Identification of a Thiamin Odor Compound from Photolysis of Thiamin

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The probable identity of a new compound, 1-methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane, with the characteristic odor of thiamine is reported. It is formed by ultraviolet radiation (2537 Å) of thiamin·HCl. Its identity was arrived at by combinations of mass spectrometry, infrared spectrometry, Raman spectrometry, ultraviolet spectrometry, proton magnetic resonance, carbon-13 nuclear magnetic resonance, and some chemical tests.

Many commercial multivitamin preparations possess a characteristic aroma associated with that of thiamin itself. The volatile compound responsible for this aroma is apparently formed by some kind of degradation of thiamin because pure thiamin has no odor. The chemistry of thiamin degradation has interested a number of researchers. A review of this subject published by Dwivedi and Arnold (1973) discussed some of the methods studied—thermal, enzymatic, chemical, radiation, and ultrasonic—to degrade thiamine and reported some of the products formed. The volatiles formed from thermal degradation or from various chemical environments have been studied extensively. Many of these compounds—the thiophenes, thiazoles, and furans—are possible contributors to food aromas. It is known that ultraviolet (UV) radiation degrades thiamin as reported by several researchers (Beral et al., 1962; Kawaski and Daira, 1962; and Button, 1968). We investigated the effect of UV radiation on thiamin degradation and the volatile components formed, especially those important to aroma, since this area has not been studied and could complement other degradation studies. This article is a report of the study of the major neutral volatile component formed from the irradiation (2537 Å) of thiamin·HCl. This compound has a highly characteristic and potent odor which appears to be identical with the aroma found in both pharmaceutical and commercial sources of thiamin used for other purposes. We have called the compound the "thiamin odor compound".

EXPERIMENTAL SECTION

Photolysis. In a typical experiment 200 g of thiamin·HCl (Roche Chemical Division of Hoffman La Roche, Inc. or Eastman Kodak No. 5180) dissolved in 270 mL of triple distilled water in a 1-L quartz flask with Teflon magnetic stirrer was irradiated 5 days in a Rayonet Photo Chemical Reactor (the Southern New England Ultraviolet Company) equipped with a circular group of 18 2537 Å Rayonet lamps at a distance of 10 cm from the center of the unit with ca. 36 W output. The flask was connected to a water-cooled condenser. A system for introducing nitrogen allowed for sweeping out air and for maintaining a static nitrogen atmosphere during photolysis. The solution at a temperature of 75 °C with the cooling fan on was stirred with a magnetic stirrer during the 5 days of irradiation. Samples ranging from 2 to 350 g were photolyzed from 3 to 7 days. Some samples were also exposed to 3500 Å radiation in air and in nitrogen atmospheres.

Extraction of Volatile Oil. Volatile oil from the photolyzed mixture was obtained by steam distillation continuous extraction using a Likens head and freshly distilled ether (150 mL). Most extracts were washed with $NaHCO_3$ to remove free acids. The ether extract was dried over anhydrous Na_2SO_4, filtered, and concentrated on a steam bath through a Vigreaux column. Yields from the photolysis at 2537 Å varied depending on conditions but were usually 0.2 to 0.3% of the weight of thiamin·HCl used.

Packed Column Gas Chromatography GLC. A 0.64 cm o.d. × 1.5 m long aluminum column, packed with 80/100 mesh Chromosorb G coated with 10% Tween 20 containing 5% by weight of Amine 220 containing a trace of Ionox, was used for initial separations. The column was usually programmed nonlinearly from 70 to 150 °C for 30 min, and then the temperature was raised to 175 °C after 15 min to elute all the peaks in 60 to 75 min. The major collected peaks were rechromatographed on a similar column but packed with 80/100 mesh Chromosorb G coated with 20% Silicone SF96-100 and 2% Igepal CO-880. The injector temperature was 200 °C as was the thermal conductivity detector system and oulet. Helium carrier gas flow was ca. 35 mL/min.

