Volatile Flavor Compounds Produced by Heat Degradation of Thiamine (Vitamin B_1)

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Thiamine hydrochloride solutions were buffered at pH 6.7 with a phosphate buffer and heated in a boiling water bath for 15 minutes. Hydrogen sulfide, 2-methylthiophene, 2-methylfuran, and 4,5-dihydro-2-methylthiophene were identified as volatile heat-degradation products of thiamine on the basis of chemical, gas chromatographic, and mass spectral data. Possible degradation schemes, as well as possible flavor implications, are discussed.

The destruction of thiamine (vitamin B_1) by heat under neutral or alkaline conditions has been well established. Booth (1943) demonstrated the marked effect of pH on the heat stability of thiamine. However, investigations related to the heat degradation of thiamine have dealt principally with the loss of biological activity of the vitamin. The identity of the heat-degradation products of thiamine has not been established.

The production of volatile flavor compounds was observed when solutions of thiamine in phosphate buffer at pH 6.7 were heated. An odor resembling that of heated or boiled milk was noted initially, while an odor similar to that of stewing chicken was noted after a few minutes of heating. The rapid detection of these odors suggested that the compounds produced were reasonably volatile. The identity of the volatile compounds resulting from the heat degradation of thiamine thus became a matter of interest.

EXPERIMENTAL

U.S.P. grade thiamine hydrochloride was recrystallized from methanol, and the crystals were washed with ethanol and ethyl ether. Thiamine solutions (0.05*M*) were prepared and buffered to pH 6.7 with 0.1*M* phosphate buffer.

Ten-milliliter quantities of the thiamine solutions were placed in screw-cap vials sealed with Teflon-lined caps and heated for 15 minutes in a boiling water bath. Bills and Keenan (1967) showed that volatile components were retained in these tubes when heated.

The heated thiamine solutions were cooled with tap water. Volatile components of the solutions were separated by gas-liquid chromatography (GLC) using the gas entrainment, on-column trapping technique of Morgan and Day (1965). The samples were submerged in a 60° C. water bath and purged with nitrogen for 10 minutes at 10 ml. per minute. A 1/8-inch o.d. × 12-foot stainless steel column packed with 20% 1,2,3-tris(2-cyanoethoxy)propane on 80- to 100-mesh Celite 545 was employed. The column flow was 25 ml. per minute, and the column temperature was 70° C.

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Control samples consisted of unheated 0.05*M* solutions of thiamine at pH 6.7 and 0.05*M* solutions of thiamine at pH 3.5 heated as previously described. Since thiamine is stable to heat at pH 3.5, peaks appearing in the chromatograms of heated pH 6.7 solutions but not in the unheated or heated pH 3.5 solutions were attributed to volatile heat-degradation products of thiamine.

Volatile compounds produced by the heat degradation of thiamine were collected from the GLC column effluent in 0.76-mm. i.d. × 15-cm. stainless steel capillary traps. Trapped components were subsequently analyzed by capillary column GLC in conjunction with mass spectrometry (MS). Trapped components were transferred directly onto the capillary column using the on-column transfer technique of Scanlan, Arnold, and Lindsay (1967). The effluent from the capillary column was directed to an Atlas CH-4 mass spectrometer equipped with a double ion source. Mass spectra were recorded with a Honeywell 1508 Visicorder. Operating parameters for the capillary GLC-MS combination were as follows.

Gas-Liquid Chromatography

Column	300 foot × 0.01 inch i.d. coated with Carbowax 20 M
Column temperature	80° C.
Carrier gas flow rate	1 ml. per minute

Mass Spectrometry

Filament current	20 e.v. source, 45 μa. 70 e.v. source, 12 μa.
Electron voltage	20 and 70 e.v.
Accelerating voltage	3000 volts
Multiplier voltage	1.6 kv.
Analyzer pressure	1.5×10^{-6} torr
Scanning speed	5 seconds from *m/e* to *m/e* 250

RESULTS AND DISCUSSION

The distinctive odor of hydrogen sulfide was noted when tubes containing 0.05*M* thiamine (pH 6.7) were opened following heating. Filter paper soaked with a solution of lead acetate turned black when held over one of these opened tubes. Further evidence of hydrogen sulfide was obtained during the purge step of the gas entrainment, on-column trapping technique. When the effluent end of the GLC column was unhooked from the detector, the odor of hydrogen sulfide was noted during purging of heated pH 6.7 thiamine solutions.

