different soy protein components. As would be expected, the most highly purified protein fractions, i.e., 7S and 11S components, contained the highest apparent protein contents. Protein fractions prepared from commerical defatted soy flakes were more readily solubilized during the protein determination, and this factor may account for the slightly higher apparent protein content values for these protein fractions compared to defatted Bragg soybean protein fractions. Additional work is in order to study the protein content and distribution among the different protein fractions by micro-Kjeldahl, biuret, or other protein determination methods.

P content values of the freeze-dried protein fractions ranged from 0.08 to 15.20% (Table I). The 11S component contained the lowest P content of all fractions, whereas the 7S component contained a similar P content as whole soy extract and soy whey. Comparison of P/protein ratios for these protein fractions (Table I) confirms that the 11S component contains the lowest P content and that the soy whey precipitate contains the highest P/protein ratios of 2 to 3.4. A similar relationship was observed for phytate/protein ratios of these fractions, where once again the 11S component contained the lowest phytate content ratio of 0.007 compared to the highest value of about 10 for soy whey precipitate. Examination of the P/phytate ratios (Table I) reveals that all are below the theoretical value of 3.546, assuming that phytate ion contains 28.2% P. Thus, most of the P contained by 11S component is nonphytate, whereas phytate accounts for a major portion of the total P of soy whey precipitate. The P and phytate content values for the above protein fractions from defatted Bragg and commercial defatted soy flakes were separately grouped and statistically analyzed (Table I). P content values were statistically different ($P < 0.05$) for the different fractions within each group, i.e., Bragg and commercial groups. Phytate values were also statistically different for the protein fractions from commercial flakes, except for whole soy extract and 7S component, which were not significantly different ($P < 0.05$).

Smith and Rackis (1957) and Hartman (1979) suggested that removal of phytate from soy proteins might alter their basic physicochemical and functional properties in food products. Studies to date (Thanh and Shibasaki, 1976b, 1978; Damodaran and Kinsella, 1982; German et al., 1982) have ignored this potentially important matter and its impact upon the nomenclature of soy proteins and their subunits. In view of the present findings, it appears that the 11S soy protein component does not bind adequate amounts of phytate ion to make this a major concern with respect to its physicochemical and functional properties. However, further consideration of this matter is required for 7S and the other soy protein fractions, since they bind substantially more phytate ion, which could readily alter their physicochemical and functional properties. Although the soy whey fractions appear interesting in the present study with respect to their high phytate contents, they are not commercially important at this time.

**Registry No.** Phytate, 83-86-3; phosphorus, 7723-14-0.

LITERATURE CITED

Allen, R. J. L. *Biochem. J.* **1940**, *34*, 858.
Bio-Rad Laboratories. "Technical Bulletin 1051"; Bio-Rad Laboratories: Richmond, CA 1976.
Brooks, J. R.; Morr, C. V. *J. Food Sci.* **1982**, *47*, 1280.
Cheryan, M. *CRC Crit. Rev. Food Sci. Nutr.* **1980**, *13*, 297.
Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1982**, *30*, 812.
Erdman, J. W., Jr. *J. Am. Oil Chem. Soc.* **1979**, *56*, 736.
Gayler, K. R.; Sykes, G. E. *Plant Physiol.* **1981**, *67*, 958.
German, B.; Damodaran, S.; Kinsella, J. E. *J. Agric. Food. Chem.* **1982**, *30*, 807.
Hartman, G. H., Jr. *J. Am. Oil Chem. Soc.* **1979**, *56*, 731.
Laemmli, U. K. *Nature (London)* **1970**, *227*, 680.
Maddaiah, V. T.; Kurnick, A. A.; Reid, B. L. *Proc. Soc. Exp. Biol. Med.* **1964**, *115*, 391.
Meinke, D. W.; Chen, J.; Beachy, R. N. *Planta* **1981**, *153*, 130.
Okubo, K.; Waldrop, G. A.; Iacobucci, G. A.; Myers, D. V. *Cereal Chem.* **1975**, *52*, 263.
Smith, A. K.; Rackis, J. J. *J. Am. Chem. Soc.* **1957**, *79*, 633.
Thanh, V. H.; Shibasaki, K. *J. Agric. Food Chem.* **1976a**, *24*, 1117.
Thanh, V. H.; Shibasaki, K. *Biochim. Biophys. Acta* **1976b**, *439*, 326.
Thanh, V. H.; Shibasaki, K. *J. Agric. Food Chem.* **1978**, *26*, 692.
Utsumi, S.; Inaba, H.; Mori, T. *Phytochemistry* **1981**, *20*, 585.

