Published in final edited form as:

Chem Res Toxicol. 2011 September 19; 24(9): 1527–1539. doi:10.1021/tx200140s.

A Structural Determinant of Chemical Reactivity and Potential Health Effects of Quinones from Natural Products

Tingting Tu, Daryl Giblin, and Michael L. Gross*

Center for Biomedical and Bioorganic Mass Spectrometry, Department of Chemistry, Washington University in St. Louis, St. Louis, MO 63130

Abstract

Although many phenols and catechols found as polyphenol natural products are antioxidants and have putative disease-preventive properties, others have deleterious health effects. One possible route to toxicity is the bioactivation of the phenolic function to quinones that are electrophilic, redox-agents capable of modifying DNA and proteins. The structure-property relationships of biologically important quinones and their precursors may help understand the balance between their health benefits and risks. We describe a mass-spectrometry-based study of four quinones produced by oxidizing flavanones and flavones. Those with a C2-C3 double bond on ring C of the flavonoid stabilize by delocalization an incipient positive charge from protonation and render the protonated quinone particularly susceptible to nucleophilic attack. We hypothesize that the absence of this double bond is one specific structural determinant that is responsible for the ability of quinones to modify biological macromolecules. Those quinones containing a C2-C3 single bond have relative higher aqueous stability and longer half-lives than those with a double bond at the same position; the latter have short half-lives at or below ~ 1 s. Quinones with a C2-C3 double bond show little ability to depurinate DNA because they are rapidly hydrated to unreactive species. Molecular-orbital calculations support that quinone hydration by a highly structure-dependent mechanism accounts for their chemical properties. The evidence taken together support a hypothesis that those flavonoids and related natural products that undergo oxidation to quinones and are then rapidly hydrated are unlikely to damage important biological macromolecules.

INTRODUCTION

Compounds containing phenolic moieties (e.g., phenols, catechols, hydroquinones) exhibit strong reducing and radical-scavenging properties. Phenol-containing natural products, found widely in fruits, vegetables, nuts, plant oils, tea, and wines, are strong antioxidants with putative disease-preventative properties [1–7]. In contrast, some phenol-containing molecules have deleterious health effects under certain conditions, fueling a controversy over their health benefits vs. their hazards [8–14]. The toxicology is often associated with a pro-oxidant property allowing the generation of quinone-related reactive intermediates that cause oxidative injury, cellular stress, cell apoptosis, and oncogenic mutations [15–24].

Quinones are often formed from phenolic precursors by two-electron oxidation carried out by monooxygenases, peroxidases, or small molecule oxidants [25]. These quinone products constitute a class of ultimate toxins responsible for a variety of cytotoxic, immunotoxic and/or genotoxic effects [9, 11, 19, 25–33]. The chemical reactivity that underpins their biological properties is largely related to their electrophilicity and redox-activity (Scheme 1). Quinones can modify nucleic acids through Michael addition reactions, giving rise to a

variety of both stable and depurinating adducts of DNA or RNA [21, 25–27, 34–40]. Quinones can also react with proteins containing reactive thiols to yield covalent conjugates [41–43]. Furthermore, redox cycling between quinones and their corresponding semiquinone radicals generates reactive oxygen species (ROS) that also cause damage to lipids, DNA and proteins [11, 15–18, 25–27, 32, 38, 41, 44–49].

Although phenolic natural products can be metabolized to quinones, many are apparently not toxic or carcinogenic [50–53]. Furthermore, some quinones themselves are used as xenobiotic drugs for antitumor therapy [54–56]. From the perspective of human health, it is imperative to understand the balance of chemo-preventive vs. toxic-carcinogenic properties of quinones and their precursors and to elucidate the molecular mechanisms that account for any significant differences in biological or physiological outcomes.

We recently reported a case study of chemical properties of the quinones from genistein (a phytoestrogenic isoflavonoid) and from estrone, and found differences that may underlie their opposite influences on breast-cancer risk [57]. We suggested that the chemical stability of quinones in physiological-relevant media is directly related to the different extents of quinone-induced carcinogenesis, the rationale being that the products of hydration are not carcinogenic. Estrone quinones have relatively long half-lives [58], allowing them to cause directly or indirectly DNA damage. Genistein quinone, on the other hand, undergoes rapid hydration (half-life of ~ 4 s), which preempts DNA modification [57]. Quinone hydration may be viewed as a “detoxification” process that competes with reactions associated with quinone-induced toxicity. We proposed that hydration of genistein quinone is favorable because it is facilitated by forming a protonated species (an oxonium ion) that is stabilized by significant conjugation made possible by the double bond in the C ring of genistein [57]. On the basis of this preliminary hypothesis, we further suggested that the rate of quinone hydration is highly structure-dependent.

We now report an extension of the scope of our inquiry to four more quinones produced in the oxidation of precursor flavanones and flavones that are of biological and pharmacological interest. We compare the chemical stabilities of these quinones under physiological-relevant conditions (pH 7.4, 37 °C) by measuring their half-lives by using a glutathione trapping approach [57, 58]. Their abilities to depurinate DNA are correlated with their reactivity with solvent water, and that reactivity may be a predictor of quinone-induced toxicity. A “conjugatable” double bond serves as the specific structural determinant to reduce any quinone-induced toxicity by delocalizing charge from protonation of the quinone and rendering it particularly susceptible to nucleophilic attack, even by water. This reaction belongs to the class of quinone methide reaction identified by Thompson et. al. [59], who earlier proposed that the reaction of quinone methides with nucleophiles competes with reactions that damage biomolecules. Our theoretical molecular orbital calculations support the structure-dependent mechanisms of the quinone hydration. This work aims to establish more firmly the relationship between molecular structure, chemical properties, and reactivity of quinones with biological macromolecules. The hypothesis may facilitate rational design of new drugs and provide a basis for assessing the safety of food products, dietary supplements, and drug metabolites.

EXPERIMENTAL METHODS

Materials

Eriodictyol, luteolin, 4'-hydroxyflavanone, 3',4'-dihydroxyflavone and genistein were purchased from Indofine Chemical Company (Hillsborough, NJ). Glutathione (GSH), adenine (Ade), guanine (Gua), formic acid (FA, LC-MS grade), trifluoacetic acid (TFA, spectrophotometric grade), phosphate-buffered saline (PBS) tablet, *N,N*-dimethylformamide

(DMF, anhydrous) and dimethyl sulfoxide (DMSO, anhydrous) were obtained from Sigma-Aldrich (St. Louis, MO). Omnisolv water, acetonitrile (ACN) and methanol (MeOH) were from EMD Chemicals (Cincinnati, OH). Ethanol (anhydrous) was from Pharmco-AAPER (Brookfield, CT). Calf thymus DNA was from USB Corp. (Cleveland, OH). The oxidant, 2-iodoxybenzoic acid (IBX), and a ^{15}N -labeled internal standard (IS) of an estrone-adenine adduct, 4-hydroxyestrone-1- $N3(\text{U-}^{15}\text{N})\text{Ade}$ (4-OH-E₁-1- $N3(\text{U-}^{15}\text{N})\text{Ade}$) were previously synthesized and purified in our lab, as reported previously [60].

