JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Structure–Reactivity Relationships of Flavan-3-ols on Product Generation in Aqueous Glucose/Glycine Model Systems

YUKO NODA AND DEVIN G. PETERSON*

Department of Food Science, The Pennsylvania State University, 327 Food Science Building, University Park, Pennsylvania 16802-2504

Ring structure−reactivity relationships of three flavan-3-ols [epicatechin (EC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG)] and three simple phenolic compounds (1,3,5-trihydroxybenzene, 1,2,3-trihydroxybenzene, and methylgallate as the analogous individual A, B, and C benzene rings of EGCG) on product generation in an aqueous glucose−glycine reaction model system (125 °C and 30 min) were investigated. The addition of EC, ECG, or EGCG to a glucose−glycine model was reported to similarly significantly reduce the formation of pyrazine, methyl-substituted pyrazines, and cyclotene. All three flavan-3-ols were also reported to generate phenolic-C_2, C_3, C_4, and C_6 sugar fragment adducts and to statistically reduce the concentration of glyoxal, glycolaldehyde, methylglyoxal, hydroxyacetone, diacetyl, acetoin, and 3-deoxyglucosone during the reaction time course, except for the EGCG reaction where 3-deoxyglucosone was not statistically different from the control after 20 min. For the simple phenolic compounds, methylgallate followed by 1,2,3-trihydroxybenzene was the least reactive, while 1,3,5-trihydroxybenzene was reported as the most reactive phenolic structure for quenching or reducing the concentration of the α-hydroxy- and α-dicarbonyl sugar fragments during the reaction time course. These results imply that the main mechanism flavan-3-ols reduced product generation was phenolic−sugar fragment carbonyl trapping reactions primarily on the A ring (the *meta*-polyhydroxylated benzene ring) or not due to the alteration of the reaction reduction potential.

KEYWORDS: Carbonyl reactions; flavan-3-ols; Maillard reaction; sugar fragments; structure−reactivity; trapping; aroma generation; polyhydroxybenzene

INTRODUCTION

The Maillard reaction is a critical food reaction, which results in the formation of compounds that affect the color, flavor, and texture attributes as well as the nutritional value or toxicity of processed foods (*1*). In general, the initial stage of this key food reaction is initiated by a condensation reaction between an amino acid and a reducing sugar followed by a series of transformations to yield an Amadori product (*N*-substituted 1-amino-1-deoxy-2-ketose), which can further degrade to 1- or 3-deoxyosones by enolization reactions (*2*). The corresponding deoxy-hexosuloses (for hexose sugars) are known to undergo further fragmentation by retroaldol condensation reactions to yield reactive C_2, C_3, C_4, and C_5 α-dicarbonyl and α-hydroxycarbonyl sugar fragments, all of which are key transient precursors of Maillard reaction products (MRPs). In addition, these carbonyl compounds are also considered to be interconnected and modulated through redox cycles (*3*). Consequently, the quality of processed food can be related to the fate of these intermediate sugar fragment MRPs.

Common dietary phenolic compounds, such as epicatechin (EC) and ferulic acid, have been previously reported to function as trapping agents of Maillard-derived sugar fragments (*4*, *5*), suggesting that phenolic chemistry may be applied to suppress the generation of undesirable MRPs (i.e., off-flavor compounds) in food products during thermal processing and storage. Furthermore, these phenolic−sugar fragment reactions provide a new mechanism for understanding Maillard product generation in plant-based food products (i.e., whole grain) and ultimately product quality. In a series of studies, Totlani and Peterson (*4*, *6*, *7*) indicated that EC formed adduct reaction products with C_2, C_3, C_4, and C_6 sugar fragment precursors, such as glyoxal, methylglyoxal, hydroxyacetone, erythrose, and 3-deoxy-2-hexosulose, in glucose−glycine model systems and likewise suppressed aroma generation. They suggested that EC reacted with these sugar fragments by electrophilic aromatic substitution reactions primarily on the A ring, which was supported by subzero temperature two-dimensional NMR analyses of an EC−methylglyoxal adducts that indicated that the C_1 position of the methylglyoxal was covalently bonded to the C_6 or C_8 position of the EC A ring (*6*). In food products, the application of EC to suppress the generation of Maillard type off-flavor compounds has also been demonstrated during ultrahigh temperature processing of bovine milk as well as during processing and storage of skim milk powder (*8*, *9*).

* To whom correspondence should be addressed. Tel: 814-865-4525. Fax: 814-863-6132. E-mail: dgp10@psu.edu.

10.1021/jf063423e CCC: $37.00
Published on Web 03/30/2007

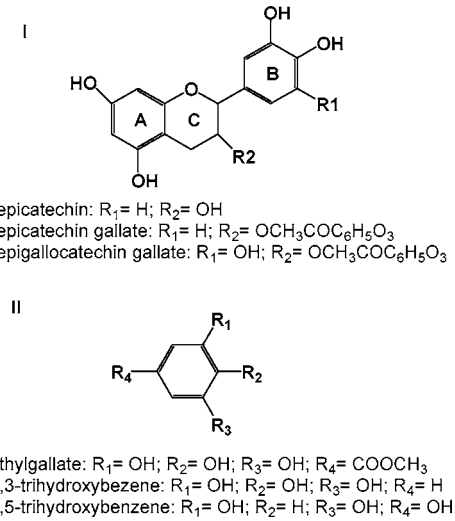

Figure 1. Chemical structure of investigated phenolic compounds: (**i**) flavan-3-ols and (**ii**) simple phenolics.

