Garlic Chemistry: Stability of S-(2-Propenyl) 2-Propene-1-sulfinothioate (Allicin) in Blood, Solvents, and Simulated Physiological Fluids

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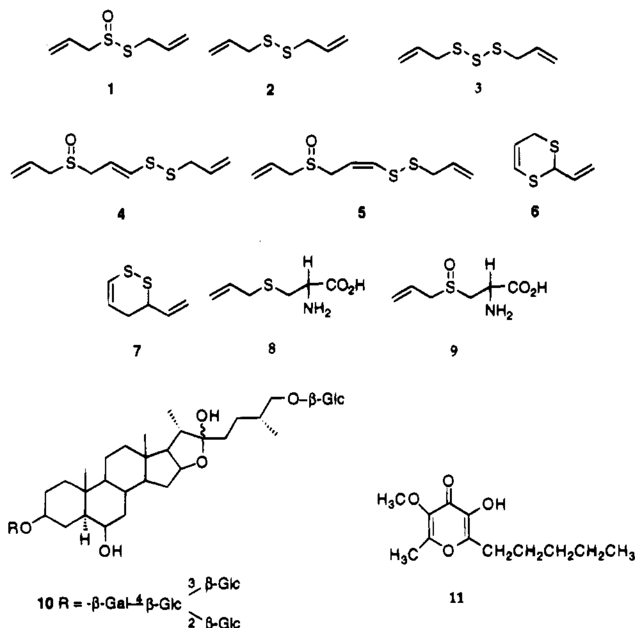
S-(2-Propenyl) 2-propene-1-sulfinothioate (allicin), which is one of the constituents of freshly crushed garlic (garlic homogenate), was synthesized, and its stability in blood, ethyl acetate, methanol, simulated gastric fluid (SGF, pH 1.2), simulated intestinal fluid (SIF, pH 7.5), and water (pH 1.2 and 7.5) and under simulated digestive conditions (sequential combination of SGF and SIF) was investigated by HPLC. Although neat allicin decomposes rapidly at 37 °C, it is more stable in protic polar methanol than in aprotic polar ethyl acetate. Approximately 90% of the allicin remained after incubation at 37 °C for 5 h in water at pH 1.2 and 7.5. Only traces of allicin could be detected after it was incubated in blood for 5 min. The allicin content and allicin-producing potential of commercial garlic preparations were also analyzed. The allicin contents in these garlic preparations were less than 1 ppm, and the allicin-producing potential was severely suppressed under simulated digestive conditions (sequential combination of SGF and SIF). The transformation products of allicin [(E)-ajoene, 2-ethenyl-4H-1,3-dithiin, diallyl disulfide] were identified.

Keywords: *(E)-Ajoene; allicin; allicin content; allicin-producing potential; allicin stability; garlic preparations; 2-ethenyl-4H-1,3-dithiin; diallyl disulfide; garlic products*

INTRODUCTION

Since ancient times, garlic (*Allium sativum* L.) has been used worldwide not only as a food but also as a medicine. As early as 3000 B.C., in ancient civilizations, including Egyptian, Greek, Indian, Roman, Babylonian, Viking, and Chinese, garlic was used for treatment of heart conditions, headache, bites, worms, wounds, ulcers, and tumors (Ariga et al., 1981; Block, 1985, 1986, 1991, 1992; Block et al., 1986, 1988, 1992; Cavallito and Bailey, 1944; Cavallito et al., 1944; Hanley and Fenwick, 1985; Lin, 1989; Mathew and Augusti, 1973; Pinto et al., 1992; Sparnins et al., 1988; Stoll and Seeback, 1949; Sumiyoshi and Wargovich, 1989, 1990; Wargovich, 1987; Wargovich et al., 1988). Recent investigations into the medical effectiveness of garlic have revealed relationships between the pharmacological effects of garlic and certain constituents of garlic or their transformation products. S-(2-Propenyl) 2-propene-1-sulfinothioate (allicin, **1**), which is one of the most widely known organosulfur compounds derived from garlic, has been reported to have antibiotic activity (Block, 1985; Cavallito and Bailey, 1944; Cavallito et al., 1944; Hanley and Fenwick, 1985) and activity against diabetes (Hanley and Fenwick, 1985; Mathew and Augusti, 1973). Pharmacological activity of other compounds found in garlic, including diallyl sulfide (Block et al., 1986, 1988; Sparnirs et al., 1988; Sumiyoshi and Wargovich, 1989, 1990; Wargovich, 1987; Wargovich et al., 1988), bis(2-propenyl) 2-propenyl disulfide (diallyl disulfide, DADS, **2**) (Block et al., 1986, 1988; Hanley and Fenwick, 1985), methyl allyl trisulfide (Ariga et al., 1981), bis(2-propenyl) 2-propenyl trisulfide (**3**) (Apitz-Castro et al., 1983); (Z)- and (E)-ajoene [4,5,9-trithiadodeca-1,6,11-triene 9-oxide, (Z)-**4** and (E)-**5**] (Apitz-Castro et al., 1983; Block et al., 1984, 1986, 1988; Gargouri et al., 1989; Yoshida et al., 1987), 2-ethenyl-4H-1,3-dithiin (**6**) (Apitz-Castro et al., 1983; Block et al., 1986; Egen-Schwind et al., 1992), 3-ethenyl-3,4-dihydro-1,2-dithiin (**7**), S-allylcysteine (SAC, **8**) (Nakagawa et al., 1988; Sumiyoshi and Wargovich, 1990), (+)-S-2-propenyl L-cysteine S-oxide



(alliin, **9**) (Itokawa et al., 1973), a new furostanol glycoside (26-O-β-glucopyranosyl 22-hydroxy-25(R)-5α-furostane-3β,6β-26-triol 3-O-β-glucopyranosyl(1→2)-[β-glucopyranosyl(1→3)]-β-glucopyranosyl(1→4)-β-galactopyranoside, **10**) (Matsuura et al., 1988), and allixin (**11**) (Nishino et al., 1990; Yamasaki et al., 1991), have been also reported. It is also known (Block, 1992; Block et al., 1984, 1986, 1988, 1992; Lin, 1989) that compounds **2**–**5** and other organosulfur compounds found in garlic are derived from allicin (**1**).

Allicin (**1**) is not present in raw garlic but is rapidly produced by the action of CS-lyase (alliinase) on alliin (**9**). Alliinase is activated by crushing or cutting the garlic cloves (Block, 1985; Cavallito and Bailey, 1944; Cavallito et al., 1944; Stoll and Seebeck, 1951). Comercially, allicin (**1**) and allicin potential (alliin and alliinase activity) have been considered as indices for

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evaluation of the medicinal value of garlic preparations. Owing to errors in previous structure determinations of compounds from garlic and in analytical analyses, quantitative HPLC methods for the analysis for allicin (**1**), alliin (**9**), and other compounds from garlic have been developed (Blania and Spangenberg, 1991; Block, 1992; Block et al., 1992; Iberl et al., 1990a,b; Jansen et al., 1987; Koch and Jager, 1989; Koch et al., 1989; Lawson et al., 1991; Miething, 1985; Mochizyki et al., 1988; Muller, 1989; Saito et al., 1988; Voigt and Wolf, 1986; Ziegler and Sticher, 1989).