Capillary GLC–Mass Spectrometry (MS). A 0.75 mm i.d. × 300 m long stainless steel capillary column coated with Tween 20 containing 5% Igepal CO-880 was used. The carrier gas, helium, had an inlet pressure of 8 psi. Hydrogen flame detection was used. The injector temperature was 175 °C. The column was programmed from 70 to 170 °C at 0.5 °C/min and held at 170 °C. A splitter allowed half of the sample to go to a modified Consolidated 21-620 cycloidal type mass spectrometer using a silicone membrane separator. Mass spectra were obtained for the major components from the whole oil and from GLC peaks collected from packed columns previously described. The "thiamin odor compound" recognized by its odor from the packed column effluent was the largest component and could be easily identified.

High-Resolution Mass Spectrometry (MS). High-resolution mass spectra were obtained for the GLC-purified thiamin odor compound on a Consolidated 21-110A double-focusing instrument.

Infrared Absorption (IR) Spectrometry. The GLC-purified samples, collected in borosilicate tubes (150 × 2 mm), were run as a film on micro-salt plates on a Perkin Elmer 237 instrument.

Ultraviolet (UV) Spectrometry. Spectra were obtained on a Cary 1115 instrument using a 1-cm cell with the thiamin odor compound in methanol (0.1 mg/3 mL).

Nuclear Magnetic Resonance (^{1}H NMR and ^{13}C NMR). ^{1}H NMR spectra were obtained on a Varian HA-100 modified by addition of a field frequency lock at 100 MHz. The ^{13}C NMR spectra were obtained using a JEOL PFT-100 spectrometer at 25 MHz. Samples were run in $CDCl_3$ and Me_4Si was used as reference.

Desulfurization. The "thiamin odor compound" (1.5 mg) was reacted with 1 g of Raney nickel [W-5 (prepared

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as described in "Organic Syntheses", Horning, 1955)] in 2 mL of ethanol stirred 18 h and then refluxed 6 h with 4 mL additional ethanol by the procedure of Mozingo et al. (1944).

RESULTS AND DISCUSSION

UV radiation (2537 Å) of thiamin·HCl and subsequent continuous steam distillation–ether extraction yielded 2000 parts/10^6 parts of volatile oil (after removal of free acids) with a potent "thiamin-like" odor. Analysis of the oil on the Tween 20 capillary GLC column showed the major peak was 19% of the whole oil as calculated from peak areas using the triangulation method. Yields of the major peak varied from ca. 10 to 20% of the whole oil depending upon amounts of thiamin·HCl irradiated, time of irradiation, and conditions of extraction (time and pH of solution). The conditions were varied to find maximum yields. The major peak was separated and collected using the 0.64 × 1.5 m long Tween 20 packed column with ca. 50% recovery from that expected from the peak areas of the chromatogram. The compound may break down in the metal thermal conductivity system and its exit port. The odor from less than 0.1 mg of the "thiamin odor compound" emerging from the GLC exit port permeated the entire laboratory. The odor of the major peak is potent and is in the author's and other experienced odor judges' opinions identical with the characteristic odor of vitamin B_1. The major peak, called the "thiamin odor compound", was rechromatographed on a 0.64 × 1.5 m long Silicone SF96-100 column and a portion of the collected material injected onto the Tween 20 capillary column to confirm its purity. A portion of the material from the capillary column passed through a silicone membrane into the Consolidated 21-620 mass spectrometer as previously described. This preliminary mass spectrum showed the compound had a molecular weight of 162.

Formation. Volatiles obtained from thiamin irradiated at 3500 Å in both air and nitrogen atmospheres and extracted under the same conditions as material irradiated at 2537 Å were also analyzed by capillary GLC–MS. The chromatograms showed a simple mixture with no detectable "thiamin odor compound". The odor of the whole extract was not characteristic of the "thiamin odor compound". Thiamin·HCl (2.5 g) carried through the whole experiment with no UV radiation showed no evidence of volatile material by capillary GLC. A yield of ca. 5 mg would be expected from irradiation at 2537 Å of 2.5 g of thiamin·HCl. The presence of an antioxidant in the thiamin·HCl prevented formation of the thiamine odor compound and gave few volatile components as seen in a capillary GLC chromatogram of the oil from an irradiated sample of thiamin·HCl. These experiments indicate that a free radical mechanism is involved in the formation of the thiamin odor compound at 2537 Å.

