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Direct and Indirect Photolysis of the Phytoestrogens Genistein and Daidzein

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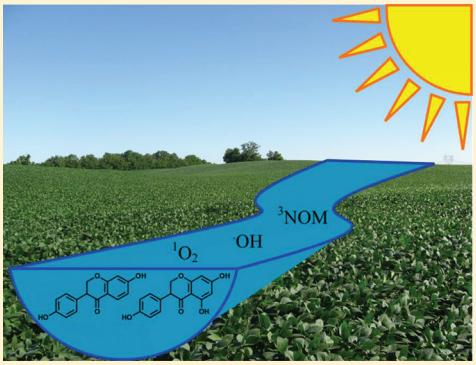
Supporting Information

ABSTRACT: Genistein and daidzein are two estrogenic compounds derived from plants, especially legumes. This research begins to explore their environmental fate, focusing on direct and indirect photolysis. UV−visible spectra for both compounds at varying pH values were taken, the pK_a values for both compounds were measured, and UV−visible spectra for each protonation state were determined. The loss of both compounds in deionized water was observed upon exposure to natural sunlight, and the quantum yields were determined for each protonation state. In Mississippi River water, direct photolysis does not account for all of the loss of genistein and daidzein. The mechanism of indirect photolysis was probed using quenchers and sensitizers, and results suggest that daidzein is transformed mainly via direct photolysis and singlet oxygenation, while genistein is transformed mainly via reaction with triplet-state natural organic matter. The parameters determined in this study will allow for estimation of the concentration of genistein and daidzein in sunlit surface waters, which will allow for assessment of any risks posed to aquatic wildlife.



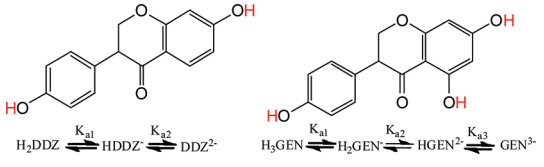
INTRODUCTION

Endocrine disrupting compounds (EDCs) are of serious concern as aquatic pollutants due to the low concentrations at which they are bioactive. Most research on the environmental presence, degradation pathways, and effects on humans and wildlife has focused on natural human estrogens, synthetic estrogens for therapeutic and contraceptive use, or industrially produced EDCs such as bisphenol A and nonylphenol.[1−4] Another class of EDCs, the phytoestrogens (plant-derived estrogens), is also gaining attention. Although typically 2 to 3 orders of magnitude less potent than the human estrogens, such as 17β-estradiol and estrone or the synthetic estrogen 17α-ethinylestradiol, several investigations have indicated that phytoestrogens may act as EDCs.[5−8] Genistein and daidzein (Scheme 1), the focus of this study, are two such compounds.

These phytoestrogens are present at high concentrations in soybean plants and other legumes. Genistein and daidzein were detected in the effluents of soy-processing industries (4.8−151 μg/L and 2.1−98.9 μg/L, respectively), other plant-processing industries (up to 10.5 μg/L and 3.5 μg/L, respectively), and other industries (up to 30.8 μg/L and 12.4 μg/L, respectively).[9,10] Genistein and daidzein have also been detected in a variety of surface waters. In Iowa streams draining large areas of corn and soybean fields and Swiss streams draining pastures and urban lands, genistein was detected at concentrations of 8 ng/L to 24.2 ng/L, and daidzein was detected at concentrations of 6.4 ng/L to 41 ng/L.[11−13] In streams draining urban or residential areas, the concentrations observed have usually been lower (2−7 ng/L daidzein and 4−7 ng/L genistein),[14,15] but in one urban Japanese stream, up to 143.4 μg/L genistein and 42.9 μg/L daidzein were observed.[16] A conclusive explanation of these extremely high concentrations was not offered, but it was noted that there are food and wood pulp factories in the area.