Owing to its instability in fatty oil and in organic solvents (Iberl et al., 1990b; Voigt and Wolf, 1986), it is doubtful whether allicin (**1**) is responsible for some of the pharmacological properties attributed to garlic (Block, 1992; Block et al., 1992; Lin, 1989). Before one can evaluate the effectiveness of allicin (**1**) in the body, it must be determined whether or not it (**1**) can actually reach the target organs. If allicin (**1**) or an allicin-containing substance is ingested, it (**1**) would be exposed to the acidic conditions of the stomach and then to the neutral conditions of the intestines. For these reasons, the relative stability of allicin (**1**) in blood, different solvents [ethyl acetate, methanol, water (pH 1.2 and 7.5)], simulated gastric fluid (SGF, pH 1.2), and simulated intestinal fluid (SIF, pH 7.5) was investigated.

Dried garlic powders also have allicin-releasing capacity which is referred to as the allicin-producing potential. Allicin (**1**) is formed from these preparations on contact with aqueous solution. Thus, the detection of allicin (**1**), the stability of **1**, and the allicin-producing potential of several commercial garlic preparations were also investigated under SGF, SIF, and simulated digestive conditions (sequential combination of SGF and SIF).

EXPERIMENTAL PROCEDURES

Materials and Reagents. Chemicals and reagents were purchased from Aldrich Chemical Co. and Fisher Scientific. The garlic products and grade A homogenized and pasteurized cow's milk were purchased at local grocery stores, and the garlic powder was purchased from the American Vegetable Co. The enzymes pepsin and pancreatin were purchased from J. T. Baker Chemical Co. Kyolic LQ (lot 1E02) and Kyolic 100 cap (lot 1K84) are distributed by Wakunaga of America Co., Mission Viejo, CA. Blood (type B) was collected in a VACUTAINER tube that contained sodium heparin from a healthy 33-year old male at the Department of Community and Environmental Medicine, University of California, Irvine. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to *The United States Pharmacopeia XXII* (1990).

Equipment. IR spectra were obtained with a Perkin-Elmer 283 FTIR spectrophotometer, calibrated with the 1601 cm^{-1} absorption of polystyrene, in CCl_4, as neat films or as KBr disks. A Shimadzo VU-160 UV–visible recording spectrophotometer was used to obtain visible spectra. High-resolution mass spectra (HRMS) were obtained with a VG 7070E-HF mass spectrometer (70 eV). Medium-resolution mass spectra were obtained with a Finnigan 9610 GC-EI-CI mass spectrometer with a Nova 3 data system operating at an ionization potential of 70 or 100 eV. Chemical ionization mass spectra (CIMS) were obtained by using 2-methylpropane as the reactant gas. ^1H NMR spectra were recorded at 250 MHz (Bruker WM-250), at 300 MHz (GE-Nicolet), and at 500 MHz (GE-Nicolet) with the solvent(s) noted. Chemical shifts (δ) are reported downfield from internal Me_4Si (~0.5% for Fourier transform) at δ 0.00. ^{13}C NMR data were obtained with a GE-Nicolet 300 MHz spectrometer. Reagents and solvents were purified according to standard procedures. Dinitrogen was dried by passing it through a column of Drierite and 5 Å molecular sieves. Thin layer chromatography (TLC) was performed on silica gel glass plates (Merck, $60F_{254}$, 250 μm thick) that were developed in the appropriate solvents. After the solvent had risen to a line near the top, the plates were analyzed with iodoplatinate reagent and ultraviolet light and/or developed in a diiodine chamber. A Fisher Marathon Series Model 6K general purpose centrifuge with a maximum speed of 9000 rpm and a maximum force of 6520g was used.

The Shimadzu HPLC LC-600 system with a TSK gel ODS 80 TM (4.6 mm i.d. × 15 cm, Tosoh) column was used for these experiments. Eluent solvents for the separation of allicin (**1**) and its products were 38% acetonitrile–62% water (eluent A) and 80% acetonitrile–20% water (eluent B), respectively. Separation was performed using a gradient of both eluents. Flow rate was 0.8 mL/min. Dimethyl phthalate was used as internal standard (IS) for the experiments on allicin stability. The amount of allicin remaining and the products derived from allicin (**1**) were calculated by comparison of their peak areas with that of the IS. Freshly prepared allicin (**1**, *vide infra*) was used as the standard for the quantitative determination of allicin (**1**) in all experiments.

Synthesis of Allicin (1). Hydrogen peroxide (30%, 11.7, g, 103 mmol) was added dropwise to a solution of diallyl disulfide (**2**, 15 g, 103 mmol) in acetic acid (100 mL) at 0 °C (Block et al., 1986; Cavallito and Bailey, 1944; Cavallito et al., 1944; Freeman et al., 1993; Vedejs et al., 1982). The reaction mixture was stirred for 5 h at 23 °C and poured into 800 mL of H_2O at 0 °C. The solution was extracted with CH_2Cl_2 (3 × 50 mL), the extract was dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue was purified by HPLC using a reversed phase column (YMC pack D-ODS-5, 20 mm i.d. × 250 mm, Yamamura Kagaku) and 38% acetonitrile–62% water as the eluent. The eluents were monitored with a Shimadzu UV monitor at 254 nm. The allicin-containing fraction was collected and extracted with dichloromethane. The extract was dried (Na_2SO_4) and the solvent removed *in vacuo* to give allicin (**1**, 93% purity), which was used in subsequent experiments and as the standard for quantitative analysis. The freshly prepared allicin (**1**) was stored at dry ice temperature. ^1H NMR ($CDCl_3$) δ 5.83–5.96 (m, 2 H, –CH=), 5.16–5.45 (m, 4 H, CH_2), 3.71–3.86 (m, 4 H, CH_2); ^{13}C NMR ($CDCl_3$) δ 34.87, 59.60, 118.91, 125.63, 125.63, 132.71.

