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A Model System Study of the Inhibition of Heterocyclic Aromatic Amine Formation by Organosulfur Compounds

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Organosulfur compounds and sodium bisulfite significantly inhibited ($P < 0.05$) heterocyclic aromatic amine (HAA) formation in model systems containing phenylalanine, creatinine, and glucose. There was, however, no inhibition by the same compounds in a model system containing only phenylalanine and creatinine. Diallyl disulfide (DAD) and dipropyl disulfide (DPD) concentrations in the model systems were significantly decreased ($P < 0.05$) after heating for 10 min at 180 °C. Only very low concentrations of sulfhydryl groups (4.19 and 4.00 μmol) were produced on heating DAD and DPD for 30 min. Reaction of glucose and DAD produced several sulfur-containing compounds. After 10 min of heating at 180 °C, HAA formation in the control model systems was increased significantly, and DAD was an effective inhibitor during this heating period. Tetrahydrothiophene-3-one (THT) and tetrahydrothiophene (THP); two products resulting from the interaction of glucose and DAD, had no direct influence on HAA formation in the model systems.

KEYWORDS: Heterocyclic aromatic amine; sulfur compound; model system; tetrahydrothiophene-3-one; tetrahydrothiophene

INTRODUCTION

When food is cooked, carbonyl and amino compounds react via the Maillard reaction to produce several hundreds of reaction products. Some of these contribute to the color and flavor of the cooked food. The Maillard reaction may also have an impact on the nutritional value of the food (*1*). Furthermore, in some cases, the Maillard reaction can lead to the formation of genotoxic compounds called heterocyclic aromatic amines (*2*−*5*).

Heterocyclic aromatic amines (HAAs) are formed in cooked meat and fish products, most likely from the reaction of proteins, sugar, and creatine (*6* and *7*). These compounds have been shown to induce cancer of the colon, breast, pancreas, and prostate (*8*). The most commonly found HAAs in foods are 2-amino-3-methylimidazo[4,5-*f*]-quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]-quinoline (MeIQ), 2-amino-3,8 dimethylimidazo[4,5-*f*]-quinoxaline (MeIQx), (2-amino-3,4,8 trimethylimidazo[4,5-*f*]-quinoxaline (4,8 DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine (PhIP) (*9*−*11*).

It has been postulated that pyrazines/pyridines, aldehydes, and creatine/creatinine are condensed to form IQx- and IQ-type compounds (*12*, *13*). It has also been determined that PhIP is formed from the reaction of creatinine and glucose with certain amino acids such as phenylalanine, isoleucine, or tyrosine (*14*). Factors that influence the formation of HAAs in foods include precursor concentrations, type of amino acid, and cooking time and temperature (*15*). Certain food ingredients can reduce HAA formation in foods, such as vitamin E (*16*), cherry tissue (*17*), tea polyphenolic compounds (*18*), soy protein concentrate (*19*), and defatted glandless cottonseed flour (*20*).

Garlic is a commonly used foodstuff. In addition, a variety of garlic-based health products are now readily available on the market. Most of their health-promoting claims are based on the presence of organosulfur compounds such as allicin, diallyl disulfide (DAD), diallyl sulfide (DAS), and dipropyl disulfide (DPD). DAD, for example, has been shown to have a positive effect on arteriosclerosis (*21*), and on serum cholesterol (*22*) and triglyceride levels (*23*). In addition, it has exhibited hypotensive (*24*), anticarcinogenic (*25*), and antidiabetic effects (*26*).

It has been established that organosulfur compounds inhibit Maillard browning reactions (*27*, *28*). The inhibition of this reaction may be the key to reducing HAA formation in foods through the addition of garlic and other organosulfur compounds. However, the mechanism by which organosulfur compounds inhibit the Maillard reaction has not been fully elucidated.

In a previous study, we established that organosulfur compounds, when added to ground beef patties before frying, inhibit or greatly reduce HAA formation and overall mutagenicity (*29*). However, the mechanism by which this occurs is unclear. The objective of this study was to better understand how the formation of HAAs is inhibited by the addition of sulfur compounds to model systems.

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MATERIALS AND METHODS

Safety. HAAs are mutagenic/carcinogenic and should be handled with appropriate safety precautions including the use of goggles, latex gloves, and efficient fume hoods.

