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.

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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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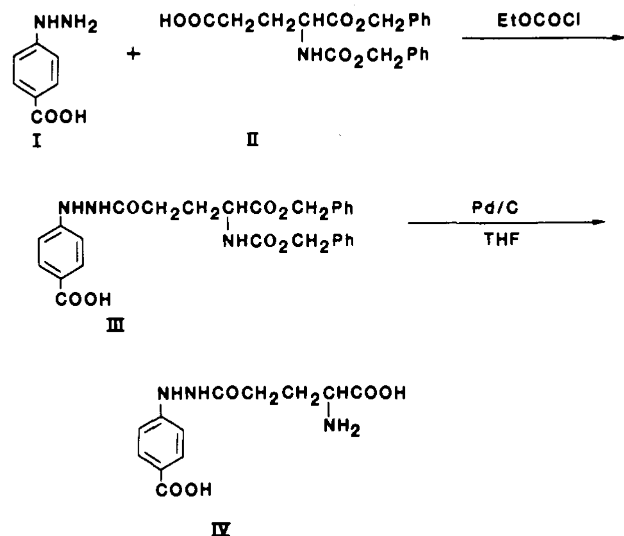

Figure 1. Chemical structures of 4-carboxyphenylhydrazine (I), *N*-benzyl-*N*-(benzyloxycarbonyl)-L-glutamate (II), the benzyl ester of *N*-(benzyloxycarbonyl)-L-glutamic acid 5-[2-(4-carboxyphenyl)]hydrazide (III), and N^2-[γ-L-(+)-glutamyl]-4-carboxyphenylhydrazine (IV).

venberg, 1962). Various nitrogen–nitrogen bond containing compounds induced cancer in several organs and tissues of mice (Toth et al., 1978, 1981 a,b). From an environmental viewpoint, these findings have practical implications, since the human population consumes large amounts of *Agaricus bisporus* and its chemical components (U.S. Department of Agriculture, 1982).

Agaritine is a naturally occuring, N–N bond containing compound that apparently is a normal biosynthetic product of intact *A. bisporus*. The aim of the present study was to synthesize and characterize a postulated agaritine precursor N^2-[γ-L-(+)-glutamyl]-4-carboxyphenylhydrazine (GCPH) (Schutte et al., 1977; LaRue, 1977), a compound in the L-glutamic acid series, for the bioassay of its possible tumorigenic properties. A recent preliminary chemical analysis in our laboratory indicates that this compound might be present in the mushroom.

EXPERIMENTAL SECTION

Palladium on activated carbon (Pd/C) was purchased from Stern Chemicals, Inc. Tetrahydrofuran (THF) was dried over lithium aluminum hydride and distilled. Thin-layer chromatography (TLC) was performed by using Brinkman Instruments precoated silica gel G-25 UV254 plates. ^{13}C NMR spectra were obtained on a Varian CFT-20 spectrophotometer. IR spectra were taken with a Beckman IR-9 spectrophotometer and melting points (uncorrected) on a Thomas-Hoover capillary apparatus. High- and low-resolution fast atom bombardment induced MS (FAB-MS-hr and FAB-MS-lr) were obtained by the National Science Foundation, Midwest Center for Mass Spectrometry at the University of Nebraska—Lincoln. Fragmentations were collisionally induced. UV was determined with a Cary 14 Varian spectrophotometer and optical rotations were determined with a Polyscience SR-6 polarimeter. Elemental analyses were carried out by Micro-Tech Laboratories, Skokie, IL. (Wallcave et al., 1979). All reactions were performed under an atmosphere of nitrogen. *N*-Benzyl-*N*-(benzyloxycarbonyl)-L-glutamate (II) (Figure 1) was synthesized by the method of Nefkens and Nivard (1964), and the *N*-benzyl ester of *N*-(benzyloxycarbonyl)-L-glutamic acid 5-[2-(4-carboxyphenyl)-hydrazide] (III) was prepared as reported earlier by our laboratory (Wallcave et al., 1979). The *N*-(benzyloxycarbonyl)-L-glutamic acid used in this synthesis was prepared by the method described earlier (Bergmann and Zervas, 1932). The overall yield of II from L-glutamic acid was 50%.