Quinone Synthesis

Four flavonoids, eriodictyol, luteolin, 4'-hydroxyflavanone and 3',4'-dihydroxyflavone (Scheme 2) were used as quinone precursors. Each was dissolved in DMF or DMSO to afford a concentration of 1 mg/mL. Equimolar IBX (solution in DMF or DMSO at 2 mg/mL) was then added, and the reaction was allowed to occur for 1 h (2 h for 4'-hydroxyflavanone). A single 3',4'-quinone product was obtained from each quinone precursor and was used for subsequent experiments. The structural assignments of the quinones were by NMR on an Inova-600 (Varian Inc., Palo Alto, CA) by using DMSO- d_6 (99.9% D, Cambridge Isotope Laboratory, Andover, MA) as solvent.

Determination of Quinone Half Lives Using GSH Trapping Method

The experimental procedure of GSH trapping was adapted from a previous report [57] with minor modifications. Generally, to initiate the quinone decay under physiologically relevant conditions, the freshly prepared quinone solution in DMF was mixed (1:20, v/v) in PBS (pH 7.4), which was pre-incubated in a 37 °C water bath. For temperature-dependent experiments, two lower temperatures were used for PBS pre-incubation: 0 °C in ice bath and 23 °C at room temperature. At various time intervals, the quinone decay reaction was quenched by removing 200 μL aliquots from the mixture and combining it with 200 μL GSH solution (10 mM in PBS) that was pre-incubated at the same temperature. For the zero decay time, 10 μL quinone in DMF was added directly to 200 μL of GSH solution (10 mM in PBS buffer), then mixed with 190 μL PBS to afford the same volume as the other samples. All the samples were diluted by 100 fold with H₂O/ACN/FA (93.4/6.5/0.1%, v/v/v), and the resulting solution was analyzed by LC/MS. It was assumed that GSH reacts substantially faster with the quinones than does water, on the basis of previous experience [57]. Genistein was spiked into each sample as an internal standard to quantify the levels of GSH-quinone conjugates.

Reaction of Quinones with Free Purine Bases

To investigate the chemistry of quinones and their reactivity with DNA bases, the reactions of different quinones with the free purines, adenine and guanine, were chosen. Two reaction conditions were employed. In the first, the inherent reactivity of quinones with free purines was investigated in a non-aqueous solvent. Freshly prepared quinones in 50 μL DMF were mixed with 1 mg Ade or Gua in 1 mL DMF and reacted for 4 h at 37 °C at 1400 rpm shaking speed. In the second, the reactivity of quinones with free purines was studied under largely physiological conditions of solvent, pH, T, and ionic strength. Freshly prepared quinones (in 50 μL DMF) were reconstituted in 50 μL acetonitrile and incubated with 1 mg Ade or Gua in PBS at 37 °C overnight at 1400 rpm shaking speed. After the incubation, the samples were centrifuged at 14 k rpm for 6 min, and the supernatants were diluted 100 times with H₂O/ACN/FA (93.4/6.5/0.1%, v/v/v). The internal standard 4-OH-E₁-1- $N3(\text{U-}^{15}\text{N})\text{Ade}$ was spiked (2 pmol for quantifying Ade adducts and 200 fmol for Gua adducts) into each sample solution, from which 5 μL was injected and analyzed by LC/MS.

Reaction of Quinones with Double-Stranded DNA

Calf thymus double-stranded DNA was dissolved in PBS at 1 mg/mL and stored at 4 °C overnight to allow full dissolution to occur. Freshly prepared quinones in 50 μ L DMF (from reactions of 20 mg/mL quinone precursors with equal molar IBX) were reconstituted in 50 μ L acetonitrile and then incubated with a 1 mL DNA solution at 37 °C overnight. Then 2.5 volumes of ethanol were added, and the samples were stored at -20 °C overnight to precipitate the DNA. The suspension was centrifuged at 14 k rpm for 20 min at 4 °C. The supernatant was then isolated, the solvent was removed under reduced pressure, and the analytes were reconstituted in 3 mL H₂O/MeOH (2/1, v/v). To calibrate the analysis, 1 pmole 4-OH-E₁-1-*N*3(U-¹⁵N)Ade was added as an internal standard. The solutions were submitted to solid-phase extraction (SPE) cleanup. Focus SPE cartridges (50 mg sorbent, 6 mL load capacity) were purchased from Varian Inc. (Palo Alto, CA). Each SPE cartridge was conditioned with 5 mL MeOH followed by 5 mL H₂O. The analytes, after solvent reconstitution, were loaded and washed with 5 mL H₂O followed by 5 mL 10% ACN. The analytes were eluted with 4 $\times$ 1 mL MeOH/ACN/H₂O/TFA (30/60/10/0.25%). After the solvent used in the elution was removed at reduced pressure, each sample was reconstituted in 20 μ L of H₂O/ACN/FA (93.4/6.5/0.1%), and a 10 μ L of the supernatant was used for each LC/MS run.

Liquid Chromatography-Mass Spectrometry

LC/MS and LC tandem MS (LC/MS/MS) were conducted on an ion-trap/FT-ICR (LTQ-FT, Thermo-Scientific, San Jose, CA) or an ion-trap/orbitrap (LTQ-Orbitrap XL, Thermo-Scientific, San Jose, CA) mass spectrometer. A 360/75 μ m OD/ID fused-silica capillary column with laser-pulled nano tip (~15 μ m) was custom-packed with Luna C18(2) reverse-phase particles (Phenomenex, Torrance, CA) to afford a column that was ~12 cm in length. The column was used for on-line LC/MS analysis; the analytes were separated by nano-LC with a flow rate of 260 nL/min by using an Eksigent NanoLC-1D HPLC (Livermore, CA). The LC gradient using solvent A (0.1% FA) and B (0.1% FA in acetonitrile) started with 97% A for 5 min, followed by a 60 min linear gradient from 3% to 97% B, and finally by 100% A for 10 min for re-equilibration. The eluents were sprayed directly from the tip of nano-column to the mass spectrometer by using a PicoView Nanospray Source (PV550, New Objective, Woburn, MA).

The mass spectrometer was operated in the positive-ion mode over a mass range of ions of *m/z* 100–1000. The spray voltage was 1.9–2.5 kV, and no nitrogen sheath or auxiliary gas was used. When accurate mass measurements in the MS mode were needed, the mass resolving power of the FT/orbitrap was set at 60,000 (for ions of *m/z* 400). For data-dependent MS² experiments, the collision energy was optimized at 40% of the maximum energy available (~5 eV), and ions within 1 Da mass window were activated by wide-band activation. When accurate mass measurements were needed for unambiguous fragmentation assignment, the mass resolving power of the FT/orbitrap for production detection was 7500.

Molecular Orbital Calculations

Initial calculations by PM3 semi-empirical [61, 62] algorithm (Spartan for Linux, Wavefunction, Inc) were performed to explore structures of the flavones studied here to examine proposed reaction products with water and protonation sites for isomers and conformers. The resultant structures were further optimized by using DFT (Density Function Theory, part of the Gaussian 03 suite, Gaussian Inc.) at the B3LYP/6-31+G(d,p) level and confirmed by vibrational frequency analysis. Single-point energies were calculated at the B3LYP/B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level, and scaled zero-point energies and thermal-energy corrections were applied [63]. The DFT methodology was selected for high-level calculations because it requires less computational overhead than *ab*

initio methods of similar accuracy [64, 65]. Results are reported in kJ/mol as relative enthalpies of protonation (negative of relative proton affinities – PA) of a particular quinone or as enthalpies of reaction for water addition.

Calculations that included solvent water molecules were performed using the polarized continuum model (SCRF=PCM) as realized in Gaussian 03 [66–68] with the UAHF set [66] of solvation radii to build the cavity.