Materials. Phenylalanine, glycine, glucose, creatinine, sodium bisulfite, cysteine, 5,5′-dithiobis(2-nitrobenzoate) (DTNB), tridecane, Tris-HCl, tetrahydrothiophene-3-one (THT), and teetrahydrothiophene (THP) were purchased from Sigma Chemical Company (St. Louis, MO). DAD, DPD, and allyl mercaptan (AM) were purchased from Fluka Chemical Co. (Buchs, Switzerland). The HAA standards (IQx, MeIQx, and PhIP) were obtained from Toronto Research Chemicals (Toronto, Canada). The HAA standard (FEMA-Flavor and Extracts Manufacturer's Association) and the internal standard, caffeine, were gifts from Dr. Mark Knize, Lawrence Livermore National Laboratory, Livermore, CA. The FEMA standard contained IQx, MeIQx, and PhIP, each at 5 ng/μL. Propyl-sulfonic acid (PRS) Bond-Elut columns (500 mg) and C18 (100 mg) cartridges were purchased from Varian Inc. (Harbor City, CA). Extrelut-20 columns and Extrelut diatomaceous earth were obtained from E. M. Separations Technology (Gibbstown, NJ). All other chemicals were of analytical grade and were obtained from Fisher Scientific (Fair Lawn, NJ).

The heating module was a Reacti-Therm III, model 18835, made by Pierce Co. (Rockford, IL). Stainless steel test tubes, 2.3-mL capacity, with threaded, self-sealing stainless steel caps were manufactured by the Enginering Research Complex Machine Shop at Michigan State University. A new set of stainless steel test tubes was used for each amino acid to avoid carryover of HAAs from one experiment to another.

Effect of Organosulfur Compounds and Sodium Bisulfite on HAA Formation in Model Systems. The control model system contained 0.6 mmol phenylalanine and 0.6 mmol creatinine in 1.5 mL water, with or without 0.3 mmol glucose. The reactants were added directly to the stainless test tubes and sealed with threaded caps wrapped with Teflon tape. Organosulfur compounds (0.67 mmol DAD, DPD, AM, and cysteine) and sodium bisulfite (0.67 mmol) were added to the model systems. The reaction was conducted in a closed hood. The Reacti-Therm heating module was preheated for a minimum of 1.5 h before heating the samples. The heating temperature was 180 $\pm$ 5 °C, and silicon oil (0.5 mL) was placed in each cavity in the heating block to facilitate heat transfer from the block to the test tubes. The stainless test tubes were heated for 30 min and then immediately cooled in an ice-bath. The contents of each test tube were quantitatively transferred to microvials (1.5-mL capacity) and stored at 5 $\pm$ 1 °C until required.

The contents of two microvials were mixed with 57 mL of 5 N NaOH. Four 10-mL aliquots were taken from the mixture and each placed in a 250-mL beaker. The remainder of the mixture was saved and used if further extractions were required. To determine extraction recoveries, two of the aliquots were spiked with 1.0 μg each of IQx, MeIQx, and PhIP dissolved in 50 μL methanol. Samples were mixed with Extrelut diatomaceous earth to fill an Extrelut 20 column. All four extractions were made with 40 mL dichloromethane containing 5% toluene using attached Bond Elut PRS extraction columns. The PRS cartridges were washed sequentially with 6 mL of 0.1 N HCl, 15 mL of 40% methanol in 0.1 N HCl, and 2 mL of water. The HAAs were then transferred to Bond Elut C18 cartridges (100 mg) with 20 mL ammonium acetate buffer solution (0.5 M, pH 8.0). The cartridges were eluted with 0.8 mL MeOH·NH_4OH (9:1, v/v). The elute was evaporated to dryness, and the residue dissolved in 50 μL methanol containing 5 ng/μL caffeine as an internal standard.

Separation of the HAAs was performed on a TSK-gel ODS80-TM column (25 cm × 4.6 mm i.d.; Tosoh Haas, Montgomeryville, PA). A precolumn (Supelguard LC-8-DB, Supelco, Bellefonte, PA) was attached between the injector port and column, and the cartridge was replaced approximately every 60 injections. The flow rate of the mobile phase was 1 mL/min. The initial ratio of acetonitrile/buffer (triethylamine phosphate, 0.01 M, pH 3.2) was 8:92, which increased to 17:83 during the first 10 min. The acetonitrile concentration continued to increase until the ratio was 25:75 (10 min), then 55:45 (10 min). During the next 5 min, the acetonitrile/buffer ratio was increased to 80:20 to facilitate elution of other compounds. After 35 min, the eluting solvent was returned to its initial ratio (8:92) for 10 min to allow the column to reequilibrate before the next injection. Samples were analyzed on a Millennium 2000 HPLC system (Millipore, Milford, MA) with a photodiode array detector (model 991) and a scanning fluorescence detector (model 474).