Other researchers have also suggested that the redox properties of phenolic compounds can alter Maillard mechanisms and color formation by effecting the reduction potential of the reaction matrix (*10*, *11*). Considering EC is a well-known redox-active compound, similar mechanisms may also be used to explain the previously observed negative influence of EC on Maillard type product generation in both model and food systems.

To further examine the reactivity of phenolic compounds on the product generation in Maillard model systems, the influence of flavan-3-ol phenolic ring chemistry or the phenolic structure−reactivity relationships was investigated using EC, epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) (**Figure 1i**). On the basis of the polyhydroxyl configuration on the benzene rings of the flavan-3-ols, the B ring is considered to be more redox-reactive than the A ring (*12*), while the A ring would be anticipated to be more reactive for carbonyl trapping reactions. Among the flavan-3-ols, structural changes in the B ring (degree of hydroxyl substitution) are also considered to impact the antioxidant activity; for example, EGCG is considered to be a stronger antioxidant than EC (*13*). Consequently, if the flavan-3-ol A ring structure was reported to be more reactive in altering the mechanisms of the Maillard reaction than the B ring, this would suggest that trapping reactions were the main reaction mechanisms that these phenolic compounds impacted in Maillard chemistry.

The goal of this study was, therefore, to determine the effect of EC, EGC, and EGCG on the time−course concentration of sugar fragments as well as on product generation in a glucose−glycine model system. The role of individual rings of the flavan-3-ol structure was suggested by investigating three simple phenols, methylgallate, 1,3,5-trihydroxybenzene, and 1,2,3-trihydroxybenzene (**Figure 1ii**), which were used to simulate the A, B, and C ring chemistry of EGCG, respectively.

MATERIALS AND METHODS

Chemicals. Hydroxyacetone, diacetyl, 2,5-dimethylpyrazine, *n*-dodecane, (−)-EC, formic acid, glycine, 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene), 2-methylpyrazine, pyrazine, 2,3,5-trimethylpyrazine, 1,3,5-trihydroxybenzene, 1,2,3-trihydroxybenzene, butylparaben, quinoline, *o*-ethoxamine hydrochloride, [$^{13}C_2$, ^{15}N]glycine (98% enrichment), and 1,2-diaminobenzene were obtained from Sigma-Aldrich Co. (St. Louis, MO). 1,2-Diaminobenzene was recrystallized twice from ethanol prior to use. (−)-EGCG (98% purity) and (−)-ECG (98% purity) were obtained from Zhejiang Yixin Pharmaceutical Co. (Zhejiang, China). Acetoin, methylgallate, and gallic acid were obtained from TCI America (Portland, OR). $^{13}C_6$-Glucose (99% enrichment) was obtained from Cambridge Isotope Laboratories (Andover, MA). Glycolaldehyde and methylglyoxal were obtained from MP Bio Medicals (Irvine, CA). Glyoxal was purchased from Alfa Aesar (Ward Hill, MA). 3-Deoxyglucosone was obtained from Toronto Research Chemicals (Ontario, Canada). Ammonium acetate, D-glucose monohydrate, high-performance liquid chromatography (HPLC) grade methanol, anhydrous sodium sulfate, and sodium phosphate were obtained from EMD Chemicals (Gibbstown, NJ). High-purity diethyl ether was obtained from Burdick & Jackson (Muskegon, MI).

Table 1. Maillard Model Reactions[a]

	reactant concentration (mM)					
reactants	model A	model B	model C	model D	model E	model F
glucose	10	10	10	5	10	5
glycine	10	10	10	10	5	10
flavan-3-ol[b]		10		10	10	
simple phenol[c]			10			10
[$^{13}C_6$]glucose				5		5
[$^{13}C_2$, ^{15}N]glycine					5	

[a] Reactants were dissolved in 0.1 M phosphate buffer, reacted at 125 °C for 30 min (does not include reactor heating time to 125 °C) and cooled down to 20 °C prior to sample preparation. [b] EC, ECG, or EGCG. [c] Methyl gallate, 1,2,3-trihydroxybenzene, or 1,3,5-trihydroxybenzene.

Model Maillard Reaction System. A 10 mM concentration of reactant Maillard model systems A−F (shown in **Table 1**) in 0.1 M phosphate buffer (pH 7.0) was reacted in a 600 mL Parr reactor (model 4563; Parr Instrument Co., Moline, IL) at 125 °C for 30 min under constant agitation (set to 40% of the maximum speed), cooled to 20 °C by an internal cooling coil, and then immediately prepared for further analysis.