RESULTS AND DISCUSSION

Hypothesis and Quinone Selection

Quinones can damage biomolecules by alkylating them via a Michael addition or by forming reactive oxygen species (ROS) via redox cycling with their corresponding semiquinone radicals [25–27]. Not all phenolic precursors of quinones, however, show significant cytotoxic/genotoxic effects *in vivo*. The reasons are complicated, but specific chemical properties of quinones may hold one key to understand the bioactivity of their precursors. In our previous study, we selected estrone and genistein quinones as two examples that exhibit contrasting health effects [57]. The chemical reactivities of the quinones from these two precursors are significantly different at physiological pH, temperature, and ionic strength. The half-life of genistein quinone, which has positive health benefits, is 4 ± 1 s, undergoing rapid conversion to a stable dihydrate. The hydration mechanism is facilitated by the C2–C3 double bond in the C-ring of genistein; the double bond stabilizes the reaction intermediate by conjugation and provides a driving force for facile protonation and hydration of the genistein quinone [57]. Our hypothesis is the presence of a conjugated double bond of a quinone undermines its stability in water by rendering it susceptible to nucleophilic attack; this rapid capture by solvent decreases its availability for reaction with cellular macromolecules. There is precedent for this hypothesis [59].

To test further this hypothesis, we chose pairs of quinones having similar structures but with the important difference that one of each pair has the relevant double bond for a more conjugated intermediate; this double bond stabilizes protonation. Flavonoids, as quinone precursors, are good candidates for study because there are many flavonoid structures with differing biological activities, and many are important chemopreventive substances [21, 69–75]. One of the four quinones precursors we used (Scheme 2) is eriodictyol, a flavanone found in citrus fruits and a potent compound that protects human cells from oxidative stress–induced cell death through induced Nrf2 activation and phase-2 gene expression [76, 77]. Luteolin, another precursor we selected, is one of the most common flavones, found in leaves and used as a nutritional supplement; it exerts a variety of anti-inflammatory properties and immuno-modulatory effects [77–82]. It is readily oxidized to a reactive quinone by peroxidase [53, 83]. Eriodictyol and luteolin quinones (quinone **a** and **b**, respectively) have similar structures (Scheme 2), the only difference being the bond at the C2–C3 position: a double bond for quinone **a** vs. a single bond for quinone **b**.

We additionally selected 4'-hydroxyflavanone and 3',4'-dihydroxyflavone as two other quinone precursors. They are synthetic flavonoids, and their corresponding quinones, **c** and **d**, respectively, also have or do not have the C2–C3 double bond.

Half Lives of Quinones under Physiological-relevant Conditions

The reactivity of quinones with solvent water may be inverse to their ability to modify biomolecules [57, 58]. We determined that reactivity by using the glutathione (GSH) trapping method, which was first introduced by Bolton and co-workers [58] who used HPLC-UV to measure the half lives of estrogen quinones. It is based on the quantitative Michael addition reaction of quinones with GSH, which is a nature's trapping and reducing

agent, to form in a rapid way covalently bound conjugates [35, 83–86]. We adapted the GSH-trapping method to LC-MS and used it to determine the half lives of quinones in aqueous solution. Although there are likely other methods to analyze quinones, we chose the GSH trapping because it can be generally applied to many quinones under various conditions of complexity and concentration.

When quinone **a** reacts with GSH, two mono glutathionyl eriodictyol conjugates are the major products: 2'-glutathionyl eriodictyol and 5'-glutathionyl eriodictyol, manifested as two LC peaks in the extracted ion chromatogram (EIC) (Figure 1). Only one mono glutathionyl luteolin conjugate from quinone **b** was produced, presumably as a 2'-glutathionyl conjugate [53, 87]. Quinone **c** formed a major mono glutathionyl conjugate and a minor one, which appeared as a shoulder in the EIC (Figure 1). We observed a single peak in the EIC from the glutathionyl conjugation of quinone **d**, indicating that a single mono glutathionyl conjugate had formed, although it is possible, albeit unlikely, that another glutathionyl conjugate eluted identically. In all the cases, little or no 2',5'-diglutathionyl conjugates were produced.

To measure the half life of each quinone under physiological-relevant conditions, the time course for the formation of the glutathionyl quinone conjugates was followed by quantifying their abundances at different time points. These abundances reflect the quinone amount that is trapped by GSH and not reacted in any other way at that time. At each time point, the peak area of glutathionyl conjugates in the EIC was integrated, divided by the peak area of internal standard, and normalized to 1.0 at $t = 0$. We assumed that the decay of the quinone is first-order.

The half-lives of quinones **a** and **c** (from two flavanones) are 33 ± 2 s and 170 ± 10 s at 37 °C, respectively, as determined by the fit of the decay curves (Figure 2). In contrast, quinones **b** and **d**, derived from the flavones with a C2-C3 double bond, exhibit a significantly more rapid reaction in water under the same conditions. In fact, most of the quinone had reacted by the first time point that we were able to measure (see the point at $t = 5$ s in Figure 3C), making it difficult to obtain accurate half-lives of quinones **b** and **d** at 37 °C.

Temperature-dependent experiments at 0 °C (ice bath) and 23 °C (room temperature) were conducted to define the reactivity of quinones **b** and **d** in water at 37 °C. At the two lower temperatures, slower rates pertain, allowing us to generate more reliable fitting results. For example, the experimental half lives of quinone **d** are 7.4 ± 0.6 s at 0 °C and 2.3 ± 0.4 s at 23 °C, respectively (Figure 3A, B). On the basis of the Arrhenius equation: $\ln(k_2) - \ln(k_1) = -E_a/R(1/T_2 - 1/T_1)$ (where E_a is the activation energy, k_1 and k_2 are the rate constants at the two temperatures T_1 and T_2 , respectively), we calculated both E_a for the reaction of quinone **d**, in water and the rate constant k at 37 °C to be ~ 0.6 s⁻¹ ($t_{1/2} \sim 1.2$ s). An inferential decay curve (dashed line in Figure 3C), generated by using the extrapolated half life, fits well with the experimental data at 37 °C. Similarly, for quinone **b**, the half lives are 2.8 ± 0.8 s at 0 °C and 1.1 ± 0.2 s at 23 °C, respectively, giving a $t_{1/2}$ at 37 °C of 0.8 s, indicating a even faster reaction rate at physiological temperature.

From the half-life measurements (summarized in Table 1), it is apparent that the absence of the C2-C3 double bond in the quinone plays an important role in stabilizing the quinone in water under physiological-relevant conditions. For quinone **b**, the double bond causes the rate constant for reaction in water to be ~ 40 times larger than that for reaction of quinone **a** at 37 °C. For quinones **c** and **d**, the difference of rate constants is more than 140 times. We propose that the C2-C3 double bond is responsible for the significant difference because it facilitates a hydration reaction of quinones by allowing more facile protonation. The

products formed in the reaction with water and the mechanisms of those reactions are discussed at the last section of **Results and Discussion**.

Reactivity of Quinones with Free Purine Nucleobases

The *o*-quinones are essentially good electrophiles owing to their α,β -unsaturated structure, which also imparts high reactivity with DNA bases via a 1,6-Michael addition reaction in these cases. On a DNA strand, the covalent bonding with adenine and guanine weakens the glycosidic bond, inducing bond cleavage and generating apurinic sites on the DNA strand (i.e., depurinates DNA) [37, 39]. Error-prone repair of apurinic sites may be an important pathway to initiate carcinogenesis [23, 24, 28]. The different rates of quinone reactions in aqueous solutions suggest that their reactions with purines and their ability to depurinate DNA may also be different. Therefore, we hypothesize that the quinone decay in water is inversely related to their toxicity and/or carcinogenicity in a cell.