The identities of the peaks were established by comparing retention times of the peaks with those of the corresponding spiked samples analyzed under the same conditions. Furthermore, UV spectral characteristics of the HPLC peaks in each sample were compared with library spectra acquired from standard HAA solutions. For each experiment, before HPLC separation of the sample extracts, four aliquots (10, 15, 20, and 25 μL) of two standard mixtures of HAAs (containing 0.5 ng/μL of each compound), the caffeine internal standard (5 ng/μL), and HAA standard FEMA (5 ng/μL) were injected. Linear regression (nanograms of compound against peak area) was performed for individual HAAs in each mixture. A correlation coefficient of 0.99 or greater was considered acceptable for FEMA internal standards, and 0.97 or greater for the laboratory mixtures of HAAs. Each peak area corresponding to an HAA was corrected with the internal standard regression line and expressed as nanograms per gram of meat. The standard addition method of Gross and Grüter (*30*) was then used for determining extraction efficiency and for quantification of HAAs. Each data point consisted of four subsamples; two spiked and two unspiked. The average area of the spiked samples minus the average of the unspiked samples allowed comparison with the regression line for the standard mixture. Each data point was then corrected for its individual extraction efficiency, or percent yield. Concentrations of each HAA formed were determined using the average of the two unspiked subsamples. The linear regression slope for FEMA was used used to determine the exact amount of each HAA present in each sample.

Effect of Heating on the Stability of Organosulfur Compounds. Organosulfur compounds (0.67 mmol DAD or DPD), with or without model system reactants (0.3 mmol glucose, 0.6 mmol phenylalanine, and 0.6 mmol creatinine), were heated at 180 °C for 30 min as described previously. Samples were taken at 10-min intervals to quantitate DAD and DPD concentrations remaining in the model system. A HP 5890 gas chromatograph (Hewlett-Packard, Avondale, PA), equipped with a fused silica capillary column (60 m × 0.25 mm i.d.; 1 μm thickness, DB-1, J&W Scientific Inc., Folsom, CA) and a flame ionization detector, was used to determine the DAD and DPD concentrations. The analytical conditions were as follows: injector temperature, 270 °C; detector temperature, 300 °C; helium carrier flow rate, 1 mL/min; temperature program, 40 °C (5 min), 2 °C/min, 260 °C (60 min). A split ratio of 50:1 was used. DAD and DPD concentrations were determined using the peak areas on the gas chromatograms. The peak area of DAD or DPD from the control system before heating was standardized as 100%, and DAD or DPD concentrations from the reaction mixture were calculated.

Effect of Heating on Formation of Sulfhydryl Groups. Each sample contained 0.67 mmol DAD or DPD in 1.5 mL water. These samples were mixed directly in the stainless test tubes and heated at 180 $\pm$ 5 °C for 30 min as described previously. An aliquot (0.1 mL) of the heated sample was diluted with 0.02 mL of 10 mM DTNB, 0.1 mL of 0.2 M Tris-HCl buffer (pH 7.5), and 0.78 mL of water, and its turbidity was measured at 412 nm using a UV spectrophotometer (Varian Inc., Harbor City, CA). Sulfhydryl concentrations (micromoles in 1.5 mL) were calculated using the Beer−Lambert law equation:

$$\Delta A = \Delta a_m \cdot c \cdot b$$

where ΔA is the absorbance at 412 nm, Δa_m is the extinction coefficient (13,600 $M^{-1}\cdot cm^{-1}$), c is the concentration, and b is the sample thickness (1 cm).

Formation of Volatile Compounds in Heated Model Systems. Samples containing 0.67 mmol DAD or 0.3 mmol glucose, or 0.67 mmol DAD and 0.3 mmol glucose in 1.5 mL water were heated in stainless steel test tubes at 180 $\pm$ 5 °C for 30 min. After cooling rapidly in an ice bath, the reaction mixtures were mixed with an internal standard (tridecane, 100 μL) and extracted four times with 10 mL dichloromethane. The combined extracts were dried over anhydrous sodium sulfate and concentrated to 5 mL in a Kuderna-Danish concentrator (Supelco, Bellefonte, PA). The extract was then concen-