The potential impact of phytoestrogens on aquatic systems merits investigation. This study seeks to determine the importance of photolysis in the environmental fate of genistein and daidzein in natural waters. Other studies have shown that estrogenic compounds with at least one phenolic group, such as nonylphenol, 17β-estradiol, and 17α-ethinylestradiol, undergo direct photolysis slowly but are efficiently transformed by hydroxyl radical (·OH) in engineered UV systems.[17−19] Similarly, bisphenol A has been shown to undergo slow removal by direct photolysis.[20,21] Degradation of bisphenol A is

Scheme 1



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enhanced by ·OH and triplet state natural organic matter (^{3}NOM).[20,21] Other wastewater-derived compounds with electron-donating groups on an aromatic ring are removed slowly by direct photolysis and more quickly by reaction with ·OH, singlet oxygen ($^1O_2$), or ^{3}NOM.[22−24]

Direct photolysis occurs when a molecule absorbs sunlight and is transformed. The first order rate expression is given by

$$-\frac{dC}{dt} = \phi_{dc} k_a C \quad (1)$$

where C is the concentration of the compound of interest, k_a is the rate at which the compound absorbs light summed over the wavelengths at which it absorbs, and ϕ_{dc} is the quantum yield.[25] The quantum yield represents the fraction of molecules absorbing a photon that are transformed. It is calculated using the equation

$$\phi_{dc} = \frac{k_{dc}}{k_{da}} \sum_{\lambda} \frac{\varepsilon_{\lambda a} L_\lambda \lambda_{range}}{\varepsilon_{\lambda c} L_\lambda \lambda_{range}} \phi_{da} \quad (2)$$

where ϕ_{da} is the quantum yield of a chemical actinometer (a compound with a known quantum yield), k_{dc} and k_{da} are the rate constants for the photolysis of the compound and the actinometer, $\varepsilon_{\lambda a}$ and $\varepsilon_{\lambda c}$ are the molar absorptivities of the actinometer and compound, respectively, at wavelength λ, L_λ is the solar irradiance at wavelength λ, and λ_{Range} is the difference between λ_n and λ_{n+1}.[25]

Sunlight interacting with the organic matter present in natural water also generates transient reactive species such as $^1O_2$, ·OH, ^{3}NOM, or other photochemically produced reactive intermediates. The reaction of these photochemically produced reactive intermediates with pollutants is known as indirect photolysis.

Given their presence in effluent streams and surface waters, photolysis is a potentially important loss process for genistein and daidzein in sunlit aquatic environments. This study sought to quantify the rates of direct and indirect photolysis of genistein and daidzein. Given their structural similarity to other estrogenic compounds and the presence of the phenolic rings, it was hypothesized that genistein and daidzein would be subject to both direct and indirect photolysis processes.

METHODS

Experimental Design. The UV−visible spectrum of each compound was measured at a variety of pH values. This allowed the determination of the pK_a values of each compound and provided information necessary for calculation of direct photolysis quantum yields. Genistein, daidzein, and an actinometer were exposed to natural and simulated sunlight in deionized (DI) water or Mississippi River water (MRW) at a variety of pH values. The actinometer allowed experiments with long exposure times to be conducted without concern for changes in cloud cover or sunlight intensity. Important indirect photolysis processes were identified by quencher and sensitizer experiments.

Chemicals. Chemicals and the MRW used in this study are described in the Supporting Information (SI).

HPLC Methods. High pressure liquid chromatography (HPLC) methods are described in the SI.

UV−Vis Spectroscopy. Stock solutions of genistein (2.5 mM) and daidzein (1 mM) were prepared in methanol. Separately, buffered aqueous solutions from pH 4.5−13.5 were prepared. Stock solutions were diluted with the aqueous buffers resulting in 100 μM daidzein with 10% methanol or 50 μM genistein with 2% methanol. The resulting samples were placed in a 1-cm path length quartz cuvette, and spectra were collected from 190 to 400 nm at each selected pH value by a Shimadzu UV-1601 spectrophotometer. An aqueous blank containing either 10% or 2% methanol was used.

