromatograph equipped with an electron capture detector and using a 6% DC-200 Chrom-Q column at 225 °C. Thus, the decline of chlorothalonil with time at the three pH levels was determined.

The sample stored at pH 9 was analyzed utilizing radiotracer techniques to determine the fate of chlorothalonil under the described conditions after 89-days storage. To determine any losses due to volatility, duplicate 1.0-ml portions of each aqueous sample were transferred to scintillation vials, 10 ml of Aquasol was added, and the samples were counted using a Searle Analytic ambient temperature counter.

To determine the formation of any water-soluble, nonpartitioned hydrolysis products, a known portion of the aqueous pH 9 sample was transferred to a separatory funnel, acidified with 10 drops of 1:1 sulfuric acid, and partitioned with 30 ml of isopropyl ether. Duplicate 1.0-ml portions of each phase were counted for radioactivity to determine the effect of storage time upon the partitioning of chlorothalonil residues. All data were corrected for background and counting efficiency.

Characterization of radioactivity partitioned into the organic phase was accomplished by concentrating a known