To a rapidly stirred solution of 0.005 mol of III in 30 mL of anhydrous THF was added a suspension of 5% Pd/C (0.8 g). A continuous flow of hydrogen was maintained over the surface of the mixture. The reaction was monitored by TLC chromatography with a solvent system consisting of 10 mL of chloroform–methanol (7:3) and 2 drops of acetic acid. After 32 h the product precipitated at room temperature and the reaction mixture was shielded from light while filtering on sintered glass and washed with THF acetonitrile. The precipitate was washed with 50 mL of water, filtered to remove the spent catalyst, and concentrated under reduced pressure at room temperature to 15 mL. This solution was cooled to 0 °C, and upon addition of ether yielded a white precipitate that was filtered and dried. The product obtained was a fine amorphorus crystalline white monohydrate, solid: yield ~60%; mp 216–218°C dec. Anal. Calcd for $C_{12}H_{15}N_3O_5 \cdot H_2O$: C, 48.52; H 5.97; N 13.90. Found: C, 48.12; H, 5.72; N, 14.03.

An anhydrous product was obtained from the hydrated material by precipitating from a saturated aqueous solution at pH 4.2 with 5 volumes of 1-butanol–ethanol (2:3) and drying under vacuum at 100 °C: mp 218–221 °C dec; $[\alpha]^{25}_D$ +6.5 (water). Anal. Calcd for $C_{12}H_{15}N_3O_5$: C, 51.25; H, 5.38; N 14.92. Found: C, 50.99; H, 5.19; N, 14.45. IR ν_{max} 3325, 3220, 2960, 2860, 1695, 1645, 1615, 1500, 1305, 1250, 770, 725 cm^{-1}; UV ν_{max} 258 (ε 11 200); ^{13}C NMR δ 177.1, 172.0, and 167.8 (C-1, C-5, and C=O, respectively), 56.5 (C-4), 29.8 (C-2), 27.1 (C-3), 110.9 (C-2 and C-6 of aromatic ring), 131.6 (C-3 and C-5 of aromatic ring), 153.0 (C-1 of aromatic ring), and 120.8 (C-4 of aromatic ring). FAB-MS-hr 282.1089 (calculated for $C_{12}H_{15}N_3O_5$, m/e^+ + H 282.1086); FAB-MS-lr 282 (base, m/e^+ + 1), 265 (m/e^+ − NH_3, 65), 219 [m/e^+ − (CO_2 + H_2O) or (CO_2H + NH_3), 35], 189 [m/e^+ − [$CH(NH_3)CO_2H$ + H_2O], 75], 152 (m/e^+ for $HO_2CC_6H_4NHNH_2^+$, 90], 137 (m/e^+ for $HO_2CC_6H_4NH_2^+$, 50], 131 [m/e^+ for $COCH_2CH_2CH(NH_3)CO_2H$, 55], 121 (m/e^+ for $C_6H_4CO_2H^+$, 25), 92 (m/e^+ for $C_6H_4NH_2$, 22), 85 (m/e^+ for $COCH_2CH_2CHNH_2^+$, 65), ≥20 plus key peaks.

RESULTS AND DISCUSSION

The condensation of the mixed anhydride derived from *N*-benzyl-*N*-(benzyloxycarbonyl)-L-glutamate (II) and ethylchloroformate was reacted with 4-carboxyphenylhydrazine (I) to yield the *N*-benzyl ester of *N*-(benzyloxycarbonyl)-L-glutamic acid 5-[2-(4-carboxyphenyl)]-hydrazide (III). Compound III was isolated in a 60% yield and used in the next step without further purification. The benzyl ester (III) was stirred with palladium on activated carbon at room temperature in THF under a hydrogen atmosphere. This removed protecting groups to give the desired product N^2-[γ-L-(+)-glutamyl]-4-carboxyphenylhydrazine (IV). The chromatographically homogeneous product was isolated in a 60% yield as a colorless, microcrystalline solid, which decomposed at 218–220°C. It was poorly soluble in ether, chloroform, tetrahydrofuran, and alcohol.

Strong evidence for the structure IV was obtained from elemental analyses and comparison of the ^{13}C NMR and mass spectral data from the precursor and agaritine. The infrared spectrum of the compound showed the presence of a benzenoid aromatic nucleus and the existence of carboxy functional groups. The carbon magnetic resonance spectrum was interpreted by using agaritine and substituted glutamic acids as model compounds (Wallcave et al., 1979).

Preliminary examination of the fast atom bombardment

induced mass spectrum of structure IV showed that a major fragment ion appears at m/e 152 and a high-resolution mass measurement on this ion establishes its composition as $HOOC–C_6H_4–NHNH_2^+$. Further work is in progress to isolate this compound from *A. bisporus*, the cultivated commerical mushroom.