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# COMMUNICATIONS

## Thiamin Odor and Bis(2-methyl-3-furyl) Disulfide

A potent odor fraction, previously isolated as a single packed column GLC peak from the products of the UV irradiation of thiamin hydrochloride (and shown to be predominantly 2,3-(methylenedithio)-2-methyltetrahydrofuran), was resolved further into eight fractions by separation on a Carbowax 20-M Pyrex glass capillary GLC column. Odor threshold determinations of these fractions showed that the most potent was one containing bis(2-methyl-3-furyl) disulfide, a minor component of the packed column peak. This compound (a known flavor compound) was found to be an extremely potent odorant with an odor threshold of 2 parts in $10^{14}$ parts of water.

The degradation products of thiamin have been studied for many years and earlier studies have been reviewed [cf. Dwivedi and Arnold (1973) and van der Linde et al. (1979)]. Some of us, after studying a number of different ways of degrading thiamin, had isolated a particular packed column gas liquid chromatography (GLC) peak (called the "thiamin odor compound" or the "thiamin odor peak") that had shown the highest odor potency (lowest

determined odor threshold) of any of the thiamin degradation products they isolated (Siefert et al., 1978; Buttery et al., 1981). It had also been determined in this work (by spectral means) that the predominant component of the thiamin odor peak was 2,3-(methylenedithio)-2-methyltetrahydrofuran (1-methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane). It was incorrectly concluded (Buttery et al., 1981) that this predominant component was responsible for the high odor potency of this thiamin odor peak. Comparison of the odor thresholds of the synthetic 2,3-(methylenedithio)-2-methyltetrahydrofuran with that of the isolated (natural) thiamin odor peak showed that this synthetic compound was a much weaker odorant (Buttery and Seifert, 1982).

The present studies were carried out to isolate other minor components of the thiamin odor peak and to determine with some certainty the identity of the compound responsible for the high odor potency.

## EXPERIMENTAL SECTION

**Materials.** Bis(2-methyl-3-furyl) disulfide was generously supplied by Dr. William Evers of International Flavors and Fragrances, Inc. It was purified by GLC separation through a 3 m long by 0.64 cm o.d. stainless steel column packed with 80–100-mesh Chromosorb G-DMCS coated with 2% Carbowax 20-M. Its purity was checked by capillary GLC, mass spectrometry, NMR spectra, and infrared spectrometry.

*Isolation of Thiamin Odor Peak.* This was carried out in the same way as described previously (Seifert et al., 1978) by using a 1.5 m long × 0.64 cm o.d. aluminum column packed with 80–100 mesh Chromosorb G coated with 10% Tween-20 (containing 5% Amine 220). This separated the thiamin odor peak.

*Capillary GLC–Mass Spectrometry (GC–MS).* The thiamin odor peak from the Tween-20 packed column was analyzed by capillary GC–MS. The capillary GLC column was a 150 m long × 0.66 mm i.d., laboratory constructed, Pyrex glass capillary, wall coated with Carbowax 20-M. The column was programmed from 50 to 170 °C at 1 °C/min holding at the upper limit. The inlet pressure was 15 psi He. Several different GC–MS runs were made, some using a VG-Micromass 70/70F double-focusing mass spectrometer and others using a modified Consolidated 21-620 cycloidal-type mass spectrometer.

*Collections of Fractions from Capillary GLC.* When the above Pyrex Carbowax 20-M capillary column was used, the GLC apparatus was set up so that ca. 50% of the outlet went to the GLC detector and 50% went to a glass-lined heated outlet. A number of fractions (A–H) were collected covering the areas shown in Figure 1. Each collector consisted of a 5-cm length of Teflon tubing that led to the surface of 0.5 mL of pure ethanol in a micro test tube. After each collection, any material trapped in the Teflon tubing was washed through with an additional 0.5 mL of ethanol into the micro test tube. Each fraction was then made up to 100 mL with water in a standard flask. Dilutions were made from these standard solutions for odor threshold determinations in order to locate the most potent odor fraction.

*Odor Threshold Determinations.* This was carried out following the procedure described by Guadagni and Buttery (1978) using odor-free Teflon squeeze bottles equipped with Teflon tubes.