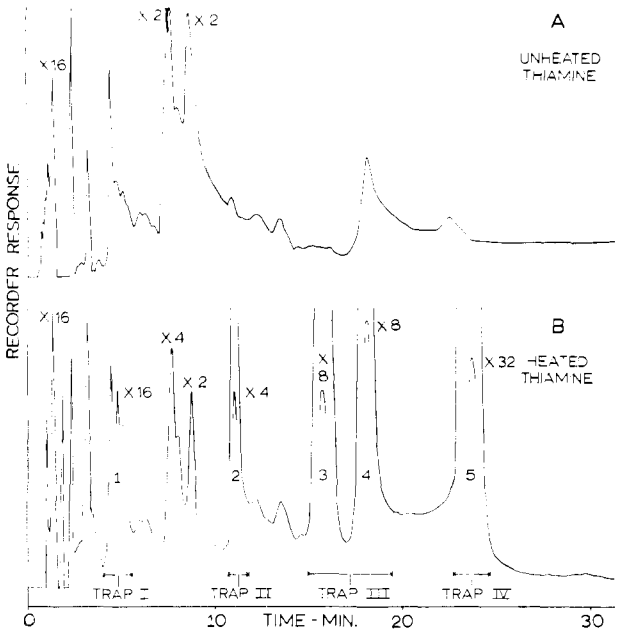

Figure 1. Chromatograms by the gas entrainment, on-column trapping technique from thiamine solutions, pH 6.7

Bubbling the effluent through a 5% solution of zinc acetate produced a white precipitate, suggesting that hydrogen sulfide was being purged from the sample but not being stopped by the cold trap section of the column. No lead acetate reaction or precipitate formation with zinc acetate was noted with unheated or heated acidified (pH 3.5) thiamine solutions. Therefore, hydrogen sulfide was concluded to be a heat-degradation product of thiamine.

Chromatograms obtained from heated and unheated 0.05*M* thiamine solutions at pH 6.7 by the gas entrainment, on-column trapping technique are shown in Figure 1. Five new or augmented peaks, 1 through 5, appear in the chromatogram of heated thiamine. Components responsible for these peaks were collected in traps I through IV, as indicated in Figure 1.

The mass spectral data obtained for peaks 1, 3, 4, and 5 are presented in Table I. The component responsible for peak 2 could not be recovered in sufficient concentration by trapping to obtain a mass spectrum.

The mass spectrum of peak 1 is in good agreement with the published spectrum of 2-methylfuran (American Petroleum Institute, 1948 *et seq.*). The ratios of the retention time of 2-methylfuran to that of the unknown component were 1.00 and 1.01, respectively, on the tris and Carbowax columns. Peak 1 was therefore concluded to be 2-methylfuran.

The mass spectrum of peak 3 could not be interpreted as due to any one compound of molecular weight 86. Therefore, it was concluded to be due to an unresolved mixture of compounds.

The mass spectrum of peak 4 is in good agreement with the published mass spectrum of 2- or 3-methylthiophene (Budzikiewicz *et al.*, 1964). The ratios of the retention times of 2-methylthiophene and 3-methylthiophene to that of the unknown component on the two columns were as follows.

	Tris Column	Carbowax Column
2-Methylthiophene	1.00	1.01
3-Methylthiophene	1.30	1.17

Peak 4 was therefore concluded to be 2-methylthiophene.

No standard spectrum similar to the mass spectrum of peak 5 could be located. The large parent (*P*) and *P*-1 fragments, together with significant *m/e* 39, 45, 58, and 59 fragments suggested that the unknown had considerable thiophene character (Budzikiewicz *et al.*, 1964). The fact that the molecular weight was two mass units higher than that of 2-methylthiophene (peak 4) suggested that the unknown was a similar compound with one less double bond. The large *m/e* 85, indicating the ready loss of a methyl group, is also consistent with a partially reduced methylthiophene structure.

None of the dihydro-2- or 3- methylthiophenes were readily available from chemical suppliers. Birch and McAllan (1951) had shown that reduction of 2- or 3-methylthiophene with sodium in liquid ammonia–methanol resulted in unreduced methylthiophene, 2,5- and 4,5-dihydromethylthiophenes, and pentanethiols. The unreduced and dihydromethylthiophenes were recovered in the neutral fraction by extraction with isopentane.

This reaction, as described by Birch and McAllan (1951), was carried out with 2- and 3-methylthiophene to determine if any of the reaction products had a retention time similar to that of the unknown component. GLC of the neutral reaction products revealed three major components from each reduction—reduced

Table I. Mass Spectra Obtained for Peaks 1, 3, 4, and 5 of Figure 1

GLC Peak	Mol. Wt.		Mass Spectral Data									
1	82	*m/e*	82	53	81	39	27	50	26	28	29	51
		Relative intensity, %	100	74	72	52	46	20	20	18	16	14
3	86	*m/e*	57	43	29	27	41	86	39	58	71	56
		Relative intensity, %	100	100	60	35	22	22	13	10	8	8
4	98	*m/e*	97	98	45	39	53	99	69	27	59	71
		Relative intensity, %	100	52	18	13	12	8	7	7	6	4
5	100	*m/e*	85	59	100	99	39	45	65	27	58	41
		Relative intensity, %	100	86	73	67	34	32	29	20	17	16

methylthiophene and the two dihydro isomers. One of the two dihydro isomers of 2-methylthiophene had a retention time identical to that of the unknown component on the tris column, while the other isomer had a slightly greater retention time. The retention times of the two dihydro-3-methylthiophenes were much greater than that of the unknown.