The possibility that another mechanism might also produce the thiamin odor was investigated. High-temperature (235 °C) treatment of thiamin·HCl for 2 h with analysis of the extracted volatiles by capillary GLC–MS showed a number of degradation products but no thiamin odor compound. Some thiazoles and a mol wt 162 compound (different from the thiamin odor MW 162 compound) were found.

IR, Raman, and UV Spectra. The infrared (IR) spectrum of the thiamin odor compound collected from the Tween 20 packed column showed absorption at 5.85 μm. Rechromatography of the same material on the Silicone SF96-100 packed column and examination of its IR spectra showed drastic reduction of the 5.85 μm absorption, indicating no C=O present. The spectrum of the repurified material in the region 5 to 14 μm is as follows: 9.71 (very strong), 8.85, 9.18, 9.39, 11.26 (strong), 6.90, 7.25, 11.97, 13.69 (medium), 5.85, 6.37, 6.39, 6.75, 7.04, 7.38, 8.03, 8.40, 10.58, 11.05 (weak), and 5.67, 5.78, 6.04, 6.19, 6.58, 7.66, 7.93, 8.30, and 10.5 (very weak). The C–H region showed typical absorption bands for $-CH_3$ at 3.36 μm and for $-CH_2$ and 3.42 and 3.48 μm with no evidence for unsaturation. Absorptions at 8.9 to 10.0 μm were indicative of aliphatic or saturated C–O–C. The absence of absorption in the 3.92 to 3.85-μm region tends to rule out –SH groups. Raman spectra supported the data found in the IR spectra. Raman spectra contained no evidence for –SH or S–S. Peaks at 22.72 and 19.72 μm indicated C–S bonding. There was also no evidence of double bonds shown in the –CH region. The ultraviolet spectrum of the thiamin odor compound in methanol also showed no evidence for double bonds. Absorption was at λ max 257 with ε max 820.

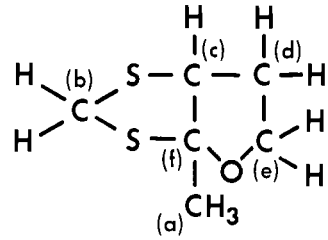

Figure 1. 1-Methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane.

Mass Spectrum. The high-resolution mass spectrum of the purified thiamin odor compound showed a molecular weight of 162.0187 which is consistent with the empirical formula $C_6H_{10}OS_2$ (162.0173). The mass spectrum is as follows with relative intensities in parentheses: 39 (9), 41 (6), 43 (100), 44 (3), 45 (14), 46 (5), 47 (3), 53 (4), 55 (7), 58 (2), 59 (3), 69 (3), 71 (4), 73 (4), 74 (2), 83 (12), 84 (34), 99 (1), 116 (1.5), 117 (1), 162 (4), 163 (0.4), 164 (0.4).

Desulfurization and Carbonyl Test. The mass spectral information indicated that the compound contained two S atoms. Attempted desulfurization of the thiamin odor compound gave almost complete recovery of the starting material except for a small quantity of an unidentified mol wt 132 compound. The recovered compound also gave a negative test for carbonyl when reacted with 2,4-dinitrophenylhydrazine.

NMR. A number of structures for the thiamin odor compound were possible considering only the data described above. The ^1H NMR spectral data described below narrowed the possibilities to a ring structure. The structure that best fit the data was 1-methylbicyclo-[3.3.0]-2,4-dithia-8-oxaoctane (Figure 1).