Identification and Quantification of Volatile Compounds. Sixty-five milliliters of a 70 mL reaction mixture of models A and B was extracted three times with 15 mL of diethyl ether spiked with *n*-dodecane (0.1 μL *n*-dodecane/120 mL diethyl ether, internal standard). The pooled organic layer was dried with anhydrous sodium sulfate, filtered, and concentrated to 0.5 mL using a spinning band distillation apparatus (model 800; B/R Instruments, Easton, MD) prior to gas chromatography (GC) and GC/mass spectrometry (MS) analysis. Positive identifications of unknowns were made using mass spectra and comparing linear retention indices of authentic compounds under identical analytical conditions. Quantification of each analyte was performed via GC using internal standard techniques (the internal standard was *n*-dodecane; standards were prepared at 5 mg/50 mL diethyl ether) except for select pyrazine derivatives and cyclotene, which were below the GC detection limit and were subsequently quantified by GC/MS in select ion monitoring (SIM) mode using external standard curves (identified in **Table 2**). External standard curves were made using 10, 5, 2, 1, 0.2, 0.1, and 0.02 mg/L standard solutions, with the exception of 2,3,5-trimethylpyrazine where 0.005 and 0.001 mg/L solutions were also included ($r^2 > 0.996$ for all compounds). For volatile quantification, select ions were monitored as follows: *m*/*z* 80, 94, 108, 122, 112, and 169 for pyrazine, methylpyrazine, 2,5-dimethylpyrazine, 2,3,5-trimethylpyrzine, cyclotene, and dodecane, respectively.

Identification of Phenolic−MRP Adducts. A 10 mL sample from 70 mL reaction mixtures (**Table 1**, models A−F) was spiked with 10 μL of 300 μg/mL butylparaben in methanol (internal standard) and loaded onto a conditioned SPE cartridge from Supelco (Supelclean LC-18, 1 g, 6 mL capacity; Bellefonte, PA) under vacuum at a flow rate of 0.9 mL/min. The cartridge was washed with 10 mL of nanopure water and then eluted with 2 mL of pure HPLC grade methanol under vacuum at a rate of 1.4 mL/min. The eluent was concentrated to approximately 1 mL under a stream of nitrogen and subsequently analyzed by LC/MS. The MS data were collected over the scan range of 120−1000 *m*/*z* with a scan time of 1.0 s (negative ion mode).

Table 2. Concentration of Volatile MRPs Generated in Models A and B[a,b]

	volatile MRPs (ng/mL)				
treatment	pyrazine	2-methylpyrazine	2,5-dimethylpyrazine	2,3,5-trimethylpyrazine	cyclotene
control	39.9 A	74.4 A	95.9 A	39.0 A	152.1 A
EC	11.1 B	2.7[c] B	2.9[c] B	0.2[c] B	7.5 B
EGCG	9.1 B	1.5[c] B	1.5[c] B	0.1[c] B	2.9[c] B
ECG	6.8[c] B	1.6[c] B	2.3[c] B	2.4 B	2.6[c] B

[a] Quantification was done using internal standards via GC/FID unless otherwise noted. Average of triplicates. [b] Different letters in the same column (A and B) are significantly different (Tukey's HSD pairwise comparison $\alpha = 0.05$). [c] Quantified using GC/MS in SIM mode.

Time−Course Study of Maillard Intermediate Reaction Products. An 8 mL aliquot of a 70 mL reaction mixture (**Table 1**, models A−C) was sampled at 5, 10, 15, 20, and 30 min from the Parr reactor via a sampling tube, cooled on ice, and then immediately prepared for further analysis. Each aliquot was further subdivided for analysis of α-dicarbonyl and α-hydroxycarbonyl MRPs.

Quantification of Glyoxal, Methylglyoxal, Diacetyl, and 3-Deoxyglucosone (α-Dicarbonyls). One milliliter of the reaction mixture sample was diluted with 1 mL of water followed by 2 mL of 1 M 1,2-diaminobenezene in methanol, and the pH was immediately adjusted to 6.5 with 0.1 M hydrochloric acid. The samples were then held at 30 °C for 3 h under constant agitation, subsequently spiked with an internal standard (1 μL of 100 μg/mL quinoline solution in methanol), filtered, and analyzed by LC/MS. The data were normalized using quinoline and quantified using external standard calibration curves. The concentrations for the standard solutions were as follows: 100, 50, 20, 10, and 0.1 μM for glyoxal; 100, 50, 20, 15, and 10 μM for methylglyoxal; 40, 10, 5, 2, and 0.5 μM for diacetyl; and 50, 20, 15, 10, and 5 μM for 3-deoxyglucosone ($r^2 > 0.98$ for all standard curves). The corresponding quinoxaline derivatives were detected in SIM mode (positive ion); *m*/*z* of 131, 145, 159, and 235 for glyoxal, methylglyoxal, diacetyl, and 3-deoxyglucosone respectively. Quinoline was also detected in SIM mode; *m*/*z* of 130.