We first examined the reactivity of free adenine and guanine with the four quinones in two solvents, DMF and PBS buffer (aqueous physiological-relevant solution). Given that quinones are relatively stable in DMF, the reactivities of the quinones in this aprotic organic solvent may be viewed as “intrinsic” (i.e., minimally affected by solvent). All four quinones in our study effectively form purine adducts as multiple isomers in the reactions in DMF. LC traces from an EIC show that a minimum of three isomers of **a**-Ade (quinone **a**-adenine adduct), eluting between 19.6–25.3 min, result from the reaction of quinone **a** with adenine. The protonated molecules have an m/z 422.1099 (within 0.9 ppm of the theoretical mass) (Figure 4). Although the product-ion spectra of each of these three isomers show similar fragmentation patterns, the lack of informative cleavages does not allow us to determine the complete structure. We also saw at least two major quinone **b**-adenine adducts (**b**-Ade) of m/z 420.0939 (within 1.1 ppm of the theoretical mass), although they were not well resolved in the chromatogram. The different EIC peak distributions, as revealed by the EIC for **a**-Ade and **b**-Ade, indicate that a C2-C3 double bond affects the binding between quinone and adenine. A minimum of four major species were found for **c**-Ade (m/z 390.1202, 1.3 ppm), and two for **d**-Ade (m/z 388.1043, 0.8 ppm).

To ensure reliable relative quantitation, the peak areas of adenine adducts from each quinone were summed and normalized to the signal of the internal standard, 4-OH-E₁-1-*N*3(U-¹⁵N)Ade, that was added to each sample. The results (Table 2) reveal that the yields of quinone-adenine adducts are similar for the four quinones provided the ionization efficiencies of these quinone-adenine adduct are comparable. Thus, the “intrinsic” reactivities of quinones **a**, **b**, **c**, **d** with free adenine are not significantly different.

We investigated the reaction of quinones with adenine in aqueous PBS buffer, and found considerably lower yields for all the quinone-adenine adducts (Table 2). The fast reaction of quinones with solvent water at physiological-relevant conditions out-competes any reaction with nucleophilic sites on adenine. The difference in yields of quinone-adenine products in two solvents DMF and PBS is consistent with the differences in the quinone rates of reaction with water as determined by the GSH trapping. For example, quinone **b**, which reacts fastest in aqueous solution, shows the most striking reactivity difference in two reaction media, more than 180 times. In contrast, quinone **c**, which reacts relatively slowly in water, shows comparable reactivity in DMF and PBS to give the **c**-Ade product; the abundance difference is only 3.5 times.

We also determined the quinone reactivity with free guanine in both reaction solvents. The quinone-guanine adducts produced in DMF eluted from 22.4 to 29.4 min, later than the corresponding adenine adducts. The distributions of the three major products from reaction of **a** and **c** with Gua are similar, as seen in the LC-MS traces, suggesting that quinones **a** and

c react similarly with guanine. The reactions of **b** and **d** with Gua in DMF yield nearly identical product distributions. No **b**-Gua or **d**-Gua products, however, were detectable when the reaction occurs in aqueous media, in accord with the short half-lives of these two quinones in their reactions with water. The abundance of **a**-Gua produced in PBS solution is ~ 52 times less than that produced in DMF, whereas the yield of **c**-Gua in PBS buffer is not significantly reduced compared with that in DMF (Table 2), a result that is consistent with quinone **c** having the longest half-life of the four quinones.

Depurinating Adducts in Reactions with Calf Thymus DNA

We further extended this investigation by examining the reactivity of quinones with calf thymus double-stranded DNA under physiological-relevant conditions. After over-night incubation of each quinone with solutions of DNA, we added an internal standard, 4-OH-E₁-1-*N*3(U-¹⁵N)Ade, before SPE cleanup, to assure good recoveries and quantification. Quinone **c**, with the longest half-life in water, does depurinate DNA. Supported by accurate mass measurement (Figure 5A), we determined that **c**-Gua eluted at 29.59 min with an *m/z* 406.1146 for [M + H]⁺ (within 1.0 ppm of the exact mass) and a **c**-Ade at 23.97 min with an *m/z* 390.1196 for [M + H]⁺ (0.3 ppm), as seen from EIC. Assuming the SPE recovery and the ionization efficiencies of two adducts are identical, we conclude from the EIC peak areas that the abundance ratio of **c**-Ade to **c**-Gua is ~ 1:9. The product-ion spectra of **c**-Gua and **c**-Ade show the same characteristic fragmentations as do the adducts formed in the reactions of the quinones with free purines, confirming that depurination to release Gua and Ade occurs in the reaction with DNA. For example, the product-ion spectra of both **c**-Gua and **c**-Ade show cross-ring fragmentations including the cleavage of O1-C2 bond on the ring C (Figure 5A). The product ion of *m/z* 280.0590, resulting from through-ring cleavage of the guanine moiety, suggests that the reaction occurs at N7 or C8 of guanine. This is in accord with previous reports that depurination occurs as a consequence of bonding at N7 or C8 of guanine [37, 39]. We note that the retention times formed by DNA depurination are slightly different than those formed in the reaction of the quinone with the free purines, likely due to variability in the custom-built nano columns used in LC/MS and the slow nL flow rates.

Under the same conditions, the reaction of quinone **a** also leads to depurination of DNA, but its reactivity is less than that of quinone **c**. As shown in Figure 5B, only a low-abundance guanine adduct (**a**-Gua) results from the reaction (26.28 min, *m/z* 438.1040 (1.0 ppm)) on the EIC. The product-ion spectrum of **a**-Gua shows cross-ring fragmentations with the cleavages on C ring (Figure 5B), in accord with its structure. We saw little or no adenine adducts (**a**-Ade).

For quinones **c** and **a**, the guanine adducts are more abundantly produced in the depurination than are the adenine adducts. This variability of the predominant depurination adducts is not unprecedented; literature reports show that is of Ade in the reactions of DNA with some other quinones (e.g. estrogen quinones) [88].

In contrast, little if any DNA depurination products were observed in the reactions of DNA with quinones **b** and **d**, in accord with the transient nature of these quinones owing to a competing hydration reaction that reduces their availability to cause DNA depurination. These results further support the importance of the C2-C3 double bond as an essential structural determinant of the quinone reactivity with biomolecules. Although other mechanisms of quinone-induced toxicity or carcinogenesis exist (e.g., ROS generation, the formation of stable adducts with DNA, and protein adduction), quinone-driven DNA depurination in competition with hydration is one consideration in understanding the biological effects of quinones.

Quinone Hydration–Theoretical Calculations and Experimental Clues

The comparisons of quinone reactivity with water and with DNA bases strongly suggest that the C2-C3 bond in quinones of the nature studied here impacts their stability and toxicity under physiological-relevant conditions. To understand further the specific role of C2-C3 double bond in quinone hydration, we carried out molecular orbital calculations on the quinones and hydration products with and without protonation for all four quinones. Although these results refer to gas-phase properties, they should be at least a guide to understanding solution reactions and, ultimately, rates.