Table 1. Effect of Organosulfur Compounds and Sodium Bisulfite on the Formation of HAAs in a Model System Containing Phenylalanine, Creatinine, and Glucose[a]

	heterocyclic aromatic amines (nmol/mmol of creatinine)		
treatment	IQx	MeIQx	PhIP
control	7.2 ± 4.2^{b}	12.9 ± 3.0^{b}	34.4 ± 8.4^{b}
DAD	1.8 ± 1.1^{c}	2.2 ± 0.7^{d}	8.2 ± 4.2^{d}
inhibition (%)	75	82	73
DPD	2.9 ± 1.8^{c}	4.9 ± 1.9^{c}	12.6 ± 3.7^{d}
inhibition (%)	61	63	56
bisulfite	1.7 ± 0.4^{c}	2.1 ± 0.7^{d}	7.8 ± 2.0^{d}
inhibition (%)	77	84	78
AM	4.4 ± 1.5^{b}	7.5 ± 2.2^{b}	19.9 ± 4.1^{c}
inhibition (%)	39	42	42
cysteine	5.6 ± 2.2^{c}	10.2 ± 3.4^{b}	29.5 ± 6.5^{b}
inhibition (%)	22	20	14

[a] Heated at 180 °C for 30 min. [b−d] Means with different superscripts are significantly different ($P < 0.05$). Comparisons are made only within the same column. Means ± standard deviations; $n = 5$ for all treatments.

trated to 1 mL under nitrogen. The generated volatile compounds were separated by gas chromatography using the same conditions as described in the preceding section.

The concentrated isolates were analyzed by GC-mass spectrometry (MS) using a HP 5890 gas chromatograph interfaced with a HP 5970 mass selective detector (MSD) mass spectrometer. The GC/MS system was equipped with a HP 59970 Chemstation Data System. The GC was operated under the same conditions as previously described. The MS was operated in the electron-impact mode with an electron energy of 70 eV and an ion source temperature of 250 °C. Compounds were introduced to the ion source directly from the capillary column in the GC using an open-split interface. A continuous scan mode with a scan time of 1 s over a mass range of 40−300 was used. The GC/MS data were monitored, stored, and analyzed using a HP Chemstation data system. Several compounds in the isolate were identified by comparing their mass spectral data with those of authentic compounds available in the NIST/EPA/MSDC Mass Spectral Database purchased from ACS Publication Co. (Washington, DC) or INRA MassSpectra Computer Library (Laboratoire de Recherche sue les Aromes, Dijon, France).

Effect of Heating Time on HAA Formation in Model Systems Containing DAD or DPD. Several model systems were evaluated. The control system contained 0.6 mmol phenylalanine, 0.6 mmol creatinine, and 0.3 mmol glucose in 1.5 mL water. Other systems contained 0.67 mmol DAD (or DPD) in addition to the three primary reactants. Samples were heated for 30 min at 180 °C, with 3-mL aliquots being taken at 10-min intervals. The effect of the sulfur compounds on HAA formation was determined by extraction, purification, HPLC analysis, and HAA peak identification as described earlier.

Effect of THT and THP on HAA Formation in Various Model Systems. The direct effect of THT and THP on HAA formation in various model systems was evaluated by adding (0.67 mmol THT or THP) to model systems containing 0.6 mmol creatinine, 0.6 mmol phenylalanine or glycine, and with or without 0.3 mmol glucose in 1.5 mL water. The reactants were heated at 180 °C for 30 min. Samples were extracted and purified as previously described and analyzed by HPLC.

Statistical Analyses. The results were analyzed by Sigma Stat 2.0 (Jandel Corp., San Rafael, CA). One way analysis of variance (ANOVA) was performed for each HAA. Appropriate comparisons were made using Student-Newman-Keuls test for one-way ANOVA analysis.

RESULTS AND DISCUSSION

Effect of Organosulfur Compounds and Sodium Bisulfite on HAA Formation in Model Systems. The major HAAs detected in the heated model systems containing glucose, phenylalanine, and creatinine were IQx, MeIQx, and PhIP (**Table 1**). The dominant HAA was PhIP (34.4 ± 8.37 nmol/mmol creatinine), followed by MeIQx (12.9 ± 6.98 nmol/mmol creatinine) and IQx (7.2 ± 4.17 nmol/mmol creatinine). These observations agree with those of Shioya et al. (*31*) and Skog and Jägerstad (*32*). The average recoveries of HAAs from the model system were 87 ± 16, 85 ± 18, and 82 ± 15% for IQx, MeIQx, and PhIP from similar model systems, respectively. These recoveries are similar to those of Scranton (*33*), who reported average recoveries of 88, 88, and 71% for IQx, MeIQx, and PhIP, respectively, from similar model system. Concentrations of PhIP were significantly reduced ($P < 0.05$) upon the addition of organosulfur compounds to the model systems. The percentage inhibition ranged from 73% for DAD to 42% for AM. MeIQx concentrations were reduced 82% by DAD and 63% by DPD. However, cysteine did not significantly reduce HAA formation in the model system.