Quantification of Acetoin, Hydroxyacetone, and Glycoaldehyde (α-Hydroxycarbonyls). Two milliliters of 1.5 M *o*-ethoxamine hydrochloric acid solution in 0.5 M phosphate buffer was added to 4 mL of the aliquot. The pH was adjusted to 7.5 with 1 M sodium hydroxide, and the solution was held at 30 °C for 3 h. The pH was lowered to 6 with 1 M hydrochloric acid. The sample was extracted three times with 9 mL of diethyl ether spiked with 3.7 μg/mL dodecane (internal standard), and the combined organic layer was dried over sodium sulfate and concentrated down to 1 mL using a spinning band distillation apparatus. The *o*-ethoxamine hydrochloric acid derivatized samples were analyzed using GC/MS−chemical ionization (CI). The data were normalized using dodecane and quantified using external standard calibration curves. The concentrations for the standard solutions were as follows: 1000, 750, 500, 250, and 1 μM for hydroxyacetone; 7, 5, 3.5, 2, and 0.01 μM for acetoin; and 800, 400, 200, 100, and 0.01 μM for glycoaldehyde ($R^2 > 0.99$ for all standard curves). The corresponding ethyloxime derivatives were detected in SIM mode (positive ion); *m*/*z* of 132, 118, 104, and 134 for acetoin, hydroxyacetone, and glycoaldehyde, respectively. Dodecane was also detected in SIM mode; *m*/*z* of 169.

GC. An Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector and a Combi-Pal autosampler (CTC 9 Analytics, Carrboro, NC) was used for the volatile analysis. The samples were analyzed on a DB-wax capillary column (30 m × 0.25 mm i.d. with 0.25 μm film thickness; Agilent Technologies). Two microliters of sample was injected in splitless mode at a constant pressure of 7 psi (H_2) with an inlet temperature of 200 °C and a detector temperature of 250 °C. The time program was as follows: held at 30 °C for 2 min, ramped by 5 °C/min to 180 °C, ramped by 35 °C/min to 230 °C, and held for 6 min.

GC/MS−Electron Impact (EI). Samples were analyzed with a Agilent 6890 gas chromatograph (Agilent Technologies) with a 5973 Agilent mass spectrometer detector operated in EI mode (Agilent Technologies). One microliter was injected by a liquid autosampler (model A200SE, CTC Analytics) onto a DB-Wax capillary column (60 m × 0.25 mm i.d. and 0.25 μm film thickness; Agilent Technologies). The samples were injected in splitless mode at a constant pressure of 12 psi (He) with the inlet set at 200 °C. The mass selective detector operational parameters were as follows: capillary direct interface temperature at 250 °C and source temperature at 150 °C. The oven time program was as follows: 30 °C for 2 min, ramped by 5 °C/min to 210 °C, ramped by 35 °C/min to 230 °C, and then held for 4 min.

GC/MS-CI. An Agilent 6890 gas chromatograph with a 5972 Hewlett-Packard mass spectrometer detector equipped with a chemical ionization source (the reagent gas was methane, positive ion mode) was used. One microliter of sample was injected with a Combi-Pal autosampler onto a Rtk-Wax capillary column (30 m × 0.25 mm i.d. and 0.50 μm film thickness; Restek, Bellefonte, PA). The samples were run on splitless mode at a constant flow of 0.8 mL/min (He) with the inlet set at 200 °C. The MS operational parameters were as follows: transfer line at 230 °C; MS temperature, 164 °C. The time program was as follows: held at 35 °C for 2 min, ramped by 5 °C/min to 230 °C, and held for 8 min.

LC/MS. Samples were analyzed in electrospray ionization mode on a Shimadzu HPLC system (Shimadzu, Columbia, MD) equipped with a degasser (DGU-14A), two pumps (LC-10ADvp), an autosampler (SIL-10vp), and a Waters column heater (model TCM; Waters, Milford, MA) with a Pursuit C18 column (particle size 5 μm, 250 mm × 4.6 mm i.d.; Varian, Lake Forest, CA) interfaced to a Waters ZMD 2000 mass spectrometer (Waters). The column heater was set at 25 °C, the column flow rate was 1.0 mL/min, and the effluent was split postcolumn (zero dead volume T-splitter; Supelco), with one part to the MS and four parts to waste. The mobile phase consisted of a linear concentration gradient of two solvents (A and B). Solvent A was either 10 mM ammonium acetate (for phenolic−Maillard adducts analysis) or 0.1% formic acid (for α-dicarbonyl time−course analysis), and solvent B was methanol. The initial mobile phase conditions were 10% B in A (0−2 min) and then increasing B to 80% over 20 min (2−22 min). B was then increased to 99% (22−24 min) and held for 5 min (24−29 min) before decreasing to 10% (29−30 min) and holding for 6 min (30−36 min). The sample volume was 50 μL for the phenolic−Maillard adducts analysis and 10 μL for α-dicarbonyl time−course analysis. The MS operational parameters were as follows: capillary voltage of 3.5 kV, cone voltage of 25 kV, source temperature of 110 °C, and desolvation temperature of 250 °C.