Results from calculation of relative enthalpies of protonation (negative of relative proton affinities) for quinone **b** (Schemes 3A) shows the oxygens on 3' (preferred), 4' and 4 carbonyls can all be protonated owing to small difference in their proton affinities. Sequential additions of two water molecules are exothermic when the initial protonation takes place on 3' carbonyl, which activates the C3 position to nucleophilic attack. Addition of the first water to quinone **b** (Scheme 3A) in its protonated form (**b_{H1}**) is significantly more exothermic than addition to the neutral quinone (**b_{N1}**): $\Delta H_{rxn} = -20$ vs. -93 kJ/mol, respectively. After hydration the resultant charge in intermediate product **b_{H2}** is now at the 4'-O and by delocalization activates the C2 position to nucleophilic attack; the second hydration proceeds with exothermicity, $\Delta H_{rxn} = -13$ kJ/mol, to yield **bW_{H2}** (Scheme 3A). Both hydrations rely on charge delocalization to activate positions on the C ring (the C2-C3 double bond) for nucleophilic attack.

In contrast, for the case of quinone **a**, less charge delocalization is possible, and the addition of water to the protonated quinone (**a_{H1}**) is slightly endothermic (4 kJ/mol) whereas it is moderately exothermic for the neutral quinone (**a_{N1}**) (-29 kJ/mol, Scheme 3B). Based on the gas-phase values for the enthalpies of water addition based upon likely protonation sites we proposed that such differences in exothermicity can serve as a guide to understanding the faster uptake of water by quinone **b** (shorter half-life) relative to quinone **a** in solution.

Similar patterns of results pertain for the calculated protonation and addition of water to quinone **d** relative to quinone **c** in the gas phase. In this case, the addition of water to protonated quinone **d** (**d_{H1}**) is significantly more exothermic than to quinone **c** whether protonated or not (**c_{H1}**, **c_{N1}**) (Scheme 4A vs. Scheme 4B). These results parallel those for quinones **b** and **a**, and also correlate with the faster hydration reaction of quinone **d** in water compared to that for quinone **c**. Likewise, addition of a second water to quinone **d** to form **dW_{H2}** in the gas-phase is somewhat exothermic as also for the case of quinone **b**. In addition, calculations of single-point energies in the presence of solvent water (method described in **Experimental**) yield similar results, which bolster confidence that the calculated relative enthalpies of reaction (gas-phase) for competing reactions do have predictive power to indicate trends in relative rates of reaction in solution for this class of reactions. In addition, the quinones **b** and **d** are an extension of the quinone methide class of compounds, which upon protonation become susceptible to nucleophilic attack [59].

Experimental results support the formation of dihydrate from quinones **b** and **d** assigned structures **bW_{H2}** (Scheme 3A) and **dW_{H2}** (Scheme 4a) based on analogy to genistein [57]. Quinone dihydrates, **b**·2H₂O (**bW_{H2}**, retention time 23.27 min, m/z 321.0603, 0.6 ppm deviation) and **d**·2H₂O (**dW_{H2}**, retention time 23.16 min, m/z 289.0703 (1.4 ppm)) could be observed from extracted ion chromatograms in the LC/MS product analyses of quinones **b** and **d** reacting in aqueous buffer. The product-ion spectra of these dihydrates show mainly successive losses of small neutral molecules (i.e., H₂O, CO and CO₂). The abundances of these dihydrates, however, are relatively low, in part because their low ionization efficiencies are low. Another reason is that the dihydrate is easily reduced back to the

catechol precursor of the quinone by formic acid in the LC mobile phase during the LC separation [57].

CONCLUSIONS

A mass spectrometry-based case study of chemical properties of four quinones from oxidized flavanones and flavones reveals an important structural feature that impacts their stability in water, their reactivity with nucleic acids, and possibly their toxicity *in vivo*. Two of the four flavone quinones that are less stable at physiological-relevant conditions, as measured by glutathione trapping, show little or no reactivity with DNA to cause depurination. The experimental observations are in good agreement with molecular orbital calculations, which further support a highly structure-dependent mechanism of quinone hydration. The key structural determinant is the C2-C3 double bond in the flavonoid structure. For quinones other than those from flavonoids, this structural determinant may be any double bond on a “conjugatable” position to a quinone function. We encourage investigations of chemical structure-property relationships for other biologically important quinones to provide a deeper understanding the balance between health benefits and risks for the quinone class of reactive materials.

Acknowledgments

FUNDING SUPPORT

National Centers for Research Resources of the NIH, Grant P41RR000954. Washington University Computational Chemistry Facility, supported by NSF grant #CHE-0443501. Schering Plough (Merck), for which M.L.G. was a consultant.

References

1. Setchell KDR, Cassidy A. Dietary isoflavones: Biological effects and relevance to human health. *J Nutr*. 1999; 129:758S–767S. [PubMed: 10082786]
2. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, De Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*. 2006; 444:337–342. [PubMed: 17086191]
3. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: The in vivo evidence. *Nat Rev Drug Discovery*. 2006; 5:493–506.
4. Yang CS, Lambert JD, Sang SM. Antioxidative and anti-carcinogenic activities of tea polyphenols. *Arch Toxicol*. 2009; 83:11–21. [PubMed: 19002670]
5. Yang CS, Wang X, Lu G, Picinich SC. Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. *Nature Rev Cancer*. 2009; 9:429–439. [PubMed: 19472429]
6. Fahey JW, Kensler TW. Role of dietary supplements/nutraceuticals in chemoprevention through induction of cytoprotective enzymes. *Chem Res Toxicol*. 2007; 20:572–576. [PubMed: 17362031]
7. RiceEvans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med*. 1996; 20:933–956. [PubMed: 8743980]
8. Azam S, Hadi N, Khan NU, Hadi SM. Prooxidant property of green tea polyphenols epicatechin and epigallocatechin-3-gallate: implications for anticancer properties. *Toxicol Vitro*. 2004; 18:555–561.
9. Dietz B, Bolton JL. Botanical dietary supplements gone bad. *Chem Res Toxicol*. 2007; 20:586–590. [PubMed: 17362034]
10. Lambert JD, Kennett MJ, Sang SM, Reuhl KR, Ju J, Yang CS. Hepatotoxicity of high oral dose (–)-epigallocatechin-3-gallate in mice. *Food Chem Toxicol*. 2010; 48:409–416. [PubMed: 19883714]