The ^1H NMR spectra and its integration (Figure 2) confirmed mass spectral evidence for 10 H as did the ^{13}C NMR off resonance CW spectrum (Figure 3). The proton noise decoupled spectra (Figure 4) shows six single peaks (triplet = 76 to 79.5 is $CDCl_3$ solvent) corresponding to six carbons. The ^1H NMR spectral information obtained from Figure 2 is summarized in Table I. The information from the ^{13}C NMR spectra in Figure 3 and Figure 4 is compiled in Table II. Both tables I and II refer to the carbon positions of 1-methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane which are arbitrarily labeled a, b, c, d, e, and f in Figure 1. The chemical shifts and splitting found in the ^1H NMR and ^{13}C NMR spectra are consistent with what one would expect from the structure of the proposed thiamine odor compound. The data in Table I and II support and are reasonably consistent with the following structural analysis. There is $-CH_3$(a) attached to a nonprotonated C(f) whose position fits bonding to C's and heteroatoms S or O. The

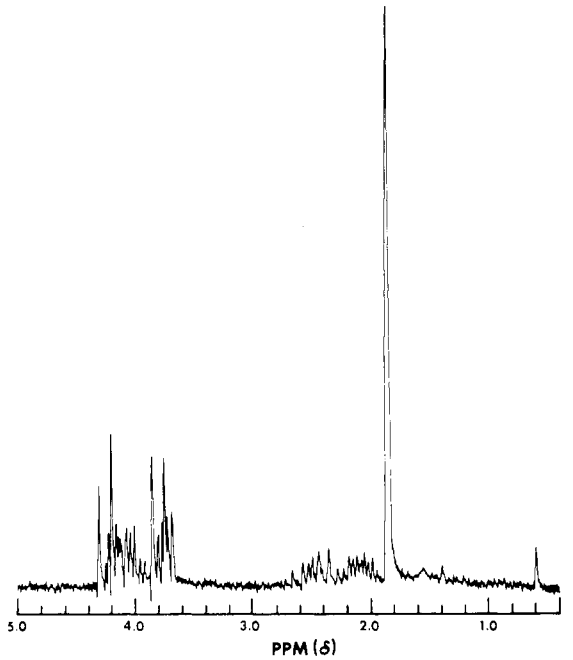

Figure 2. ^1H NMR spectrum of thiamin odor compound in $CDCl_3$ at 100 MHz.

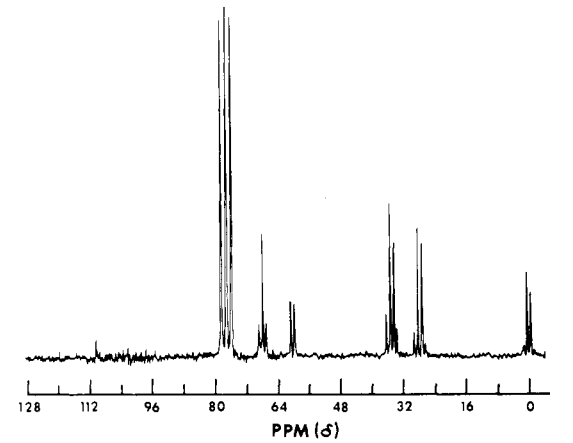

Figure 3. ^{13}C NMR off resonance decoupled spectrum of thiamin odor compound in $CDCl_3$ at 25 MHz.

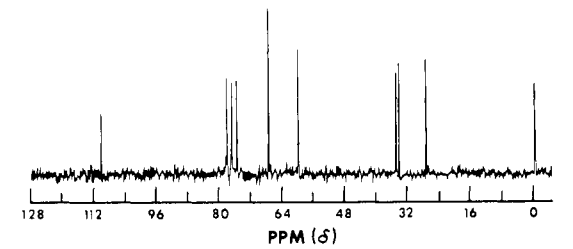

Figure 4. ^{13}C NMR proton noise decoupled spectrum of thiamin odor compound at 25 MHz.

CH_2(d) is adjacent to methylenes or CH_2 and CH (which could be bound to S). The CH_2(e) is adjacent to ring O and CH_2. The CH(c) position is difficult to predict but is reasonably compatible with bonding to S, CH2, and nonprotonated C. The CH_2(b) fits fairly well for bonding to adjacent S's.

Attempted Synthesis. We attempted to synthesize this compound by several routes starting with commercially (Aldrich Chemical Co., Inc.) available 2-methyl-4,5-dihydrofuran from which we hoped to synthesize the 2,3-dimercapto-2-methyltetrahydrofuran and ring close its sodium salt with dibromomethane.