RESULTS AND DISCUSSION

The effect of flavan-3-ol ring chemistry on the generation of volatile MRPs in an aqueous glucose−glycine model system was studied. The concentrations of the most abundant volatile MRPs for glucose−glycine (model A) and glucose−glycine−flavan-3-ol (model B) reactions are reported in **Table 2** excluding two compounds, diacetyl and hydroxyacetone, which are presented below with the time−course generation data consisting of α-hydroxycarbonyl and α-dicarbonyl compounds. Overall, the addition of flavan-3-ols significantly ($\alpha = 0.05$) reduced the formation of these compounds; the concentration of 2,3,5-trimethylpyrazine, 2,5-dimethylpyrazine, 2-methylpyrazine, and cyclotene was reduced greater than 10-fold in comparison to the glucose−glycine model. Furthermore, there were no significant differences between the three flavan-3-ol

Table 3. Major LC/MS Adducts $[M - H]^-$ from Models D and F

	intact sugar fragment moiety					
phenolic moiety	C_2	C_3	C_4	C_6	$C_3 + C_4$	$C_2 + C_2 + C_2$
EC	347, 621	343, 359, 361, 633	371	451	N/A	N/A
EGCG	538	341, 359, 499, 511, 527, 529	539	619	N/A	N/A
ECG	499	343, 359, 361, 495, 511, 513, 529, 647	523, 543	567, 603	N/A	N/A
methylgallate	N/A	221, 254	N/A	N/A	N/A	N/A
1,2,3-trihydroxybenzene	N/A	181, 197	211	233	N/A	N/A
1,3,5-trihydroxybenzene	149, 165, 183	179, 197, 287, 305, 360	191, 207, 209, 225	251, 395	261	247

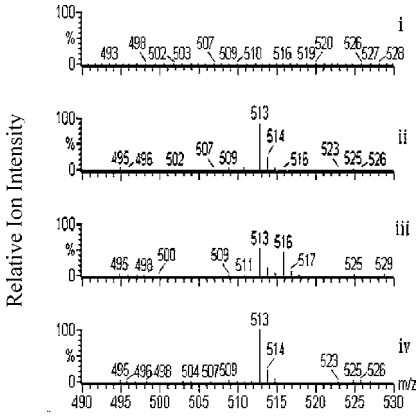

Figure 2. Measured isotopomers of analyte *m/z* $[(M - H)^-]$ 513 for ECG from (**i**) model A, (**ii**) model B, (**iii**) model D, and (**iv**) model E; the retention time was 19.95 min.

structures (EC, ECG, and EGCG) on the generation of these volatile MRPs. The A ring structure was the same for all three flavan-3-ols (**Figure 1**), indicating that the modification of the B ring (di- vs trihydroxybenzene) or C ring (gallate ester or hydroxyl group) did not significantly alter the phenolic reactions that influenced the generation of these MRPs.

All three flavan-3-ol structures also reported similar phenolic trapping reactions of key transient sugar fragment intermediate precursors of MRPs. Using a 50% labeled glucose (CAMOLA technique) or glycine (AAMOLA technique) as previously described (*4*), each flavan-3-ol was reported to form adducts with C_2, C_3, C_4, and C_6 sugar fragments (see **Table 3**) and did not report any reactivity with glycine. An example of an ECG−sugar fragment adduct reaction product corresponding to LC/MS *m/z* $[(M - H)^-]$ 513 is shown on **Figure 2**. The analyte *m/z* $[(M - H)^-]$ 513 was not found in the glucose−glycine control system (**Figure 2i**; model A) but was present in the glucose−glycine−flavan-3-ol system (**Figure 2ii**; model B). In CAMOLA reaction, 50% of the glucose was replaced with labeled glucose, leading to equal amounts of an adduct containing labeled and unlabeled sugar fragments or two isotopomers with equivalent intensities (**Figure 2iii**; model D). The mass ratio difference between the isotopomers indicated that this analyte *m/z* $[(M - H)^-]$ 513 consisted of a C_3 sugar fragment. For the AAMOLA reaction, only the analyte *m/z* $[(M - H)^-]$ 513 was reported and therefore indicated that glycine was not part of this reaction product (**Figure 2iv**; model E). On the basis of the predicted molecular weight of 514 for this reaction product, an adduct reaction product between ECG (MW 442)