## RESULTS AND DISCUSSION

Thiamin hydrochloride was irradiated with UV light (2537 Å) under nitrogen. The resulting volatile oil was isolated by steam distillation continuous extraction and

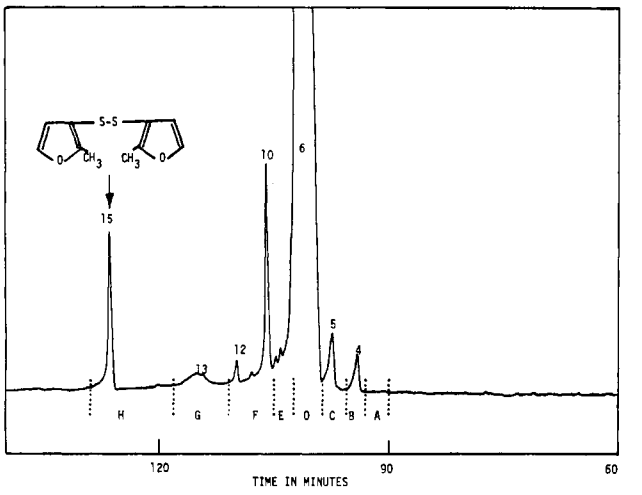


**Figure 1.** A portion of the Carbowax 20-M capillary GLC analysis showing resolution of the thiamin odor peak into fractions. Peak 6 is 2,3-(methylenedithio)-2-methyltetrahydrofuran and peak 15 bis(2-methyl-3-furyl) disulfide.

resolved into its components by preparative GLC using a Tween-20 stationary-phase packed column as previously described (Seifert et al., 1978). A GLC peak that had been determined to be the most potent odorant peak and previously called the thiamin odor peak was collected. This peak was then further resolved in the present work by using a Pyrex capillary column coated with Carbowax 20-M. The portion of the capillary GLC chromatogram showing the resolved peaks is shown in Figure 1. Fractions A–H were collected covering the areas shown in Figure 1. Dilutions were then made from these fractions (A–H) for odor threshold determinations (using 16–20 judges). Fraction H was found to be by far the most potent fraction, having an odor threshold more than 200 times lower than that of fraction D, which contained the major component 2,3-(methylenedithio)-2-methyltetrahydrofuran (peak 6).

Capillary GLC–MS analysis of the components in Figure 1 showed that the major peak in fraction H, peak 15, was bis(2-methyl-3-furyl) disulfide [molecular ion at $m/z$ 226 (39) and major ions at 113 (100), 43 (64), 45 (38), 51 (27), 69 (13), 85 (12), 59 (9), 155 (3), and 183 (2)]. Enough of peak 15 was also isolated for a $^1$H NMR spectrum, which confirmed its identity. Direct comparison of the spectra was possible with a synthetic sample of bis(2-methyl-3-furyl) disulfide. The capillary GLC retention data of peak 15 was also identical with that of the synthetic sample (Kovats index = 2070 on the Carbowax 20-M Pyrex capillary column).

Bis(2-methyl-3-furyl) disulfide had been found previously by Evers et al. (1976) among the volatile products from heating thiamin hydrochloride with L-cysteine hydrochloride and hydrolyzed vegetable protein. Evers et al. (1976) considered this compound to have potential as a synthetic meat flavor component. They, however, had not reported any determination of an odor threshold of this compound.

We carried out a study to determine the odor threshold of the GLC-purified synthetic bis(2-methyl-3-furyl) disulfide in water solution. The results of the determination are shown in Table I. Plotting these data as outlined by Guadagni et al. (1973) gave an odor threshold of 2 parts (mL) of bis(2-methyl-3-furyl) disulfide in $10^{14}$ parts (mL) of water.

The complete threshold determination was repeated 3 times at different dates over several months and gave essentially the same threshold value each time. Special care was taken each time in the preparation of the solu-

Table I. Odor Threshold Determination of Bis(2-methyl-3-furyl) Disulfide in Water

| concn, parts of compound/$10^9$ parts of water | % correct judgments | total no.[a] of judgments |
|---|---|---|
| 0.1 | 100 | 20 |
| 0.01 | 100 | 20 |
| 0.001 | 93 | 73 |
| 0.0005 | 92 | 53 |
| 0.0002 | 94 | 78 |
| 0.0001 | 89 | 118 |
| 0.00005 | 83 | 100 |
| 0.000025 | 82 | 118 |
| 0.00001 | 72 | 136 |
| 0.000005 | 62 | 119 |
| 0.0000025 | 60 | 119 |
| 0.000001 | 46 | 81 |
| 0.0000005 | 60 | 20 |
| 0.00000025 | 55 | 20 |

[a] Seventeen to twenty judges.

tions of the compound and in thoroughly checking the arithmetic of the dilutions.

This threshold is among the lowest ever reported for an organic compound in water solution. For example, it is of the order of 100 times lower than that reported for 2-isobutyl-3-methoxypyrazine (Buttery et al., 1969), the potent aroma component of bell peppers.