Sodium bisulfite also significantly ($P < 0.05$) inhibited HAA formation, with reductions of 77, 84, and 78% being achieved for IQx, MeIQx, and PhIP, respectively. The inhibitory effect of sodium bisulfite has been reported previously by Krone and Iwaoka (*34*), who observed a reduction in the mutagenicity of canned salmon upon the addition of bisulfite. Chen (*35*) also demonstrated sodium bisulfite inhibition of IQx, MeIQx, and DiMeIQx formation in fried ground beef patties, although he did not offer an explanation for its inhibitory action.

The inhibition of the Maillard reaction by sodium bisulfite has been established through its interaction with reducing sugars (*36*). Several chemical mechanisms have been proposed to explain its inhibitory action. One of the most important involves its addition to the carbonyl group of reducing sugars and other carbonyl compounds participating in the Maillard reaction. The reaction between sodium bisulfite and the carbonyl group of reducing sugars produces a 3,4-dideoxy-4-sulfohexosulose, a result of nucleophilic attack by the sulfite ion on the α,β-unsaturated carbonyl moiety of 3,4-dideoxyhexosulos-3-ene. This reaction may compete with pathways that are involved in further Maillard reactions.

It has been speculated that HAA formation occurs through intermediates of the Maillard reaction. Jägerstad et al. (*12*) proposed that pyridines and pyrazines, formed via the Maillard reaction, react with an aldehyde to form a quinoline or quinoxaline. Such structures are integral parts of the HAA molecule. Creatine undergoes dehydration and cyclization to form creatinine when heated, which then reacts with an aldehyde to form an IQ- or IQx-type HAA. Inhibition of the Maillard reaction by sodium bisulfite thus seems to be a possible mechanism by which HAA formation is inhibited/reduced in those systems containing bisulfite.

To further establish that the inhibitory action of sodium bisulfite, and possibly that of the organosulfur compounds under evaluation, is by means of reaction with glucose, a study was designed to investigate HAA formation in model systems containing only phenylalanine and creatinine. The only HAA detected in this system was PhIP (**Table 2**). These results agree with those of Overvik et al. (*37*) and Skog and Jägerstad (*32*). Skog and Jägerstad further demonstrated that the yield of PhIP increased 3-fold when glucose was added to the reaction mixture and heated under similar conditions. Furthermore, small concentrations of the IQ-type HAAs were present in the model systems. Our results (**Tables 1** and **2**) confirmed these observations, both with respect to the effect of glucose on HAA formation (3- to 4-fold increases in PhIP concentrations) and to the quantities of the other HAAs produced.

Sodium bisulfite and organosulfur compounds did not inhibit PhIP formation in the model systems that did not contain glucose

Table 2. Effect of Organosulfur Compounds and Sodium Bisulfite on the Formation of HAAs in a Model System Containing Phenylalanine and Creatinine[a]

	heterocyclic aromatic amines (nmol/mmol of creatinine)		
treatment	IQx	MeIQx	PhIP
control	ND	ND	10.2 ± 2.2^b
DAD	ND	ND	10.5 ± 1.5^b
DPD	ND	ND	11.1 ± 1.7^b
bisulfite	ND	ND	10.2 ± 1.7^b

[a] Heated at 180 °C for 30 min. ND, not detectable. Limit of detection is 0.4 ng. [b] Means with different superscripts are significantly different ($P < 0.05$). Comparisons are made only within the same column. Means ± standard deviations; $n = 5$ for all treatments.

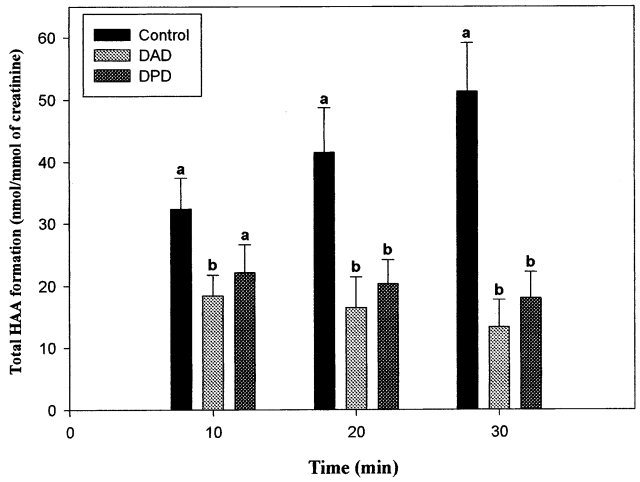

Figure 1. Effect of heating at 180 °C for 30 min on the concentrations of organosulfur compounds in model system containing phenylalanine, creatinine, and glucose. All treatments are made in triplicate.