The two dihydro-2-methylthiophene isomers were collected and mass spectra were obtained, as follows:

First Isomer: *m/e* 85 (100%), 59 (82%), 100 (molecular ion, 77%), 99 (67%), 39 (41%), 45 (38%), 65 (28%), 58 (23%), 41 (21%), 53 (18%), 27 (18%), 101 (10%), 97 (10%), 71 (10%), 55 (7%), 98 (5%), 102 (2.5%).

Second Isomer: *m/e* 85 (100%), 100 (molecular ion, 40%), 45 (34%), 39 (34%), 59 (27%), 65 (15%), 41 (15%), 27 (15%), 99 (11%), 97 (8%), 67 (8%), 58 (8%), 53 (8%), 98 (4%), 71 (4%), 101 (2%), 102 (1%).

The mass spectrum of the first isomer is in excellent agreement with that of the unknown compound (peak 5). On the basis of this information and the agreement of retention times, the unknown was concluded to be a dihydro-2-methylthiophene.

The position of the double bond is difficult to establish from the mass spectral data. The *m/e* 85 fragment, resulting from the loss of the methyl group, is the base peak of both isomers. The fact that the first isomer does not lose methyl as readily as the second isomer suggests that the first isomer is the reaction product with the double bond in the 2,3-position (4,5-dihydro-2-methylthiophene). Birch and McAllan (1951) reported that the boiling point of the 4,5-dihydro isomer of 3-methylthiophene is 8° C. less than that of the 2,3-dihydro isomer. Assuming that the isomers of dihydro-2-methylthiophene show a similar pattern, the 4,5-dihydro-2-methylthiophene would be expected to have the lower boiling point and would therefore be the first isomer to elute from the GLC column. On the basis of this reasoning, the unknown was tentatively identified as 4,5-dihydro-2-methylthiophene.

Summarizing the identifications, the following compounds have been shown to arise from heat degradation of thiamine at pH 6.7: hydrogen sulfide, 2-methylfuran, 2-methylthiophene, and 4,5-dihydro-2-methylthiophene. The latter compound appears to be the volatile product of greatest abundance, excluding hydrogen sulfide, in that it produces the largest peak with the gas entrainment, on-column GLC technique. Relative amounts of hydrogen sulfide were not compared, as the hydrogen flame detector does not respond to this compound. From the strong hydrogen sulfide odor produced, and the amount of zinc sulfide precipitate formed when the column effluent was bubbled through the zinc acetate solution during the purge step, it appears that significant amounts of hydrogen sulfide are produced by heat degradation of thiamine.

Mechanism of Thiamine Degradation. The mechanisms involved in the heat degradation of thiamine present an interesting problem for the theoretical organic chemist. The heat degradation of thiamine has a drastic effect on the thiazole portion of the thiamine molecule. The volatile products identified might be explained as thermal rearrangement and elimination products of the thiazole moiety of thiamine. Whether the thiazole ring would open first or the thiazole moiety would be cleaved from the pyrimidine moiety and then undergo further degradation is a matter of speculation.

Flavor Significance. The flavor significance of the volatile components arising from heat degradation of thiamine is of interest. Hydrogen sulfide is considered an important flavor compound in a number of foods. It has been implicated as a cause of the cooked flavor of heated milk.

Hodge (1967) describes the odor of 2-methylfuran as ethereal. This compound has been identified in coffee volatiles by a number of workers (Gianturco, 1967). Merritt *et al.* (1963) suggested that 2-methylfuran is among the more significant compounds contributing to the characteristic coffee aroma. The odor of 2-methylthiophene can be described as heated onion or sulfury and the 4,5-dihydro-2-methylthiophene possesses a similar, but much sharper, odor. The odor characteristics of these two compounds suggest that they could be important contributors to certain food flavors. Two recent literature reports are of interest in view of the authors' observations on the appearance of an odor resembling stewing chicken after a few minutes of heating thiamine at pH 6.7. A recent patent (Giancino, 1968) describes a process for imparting poultry flavoring to foodstuffs that involves reacting mixtures of sulfur-containing polypeptides or amino acids with thiamine. Nonaka, Black, and Pippen (1967) have identified 2-methylthiophene and 2-methylfuran from an odorous fraction isolated from boiling chicken meat.

The volatile compounds produced by heat degradation of thiamine contribute to food flavors. These compounds may be of considerable importance in the flavors imparted during cooking of foods high in thiamine.

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Received for review November 3, 1967. Accepted December 16, 1968. Technical Paper 2384, Oregon Agricultural Station. Investigation supported in part by Public Health Service Research Grant UI-142 from the National Center for Urban and Industrial Health.