Solar Exposure. To conduct solar irradiation experiments, phytoestrogen and actinometer solutions in quartz tubes (o.d. = 1.3 cm, i.d. = 1.1 cm, V = 10 mL) were set on the roof of the Civil Engineering building at the University of Minnesota in Minneapolis, MN (latitude 45°N), which provided no shadows for most of the day. Exposures of daidzein were conducted at pH 8.75 on Jul 12, 2009 and pH 5.1 and pH 12 on Jul 20, 2009. Experiments with genistein were conducted at pH 8.7 and pH 12 on Jul 20, 2009, pH 11 on Nov 4, 2009, and pH 5 on Nov 6, 2009. Actinometer solutions consisted of 10 μM p-nitroacetophenone (PNAP) with 80.2 mM or 22 mM pyridine or 10.5 μM p-nitroanisole (PNA) with 0.39 mM or 1.41 mM pyridine. Actinometers were selected to have a half-life longer than that of the reaction of interest, using the equations in Leifer.[25] Subsamples were taken throughout the exposure and analyzed by HPLC.

Identifying the Indirect Photolysis Processes. Sensitizers and inhibitors were added to the MRW samples at pH 12 and 8.7 to investigate indirect photolysis processes. Sorbic acid (8.9 mM) was added to quench ^{3}NOM, sodium azide (1.5 mM) was added to quench $^1O_2$, isopropyl alcohol (1% = 131 mM) was added to quench ·OH, or the solution was sparged with nitrogen or argon gas for 5 min to inhibit the formation of $^1O_2$ and/or to potentially allow enhanced formation of ^{3}NOM. Each sample was placed in a quartz tube and exposed to simulated sunlight in an Atlas CPS+ solar simulator with the intensity set to 765 W/m^2. For deoxygenated solutions, the tubes were capped with septa, and the solutions were sampled with a needle and syringe to prevent introduction of oxygen to the system. Subsamples were taken periodically and analyzed by HPLC.

Singlet Oxygen Rate Constant and Sensitization. To quantify the rate constant for the reaction between $^1O_2$ and genistein or daidzein, solutions of 7 μM genistein at pH 8.5, 5.9 μM daidzein at pH 8.75, or 20 μM furfuryl alcohol (FFA; a reference compound with a known rate constant, $k_{1O_2} = 1.2 \times 10^8$ M^{-1} s^{-1}) and 6 μM Rose Bengal, a $^1O_2$ sensitizer, were photolyzed simultaneously.[26,27] Subsamples were taken throughout the experiment and analyzed by HPLC.

To investigate whether genistein and daidzein produce singlet oxygen, solutions of 20 μM FFA were made in either DI water, DI water with 4.1 μM genistein, DI water with 4.3 μM daidzein, MRW, MRW with 6.7 μM genistein, or MRW with 7.1 μM daidzein. The solutions were irradiated in quatz tubes in the solar simulator for 2h, with subsamples removed every 20 min.

Hydroxyl Radical Rate Constant. To quantify the rate constant for the reaction between ·OH and genistein or daidzein, Fenton's reagent (40 μM $FeSO_4 \cdot 7H_2O$ and 1 mM H_2O_2) was added to solutions containing the compound of interest and 20 μM acetophenone ($k_{OH} = 5.9 \times 10^9$ M^{-1} s^{-1}), as a reference compound.[28,29] The solutions were adjusted to pH 3 with sulfuric acid. Samples (0.5 mL) were taken frequently, quenched with 0.5 mL methanol, and analyzed by HPLC.