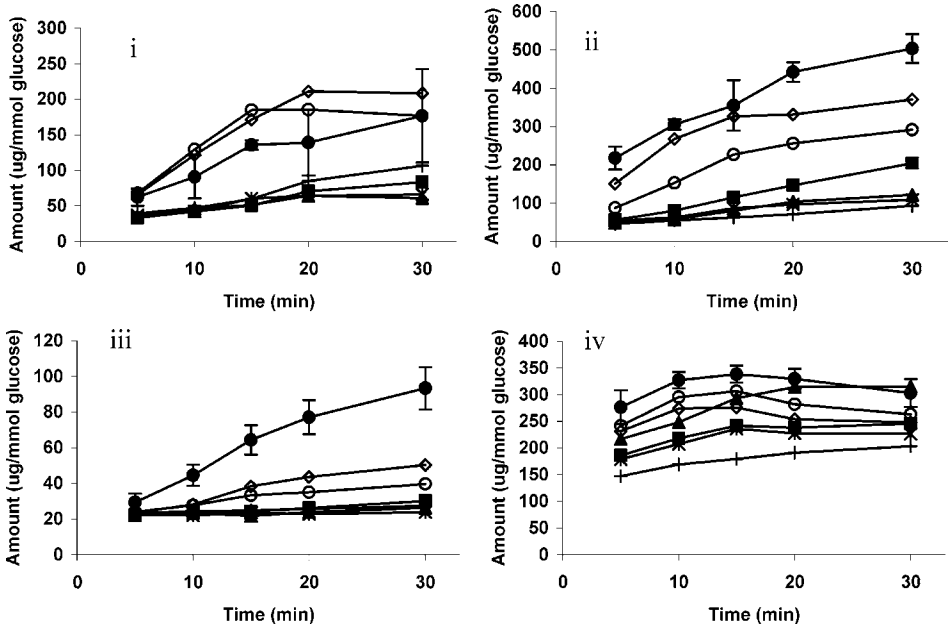

Figure 3. Time–course formation of (**i**) glyoxal, (**ii**) methylglyoxal, (**iii**) diacetyl, and (**iv**) 3-deoxyglucosone for model A, glucose + glycine control (●), and model B, EC (■), EGCG (▲), ECG (*), methylgallate (◇), 1,2,3-trihydroxybenzene (○), and 1,3,5-trihydroxybenzene (+); average of triplicate analysis, 95% CI shown for control samples.

and methylglyoxal (MW 72) could yield this product via analogous reactions previously reported for EC (*6*).

In general, the flavan-3-ol compounds predominantly generated more C_3 glucose fragment adducts than with C_2, C_4, or C_6 (**Table 3**). For ECG and EGCG, a few of the adduct analytes contained a flavan-3-ol moiety without the gallate ring. For example, the analyte *m*/*z* [(M − H)$^-$] 359 was observed for all three flavan-3-ols and the analyte *m*/*z* [(M − H)$^-$] 343 was observed for both EC and ECG.

To further examine the influence of ring chemistry on flavan-3-ol−sugar fragment adduct reactions, sugar fragment adduct formation for the three simple phenolics, methylgallate, 1,2,3-trihydroxybenzene, and 1,3,5-trihydroxybenzene, were investigated as analogous ring structures [configuration of activating (hydroxyl) and deactivating groups (ester) around benzene ring] of the individual A, B, and C ring components of EGCG (**Figure 1**). It should be noted that the reactivity of the three polyhydroxylated benzene rings of EGCG is likely influenced by the entire phenolic chemical structure; however, it would be anticipated that the reactivity of the three benzene rings would still be analogous to these simple phenolic compounds. The pKa_1 values of 1,2,3-trihydroxybenzene and 1,3,5-trihydroxybenzene are 8.99 (*14*) and 9.01 (*15*), respectively, indicating that these analytes would be similarly ionized (around 1%) at the reaction conditions used in this study (pH 7). Considering that phenolate ion would be more nucleophilic than the nonionized phenolic compound, this indicated that the reactivity of these two analytes with sugar fragment were not influenced by related pH effects.

Overall, methylgallate only reacted with a C_3 fragments whereas 1,2,3-trihydroxybenzene also reacted with C_4 and C_6 fragments. The greatest number of adducts was observed for 1,3,5-trihydroxybenzene, which quenched C_2, C_3, C_4, and C_6 sugar fragments as well as a combination of both C_3 and C_4 fragments and three C_2 fragments. The relatively higher number of sugar fragment adduct reaction products for 1,3,5-trihydroxybenzene would be anticipated based on the *meta*-polyhydroxyl configuration (the benzene ring would be more activated) and therefore would be predicted to be a stronger nucleophile.

To quantitatively investigate flavan-3-ol structure−reactivity on the mechanisms of the Maillard reactions, the influence of EC, ECG, and EGCG as well as methylgallate, 1,2,3-trihydroxybenzene, and 1,3,5-trihydroxybenzene on the time−course concentrations of α-dicarbonyls and α-hydroxycarbonyls in a glucose−glycine model reaction was investigated (see **Figures 3** and **4**). With the exception of 3-deoxyglucosone (C_6) in the EGCG model system at 30 min, the concentrations of α-dicarbonyl and α-hydroxycarbonyl precursors in the flavan-3-ol samples were significantly lower than the control. In general, the three flavan-3-ols exhibited similar effects throughout the reaction on the concentrations of α-dicarbonyl precursors, glyoxal (C_2), methylglyoxal (C_3), and diacetyl (C_4) (**Figure 3i**−**iii**) as well as for the α-hydroxycarbonyl precursors, glycolaldehyde (C_2), hydroxyacetone (C_3), and acetoin (C_4) (**Figure 4i**−**iii**). However, the time−course generation of 3-deoxyglucosone in EGCG model reaction was consistently higher than EC or ECG and, after 20 min, was not significantly different from the control sample (**Figure 3iv**). Recently, Davidek et al. (*16*) reported that a minor degradation pathway of C_6-α-dicarbonyls (i.e., deoxyglucosones) occurred by oxidative cleavage, which was induced by oxidizing species. EGCG has been reported to be a more reactive antioxidant than EC or ECG based on TEAC or Trolox assays (*17*). Possibly the higher antioxidant activity of EGCG was more effective in decreasing the oxidative degradation of 3-deoxyglucosone in comparison with that of