Synthesis of (*E*)-Ajoene (4). (*E*)-Ajoene (**4**) was synthesized as previously described (Block et al., 1986; Iberl et al., 1990a), purified (93% pure) by HPLC using the method described above for allicin (**1**), and stored at −16 °C. ^1H NMR ($CDCl_3$) δ 6.35 (d, 1 H, J = 14.83 Hz, =CHSS), 5.74–5.95 (m, 3 H, =CHC), 5.36–5.47 [m, 2, H, CH_2=CCS(O)–], 5.13–5.19 (m, 2 H, CH_2=CCSS), 3.37–3.57 [m, 4 H, $CH_2S(O)CH_2$], 3.33 (d, 2 H, J = 7.09 Hz, $SSCH_2$–); ^{13}C NMR ($CDCl_3$) δ 41.27, 52.90, 54.29, 116.64, 119.28, 123.86, 125.53, 132.47, 134.69.

Synthesis of 2-Ethenyl-4*H*-1,3-dithiin (6). 2-Ethenyl-4*H*-1,3-dithiin (**6**) was synthesized as previously described (Block et al., 1986; Breslin, 1983; Iberl et al., 1990a; Vedejs et al., 1982). The 1,3-dithiin **6** was purified using a silica gel column equilibrated with hexanes. The eluted fractions were examined by TLC, the fractions containing the dithiin **6** were collected, and the solvent was removed under reduced pressure. The 97.3% pure dithiin (**6**) was stored at −16 °C. ^1H NMR ($CDCl_3$) δ 6.25 (d, 1 H, J = 10.63 Hz, SCH=C–), 5.87–5.98 (m, 2 H, –CH=), 5.23–5.37 (q, 2 H, CH_2=), 4.69 (d, 1 H, J = 6.86 Hz, –SCHS–), 3.14–3.38 (m, 2 H, ABXY, $-CH_2S$); ^{13}C NMR ($CDCl_3$) δ 133.85, 121.72, 118.11, 116.84, 44.78, 24.75; HRCIMS m/z 145.0123, calcd for $C_6H_8S_2H^+$ 145.0143.

Determination of Allicin Stability in Organic Solvents. A methanol solution (100 mL) containing 43 mg of allicin (**1**) and 35 mg of internal standard (IS, dimethyl phthalate) was prepared, divided into three portions, and stored at −16, 6, and 23 °C. An ethyl acetate solution (50 mL) containing about 60 mg of allicin (**1**) and 100 mg of IS was prepared, divided into three portions, and stored at the same temperatures as the methanol solution. Aliquots were taken at specified times from each solution and analyzed by HPLC for the concentration of allicin. The amount of allicin (**1**) left was calculated by comparison of the ratio of the peak areas of IS and allicin.

Determination of Allicin (1) Stability in an Aqueous Solution at pH 1.2. Allicin (**1**, 60 mg) and IS (113 mg) were

added to a 100 mL volumetric flask, and an aqueous solution of pH 1.2 was added to volume. The mixture was incubated at 37 °C. Aliquots were taken at specified times from each solution and analyzed by HPLC for the concentration of allicin. The amount of allicin left was calculated by comparison of the ratio of the peak areas of IS and allicin. The aqueous solution of pH 1.2 was prepared according to the simulated gastric fluid (SGF) specifications of *The United States Pharmacopeia XXII*, with the exception that pepsin was omitted.

Determination of Allicin Stability in an Aqueous Solution at pH 7.5. Allicin (**1**, 60 mg) and IS (106 mg) were added to a 100 mL volumetric flask, and an aqueous solution of pH 7.5 was added to volume. The mixture was incubated at 37 °C. Aliquots were taken at specified times from each solution and analyzed by HPLC for the concentration of allicin. The amount of allicin left was calculated by comparison of the ratio of the peak areas of IS and allicin. The aqueous solution of pH 7.5 was prepared according to the simulated intestinal fluid (SIF) specifications of *The United States Pharmacopeia XXII*, with the exception that pancreatin was omitted.

Determination of Allicin Stability in Blood. A dilute allicin solution in saline (1.04 mg/mL) was prepared by pipetting 2 mL of an allicin solution (52 mg/mL in EtOH) into a 100 mL volumetric flask and adding saline (0.9 w/v % of NaCl in water) to volume. One milliliter of this diluted solution was transferred to a 10 mL volumetric flask, heparinized blood was added to volume, and the mixture was incubated at 37 °C. One milliliter aliquots were pipetted from the incubating mixture at 5, 12, and 30 min into a 10 mL volumetric flask. After 7 mL of methanol and 1 mL of IS solution (0.58 mg/mL) were added to the flask, methanol was added to volume. This mixture was shaken vigorously and transferred to a centrifuge tube which was centrifuged at 3000 rpm for 5 min. The supernatant was used as the sample solution. The control experiment was performed in the same manner using saline in place of the heparinized blood.

The denatured blood sample solution containing allicin (**1**) was prepared as follows. Heparinized blood (9 mL) was pipetted into a 100 mL volumetric flask, 80 mL of methanol was added, the mixture was gently shaken, 10 mL of IS solution (0.58 mg/mL) and 1 mL of the dilute allicin solution in saline (1.04 mg/mL) were added, and methanol was added to volume. The mixture was shaken vigorously and transferred to a centrifuge tube which was centrifuged at 3000 rpm for 5 min. The supernatant was used as the sample solution.

Measurement of Visible Spectra after Addition of Allicin to Blood. One milliliter of an allicin solution (53 mg/mL in EtOH) was pipetted into a 50 mL volumetric flask, and saline was added to volume. Heparinized blood (1 mL) was pipetted into a 50 mL volumetric flask, and saline was added to volume. Five milliliters of this solution were transferred to a 25 mL volumetric flask, 5 mL of the diluted allicin solution was added, and saline was added to volume. The visible spectrum between 500 and 650 nm was obtained after the mixture was allowed to stand at 23 °C for 5 min. The control experiment was conducted in the same manner using saline instead of the diluted allicin solution.

Sample Preparation of Plasma Fraction and Blood Cell Fraction. Heparinized blood (10 mL) was centrifuged at 1800 rpm for 10 min, and the upper phase was collected with a pipet as the plasma fraction. Saline was added to the lower phase to initial volume. This mixture was shaken gently and centrifuged at 1800 rpm for 10 min, and the upper phase was removed with a pipet and discarded. This washing with saline was repeated twice, and the lower phase was designated the blood cell fraction.

Determination of Allicin Stability in the Plasma Fraction and in the Blood Cell Fraction. Two milliliters of an allicin solution (53 mg/mL in EtOH) was pipetted into a 100 mL volumetric flask, saline was added to volume, and 2 mL of this solution was transferred to a 10 mL volumetric flask. The plasma fraction was added to volume, and the mixture was incubated at 37 °C. A 2 mL aliquot of the mixture was taken at 4, 36, 63, and 120 min and pipetted into a 10 mL volumetric flask. About 6 mL of methanol and 1 mL of IS solution (0.65 mg/mL) were added, followed by addition of methanol to volume. Each mixture was shaken vigorously and transferred to a centrifuge tube which was centrifuged at 3000 rpm for 5 min. The supernatant was used as the sample solution for the determination of allicin stability by HPLC. The control experiment was performed in the same manner, using saline instead of the plasma fraction, with sampling at 0, 30, and 123 min.