11. Lambert JD, Sang SM, Yang CS. Possible controversy over dietary polyphenols: Benefits vs risks. *Chem Res Toxicol.* 2007; 20:583–585. [PubMed: 17362033]
12. Schilter B, Andersson C, Anton R, Constable A, Kleiner J, O'Brien J, Renwick AG, Korver O, Smit F, Walker R. Guidance for the safety assessment of botanicals and botanical preparations for use in food and food supplements. *Food Chem Toxicol.* 2003; 41:1625–1649. [PubMed: 14563389]
13. van Breemen RB, Fong HHS, Farnsworth NR. The role of quality assurance and standardization in the safety of botanical dietary supplements. *Chem Res Toxicol.* 2007; 20:577–582. [PubMed: 17362032]
14. Yang CS, Hong J, Hou Z, Sang SM. Green tea polyphenols: Antioxidative and prooxidative effects. *J Nutr.* 2004; 134:3181S. [PubMed: 15514297]
15. Galati G, Chan T, Wu B, O'Brien PJ. Glutathione-dependent generation of reactive oxygen species by the peroxidase-catalyzed redox cycling of flavonoids. *Chem Res Toxicol.* 1999; 12:521–525. [PubMed: 10368315]
16. Galati G, Sabzevari O, Wilson JX, O'Brien PJ. Prooxidant activity and cellular effects of the phenoxyl radicals of dietary flavonoids and other polyphenolics. *Toxicology.* 2002; 177:91–104. [PubMed: 12126798]
17. Metodiowa D, Jaiswal AK, Cenas N, Dickancaite E, Segura-Aguilar J. Quercetin may act as a cytotoxic prooxidant after its metabolic activation to semiquinone and quinoidal product. *Free Radic Biol Med.* 1999; 26:107–116. [PubMed: 9890646]
18. Suh KS, Chon S, Oh S, Kim SW, Kim JW, Kim YS, Woo JT. Prooxidative effects of green tea polyphenol (–)-epigallocatechin-3-gallate on the HIT-T15 pancreatic beta cell line. *Cell Biol Toxicol.* 2010; 26:189–199. [PubMed: 19757103]
19. Sang SM, Yang I, Buckley B, Ho CT, Yang CS. Autoxidative quinone formation in vitro and metabolite formation in vivo from tea polyphenol (–)-epigallocatechin-3-gallate: Studied by real-time mass spectrometry combined with tandem mass ion mapping. *Free Radic Biol Med.* 2007; 43:362–371. [PubMed: 17602952]
20. Li GX, Chen YK, Hou Z, Xiao H, Jin HY, Lu G, Lee MJ, Liu B, Guan F, Yang ZH, Yu A, Yang CS. Pro-oxidative activities and dose-response relationship of (–)-epigallocatechin-3-gallate in the inhibition of lung cancer cell growth: a comparative study in vivo and in vitro. *Carcinogenesis.* 2010; 31:902–910. [PubMed: 20159951]
21. Rietjens IMCM, Boersma MG, van der Woude H, Jeurissen SMF, Schutte ME, Alink GM. Flavonoids and alkenylbenzenes: Mechanisms of mutagenic action and carcinogenic risk. *Mutat Res-Fundam Mol Mech Mutagen.* 2005; 574:124–138.
22. Rietjens IMCM, Martena MJ, Boersma MG, Spiegelberg W, Alink GM. Molecular mechanisms of toxicity of important food-borne phytotoxins. *Mol Nutr Food Res.* 2005; 49:131–158. [PubMed: 15635687]
23. Mailander PC, Meza JL, Higginbotham S, Chakravarti D. Induction of A·T to G·C mutations by erroneous repair of depurinated DNA following estrogen treatment of the mammary gland of ACI rats. *J Steroid Biochem Mol Biol.* 2006; 101:204–215. [PubMed: 16982187]
24. Chakravarti D, Mailander PC, Li KM, Higginbotham S, Zhang HL, Gross ML, Meza JL, Cavalieri EL, Rogan EG. Evidence that a burst of DNA depurination in SENCAR mouse skin induces error-prone repair and forms mutations in the H-ras gene. *Oncogene.* 2001; 20:7945–7953. [PubMed: 11753677]
25. Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. Role of quinones in toxicology. *Chem Res Toxicol.* 2000; 13:135–160. [PubMed: 10725110]
26. Monks TJ, Hanzlik RP, Cohen GM, Ross D, Graham DG. Quinone Chemistry and Toxicity. *Toxicol Appl Pharmacol.* 1992; 112:2–16. [PubMed: 1733045]
27. O'Brien PJ. Molecular Mechanisms of Quinone Cytotoxicity. *Chem-Biol Interact.* 1991; 80:1–41. [PubMed: 1913977]
28. Cavalieri E, Chakravarti D, Gattenplan J, Hart E, Ingle J, Jankowiak R, Muti P, Rogan E, Russo J, Santen R, Sutter T. Catechol estrogen quinones as initiators of breast and other human cancers: Implications for biomarkers of susceptibility and cancer prevention. *Biochim Biophys Acta-Rev Cancer.* 2006; 1766:63–78.

29. Bailey LR, Roodi N, Dupont WD, Parl FF. Association of cytochrome P450 1B1 (CYP1B1) polymorphism with steroid receptor status in breast cancer. *Cancer Res.* 1998; 58:5038–5041. [PubMed: 9823305]
30. Belous AR, Hachey DL, Dawling S, Roodi N, Parl FF. Cytochrome P4501B1-mediated estrogen metabolism results in estrogen-deoxyribonucleoside adduct formation. *Cancer Res.* 2007; 67:812–817. [PubMed: 17234793]
31. Awad HM, Boersma MG, Vervoort J, Rietjens IMCM. Peroxidase-catalyzed formation of quercetin quinone methide-glutathione adducts. *Arch Biochem Biophys.* 2000; 378:224–233. [PubMed: 10860540]
32. Bolton JL. Quinoids, quinoid radicals, and phenoxyl radicals formed from estrogens and antiestrogens. *Toxicology.* 2002; 177:55–65. [PubMed: 12126795]
33. Cavalieri EL, Rogan EG. Depurinating estrogen-DNA adducts in the etiology and prevention of breast and other human cancers. *Future Oncology.* 2010; 6:75–91. [PubMed: 20021210]
34. Cavalieri EL, Stack DE, Devanesan PD, Todorovic R, Dwivedy I, Higginbotham S, Johansson SL, Patil KD, Gross ML, Gooden JK, Ramanathan R, Cerny RL, Rogan EG. Molecular origin of cancer: Catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc Natl Acad Sci U S A.* 1997; 94:10937–10942. [PubMed: 9380738]
35. Bolton JL, Pisha E, Zhang FG, Qiu SX. Role of quinoids in estrogen carcinogenesis. *Chem Res Toxicol.* 1998; 11:1113–1127. [PubMed: 9778307]
36. Bolton JL, Thatcher GRJ. Potential mechanisms of estrogen quinone carcinogenesis. *Chem Res Toxicol.* 2008; 21:93–101. [PubMed: 18052105]
37. Cavalieri EL, Rogan EG, Chakravarti D. Initiation of cancer and other diseases by catechol ortho-quinones: a unifying mechanism. *Cell Mol Life Sci.* 2002; 59:665–681. [PubMed: 12022473]
38. Pfohl-Leszkowicz A, Manderville RA. Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. *Mol Nutr Food Res.* 2007; 51:61–99. [PubMed: 17195275]
39. Li KM, Todorovic R, Devanesan P, Higginbotham S, Kofeler H, Ramanathan R, Gross ML, Rogan EG, Cavalieri EL. Metabolism and DNA binding studies of 4-hydroxyestradiol and estradiol-3,4-quinone in vitro and in female ACI rat mammary gland in vivo. *Carcinogenesis.* 2004; 25:289–297. [PubMed: 14578156]
40. Zahid M, Kohli E, Saeed M, Rogan E, Cavalieri E. The greater reactivity of estradiol-3,4-quinone vs estradiol-2,3-quinone with DNA in the formation of depurinating adducts: Implications for tumor-initiating activity. *Chem Res Toxicol.* 2006; 19:164–172. [PubMed: 16411670]
41. Davies MJ. The oxidative environment and protein damage. *BBA-Proteins Proteomics.* 2005; 1703:93–109.
42. Ishii T, Ishikawa M, Miyoshi N, Yasunaga M, Akagawa M, Uchida K, Nakamura Y. Catechol Type Polyphenol Is a Potential Modifier of Protein Sulfhydryls: Development and Application of a New Probe for Understanding the Dietary Polyphenol Actions. *Chem Res Toxicol.* 2009; 22:1689–1698. [PubMed: 19743802]
43. Wong LSN, Lame MW, Jones AD, Wilson DW. Differential Cellular Responses to Protein Adducts of Naphthoquinone and Monocrotaline Pyrrole. *Chem Res Toxicol.* 2010; 23:1504–1513. [PubMed: 20695460]
44. Akanni A, AbulHajj YJ. Estrogen nucleic acid adducts: Reaction of 3,4-estrone-o-quinone radical anion with deoxyribonucleosides. *Chem Res Toxicol.* 1997; 10:760–766. [PubMed: 9250409]
45. Han XL, Liehr JG. Microsome-Mediated 8-Hydroxylation of Guanine Bases of DNA by Steroid Estrogens - Correlation of DNA-Damage by Free-Radicals with Metabolic-Activation to Quinones. *Carcinogenesis.* 1995; 16:2571–2574. [PubMed: 7586168]
46. Kalyanaraman B, Sealy RC, Liehr JG. Characterization Of Semiquinone Free-Radicals Formed From Stilbene Catechol Estrogens - An ESR Spin Stabilization And Spin Trapping Study. *J Biol Chem.* 1989; 264:11014–11019. [PubMed: 2544580]
47. Liehr JG, Roy D. Free-Radical Generation by Redox Cycling of Estrogens. *Free Radic Biol Med.* 1990; 8:415–423. [PubMed: 2199344]
48. Powis G. Free-Radical Formation by Antitumor Quinones. *Free Radic Biol Med.* 1989; 6:63–101. [PubMed: 2492250]