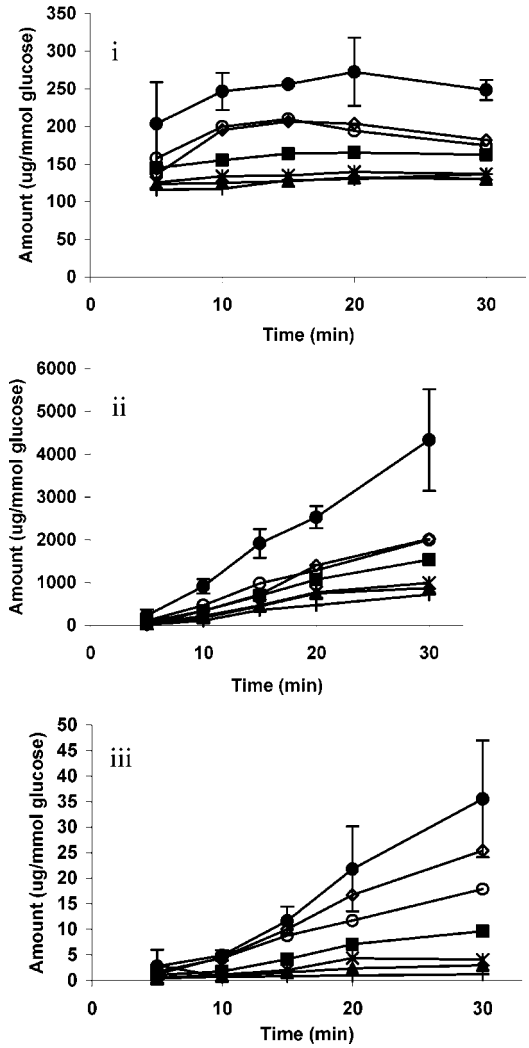

Figure 4. Time−course formation of (**i**) glycolaldehyde, (**ii**) hydroxyacetone, and (**iii**) acetoin for model A, glucose + glycine control (●), and model B, EC (■), EGCG (▲), ECG (*), methylgallate (◇), 1,2,3-trihydroxybenzene (○), and 1,3,5-trihydroxybenzene (+); average of triplicate analysis, 95% CI shown for control samples.

EC or ECG. Consequently, even though the concentration of 3-deoxyglucosone may have been reduced by flavan-3-ol trapping reactions (flavan-3-ol-C_6 adducts were reported in **Table 3**), a decreased oxidative degradation would promote accumulation and likewise may explain why the concentration of this compound was less affected by EGCG during the time course of the model reaction.

Further review of the time−course analyses for the glucose−glycine models (**Figures 3** and **4**) also indicated that hydroxyacetone (C_3) quantitatively dominated the sugar fragments monitored (>10-fold) and suggested why the flavan-3-ol-C_3−sugar adducts were more frequently detected as compared to adducts containing other sugar fragments (**Table 3**). This finding was also in agreement with Totlani and Peterson (*6*) who reported that hydroxyacetone was the main C_3 sugar fragment precursor responsible for the generation of EC−C_3 adducts detected in a glucose−glycine model system.

For the simple phenolic compounds, methylgallate followed by 1,2,3-trihydroxybenzene were reported as the least reactive, while 1,3,5-trihydroxybenzene was the most reactive compound in quenching these sugar fragment reaction products and the most similar in reactivity to the flavan-3-ols (**Figures 3** and **4**). For example, both methylgallate and 1,2,3-trihydroxybenzene

did not reduce the time−course formation of glyoxal (C_4, **Figure 3i**) in comparison to the glucose−glycine reaction, which was in agreement with the phenolic−sugar fragment adduct analysis as no C_2 sugar fragment adduct reaction products were detected (**Table 3**). Likewise, methylgallate did not report any C_4 sugar fragment adducts reaction products (**Table 3**), and as anticipated, this compound did not significantly alter the time course of the generation of acetoin (C_4, **Figure 4iii**); however, the concentration of diacetyl was significantly reduced (C_4, **Figure 3iii**). On the basis of the structure−reactivity of these simple phenolics, 1,2,3-trihydroxybenzene would be more redox-active than 1,3,5-trihydroxybenzene as predicted from the homolytic bond dissociation energies of 14.619 and 14.931 eV, respectively (*18*), whereas 1,3,5-trihydroxybenzene would be a more reactive trapping agent of sugar fragments than 1,2,3-trihydroxybenzene based on the polyhydroxyl ring configuration. 1,3,5-Trihydroxybezene has three electron-donating hydroxyl groups in the meta configuration on a benzene ring, which activate the ortho and para positions and result in a pronounced reactivity of the benzene ring for electrophilic aromatic substitutions reactions at the three unsubstituted carbon sites (e.g., with sugar fragment carbonyl compounds). Consequently, the noted higher reactivity of 1,3,5-trihydroxybenzene than 1,2,3-trihydroxybenzene for reducing the concentration of these sugar fragments suggested that phenolic−sugar trapping reactions were the main mechanism flavan-3-ols (primarily on the A ring) or these simple phenolic structures impacted Maillard chemistry (rather than the alteration of the matrix reduction potential).