The same procedure was used to ascertain the effect of the blood cell fraction on allicin stability, with the exception that the blood cell fraction was used instead of the plasma fraction and the samples were taken at 3 and 30 min.

Determination of the Stability of Diallyl Disulfide (2) in Blood. Diallyl disulfide (**2**, 62 mg) and IS (52 mg) were placed in a 10 mL volumetric flask, and ethanol was added to volume. One milliliter of this solution was diluted to 100 mL with saline. One milliliter of this diluted solution was mixed with 3 mL of heparinized blood, and the mixture was incubated at 37 °C. An aliquot (0.4 mL) of this mixture was removed at 0, 20, and 60 min and diluted with 0.8 mL of methanol. Each mixture was shaken vigorously and transferred to a centrifuge tube which was centrifuged at 3000 rpm for 5 min. The supernatant was used as the sample solution for the determination of diallyl disulfide (**2**) stability. The control experiment was performed in the same way with the exception that saline was used in place of blood and samples were taken at 0 and 60 min.

Determination of Allicin Content in Garlic Products. A sample of each garlic product was prepared as follows. When necessary, a garlic product was ground into a fine powder. About 5 g of garlic product was placed in a 50 mL centrifuge tube, and 8 mL of ethyl acetate was added. The tube was shaken vigorously and centrifuged at 3000 rpm for 5 min, and the supernatant was transferred to a test tube. The volume of the supernatant was reduced to 1−2 mL using a gentle dinitrogen stream. The residue was extracted again in the same manner, the supernatant was added to the first supernatant, and the volume was again reduced to 1−2 mL. The residue was extracted a third time in the same way, the supernatant was added to the previous supernatants, and the volume was again reduced to 1−2 mL. The concentrated supernatant solution was transferred to a 10 mL volumetric flask. The test tube was rinsed with a small amount of methanol which was added to the flask, followed by addition of methanol to volume. This solution was used as the sample solution and was centrifuged before use as necessary.

Determination of Allicin-Producing Potential from Garlic Products. A sample of each garlic product was prepared as follows. When necessary, a garlic product was ground into a fine powder. About 1−2 g of garlic product was placed in a 50 mL of volumetric flask, and water was added to volume. The mixture was allowed to stand for 30 min at 23 °C and transferred to a centrifuge tube which was centrifuged at 3000 rpm for 5 min. The supernatant was used as the sample solution.

Determination of Allicin-Producing Potential of Garlic Powder in Simulated Gastric Fluid (SGF) and in Simulated Intestinal Fluid (SIF). Garlic powder (2 g) was placed in a 50 mL volumetric flask, and SGF or SIF was added to volume. After incubation for 1 h at 37 °C, the mixture was centrifuged at 4000 rpm for 5 min. The supernatant was used as the sample solution.

Determination of Allicin-Producing Potential from Garlic Powder under Simulated Digestive Conditions. Garlic powder (2 g) was placed in a 50 mL centrifuge tube, 20 mL of SGF was added, and the tube was capped tightly, shaken thoroughly, and then incubated for 1 h at 37 °C. An aliquot (0.2 mL) was removed from the mixture and centrifuged at 4000 rpm for 5 min, and the supernant was used for allicin assay. The remaining mixture was neutralized with 0.2 M NaOH solution and then transferred to a 50 mL volumetric flask. About 20 mL of SIF was added to volume and then incubated at 37 °C. Aliquot (1 mL) were removed at 60 and 120 min and centrifuged at 4000 rpm for 5 min. The supernatant was used as the sample solution.

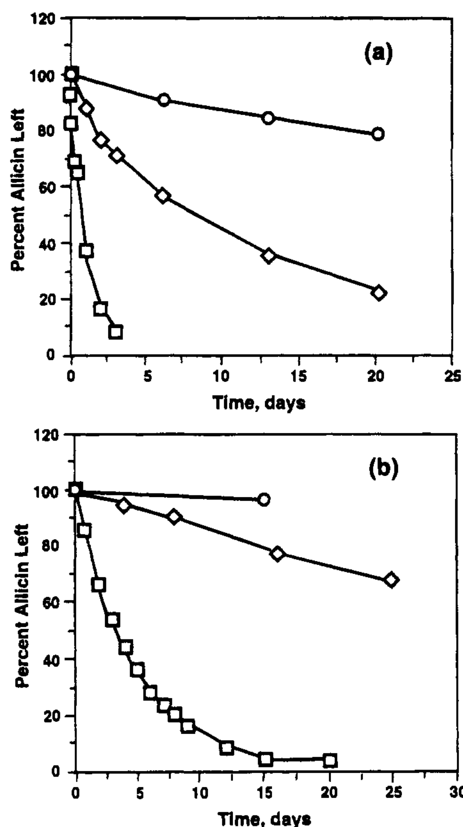

Figure 1. Allicin (**1**) stability in organic solvents: (○) at −16 °C; (◇) at 6 °C; (□) at room temperature in ethyl acetate (a) and in methanol (b). Percent allicin (**1**) remaining was calculated by comparison of the ratios of peak areas of internal standard (IS) and **1**. See Figure 2 and Experimental Procedures for HPLC conditions.

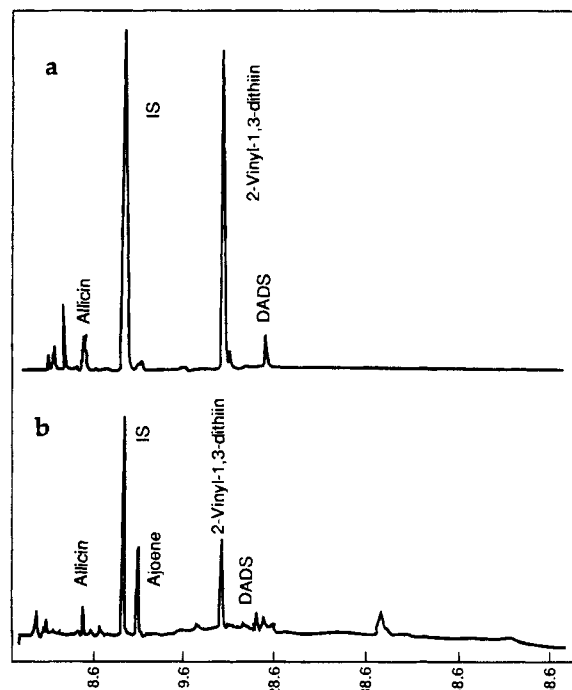

Figure 2. Chromatograms of allicin (**1**) in organic solvents at room temperature: (a) in ethyl acetate for 3 days; (b) in methanol for 15 days. HPLC gradient conditions: 0−10 min, 0% eluant B; 10−20 min, 0−45% eluant B linear gradient; 20−50 min, 45% eluant B; 50−60 min 0% eluant B.