Data Analysis. Data fitting to determine pK_a values was carried out with Scientist for Windows (v.2.1; Micromath

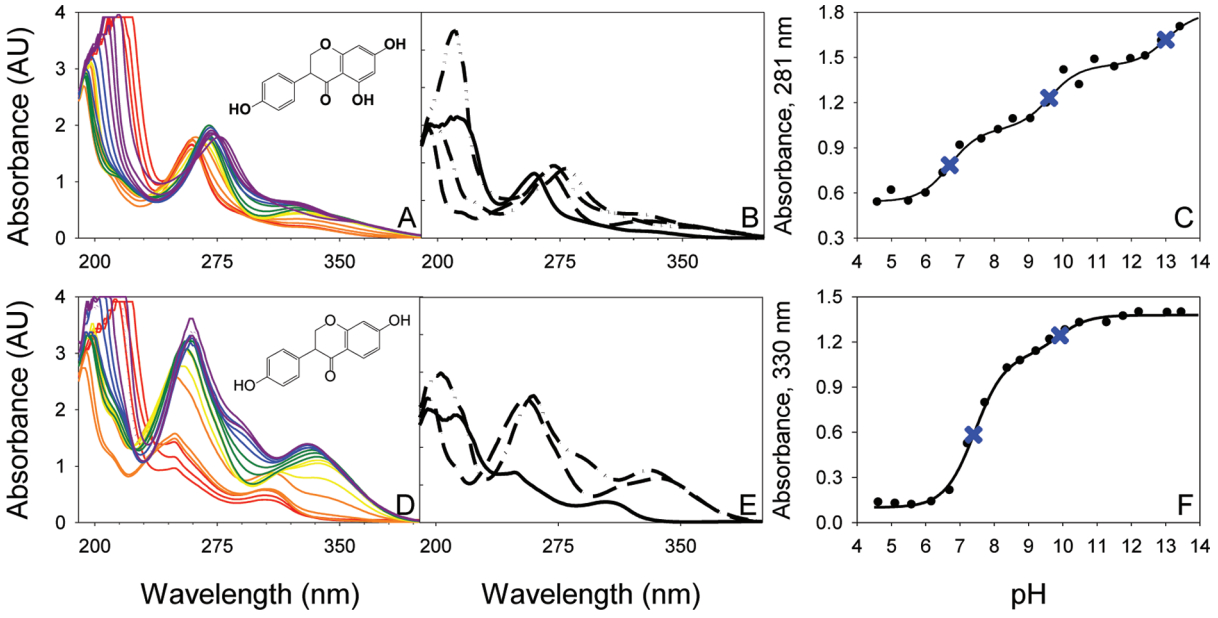

Figure 1. UV−visible spectra collected as a function of pH (panel A for genistein and D for daidzein, pH increases from red to purple) used to determine pK_a values. The pK_a values determined were 6.70 ± 0.39, 9.62 ± 0.45, and 13.0 ± 1.1 for genistein and 7.43 ± 0.10 and 9.88 ± 0.35 for daidzein (indicated by × in C and F), respectively, by observing shifts in the UV−vis spectra. The component spectra (panels B and E) for each protonation state of genistein and daidzein (H_3GEN or H_2DDZ: solid line; H_2GEN^- or $HDDZ^-$: dashed line; $HGEN^{2-}$ or DDZ^{2-}: dash-dot line; GEN^{3-}: dash-dot-dot line) were then determined using a matrix transformation, which is detailed in the Supporting Information.

Scientific Software). Protonation state spectra were determined using MATLAB (v R2009b, Mathworks). Solar spectra were generated using SMARTS (v 2.9.5, http://www.nrel.gov/rredc/smarts/).[30,31] Quantum yields and rate constants were determined via linear regressions using Microsoft Excel 2007.

RESULTS AND DISCUSSION

UV−Vis Spectra. The absorbance spectra obtained as a function of pH are shown in Figure 1. The spectra were used to determine the pK_a values of genistein and daidzein by fitting spectral data to eqs 3−5

$$(\chi_1)(A_{1,\lambda}) + (\chi_2)(A_{2,\lambda}) + (\chi_3)(A_{3,\lambda}) + (\chi_4)(A_{4,\lambda}) = A_\lambda \quad (3)$$