49. Shigenaga MK, Ames BN. Assays for 8-Hydroxy-2'-Deoxyguanosine - a Biomarker of In Vivo Oxidative DNA Damage. *Free Radic Biol Med*. 1991; 10:211–216. [PubMed: 1650737]
50. Kulling SE, Honig DM, Simat TJ, Metzler M. Oxidative in vitro metabolism of the soy phytoestrogens daidzein and genistein. *J Agric Food Chem*. 2000; 48:4963–4972. [PubMed: 11052763]
51. Kulling SE, Honig DM, Metzler M. Oxidative metabolism of the soy isoflavones daidzein and genistein in humans in vitro and in vivo. *J Agric Food Chem*. 2001; 49:3024–3033. [PubMed: 11410004]
52. Kulling SE, Lehmann L, Metzler M. Oxidative metabolism and genotoxic potential of major isoflavone phytoestrogens. *J Chromatogr B*. 2002; 777:211–218.
53. Awad HM, Boersma MG, Boeren S, van Bladeren PJ, Vervoort J, Rietjens IMCM. Structure-activity study on the quinone/quinone methide chemistry of flavonoids. *Chem Res Toxicol*. 2001; 14:398–408. [PubMed: 11304128]
54. Beall HD, Winski S, Swann E, Hudnott AR, Cotterill AS, O'Sullivan N, Green SJ, Bien R, Siegel D, Ross D, Moody CJ. Indolequinone antitumor agents: Correlation between quinone structure, rate of metabolism by recombinant human NAD(P)H: quinone oxidoreductase, and in vitro cytotoxicity. *J Med Chem*. 1998; 41:4755–4766. [PubMed: 9822546]
55. Gutierrez PL. The role of NAD(P)H oxidoreductase (DT-diaphorase) in the bioactivation of quinone-containing antitumor agents: A review. *Free Radic Biol Med*. 2000; 29:263–275. [PubMed: 11035255]
56. Asche C. Antitumour quinones. *Mini-Rev Med Chem*. 2005; 5:449–467. [PubMed: 15892687]
57. Zhang Q, Tu T, d'Avignon DA, Gross ML. Balance of Beneficial and Deleterious Health Effects of Quinones: A Case Study of the Chemical Properties of Genistein and Estrone Quinones. *J Am Chem Soc*. 2009; 131:1067–1076. [PubMed: 19115854]
58. Iverson SL, Shen L, Anlar N, Bolton JL. Bioactivation of estrone and its catechol metabolites to quinoid-glutathione conjugates in rat liver microsomes. *Chem Res Toxicol*. 1996; 9:492–499. [PubMed: 8839054]
59. Thompson DC, Thompson JA, Sugumaran M, Moldeus P. Biological and Toxicological Consequences of Quinone Methide Formation. *Chem Biol Interact*. 1992; 86:129–162. [PubMed: 8448810]
60. Zhang Q, Gross ML. Efficient Synthesis, Liquid-Chromatography Purification, and Tandem Mass Spectrometric Characterization of Estrogen-Modified DNA Bases. *Chem Res Tox*. 2008
61. Stewart JJP. Optimization of parameters for semiempirical methods. 1 Method. *J Comp Chem*. 1989; 10:209–220.
62. Stewart JJP. Optimization of parameters for semiempirical methods. 2 Application. *J Comp Chem*. 1989; 10:221–264.
63. Scott AP, Radom L. Harmonic vibrational frequencies: An evaluation of Hartree-Fock, Moller-Plesset, quadratic configuration interaction, density functional theory, and semiempirical scale factors. *J Phys Chem*. 1996; 100:16502–16513.
64. Shephard MJ, Paddonrow MN. Gas Phase Structure of the Bicyclo[2.2.1]heptane (Norbornane) Cation Radical: A Combined ab Initio MO and Density Functional Study. *J Phys Chem*. 1995; 99:3101–3108.
65. Nicolaides A, Smith DM, Jensen F, Radom L. Phenyl radical, cation, and anion. The triplet-singlet gap and higher excited states of the phenyl cation. *J Am Chem Soc*. 1997; 119:8083–8088.
66. Barone V, Cossi M, Tomasi J. A new definition of cavities for the computation of solvation free energies by the polarizable continuum model. *J Chem Phys*. 1997; 107:3210–3221.
67. Cossi M, Barone V, Cammi R, Tomasi J. Ab initio study of solvated molecules: A new implementation of the polarizable continuum model. *Chem Phys Lett*. 1996; 255:327–335.
68. Miertus S, Tomasi J. Approximate evaluations of the electrostatic free energy and internal energy changes in solution processes. *Chem Phys*. 1982; 65:239–245.
69. Arora A, Nair MG, Strasburg GM. Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. *Free Radic Biol Med*. 1998; 24:1355–1363. [PubMed: 9641252]