Table I. ^1H NMR Spectra (Figure 2)

carbon position[a]	splitting[b]	δ[b]	no. of H[b]
a	s	1.86	3
b	d	3.87	
	d	4.25	2
c	mult	3.71	
	mult	3.75	1
d	mult	2.08	
	mult	2.45	2
e	mult	4.02	
		4.18	2
f			0

[a] Figure 1. [b] Figure 2.

Table II. ^{13}C NMR Spectra (Figures 3 and 4)

carbon position[a]	splitting[b]	δ[b]	no. of H[c]
a	q	27.7	3
b	t	35.1	2
c	d	59.8	1
d	t	34.3	2
e	t	67.4	2
f	s	109.6	0

[a] Figure 1. [b] Figure 3. [c] Figure 4.

The two major routes from the dihydrofuran starting compound to the dimercaptan were formation of a 2,3-dibromo or 2,3-dihydroxy compound. Synthesis of the 2,3-dihydroxy compound was unsuccessful with $KMnO_4$ alone or with the phase transfer reagent benzyltriethylammonium chloride described by Weber and Sheperd (1972). In another approach the product from a cis-hydroxylation procedure of Woodward and Brutcher (1958) using iodine and silver acetate in wet acetic acid was first reacted with tosyl chloride and then with potassium thioacetate to form the S-acetyl derivative as described by Morell et al. (1977). Reduction of the S-acetyl derivative with $LiAlH_4$ did not yield the expected dimercaptan. No intermediates from the various steps were characterized, so it is difficult to predict which reaction was unsuccessful. However, the sterically inhibiting methyl group on the dihydrofuran ring would make these reactions difficult. The synthesis of a similar model compound without the methyl group and starting from tetrahydrofuran was also tried. Chlorination of tetrahydrofuran by the method of Crombie and Harper (1950) and subsequent reaction with Na_2S and H_2S or with H_2S alone failed to give the dimercaptan. The results of these attempted syntheses indicated that bromination of the sterically hindered 2-methyl-4,5-dihydrofuran would likely fail. Because of the difficulty in finding a successful synthetic route, it seemed more fruitful to study the structure by both ^1H NMR and ^{13}C NMR in conjunction with data obtained by other methods.

The proposed structure for the "thiamin odor compound" seems consistent with the NMR data and with MS, IR, Raman, and UV spectral evidence and the chemical tests. It is not known why desulfurization was unsuccessful. Although the compound has not been synthesized for absolute confirmation, the evidence presented for its probable identity is comprehensive. It has a unique structure and composition with few models available for comparing structural similarities. The isolated "thiamin odor compound" has a unique, potent odor characteristic of thiamin. Its probable identity and source of formation have not been previously reported. The use of an antioxidant to stop free radical formation during thiamin manufacture and storage may prevent its for-

mation and the resultant characteristic and perhaps objectionable odor found in thiamin and thiamin containing vitamin preparations.

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LITERATURE CITED

Beral, H., Murea, L., Russu, C., Jacob, A., *Chem. Abstr.* **56**, 7440e (1962).
Button, D. K., *Appl. Microbiol.* **16**(3), 530 (1968).
Crombie, L., Harper, S. H., *J. Chem. Soc.*, 1714 (1950).
Dwivedi, B. K., Arnold, R. G., *J. Agric. Food Chem.* **21**, 54 (1973).
Horning, E. C., Ed., "Organic Syntheses", Collect. Vol. 3, Wiley, New York, N.Y., 1955.
Kawaski, C., Daira, I., *Bitumin* **26**, 462 (1962).
Morell, J. L., Fleckenstein, P., Gross, E., *J. Org. Chem.* **42**, 335 (1977).
Mozingo, R., Spencer, C., Folkers, C., *J. Am. Chem. Soc.* **66**, 1889 (1944).
Weber, W. P., Sheperd, J. P., *Tetrahedron Lett*, **48**, 4908 (1972).
Woodward, R. B., Brutcher, Jr., *J. Am. Chem. Soc.* **80**, 209 (1958).