$$A_\lambda = \frac{A_1[H^+]^3 + A_2[H^+]^2K_{a1} + A_3[H^+]K_{a1}K_{a2} + A_4K_{a1}K_{a2}K_{a3}}{[H^+]^3 + [H^+]^2K_{a1} + [H^+]K_{a1}K_{a2} + K_{a1}K_{a2}K_{a3}} \quad (4)$$

$$A_\lambda = \frac{A_1[H^+]^2 + A_2[H^+]K_{a1} + A_3K_{a1}K_{a2}}{[H^+]^2 + [H^+]K_{a1} + K_{a1}K_{a2}} \quad (5)$$

where χ_i is the molar fraction of genistein in protonation state i at a given pH, $A_{i,\lambda}$ is the absorbance of that protonation state at the given pH, and A_λ is the total absorbance at wavelength λ at that pH. Eqs 4 and 5 are expanded forms of eq 3, where A_1, A_2, A_3, and A_4 are the absorbances of each of the protonation states, K_{a1}, K_{a2}, and K_{a3}, are the acid−base equilibrium constants, and $[H^+]$ is the concentration of hydrogen ions.[24]

By fitting of eqs 4 (for genistein) and 5 (for daidzein) to the UV−vis spectra of the respective phytoestrogens at various pH values shown in Figure 1, the pK_a values for each compound were determined.[24] The wavelength for the fitting, selected by trial and error, was 330 nm for daidzein and 281 nm for genistein. As shown in Figure 1, the spectrophotometric titration curves at these wavelengths give pK_a values of 6.70 ± 0.39, 9.62 ± 0.45, and 13.0 ± 1.1 for genistein and 7.43 ± 0.10 and 9.88 ± 0.35 for daidzein. Reported errors are 95% confidence intervals.

Determination of Quantum Yields. The quantum yield of photolysis was determined at each pH for each compound using eq 2. The first term in eq 2, $((k_{dc})/(k_{da}))$, was calculated by plotting the concentration of substrate $([C]/[C_0])$ versus the concentration of actinometer $([\text{actinometer}]/[\text{actinometer}]_0)$ on a log−log scale and taking the slope of the resulting plot as $((k_{dc})/(k_{da}))$. These plots, generated using data from the solar exposure experiments, are presented in Figure S1 of the SI. Spectra of genistein and daidzein at each experimental pH were calculated using the component spectra (Figure 1, panels B and E, determined using the method of Boreen et al.[24,32] as described in the SI). This was done because the samples for photolysis and the samples for the UV−vis spectra were not adjusted to exactly the same pH values. Spectra and quantum yields of the actinometers were those reported by Leifer.[25] Solar irradiance spectra were generated for each experimental date using SMARTS,[30,31] and the overlap integral of the solar spectra with genistein, daidzein, or the actinometer spectrum was calculated.

The quantum yields determined at each experimental pH are found in Table 1. Genistein reacted more quickly at high pH and exhibited the largest quantum yield at pH 12 (2.9×10^{-4}). The spectral overlap integral for genistein, however, also increases with increasing pH, so the next highest quantum yield was at pH 5 (1.3×10^{-4}). The lowest quantum yield for genistein was observed at pH 8.7 (9.4×10^{-6}). Daidzein reacted most quickly at pH 8.7 and also exhibited the largest quantum yield at pH 8.7 (6.6×10^{-4}). At pH 12, daidzein exhibited its smallest quantum yield (1.4×10^{-4}). Reaction rate depends on both light absorbance rate and quantum yield, and light absorbance depends strongly on pH. Thus, at a given pH, it is possible for a protonation state present as a minor fraction

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Table 1. Quantum Yields for Direct Photolysis at Tested pH Values, Second-Order Rate Constants for Reactions with Hydroxyl Radical and Singlet Oxygen, and Calculated Quantum Yields for Individual Protonation States