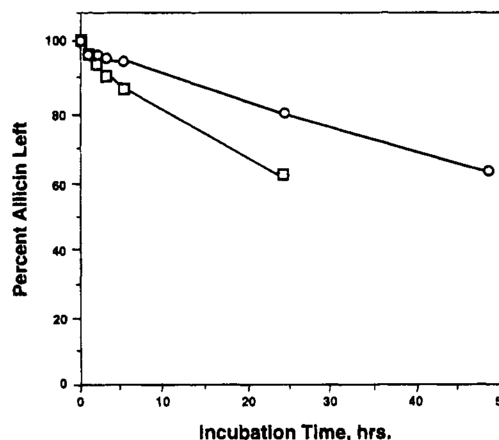

Figure 3. Allicin (**1**) stability in water at gastric (1.2) and intestinal (7.5) pH at 37 °C; (○) pH 1.2; (□) pH 7.5. Percent allicin (**1**) remaining was calculated from the ratios of peak areas of internal standard (IS) and **1**. See Figure 2 and Experimental Procedures for HPLC conditions.

RESULTS AND DISCUSSION

Figure 1 shows the greater stability of allicin (**1**) in methanol than in ethyl acetate. These results are consistent with the report (Iberl et al., 1990a) that allicin (**1**) is more stable in solvents capable of hydrogen bonding.

Figure 2 shows the chromatograms of the products (Block, 1985; Cavallito and Bailey, 1944; Cavallito et al., 1944; Iberl et al., 1990a; Lawson et al., 1991; Vedejs et al., 1982; Voigt and Wolf, 1986) obtained from allicin (**1**) in ethyl acetate and in methanol. In methanol, allicin (**1**) was transformed mainly to (*E*)-ajoene (**5**) and 2-ethenyl-4*H*-1,3-dithiin (**6**) (approximate ratio **5**:**6** = 1.0:1.1), and in ethyl acetate, it was transformed mainly to dially disulfide (**2**) and dithiin (**6**) (approximate ratio **2**:**6** = 1.0:8.8).

Figure 3 shows the greater stability of allicin (**1**) after incubation at gastric pH (pH 1.2) than at intestinal pH (pH 7.5). After 1 day at pH 1.2, about 80% of allicin remained and, at pH 7.5, about 62% of allicin remained. Interestingly, allicin does not appear to generate its normal transformation products at these pH values since an increase in the concentration of diallyl disulfide (**2**), (*E*)-ajoene (**5**), and dithiin (**6**) was not observed. Therefore, gastric or intestinal pH may not be a significant factor affecting allicin availability or decomposition in the body during the digestive period. Since food substances are normally present in the gastrointestinal tract, interaction of allicin with cow's milk was examined. After exposure of milk for 1 h, almost all allicin was recovered.

Figures 4 and 5 show that 83% of allicin (**1**) was lost during mixing with methanol-denatured heparinized blood and centrifugation without incubation. No allicin could be detected after it was incubated in heparinized blood for 5 min. Concurrent with the disappearance of allicin, the presence of diallyl disulfide (**2**) was observed in the mixture of allicin and blood. Figure 6 shows the ability of diallyl disulfide in blood. The concentration of diallyl disulfide remained unchanged after 1 h.

Figure 7 shows that allicin (**1**) is more reactive to the blood cell fraction than to the plasma fraction. No allicin was detected after 3 min when it was incubated in the blood cell fraction, but in the plasma fraction, the concentraion of allicin decreased gradually and the half-life of allicin was estimated to be about 50 min.

Another notable observation was the rapid change in color of the red blood cells to a dark red color after addition of allicin (**1**). Hemoglobin has the potential to bind to dioxygen since the iron is divalent. Hemoglobin bound to dioxygen, which is called oxyhemoglobin, has

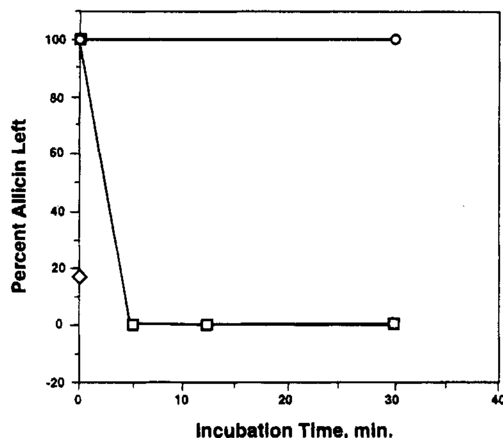

Figure 4. Allicin (**1**) stability in blood: (○) control; (□) blood sample; (◇) denatured blood sample. Percent allicin (**1**) remaining was calculated from the ratios of peak areas of internal standard (IS) and **1**. See Figure 2 and Experimental Procedures for HPLC conditions.

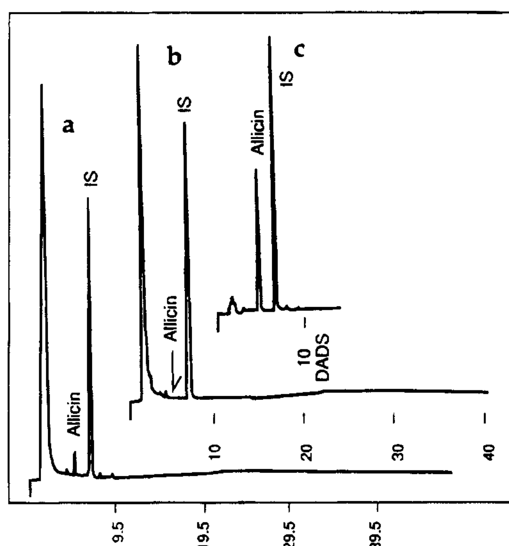

Figure 5. Chromatogram of allicin (**1**) incubated with blood: (a) sample of denatured blood in methanol; (b) blood sample incubated for 5 min at 37 °C; (c) control solution (0 min). See Figure 2 and Experimental Procedures for HPLC conditions.

absorption maxima at 541 and 577 nm (French et al., 1978; Kawanishi et al., 1985; Winterbourn et al., 1976, 1979; Winterbourn, 1990). When the iron in hemoglobin is oxidized to the trivalent state, methemoglobin, which does not bind dioxygen and has an absorption maximum at 630 nm in acidic solution, is formed (Winterbourn et al., 1976, 1979; Winterbourn, 1990). Figure 8 shows that the 541 and 577 nm absorbances of hemoglobin decreased and the 630 nm absorbance of methemoglobin appeared after addition of allicin. These results suggest that the iron in hemoglobin may be rapidly oxidized by interaction with allicin (see Figures 4–7).