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A New Method of Pyrazine Synthesis for Flavor Use

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Synthesis of alkylpyrazines from α-diketones and α-diamines through the corresponding 2,3-dihydropyrazines was studied. Dehydrogenation of dihydropyrazines in an ethanol–KOH solution with the addition of metal oxides (MnO_2, CuO) gave nearly 80% yields of pyrazines. The pyrazines obtained from this study were over 99% pure and did not contain any unreacted dihydropyrazines or by-products which cause changes in either the color or odor of the pyrazines. A kinetic study of trimethyl- and 2,3-dimethylpyrazine formation from corresponding pyrazines was also conducted.

Pyrazine compounds have been well characterized as the compounds which directly contribute to roasted or smoky flavors. There have been many reports concerning these flavor compounds (Bondarovich et al., 1967; Rizzi, 1967; Watanabe and Sato, 1971; Buttery et al., 1971). Formation mechanisms of pyrazines in foods have been investigated by several researchers (Koehler and Odell, 1970; Rizzi, 1972; Wang and Odell, 1973; Shibamoto and Bernhard, 1976).

The most common method for deriving pyrazines from 2,3-dihydropyrazines, which are obtained from the condensation reaction of α-dicarbonyl compounds and α-diamines, is to heat the 2,3-dihydropyrazines in an alcoholic solution under basic conditions in order to dehydrogenate them. For example, Flament and Stoll (1967) obtained 3-alkyl-2-methylpyrazine from the reaction of 2,3-dioxoalkanes and ethylenediamine through 2,3-dihydropyrazines. Their method gives a 55–100% yield. This experimental procedure is, however, somewhat difficult and requires a high temperature, which may cause the production of unpleasant odor. Nakatani and Yanatori (1973) obtained dialkylpyrazines from corresponding dihydropyrazines in 40–75% yields using KOH or NaOH in ethylene glycol. It is, however, difficult to separate pyrazines from ethylene glycol and this method produces a certain amount of by-products which are not suitable for flavor use. Ishiguro and Matsumura (1958) obtained 2,3-dimethylpyrazine from the reaction of diacetyl and ethylenediamine through 2,3-dimethyl-5,6-dihydropyrazine. They treated 2,3-dimethyl-5,6-dihydropyrazine with potassium hydroxide in ethanol at high temperature to dehydrogenate it. Cornforth (1958) reported the syntheses of symmetric alkylpyrazines from the self-condensation reaction of α-amino ketones through dihydropyrazines. Dihydropyrazines were either dehydrogenated by an oxidizing agent (H_2O_2, $HgCl_2$, etc.) or oxidized under aerobic conditions. Tutin (1910) obtained a mixture of equal amounts of 2,5- and 2,6-diphenylpyrazine through corresponding dihydropyrazines from the reaction of ω-chloroacetophenone with alcoholic ammonia. These methods either produced low yields or the pyrazines formed could not be easily separated from the unreacted dihydropyrazines. It is, therefore, very difficult to obtain high-purity pyrazines for flavor usage via these methods.

We used a modification of Ishiguro's method in order to obtain high yields of alkylpyrazines of very high purity.

EXPERIMENTAL SECTION

Materials. Diacetyl (Naarden), ethylenediamine, propylenediamine (Nakarai Chemicals, Ltd.), metal oxides, and metal salts (Wako Pure Chemical Industries) were obtained commercially. Acetylpropionyl was synthesized using the method reported by Semon and Damerell (1930). Dihydropyrazines were synthesized by the procedures described by Ishiguro and Matsumura (1958).

Synthesis of Pyrazines from Corresponding Dihydropyrazines. Five grams of corresponding dihydropyrazine was dissolved in 63 mL of ethanol for each experiment. *n*-Nonanol (2 g) was added to each of these solutions as the internal standard for gas chromatographic quantitation. The above solutions were refluxed with the materials and under the conditions as described in Table I. After refluxing, each reaction solution was filtered, and 5 mL of each filtrate was mixed with 20 mL of benzene and 5 mL of saturated brine water and shaken vigorously. The quantitative analysis of the pyrazine in the benzene layer of each of the above solutions was conducted by gas chromatography.

Preparation of Standard Trimethylpyrazine from Trimethyl-5,6,-dihydropyrazine for Flavor Use.

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