Observed				
	pH	ϕ_{dc} (-)	k_{OH} (M^{-1} s^{-1})	$k_{^1O_2}$ (M^{-1} s^{-1})
genistein	5	1.3×10^{-4}	$8.73 \pm 0.31^{a,b} \times 10^9$	$3.57 \pm 0.08 \times 10^{7c}$
	8.7	9.4×10^{-6}		
	11	7.2×10^{-5}		
	12	2.9×10^{-4}		
daidzein	5	4.7×10^{-4}	$6.94 \pm 0.09^{b} \times 10^9$	$1.84 \pm 0.03 \times 10^{7c}$
	8.7	6.6×10^{-4}		
	12	1.4×10^{-4}		

Calculated			
protonation state	ϕ_{dc} (-)	protonation state	ϕ_{dc} (-)
H_3GEN	6.9×10^{-5}	H_2DDZ	3.9×10^{-4}
H_2GEN^-	3.3×10^{-6}	$HDDZ^-$	7.5×10^{-4}
$HGEN^{2-}$	5.0×10^{-5}	DDZ^{2-}	3.1×10^{-5}
GEN^{3-}	3.2×10^{-3}		

[a]Reported errors are 95% confidence intervals. [b]Reactions with Fenton's reagent to determine the hydroxyl radical rate constant were conducted at pH 3. [c]Photolyses with Rose Bengal to determine the singlet oxygen rate constant were conducted at pH 8.5.

to strongly influence the total quantum yield of genistein or daidzein if the product of light absorbance rate and quantum yield is dramatically greater than that of the major protonation state present.

Determination of Component Quantum Yields. Because genistein and daidzein both consist of more than one protonation state at environmentally relevant pH values, it is useful to consider that the total rate constant of transformation is equal to the sum of the rate constants for each protonation state

$$k_{dc,total} = k_{dc,\chi_1} + k_{dc,\chi_2} + k_{dc,\chi_3} + k_{dc,\chi_4} \quad (6)$$

These values can all be divided by k_{da}, which yields a relative rate value $(k_{dc,\chi_i})/(k_{da})$ that can be used to calculate the quantum yield of each protonation state. Eq 7 was used to determine $(k_{dc,\chi_i})/(k_{da})$

$$\begin{bmatrix} \chi_{1,\,pH\,5} & \cdots & \chi_{4,pH\,5} \\ \vdots & \ddots & \vdots \\ \chi_{1,pH\,12} & \cdots & \chi_{4,pH\,12} \end{bmatrix} \begin{bmatrix} \frac{k_{dc,\,\chi_1}}{k_{da}} \\ \vdots \\ \frac{k_{dc,\,\chi_4}}{k_{da}} \end{bmatrix} = \begin{bmatrix} \frac{k_{dc,\,total,\,pH\,5}}{k_{da}} \\ \vdots \\ \frac{k_{dc,\,total,\,pH\,12}}{k_{da}} \end{bmatrix} \quad (7)$$

where $(k_{dc,\chi_i})/k_{da}$ is the rate of transformation of protonation state i relative to the transformation of the actinometer used to measure $(k_{dc,total})/k_{da}$, the observed rate of transformation of genistein or daidzein at the indicated pH normalized to the actinometer.[24] For this analysis, solar exposures needed to be conducted using the same actinometer for all pH values to compare $(k_{dc,total})/k_{da}$ values. At pH 5, however, genistein did not react quickly enough to be compared to the same actinometer as the other pH values. To estimate the $((k_{dc})/(k_{da}))$ value for genistein at pH 5, k_{da} was calculated for the PNAP actinometer with 22 mM pyridine, and for the PNA actinometer with 0.39 mM pyridine according to the equation

$$k_{da} = \phi_{da} \sum_{\lambda} \varepsilon_\lambda L_\lambda \quad (8)$$

where $\Sigma_\lambda \varepsilon_\lambda L_\lambda$ is the overlap of the absorbance spectrum of the chemical and the solar spectrum.[25] The ratio of the two k_{da} values can be used to convert the $((k_{dc})/(k_{da}))$ for genistein at pH 5 from a PNAP actinometer basis to a PNA actinometer basis. The $((k_{dc})/(k_{da}))$ value for genistein at pH 5 normalized to the PNA actinometer is then compared to those for genistein at the other pH values tested. The solar spectrum from Nov 6, 2009 was used for the PNAP actinometer, and the solar spectrum from Jul 20, 2009 was used for the PNA actinometer. The $k_{da,PNAP}/k_{da,PNA}$ conversion factor was calculated to be 0.0405. The quantum yields for each component species are found in Table 1. Using the component spectra and the component quantum yields, the fraction of direct photolysis attributable to each protonation state can be determined, according to eq 1.