Considering the extreme potent odor properties of bis-(2-methyl-3-furyl) disulfide it would seem highly probable that this compound is a major contributor to the characteristic odor of thiamin preparations. Other compounds may also contribute but none have yet been shown to have the same order of odor potency as this disulfide. One might expect that compounds with a related structure [e.g., 2-methyl-3-furanthiol; cf. van der Linde et al. (1979)] would also be very potent odorants.

The threshold of the thiamin odor peak isolated previously (Buttery et al., 1981) was so low (4 parts in $10^{13}$ parts of water) that they had found incredible the idea that the low figure could be due to a minor "impurity". That threshold, though, is approximately the right value (20 times higher) that should be obtained from the 5% (1/20th) of bis(2-methyl-3-furyl) disulfide found in the thiamin odor peak. The odor contribution of the major component of the thiamin odor peak, 2,3-(methylenedithio)-2-methyltetrahydrofuran, is of course relatively insignificant (odor threshold of the synthetic form is 6 parts/$10^9$ parts of water; Buttery and Seifert, 1982).

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**Registry No.** Thiamin hydrochloride, 67-03-8; 2,3-(methylenedithio)-2-methyltetrahydrofuran, 67411-25-0; bis(2-methyl-3-furyl) disulfide, 28588-75-2.

LITERATURE CITED

Buttery, R. G.; Seifert, R. M. *J. Agric. Food Chem.* **1982**, *30*, 1261.
Buttery, R. G.; Seifert, R. M.; Lundin, R. E.; Guadagni, D. G.; Ling, L. C. *Chem. Ind. (London)* **1969**, 490.
Buttery, R. G.; Seifert, R. M.; Turnbaugh, J. G.; Guadagni, D. G.; Ling, L. C. *J. Agric. Food Chem.* **1981**, *29*, 183.
Dwivedi, B. K.; Arnold, R. G. *J. Agric. Food Chem.* **1973**, *21*, 54.
Evers, W. J.; Heinsohn, H. H.; Mayers, B. J.; Sanderson, A. "Phenolic, Sulfur and Nitrogen Compounds in Food"; Charalambous, G.; Katz, I., Eds.; American Chemical Society: Washington, DC, 1976; ACS Symp. Ser. No. 26, p 184.
Guadagni, D. G.; Buttery, R. G. *J. Food Sci.* **1978**, *43*, 1346.
Guadagni, D. G.; Maier, V. P.; Turnbaugh, J. G. *J. Food Sci.* **1973**, *24*, 1277.
Seifert, R. M.; Buttery, R. G.; Lundin, R. E.; Haddon, W. F.; Benson, M. *J. Agric. Food Chem.* **1978**, *26*, 1173.
van der Linde, L. M.; Van Dort, J. M.; De Valois, P.; Boelens, H.; De Rijke, D. "Progress in Flavour Research"; Land, G. D.; Nursten, H. E., Eds.; Applied Science Publishers: London, 1979; p 219.

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# Synthesis of $N^2$-[γ-L-(+)-Glutamyl]-4-carboxyphenylhydrazine, a Postulated Precursor of Agaritine of *Agaricus bisporus*

A procedure for the synthesis of $N^2$-[γ-L-(+)-glutamyl]-4-carboxyphenylhydrazine (GCPH) has been developed. The availability of GCPH will permit the bioassay of its possible tumorigenic properties. Condensation of the mixed anhydride, derived from *N*-benzyl-*N*-(benzyloxycarbonyl)-L-glutamate with 4-carboxyphenylhydrazine, gave the benzyl ester of *N*-(benzyloxycarbonyl)-L-glutamic acid 5-[2-(4-carboxyphenyl)]hydrazide, which was hydrogenated to yield $N^2$-[γ-L-(+)-glutamyl]-4-carboxyphenylhydrazine. The properties (IR, UV, $^{13}$C NMR, and MS) of the new compound are compared with those of a closely related compound agaritine, a component present in cultivated commerical edible mushroom *Agaricus bisporus*.

The cultivated mushroom of the Western Hemisphere, *Agaricus bisporus*, contains several nitrogen–nitrogen bond containing chemicals. Among these, the most important are $N^2$-[γ-L-(+)-glutamyl]-4-(hydroxymethyl)phenylhydrazine (synonym: agaritine) and its breakdown product, 4-(hydroxymethyl)phenylhydrazine (Kelly et al., 1962; Gigliotti, 1963; Levenberg, 1964; LaRue, 1977; Wallcave et al., 1979). Another chemical, the 4-(hydroxymethyl)-benzenediazonium ion, also has been found in this fungus and may be generated enzymatically from agaritine (Le-

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