70. Fink BN, Steck SE, Wolff MS, Britton JA, Kabat GC, Schroeder JC, Teitelbaum SL, Neugut AI, Gammon MD. Dietary flavonoid intake and breast cancer risk among women on long island. *Am J Epidemiol*. 2007; 165:514–523. [PubMed: 17158855]
71. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem*. 2002; 13:572–584. [PubMed: 12550068]
72. Hodgson JM, Croft KD, Puddey IB, Mori TA, Beilin LJ. Soybean isoflavonoids and their metabolic products inhibit in vitro lipoprotein oxidation in serum. *J Nutr Biochem*. 1996; 7:664–669.
73. Moon YJ, Brazeau DA, Morris ME. Effects of flavonoids genistein and biochanin A on gene expression and their metabolism in human mammary cells. *Nutr Cancer*. 2007; 57:48–58. [PubMed: 17516862]
74. Moon YJ, Wang XD, Morris ME. Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. *Toxicol Vitro*. 2006; 20:187–210.
75. Ramos S. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. *J Nutr Biochem*. 2007; 18:427–442. [PubMed: 17321735]
76. Johnson J, Maher P, Hanneken A. The Flavonoid, Eriodictyol, Induces Long-term Protection in ARPE-19 Cells through Its Effects on Nrf2 Activation and Phase 2 Gene Expression. *Invest Ophthalmol Vis Sci*. 2009; 50:2398–2406. [PubMed: 19117929]
77. Hanneken A, Lin FF, Johnson J, Maher P. Flavonoids protect human retinal pigment epithelial cells from oxidative-stress-induced death. *Invest Ophthalmol Vis Sci*. 2006; 47:3164–3177. [PubMed: 16799064]
78. Dirscherl K, Karlstetter M, Ebert S, Kraus D, Hlawatsch J, Walczak Y, Moehle C, Fuchshofer R, Langmann T. Luteolin triggers global changes in the microglial transcriptome leading to a unique anti-inflammatory and neuroprotective phenotype. *J Neuroinflamm*. 2010;7.
79. Lopez-Lazaro M. Distribution and Biological Activities of the Flavonoid Luteolin. *Mini Rev Med Chem*. 2009; 9:31–59. [PubMed: 19149659]
80. Lleo A, Galea E, Sastre M. Molecular targets of non-steroidal anti-inflammatory drugs in neurodegenerative diseases. *Cell Mol Life Sci*. 2007; 64:1403–1418. [PubMed: 17447008]
81. Jang S, Kelley KW, Johnson RW. Luteolin reduces IL-6 production in microglia by inhibiting JNK phosphorylation and activation of AP-1. *Proc Natl Acad Sci U S A*. 2008; 105:7534–7539. [PubMed: 18490655]
82. Chen CY, Peng WH, Tsai KD, Hsu SL. Luteolin suppresses inflammation-associated gene expression by blocking NF-kappa B and AP-1 activation pathway in mouse alveolar macrophages. *Life Sci*. 2007; 81:1602–1614. [PubMed: 17977562]
83. Boersma MG, Vervoort J, Szymusiak H, Lemanska K, Tyrakowska B, Cenas N, Segura-Aguilar J, Rietjens I. Regioselectivity and reversibility of the glutathione conjugation of quercetin quinone methide. *Chem Res Toxicol*. 2000; 13:185–191. [PubMed: 10725115]
84. Yu LN, Liu H, Li WK, Zhang FG, Luckie C, van Breemen RB, Thatcher GRJ, Bolton JL. Oxidation of raloxifene to quinoids: Potential toxic pathways via a diquinone methide and o-quinones. *Chem Res Toxicol*. 2004; 17:879–888. [PubMed: 15257612]
85. Butterworth M, Lau SS, Monks TJ. 17 beta-Estradiol metabolism by hamster hepatic microsomes: Comparison of catechol estrogen O-methylation with catechol estrogen oxidation and glutathione conjugation. *Chem Res Toxicol*. 1996; 9:793–799. [PubMed: 8831825]
86. Butterworth M, Lau SS, Monks TJ. Formation of catechol estrogen glutathione conjugates and gamma-glutamyl transpeptidase-dependent nephrotoxicity of 17 beta-estradiol in the golden Syrian hamster. *Carcinogenesis*. 1997; 18:561–567. [PubMed: 9067557]
87. Awad HM, Boersma MG, Boeren S, van Bladeren PJ, Vervoort J, Rietjens I. The regioselectivity of glutathione adduct formation with flavonoid quinone/quinone methides is pH-dependent. *Chem Res Toxicol*. 2002; 15:343–351. [PubMed: 11896681]
88. Chakravarti D, Pelling JC, Cavalieri EL, Rogan EG. Relating aromatic hydrocarbon-induced DNA adducts and c-H-ras mutations in mouse skin papillomas: the role of apurinic sites. *Proc Natl Acad Sci USA*. 1995; 92:10422–10426. [PubMed: 7479797]

Figure 1. Extracted Ion Chromatograms (EICs) of GSH conjugates with quinone a (A), b (B), c (C), d (D).

Figure 2.

Half life determination of quinone a (A) and quinone c (B) by using GSH trapping method (pH 7.4, 37 °C). Data were fit using a first-order exponential decay function.

Figure 3.

Half life determination of quinone **d** at 0 °C (A), 23 °C (B) by using the GSH trapping method (pH 7.4) and 37 °C (C) using the inference by Arrhenius equation. Solid curves in (A) and (B) were from data fitting using a first-order exponential decay function. The dashed curve in (C) was generated from the extrapolated half life at 37 °C.

Figure 4. Extracted Ion Chromatograms (EICs) of adenine adducts (left column) formed upon adenine incubation with quinone a (A), b (B), c (C), d (D) and guanine adducts (right column) from guanine incubation with quinone a (E), b (F), c (G), d (H).

Figure 5. Extracted Ion Chromatograms (EICs) of DNA depurination adducts formed in the reactions of quinone c (A), a (B) with calf thymus DNA. Proposed fragmentations for several characteristic product ions with implied proton transfers are consistent with the structures.

Scheme 1.

Representative mechanisms of quinone-induced carcinogenesis through ROS generation and direct DNA modification *via* Michael addition. The structures are simplified so that representative species are seen. The major adenine adduct is shown here as an example for DNA depurinating adducts.

Scheme 2.

Structures of the four flavonoids selected in this study and their corresponding quinones (potentially important single/double bonds are shown in red)

Scheme 3.

Results from theoretical calculations for proposed hydrations of (A) quinone **b** (luteolin) and (B) quinone **a** (eriodictyol) in kJ/mol.

Scheme 4.

Results from theoretical calculations for proposed hydrations of (A) quinone **d** and (B) quinone **c** in kJ/mol. **Enthalpies:** Gas-phase values in black, solution phase in magenta.

Table 1

Summary of the structural feature and half-lives (pH 7.4, 37 °C) of quinone a, b, c, d.

Quinones	Bond Format on C2-C3 position	Prensence of 5-, 7- OH groups (A ring)	Quinone Half Lives at Physiological-relevant Conditions (pH 7.4, 37 °C)
<u>a</u>	Single Bond	+	33 ± 2 s
<u>b</u>	Double Bond	+	~ 0.8 s (extrapolated)
<u>c</u>	Single Bond	–	170 ± 10 s
<u>d</u>	Double Bond	–	~ 1.2 s (extrapolated)

Table 2

Relative abundances of Ade (**A**) and Gua (**B**) adducts in two different reaction media. Abundance Ratio is ratio of abundances of adducts formed in DMF to those formed in aqueous PBS.

(A)			
Quinone-Guanine Adducts	Reaction Media	Relative Adduct Abundance (to Internal Standard)	Abundance Ratio
<u>a</u> -Ade	DMF	12.5 ± 0.9	11.4
	PBS	1.1 ± 0.1	
<u>b</u> -Ade	DMF	8.2 ± 0.7	186.4
	PBS	0.044 ± 0.007	
<u>c</u> -Ade	DMF	11 ± 2	3.5
	PBS	3.1 ± 0.6	
<u>d</u> -Ade	DMF	13 ± 1	118.2
	PBS	0.11 ± 0.02	
(B)			
Quinone-Guanine Adducts	Reaction Media	Relative Adduct Abundance (to Internal Standard)	Abundance Ratio
<u>a</u> -Gua	DMF	1.1 ± 0.1	52.4
	PBS	0.021 ± 0.008	
<u>b</u> -Gua	DMF	1.6 ± 0.4	∞
	PBS	n.d. ^I	
<u>c</u> -Gua	DMF	1.26 ± 0.03	1.15
	PBS	1.1 ± 0.1	
<u>d</u> -Gua	DMF	1.9 ± 0.4	∞
	PBS	n.d. ^I	

^I n.d.= not detectable