Overall, the data presented in this study demonstrated that the polyhydroxyl benzene ring configuration (or the configuration of electron donating groups) were a key parameter that influenced the reactivity of phenolic compounds on the mechanisms of the Maillard reaction. A benzene ring structure with the electron-donating groups in a meta configuration was more reactive (than the ortho configuration) in suppressing the concentration of key Maillard intermediate reaction products (α-hydroxycarbonyl and α-dicarbonyl compounds) presumably by electrophilic aromatic substitution reactions. Consequently, the A ring of flavan-3-ols was suggested to be the main reactive phenolic site that impacted Maillard chemistry in these model systems.

LITERATURE CITED

(1) Nursten, H. E. *The Maillard Reaction: Chemistry, Biochemistry and Implications*; Royal Society of Chemistry: Cambridge, 2005.

(2) Namiki, M. Chemistry of Maillard reactions: Recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. *Adv. Food Res.* **1988**, *32*, 115−184.

(3) Yaylayan, V. A. Recent advances in the chemistry of Strecker degradation and Amadori rearrangement: Implications to aroma and color formation. *Food. Sci. Technol. Res.* **2003**, *9*, 1−6.

(4) Totlani, V. M.; Peterson, D. G. Reactivity of epicatechin in aqueous glycine and glucose Maillard reaction models: Quenching of C_2, C_3 and C_4-sugar fragments. *J. Agric. Food Chem.* **2005**, *53*, 4130−4135.

(5) Peterson, D. G.; Schwambach, S. L.; Totlani, V. M. Effects of phenolic content on the generation of Maillard-type aroma compounds in toasted oat groats. The 230th ACS National Meeting, in Washington, DC, Aug 28−Sept 1, 2005. Accessed November 15, 2006; http://oasys2.confex.com/acs/230nm/techprogram/P899154.HTM.

(6) Totlani, V. M.; Peterson, D. G. Epicatechin carbonyl-trapping reactions in aqueous Maillard systems: Identification and structural elucidation. *J. Agric. Food Chem.* **2006**, *54*, 7311−7318.

(7) Totlani, V. M.; Peterson, D. G. Influence of epicatechin reactions on the mechanisms of Maillard product formation in low moisture model systems. *J. Agric. Food Chem.* **2007**, *55*, 414−420.

(8) Colahan-Sederstrom, P. M.; Peterson, D. G. Inhibition of key aroma compound generated during ultrahigh-temperature processing of bovine milk via epicatechin addition. *J. Agric. Food Chem.* **2005**, *53*, 398−402.

(9) Schwambach, S. L.; Peterson, D. G. Reduction of stale flavor development in low-heat skim milk powder via epicatechin addition. *J. Agric. Food Chem.* **2006**, *54*, 502−508.

(10) Haffenden, L. J. W.; Yaylayan, V. A. Mechanism of formation of redox-active hydroxylated benzenes and pyrazine in ^{13}C-labeled glycince/*d*-glucose model systems. *J. Agric. Food Chem.* **2005**, *53*, 9742−9746.

(11) Wang, Y. Effects of naturally occurring phenolic compounds on the formation of Maillard aromas. Ph.D. Thesis, Rutgers University, 2000.

(12) Butkovic, V.; Klasinc, L.; Bors, W. Kinetic study of flavonoid reactions with stable radicals. *J. Agric. Food Chem.* **2004**, *52*, 2816−2820.

(13) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

(14) Mitsunaga, T. C. A. H.; Hill, C. G. J. Predicting the hydroxymethylation rate of phenols with formaldehyde by molecular orbital calculation. *J. Wood Sci.* **2002**, *48*, 153−158.

(15) *Hazardous Substance Data Bank*; United States National Library of Medicine, Rockville Pike: Bethesda, MD, 2002.

(16) Davidek, T.; Robert, F.; Devaud, S.; Vera, F. A.; Blank, I. Sugar fragmentation in the Maillard reaction cascade: Formation of short-chain carboxylic acids by a new oxidative α-dicarbonyl cleaveage pathway. *J. Agric. Food Chem.* **2006**, *54*, 6677−6684.

(17) Pannala, A. S.; Chan, T. S.; O'Brien, P. J.; Rice-Evans, C. A. Flavonoid B-ring chemistry and antioxidant activity: Fast reaction kinetics. *Biochem. Biophys. Res. Commun.* **2001**, *282*, 1161−1168.

(18) Netzeva, T.; Aptula, A. O.; Chaudary, S. H.; Duffy, J. C.; Schultz, W. T.; Schuurmann, G.; Cronin, M. T. D. Structure-activity relationships for the toxicity of substituted poly-hydroxylated benzenes to *Tetrahymena pyriformis*: Influence of free radical formation. *QSAR Comb. Sci.* **2003**, *22*, 575−582.

Received for review November 26, 2006. Revised manuscript received February 20, 2007. Accepted February 24, 2007. The project was supported by the National Research Initiative of the U.S. Department of Agriculture Cooperative State Research, Education and Extension Service, Grant 2004-35503-14794.

JF063423E