After incubation for 3 min in liver homogenate, a decrease of 90% of the initial allicin (**1**) concentration was observed and the decrease of allicin (**1**) was 99% after 6 min (Egen-Shwind et al., 1992). This report and our obtained results (see Figures 408) suggest that allicin is highly interactive with blood cell fraction and liver cell. On the other hand (Pushpendran et al., 1980), it was observed that the metabolism of ^{35}S-labeled diallyl disulfide in mice reached a maximal concentration in the liver 90 min after ip injection. Although allicin disappears rapidly in the body after absorption,

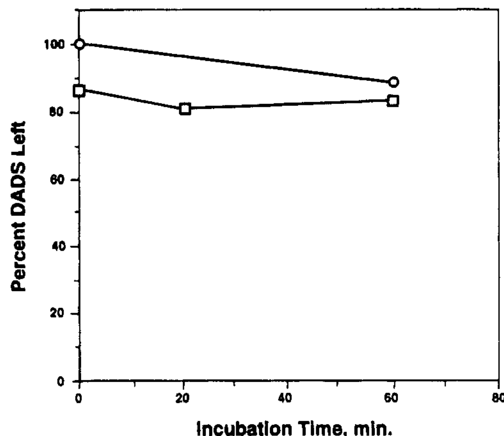

Figure 6. Stability of bis(diallyl) disulfide (DADS, **2**) in blood: (○) control; (□) blood sample. Percent DADS (**2**) remaining was calculated from the ratios of peak areas of internal standard (IS) and **2**. See Figure 2 and Experimental Procedures for HPLC conditions.

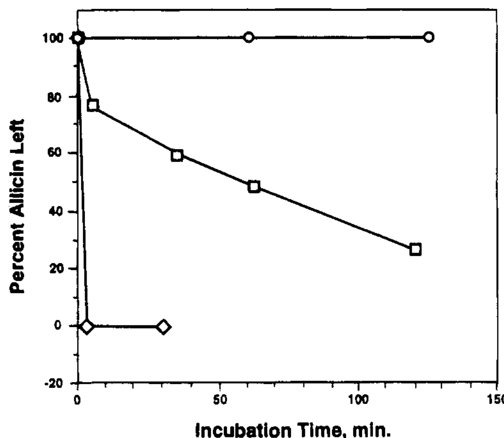

Figure 7. Allicin (**1**) stability in the plasma fraction and blood cell fraction: (○) control; (□) plasma fraction sample; (◇) blood cell fraction. Percent allicin (**1**) remaining was calculated from the ratios of peak areas of internal standard (IS) and **1**. See Figure 2 and Experimental Procedures for HPLC conditions.

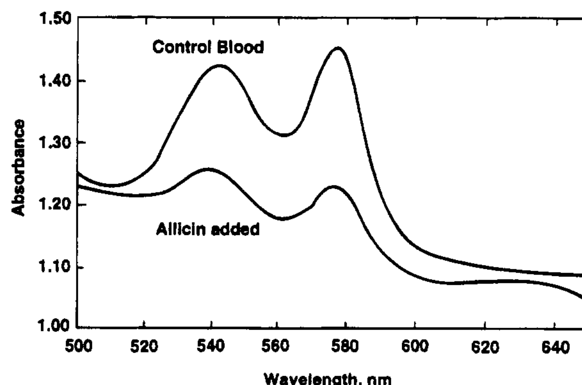

Figure 8. Visible spectrum of blood containing allicin (**1**). The sample was diluted with saline solution.

diallyl disulfide may be absorbed and delivered to organs (Egen-Shwind et al., 1992; Pushpendean et al., 1980).

The allicin content and allicin potential of commercial garlic preparations, labeled as containing allicin/allicin potential, were determined. As shown in Table 1, the allicin content of all of these garlic preparations was less than 1 ppm (the limit of detection). Variable allicin potentials were observed in different brands of products as well as among different lots of the same brand. Kyolic LQ (lot 1E02) and Kyolic 100 cap (lot 1K84),

Table 1. Allicin Content and Allicin-Producing Potential of Various Types of Garlic Preparations

product[a]	allicin content (μg/g of product)	allicin potential (μg/g of product)
A, sample 1	ND[b]	1290
A, sample 2	ND	1620
B, sample 1	ND	700
B, sample 2	ND	340
B, sample 3	ND	550
C	ND	560
D	ND	720
E	ND	280
F	ND	320
G	ND	640

[a] The following products were tested in this study: A, brand name Kwai, which is distributed by Lichtwer-Pharma (Berlin); sample 1, lot 90070403; sample 2, lot 90092003. B, brand name Ilja Rogoff, which is distributed by Woelm Pharma (Eschwege); sample 1, 281990; sample 2, lot 213128; sample 3, lot 2101090. C, brand name Garlicin, which is distributed by Natur's Way Product Inc. (Springville); lot 094061. D, brand name Garlicin HC, which is distributed by the same company as product C; lot 093688. E, brand name Garlicin CF, which is distributed by the same company as product C; lot 093710. F, brand name Quintessence, which is distributed by Pur-Gar Inc. (Tacoma); lot 2246. G, brand name Allirich, which is distributed by Arizona Natural Product Inc. This product has no lot no. These products were purchased from stores. [b] Not detected. Limit of detection is 1 μg/g of product.

Table 2. Allicin-Producing Potential of Garlic Powder[a] in Water, Simulated Gastric Fluid (SGF), and Simulated Intestinal Fluid (SIF)

incubation conditions	allicin-producing potential (μg/g of garlic powder)
water, 30 min, 37 °C	4640 (100%)[b]
SGF, 60 min, 37 °C	190 (4.1%)
SIF, 60 min, 37 °C	2863 (61.7%)

[a] Snow White brand garlic powder which is distributed by American Vegetable Co., was used for this study. [b] Numbers in parentheses are percentage of produced allicin against incubated with water.

which were prepared with aged garlic extract and were not labeled as containing allicin/allicin potential, were also studied. Neither preparation contained a detectable amount of allicin or allicin potential.

All but one of the commercial garlic products tested showed production of allicin upon incubation in water. However, such an observation cannot be assumed to be indicative in allicin production after ingestion of garlic preparations, considering the conditions in the gastric and intestinal tracts which are different from those in a flask containing only water. Therefore, allicin production of a commercially available garlic powder under simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) was investigated (Tables 2 and 3).

After 1 h under SIF conditions, about 62% of the amount of allicin produced with water was observed, and after 1 h under SGF condition, only 4% could be found. This low level of allicin production under SGF condition may be due to hydrolysis of alliinase by acid and the enzyme pepsin. Under simulated digestive conditions (sequential combination of SGF and SIF), only about 1% of allicin was observed. The small amount of allicin observed is not due to its decomposition during the incubation period because it was shown to be stable at gastric and intestinal pH for 2−3 h (Figure 3; Table 3).