(**Table 2**). Because sodium bisulfite reacts directly with glucose, it may be inferred that HAA inhibition by DAD and DPD in model systems containing glucose could also be through their interaction with glucose.

Effect of Heating on the Stability of Organosulfur Compounds. To determine whether DAD or DPD undergoes decomposition in the model systems, a study was designed to quantitate DAD or DPD concentrations after 10, 20, and 30 min of heating at 180 °C. Concentrations of DAD and DPD concentrations in the model system containing glucose, creatinine, and phenylalanine decreased by 57 and 46%, respectively, after only 10 min of heating at 180 °C (**Figure 1**). These decreases may be due to their interaction with components of the model system and/or by their own thermal decomposition. When DAD (or DPD) was heated alone under similar conditions (30 min at 180 °C), markedly smaller decreases in concentration (24−43%) were observed (**Figure 1**). These decreases may be attributed to decomposition of the sulfur compounds or by their vaporization from the sealed tubes. The differences in the percentage losses of DAD and DPD in the various model systems may be explained by their interaction with other model system reagents, namely glucose, creatinine, and phenylalanine.

A possible mode of loss of the disulfide compounds is through dissociation to sulfhydryl compounds on heating. Friedman and Molnar-Perl (*38*) proposed that sulfhydryl groups inhibit the Maillard reaction through their interaction with intermediates formed during the Maillard reaction and by suppression of free-radical formation. Heating DAD and DPD at 180 °C for 30 min produced low concentrations of sulfhydryl compounds, 4.19 and 4.00 μmol, respectively. These numbers represent conversion of 0.63% of DAD and 0.60% of DPD to their respective thiols. Thus, free sulfhydryl involvement in the inhibition of HAA formation is unlikely. It is more plausible that HAA inhibition by the organosulfur compounds occurs by their direct interaction with glucose.

Table 3. Compounds Tentatively Identified on Heating Glucose and Diallyl Disulfide at 180 °C for 30 min

compounds[a]	RT (min)[b]	MW[c]
Compounds Generated on Heating Glucose		
5-(hydroxymethyl) 2-furancarboxaldehyde	16.7	126
2-furancarboxaldehyde	23.3	96
1,3-dihydroxy 2-propanone	31.1	90
methyl 2-furoate	53.4	126
Compounds Generated on Heating Diallyl Disulfide		
diallyl disulfide	16.8	146
diallyl sulfide	28.1	114
3-vinyl-1,2-dithiocyclohex-5-ene	39.3	144
diallyl trisulfide	44.3	178
3-(2,3-dithia-5-hexenyl)3,4-dihydro-2H-thiopyran	48.7	218
6-methyl-4,5,8,9-tetrathiadodeca-1,11-diene	55.6	252
3-(2,3,4-trithia-6-heptenyl)3,4-dihydro-2H-thiopyran	58.7	250
Compounds Only Generated on Heating Glucose and Diallyl Disulfide[d]		
tetrahydrothiophene (THP)	26.5	88
3,5-diethyl-1,2,4-trithiolane	44.7	180
3-(allylthio)-propionic acid	50.4	146
tetrahydrothiophene-3-one (THT)	52.3	102
5-methyl-2-thiophene carboxaldehyde	81.4	126
9-thianoradamantane	83.1	140

[a] Identification of the volatile compounds was based on GC/MS analysis. [b] Retention time. [c] Molecular weight. [d] Compounds identified from the heating of glucose and diallyl disulfide together included compounds identified from heating glucose or diallyl disulfide alone.