As shown in Table 1, the fully deprotonated species of genistein has the highest quantum yield, but under typical environmental pH conditions, it is not present at high enough concentrations to contribute substantially to the rate of direct photolysis (Figure 2A). The fully protonated species of genistein dominates the contribution to the rate of direct photolysis at pH values of 7 and below. The singly deprotonated species of genistein dominates the direct photolysis at pH values near its maximum molar fraction. The singly protonated species of genistein dominated at pH values from 9 to 11.

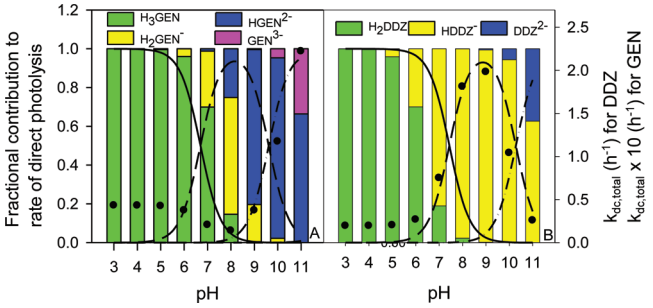

Figure 2. Contributions by each protonation state of genistein (A) and daidzein (B) to the overall direct photolysis rate (colored bars) and fractional concentrations of each as a function of pH (H_3GEN or H_2DDZ: solid line; H_2GEN^- or $HDDZ^-$: dashed line; $HGEN^{2-}$ or DDZ^{2-}: dash-dot line; GEN^{3-}: not visible, fractional concentration < 0.01 at pH 11). The circles are the overall rate constant $(k_{dc,total})$ calculated based on the quantum yields and absorbance spectra of each protonation state as a function of pH using eq 6 and the solar spectrum from ref 25 for summer 40°N.

The singly deprotonated species of daidzein has the highest quantum yield and dominates direct photolysis rates at pH 7 and above (Figure 2B). The fully protonated species of daidzein dominates direct photolysis below pH 7. The contribution of each species is mainly dependent on the pH of the solution but is also influenced to a lesser extent by the quantum yield of each species. Also shown in Figure 2 are the calculated rate constants of direct photolysis $(k_{dc,total})$ at each pH value based on the quantum yields and absorbance spectra for each protonation state substituted into eq 6 and using the solar spectrum for summer 40°N from ref 25.

Hydroxyl Radical Rate Constant. Concentrations of genistein, daidzein, and acetophenone were monitored during

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their reaction with Fenton's reagent. These experiments were carried out at pH 3 to prevent iron precipitation. The rate constant was determined by plotting the fractional concentration of genistein or daidzein against the fractional concentration of the acetophenone on a log−log scale (Figure S2) and taking the slope to be $(\ln([C]/[C_0]))/(\ln([R]/[R_0]))$ in the equation

$$k_{ROS,C} = \frac{\ln\left(\frac{[C]}{[C_0]}\right)}{\ln\left(\frac{[R]}{[R_0]}\right)} k_{ROS,R} \quad (9)$$

where $k_{ROS,R}$ is the rate constant for the reaction between acetophenone and the hydroxyl radical, and $k_{ROS,C}$ is the rate constant for the reaction between genistein or daidzein and the hydroxyl radical. The rate constants for the reactions of hydroxyl radical and genistein or daidzein were determined to be $8.71 \pm 0.31 \times 10^9$ M^{-1} s^{-1} and $6.94 \pm 0.09 \times 10^9$ M^{-1} s^{-1}, respectively (Table 1; Figure S2, SI). These values are comparable to those observed for other phenolic EDCs (bisphenol A, 17α-ethinylestradiol, and 17β-estradiol; 3.3×10^9 M^{-1} s^{-1}−1.14×10^{10} M^{-1} s^{-1}, pH 6.8).[18,33,34]