Although allicin (**1**) is stable under the gastric–intestinal condition, allicin could not reach target organs after absorption according to a study of Egen-Shwind et al. and our obtained results. These results suggest that allicin may not directly give medical effects to

Table 3. Amount of Allicin Obtained from Garlic Powder[a] under Simulated Digestive Conditions (Sequential Combination of SGF and SIF)

incubation conditions	amount of allicin (μg/g of garlic powder)
SGF, 60 min, 37 °C	90
SIF, 60 min, 37 °C[b]	64
SIF, 120 min, 37 °C[c]	54

[a] Snow White brand garlic powder, which is distributed by American Vegetable Co., was used for this study. [b] After incubation with SGF for 60 min and neutralization with NaOH. [c] After incubation with SGF for 60 min and neutralization with NaOH.

target organs as an active principle inside the body. Metabolism of allicin and the identification of other medical effective compounds derived from garlic must be further investigated.

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LITERATURE CITED

Apitz-Castro, R.; Cabrera, S.; Cruz, M. R.; Ledezma, E.; Jain, M. K. Effects of garlic extract and of three pure components isolated from it on human platelet aggregation, arachidonate metabolism, release reaction and platelet ultrastructure. *Thromb. Res.* **1983**, *32*, 155−169.

Ariga, T.; Oshiba, S.; Tamada, T. Platelet aggregation inhibitor in garlic. *Lancet* **1981**, 150−151.

Beslin, P. A facile synthesis of two thioacrolein dimers. A new entry to a flavor component in asparagus. *J. Heterocycl. Chem.* **1983**, *20*, 1753−1754.

Blania, G.; Spangenberg, B. Formation of allicin from dried garlic (*Allium sativum*): a simple HPTLC method for simultaneous determination of allicin and ajoene in dried garlic and garlic preparations. *Planta Med.* **1991**, *57*, 371−375.

Block, E. The chemistry of garlic and onions. *Sci. Am.* **1985**, *252*, 114−119.

Block, E. Antithrombotic agents from garlic: a lesson from 5000 years of folk medicine. In *Folk Medicine: The Art and the Science*; Steiner, R. P., Ed.; American Chemical Society: Washington, DC, 1986; pp 125−137.

Block, E. Biologically active organosulfur compounds from garlic and onions: the search for new drugs. In *Sulfur-Centered Reactive Intermediates in Chemistry and Biology*; Chatgilialoglu, C., Asmus, D., Eds.; Plenum Press: New York, 1991; pp 282−289 (NATO Conference Series).

Block, E. The organosulfur chemistry of the genus *Allium* implications for organic sulfur chemistry. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1135−1178.

Block, E.; Ahmad, S.; Jain, M. K.; Crecely, R. W.; Apitz-Castro, R.; Cruz, M. R. (*E,Z*)-Ajoene: a potent antithrombotic agent from garlic. *J. Am. Chem. Soc.* **1984**, *106*, 8295−8296.

Block, E.; Ahmad, S.; Catalfamo, J. L.; Jain, M. K.; Apitz-Castro, R. Antithrombotic organosulfur compounds from garlic: structural, mechanistic, and synthetic studies. *J. Am. Chem. Soc.* **1986**, *108*, 7045−7055.

Block, E.; Iyer, R.; Saha, C.; Grisoni, S.; Belman, S.; Lossing, F. Lipoxygenase inhibitors from the essential oil of garlic. Markovnikov addition of the allyldithio radical to olefins. *J. Am. Chem. Soc.* **1988**, *110*, 7813−7827.

Block, E.; Naganathan, S.; Putman, D.; Zhao, S.-H. *Allium* chemistry: HPLC analysis of thiosulfinates from onion, garlic, wild garlic (ramsons), leek, scallion, shallot, elephant (great head) garlic, chive, and Chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. *J. Agric. Food Chem.* **1992**, *40*, 2418−2430.

Cavallito, C. J.; Bailey, J. H. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. *J. Am. Chem. Soc.* **1944**, *66*, 1950−1951.

Cavallito, C. J.; Buck, J. S.; Suter, C. M. Allicin, the antibacterial principle of *Allium sativum*. II. Determination of the chemical structure. *J. Am. Chem. Soc.* **1944**, *66*, 1952–1954.

Egen-Schwind, C.; Eckard, R.; Jekat, F. W.; Winterhoff, H. Pharmacokinetics of vinyldithiins, transformation products of allicin. *Planta Med.* **1992**, *58*, 8–13.

Freeman, F.; Lin, R. I.-S.; Ma, X. Garlic chemistry: peroxidation of bis(2-propenyl) disulfide, bis(2-propenyl) trisulfide, and their monoxide derivatives. *Sulfur Lett.* **1993**, *15*, 253–262.

French, J. K.; Winterbourn, C. C.; Carrell, B. W. Mechanisum of oxyhemoglobin breakdown on reaction with acetylphenylhydrazine. *Biochem. J.* **1978**, *173*, 19–26.

Gargouri, Y.; Moreau, H.; Jain, M. K.; de Haas, G. H.; Verger, R. Ajoene prevents fat digestion by human gastric lipase *in vitro*. *Biochim. Biophys. Acta* **1989**, *1006*, 137–139.

Hanley, A. B.; Fenwick, G. R. Cultivated *Alliums*. *J. Plant Foods* **1985**, *6*, 211–238.

Iberl, B.; Winkler, G.; Knobloch, K. Products of allicin transformation: ajoenes and dithiins characterization and their determination by HPLC. *Planta Med.* **1990a**, *56*, 202–211.

Iberl, B.; Winkler, G.; Muller, B.; Knobloch, K. Quantitative determination of allicin and alliin from garlic by HPLC. *Planta Med.* **1990b**, *58*, 320–326.

Itokawa, Y.; Inoue, K.; Sasagawa, S.; Fujiwara, M. Effect of S-methylcysteine sulfoxide, S-allylcysteine sulfoxide and related sulfur-containing amino acids on lipid metabolism of experimental hypercholesterolemic rats. *J. Nutr.* **1973**, *103*, 88–92.

Jansen, H.; Muller, B.; Knobloch, K. Allicin characterization and its determination by HPLC. *Planta Med.* **1987**, *53*, 559–562.

Kawanishi, S.; Caughey, W. S. Mechanism of electron transfer to coordinated dioxygen of oxyhemoglobins to yield peroxide and methemoglobin. *J. Biol. Chem.* **1985**, *260*, 4622–4631.

Koch, H. P.; Jäger, W. Garlic. Allicin release from fresh and dried garlic and products thereof. *Dtsch. Apoth. Ztg.* **1989**, *129*, 273–276.