Formation of Volatile Compounds in Heated Model Systems. To further determine whether the reaction between DAD and glucose could produce compounds with the potential to inhibit HAA formation, a study was designed to identify some of the principal volatile compounds produced on heating glucose and DAD singly and an combination, at 180 °C for 30 min. Volatile compounds tentatively identified by mass spectrometry from the thermal degradation of glucose include methyl 2-furoate, 5-(hydroxymethyl) 2-furancarboxaldehyde, 2-furancarboxaldehyde, and 1,3-dihydroxy 2-propanone (**Table 3**). These results generally agree with those of Tai and Ho (*39*) and Yu et al. (*40*) who reported that 2-furancarboxaldehyde was the major thermal degradation product of glucose. The furfural group is mainly derived by sugar caramelization. Volatile compounds identified from the thermal degradation of DAD include diallyl sulfide, 3-vinyl-1,2-dithiocyclohex-5-ene, diallyl trisulfide, 3-(2,3-dithia-5-hexenyl)-3,4-dihydro-2H-thiopyran, 6-methyl-4,5,8,9-tetrathiadodeca-1,11-diene, and 3-(2,3,4,trithia-6-heptenyl)-3,4-dihydro-2H-thiopyran (**Table 3**). Block et al. (*41*) identified several sulfur-containing compounds produced on heating DAD at 80 °C for 2−10 days, including thioacrolein dimer 3-vinyl-4H-[1,2]-dithin, 2-vinyl-4-H-[1,3]-dithin, diallyl sulfide, diallyl tetrasulfide, 6-methyl-4,5,8,9-tetrathiadodeca-1,11-diene, a mixture of 2- and 3-(2′,3′-dithia-5′-hexenyl)-3,4-dihydro-2H-thiopyran, and 4,5,9,10-tetrathiatrideca-1,12-diene.

Compounds identified from the heating of glucose and DAD together at 180 °C for 30 min included compounds identified from heating glucose or DAD singly, and THP, 5-methyl-2-thiophene carboxaldehyde, and THT (**Table 3**). Thiophene and thiophene-3-one formation from the reaction of glucose and DAD can be explained by the exchange of S and O in the furan ring during heating (*3*). As indicated previously, furan ring

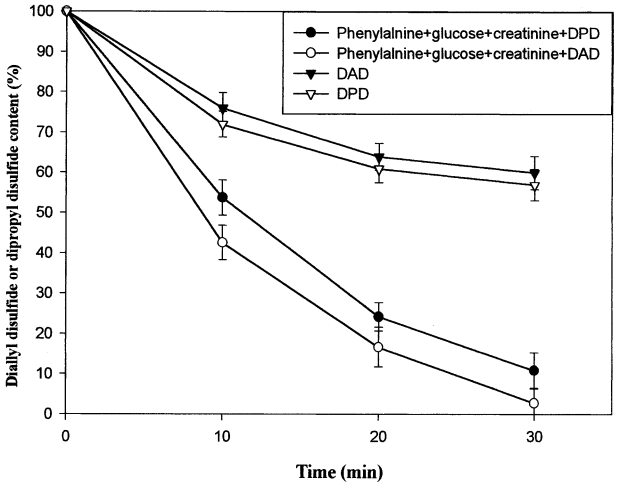

Figure 2. Inhibition of total HAA formation by DAD and DPD in a model system containing phenylalanine, creatinine, and glucose heated at 180 °C for 30 min. Bars with different letters are significantly different ($P < 0.05$). Comparisons are made only within the same column. $n = 3$ for all treatments.

products such as 2-furancarboxaldehyde, methyl 2-furoate, and 5-(hydroxymethyl) 2-furancarboxaldehyde are thermal degradation products of glucose. Because glucose is viewed as a major contributor to HAA formation, it is possible that the reaction of DAD with glucose reduces the availability of glucose to participate in the Maillard reaction, that is, the carbonylamino reaction.

Effect of Heating Time on HAA Formation and Inhibition of HAA Formation in Model Systems Containing DAD and DPD. To gain further insight into the inhibition of HAA formation by organosulfur compounds, a study was designed to determine the effect of heating time on HAA formation and inhibition by DAD and DPD in model systems containing phenylalanine, creatinine, and glucose **(Figure 2)**. When HAA formation was evaluated throughout the entire heating period (30 min), approximately 63% of the HAAs were produced in the initial 10 min of heating. This observation could be explained by the very fast and efficient heat transfer through the wall of the test tube and by the relatively low activation energies (68.9−134.4 kJ/mol) of HAA formation. These results generally agree with those of Arvidson et al. (*42*) and Trompeta and O'Brien (*28*), who demonstrated a rapid depletion of glucose in the early stages of the reaction involving phenylalanine, glucose, and creatinine. These investigators concluded that glucose was a limiting precursor and actively participated in the formation of HAAs. The retention of creatinine and amino acids was >20%, even after 15 min of heating, while all glucose had reacted after 2.5 min. Chen and Meng (*43*) also observed rapid formation of HAAs within the first 5−10 min of heating a model system containing glucose, phenylalanine, and creatinine at 150 °C and 200 °C. After this time, only a steady increase in HAA formation was observed. They concluded that this occurred possibly through the rapid exhaustion of all the HAA precursors in the reaction system.