Registry No. I, 619-67-0; II, 3705-42-8; III, 71426-47-6; IV, 69644-85-5; agaritine, 2757-90-6.

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Analysis of Glyphosate Residues in Kiwi Fruit and Asparagus Using High-Performance Liquid Chromatography of Derivatized Glyphosate as a Cleanup Step

A procedure was developed for the analysis of glyphosate herbicide in kiwi fruit and asparagus. The method consisted of extraction with a water–chloroform mixture, cleanup of the water extract by anion-exchange and gel permeation chromatography, derivatization with trifluoroacetic anhydride followed by diazomethane, further cleanup of derivatized glyphosate by silica gel HPLC using a methyl *tert*-butyl ether–tetrahydrofuran solvent gradient, and determination of the appropriate HPLC fraction by GLC using a phosphorus flame photometric detector. Recoveries averaged 87% for kiwi fruit fortified at 0.10–0.25 ppm and 80% for asparagus fortified at 0.05–0.10 ppm.

Glyphosate [*N*-(phosphonomethyl)glycine] is a broad-spectrum postemergent herbicide having wide utility for weed control. To support applications for U.S. registrations on specialty food crops, the national IR-4 pesticide clearance program must obtain residue data for glyphosate on each crop for which registration is sought. In carrying out residue analysis on kiwi fruit and asparagus, interferences were encountered that precluded straightforward adaption of GLC-based residue analytical methods for glyphosate ("Pesticide Analytical Manual", 1977; Guinavan et al., 1982) at the required limits of detections. We thus made several modifications in the sample preparation steps of published procedures and added a cleanup of the *N*-trifluoroacetyl *O,O,O*-trimethyl derivative of glyphosate using silica gel adsorption HPLC (Wehner et al., 1984) in order to decrease GLC interferences. With these changes a detection limit of approximately 0.05 ppm was achieved with both kiwi fruit and asparagus.

MATERIALS AND METHODS

Glassware and Reagents. Glass distilled water and nanograde or equivalent distilled-in-glass solvents were used throughout, with the exception of methyl *tert*-butyl ether and tetrahydrofuran (THF), which were UV grade (Burdick and Jackson, Muskegon, MI). Anion-exchange chromatography used Amberlite IRA-401S C.P. resin (Mallinckrodt, St. Louis, MO) converted from the chloride form to the bicarbonate form by washing 0.75 kg of resin in a suitable column with 15 L of 1 M ammonium bicarbonate (flow rate 10 mL/min) followed by 2 L of distilled water. Prepared resin (20 g) was added to 15 mL of distilled water in a 1.8 × 30 cm glass column equipped with a stopcock and a 2.5 cm diameter flaired section at the column head to serve as a solvent reservoir. The column was prewashed with 300 mL of 1 M ammonium bicarbonate solution at 5 mL/min followed by 300 mL of distilled water at 10 mL/min. An 8-cm head of water was left on top of the resin and then a 1-L separatory funnel was attached to the column inlet with a rubber stopper. Gel permeation chromatography used Bio-Gel P-2 resin (50–100 mesh, Bio-Rad, Richmond, CA) preswelled by soaking in distilled water. A 2.6 × 40 cm SR-25 glass column equipped with two SR-25 adapters (Pharmacia, Piscataway, NJ) was packed completely with a resin–water slurry. The column was then connected via 1.14 mm (i.d.) P.T.F.E. tubing to a Milton-Roy minipump and a Pharmacia SRV-3 injection valve. The column flow rate was maintained at 3 mL/min with pH 2.1 distilled water (ca. 1.2 mL of concentrated hydrochloric acid in 2 L of water) and calibrated each week by injecting 100 000 cpm of [^{14}C]glyphosate (Monsanto Chemical Co., St. Louis, MO, who also supplied unlabeled glyphosate standard) in 5 g of kiwi fruit or asparagus extract and collecting and counting 25-mL fractions. The usual elution pattern for a 5-g extract involved discarding the first 100 mL of eluate and collecting the next 45 mL. The column was flushed with distilled water, previously adjusted to pH 2.1 with concentrated hydrochloric acid, for at least 3 h after sample collection and kept on standby with a flow of 0.25 mL/min when not in use. Diazomethane, prepared in ether solution by a standard procedure from Diazald (Aldrich, Milwaukee, WI), was kept at −10 °C prior to use.

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