Koch, J.; Berger, L.; Vieregge-Reiter, C. Allicin in garlic (*Allium sativum* L.) and garlic preparations: determination by headspace gas chromatography. *Planta Med.* **1989**, *55*, 327–331.

Lawson, L. D.; Wood, S. G.; Hughes, B. G. HPLC analysis of allicin and other thiosulfinates in garlic clove homogenates. *Planta Med.* **1991**, *57*, 263–270.

Lin, R. I-S. *Garlic in Nutrition & Medicine*; Nutrition International: Irvine, CA, 1989.

Mathew, P. T.; Augusti, K. T. Studies on the effect of allicin (diallyl disulphide-oxide) on alloxan diabetes: part I—hypoglycaemic action and enhancement of serum insulin effect and glycogen synthesis. *Indian J. Biochem. Biophys.* **1973**, *10*, 209–212.

Matsuura, H.; Ushiroguchi, T.; Itakura, Y.; Hayashi, N.; Fuwa, T. A Furostanol glycoside from garlic bulbs of *Allium sativum L. Chem. Pharm. Bull.* **1988**, *36*, 3659–3663.

Miething, H. Allicin and oil in garlic bulbs—HPLC quantitative determination. *Dtsch. Apoth. Zg.* **1985**, *125*, 2049–2050.

Mochizuki, E.; Nakayama, A.; Kitada, Y.; Saito, K.; Nakazawa, H.; Suzuki, Fujita, M. Liquid chromatographic determination of alliin in garlic and garlic products. *J. Chromatogr.* **1988**, *55*, 271–278.

Müller, B. Analytical estimation of garlic preparations. *Dtsch. Apoth. Ztg.* **1989**, *129*, 2500–2504.

Nakagawa, S.; Kasuga, S.; Matsuura, H. Prevention of liver damage by aged garlic extract and its components in mice. *Phytother. Res.* **1988**, *1*, 1–4.

Nishino, N.; Nishino, A.; Takayasu, J.; Iwashima, A.; Itakura, Y.; Kodera, Y.; Matsuura, H.; Fuwa, T. Antitumor-promoting activity of allixin: a stress compound produced by garlic. *Cancer J.* **1990**, *3*, 20–21.

Pinto, J. T.; Tiwari, R. K.; Qiao, C. H.; Osborne, M.; Lin, R. I-S.; Freeman, F.; Ma, X. Presented at the Fourth International Conference on Prevention of Human Cancer: Nutrition and Chemoprevention Controversies, Tucson, AZ, June 3–6, 1992.

Pushpendran, C. K.; Devasagayam, T. P. A.; Chintalwar, G. J.; Benerji, A.; Eapen, J. The metabolic fate of [^{35}S]-diallyl disulphide in mice. *Experientia* **1980**, *36*, 1000–1001.

Saito, K.; Horie, M.; Hosino, Y.; Nose, N.; Mochizuki, E.; Nakazawa, H. Gas chromatographic analysis of alliin in garlic products. *Eisei Kagaku, Jpn. J. Toxicol. Environ. Health* **1988**, *34*, 536–541.

Sparnins, V. L.; Barany, G.; Wattenberg, L. W. Effects of organosulfur compounds from garlic and onions on benzo[a]pyrene-induced neoplasia and glutathione S-transferase activity in the mouse. *Carcinogenesis* **1988**, *9*, 131–134.

Stoll, A.; Seebeck, E. *Allium* compounds. II. Enzymic degradation of alliins and the properties of allinase. *Helv. Chim. Acta* **1949**, *32*, 197–205.

Stoll, A.; Seebeck, E. Chemical investigations on alliin, the specific principle of garlic. *Adv. Enzymol.* **1951**, *11*, 377–400.

Sumiyoshi, H.; Wargovich, M. J. Garlic (*Allium sativum*): a review of its relationship to cancer. *Asia Pacific J. Pharmacol.* **1989**, *4*, 133–140.

Sumiyoshi, H.; Wargovich, M. J. Chemoprevention of 1,2-dimethylhydrazine-induced colon cancer in mice by naturally occurring organosulfur compounds. *Cancer Res.* **1990**, *50*, 5084–5087.

The United States Pharmacopeia XXII; United States Pharmacopeial Convention: Rockville, MD, 1990.

Vedejs, E.; Eberlein, T. H.; Varie, D. L. Dienophilic thioaldehydes. *J. Am. Chem. Soc.* **1982**, *104*, 1445–1447.

Voigt, M.; Wolf, E. Garlic. HPLC determination of garlic components in extracts, powder, and pharmaceuticals. *Dtsch. Apoth. Ztg.* **1986**, *126*, 591–593.

Wargovich, M. J. Diallyl sulfide, a flavor component of garlic (*Allium sativum*), inhibits dimethylhydrazine-induced colon cancer. *Carcinogenesis* **1987**, *8*, 487–489.

Wargovich, M. J.; Woods, C.; Erg. V. W. S.; Stephens, L. C.; Gray, K. N. Chemoprevention of N-nitrosomethylbenzylamine-induced esophageal cancer in rats by the naturally occurring thioether, diallyl sulfide. *Cancer Res.* **1988**, *48*, 6872–6875.

Weinberg, D. S.; Manier, M. L.; Richardson, M. D.; Haibach, F. G. Identification and quantification of organosulfur compliance markers in a garlic extract. *J. Agric. Chem.* **1993**, *41*, 37–43.

Winterbourn, C. C. Oxidative reactions of hemoglobin. *Methods Enzymol.* **1990**, *186*, 265–272.

Winterbourn, C. C.; McGrath, B. M.; Carrell, R. W. Reactions involving superoxide and normal and unstable hemoglobins. *Biochem. J.* **1976**, *155*, 493–502.

Winterbourn, C. C.; French, J. K.; Claridge, R. F. C. The reaction of menadione with hemoglobin. *Biochem. J.* **1979**, *179*, 665–673.

Yamasaki, T.; Tool, R. W.; Law, B. H. S. Effect of allixin, a phytoalexin produced by garlic, on mutagenesis, DNA-binding and metabolism of aflatoxin B_1. *Cancer Lett.* **1991**, *59*, 89–94.

Yoshida, S.; Kasuga, S.; Hayashi, N.; Ushiroguchi, T.; Matsuura, H.; Nakagawa, S. Antifungal activity of ajoene derived from garlic. *Appl. Environ. Microbiol.* **1987**, *53*, 615–617.

Ziegler, S. J.; Sticher, O. HPLC of *S*-alk(en)yl-L-cysteine derivatives in garlic including quantitative determination of (+)-*S*-allyl-L-cysteine sulfoxide (alliin). *Planta Med.* **1989**, *55*, 372–378.

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