When DAD was added to the system, HAA formation during the first 10 min was significantly ($P < 0.05$) decreased (43% reduction). When the model system was heated for 20 min, the HAA concentration was approximately 81% of that produced after 30 min of heating. However, the HAA concentrations formed after 10 and 20 min were not significantly different. The addition of DAD and DPD to the model system reduced HAA formation by 60% and 51%, respectively. These results

Table 4. Effect of THT and THP on Formation of HAAs in a Model System Containing Phenylalanine, Creatinine, and Glucose[a]

	heterocyclic aromatic amines (nmol/mmol of creatinine)		
treatment	IQx	MeIQx	PhIP
control	6.7 ± 2.4^b	14.6 ± 5.4^b	30.9 ± 8.7^b
THT	6.1 ± 2.1^b	15.7 ± 4.9^b	31.1 ± 9.1^b
THP	7.0 ± 2.4^b	16.0 ± 4.4^b	33.2 ± 8.5^b

[a] Heated at 180 °C for 30 min. [b] Means with different superscripts are significantly different ($P < 0.05$). Comparisons are made only within the same column. Means ± standard deviations; $n = 3$ for all treatments.

Table 5. Effect of THT and THP on Formation of HAAs in a Model System Containing Phenylalanine and Creatinine[a]

	heterocyclic aromatic amines (nmol/mmol of creatinine)		
treatment	IQx	MeIQx	PhIP
control	ND	ND	11.4 ± 2.7^b
THT	ND	ND	10.2 ± 2.1^b
THP	ND	ND	12.8 ± 2.9^b

[a] Heated at 180 °C for 30 min. ND, not detectable. Limit of detection is 0.4 ng. [b] Means with different superscripts are significantly different ($P < 0.05$). Comparisons are made only within the same column. Means ± standard deviations; $n = 3$ for all treatments.

Table 6. Effect of THT and THP on Formation of HAAs in a Model System Containing Glycine, Creatinine, and Glucose[a]

	heterocyclic aromatic amines (nmol/mmol of creatinine)	
treatment	IQx	MeIQx
control	8.5 ± 1.9^b	18.3 ± 4.3^b
DAD	2.3 ± 0.7^c	5.5 ± 1.7^c
THT	7.1 ± 2.3^b	17.4 ± 3.9^b
THP	8.7 ± 2.7^b	17.6 ± 4.5^b

[a] Heated at 180 °C for 30 min. [b,c] Means with different superscripts are significantly different ($P < 0.05$). Comparisons are made only within the same column. Means ± standard deviations; $n = 3$ for all treatments.

and those of previous studies suggest that HAA inhibition by DAD seems to be through its active interaction with glucose during the first 10 min of heating at 180 °C.

Effect of THT and THP on HAA Formation in Various Model Systems. It has been postulated by Tsai et al. (*44*) that THT might play an important role in HAA formation. Thus, a study was conducted to investigate the possible roles in HAA formation in model systems containing phenylalanine, creatinine, and glucose, and glycine, creatinine, and glucose.

The principal HAAs formed in the model systems containing glucose, phenylalanine, and creatinine were PhIP, IQx, and MeIQx, whereas PhIP was the only HAA produced in the control model system containing phenylalnine and creatinine (**Tables 4** and **5**). These results confirm our previous data and also show that THT and THP had no effect on HAA formation in the model systems, regardless of whether glucose was present or not. The major HAAs in the model systems containing glycine, creatinine, and glucose were IQx and MeIQx (**Table 6**). Scranton (*33*) also reported that IQx and MeIQx were the dominant HAAs formed in the same model system under similar heating conditions. The addition of DAD significantly ($P < 0.05$) inhibited IQx and MeIQx formation, while THT and THP had no effect on HAA formation. Tsai et al. (*44*) postulated that THT might play an important role in IQ-mutagen formation in the reflux boiling of pork juice extracts. They summarized that reductions in the concentrations of THT and the four major

Maillard reaction products (pyridines, pyrazines, thiophenes, thiazoles) correlated with a reduction in mutagenicity, even though there was no correlation with mutagenicity when each Maillard reaction product was examined alone. However, our data indicate that THT and THP are merely reaction products between glucose and DAD, and do not influence HAA formation.

Conclusions. Although this study points to a competitive reaction between organosulfur compounds and amino acids for glucose, the mechanism by which these compounds inhibit HAA formation is still not clarified. However, the observation that DAD has no effect on HAA formation in model systems without glucose provides supporting evidence that the interaction of DAD with glucose is a possible key element in its inhibition of HAA formation. It is also apparent that the products of interaction of glucose and DAD are not directly involved in the inhibition reaction.

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