Singlet Oxygen Rate Constant. To determine the singlet oxygenation rate constants, the concentrations of genistein, daidzein, and FFA were monitored during irradiation in the presence of Rose Bengal at pH 8.5. Instead of acetophenone, the concentration of FFA was used to determine the value of $(\ln([C]/[C_0]))/(\ln([R]/[R_0]))$. The measured rate constants for the reactions of singlet oxygen with genistein or daidzein were $3.57 \pm 0.08 \times 10^7$ M^{-1} s^{-1} and $1.84 \pm 0.03 \times 10^7$ M^{-1} s^{-1}, respectively (Table 1; Figure S3, SI). These values are nearly an order of magnitude lower than the rate constant reported for bisphenol A in pH 10 water, 1.01×10^8 M^{-1} s^{-1}.[35] Bisphenol A at pH 10 would consist of 27% of the fully protonated form, 70% of the singly deprotonated form, and 3% of the fully deprotonated form. These experiments were carried out at pH ∼8.5, where the singly deprotonated species is the dominant protonation state for both genistein and daidzein. That the rate constant for bisphenol A is nearly an order of magnitude greater suggests that the singly deprotonated form of bisphenol A is more reactive toward singlet oxygen than that of either genistein or daidzein. As pH increases, the singlet oxygen rate constant may also increase, because the aromatic rings become more electron-rich when additional deprotonation steps occur.

Importance of Indirect Photolysis Processes. Side-by-side irradiation of solutions of genistein or daidzein in DI water and MRW showed that direct photolysis could not account for all observed losses in MRW at all pH values tested, as shown in Figure 3 for pH 8.5 and in Figure S1 for other pH values. To determine the mechanism of indirect photolysis, genistein and daidzein were exposed to simulated sunlight in the presence of probes and quenchers (Figure 3). When added to a pH 8.5 solution of MRW and genistein, isopropanol slightly slowed the loss of genistein ($k_{isopropanol}/k_{MRW} = 0.85$). Deoxygenation also slightly slowed the reaction ($k_{deoxygenated}/k_{MRW} = 0.86$), but adding sodium azide caused genistein transformation to occur more rapidly ($k_{sodium\ azide}/k_{MRW} = 1.46$), an unexpected effect seen previously for mefenamic acid.[22] The reaction of genistein in MRW in the presence of sorbic acid ($k_{sorbic\ acid}/k_{MRW} = 0.04$) slowed dramatically, and the resulting rate of loss was slower than that in DI water ($k_{DI}/k_{MRW} = 0.11$). The reaction of daidzein in MRW was unaffected by sodium azide ($k_{sodium\ azide}/k_{MRW} = 0.92$) and isopropanol ($k_{isopropanol}/k_{MRW} = 1$).

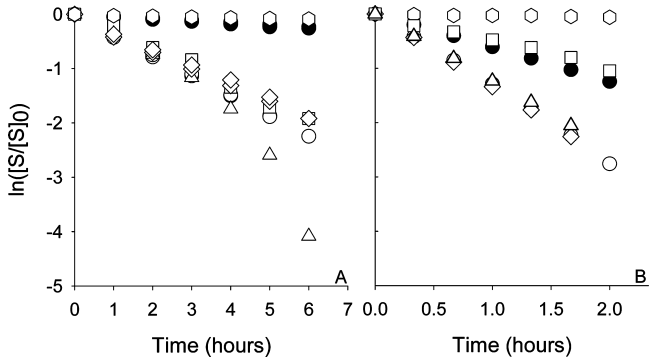

Figure 3. Loss of genistein (A) and daidzein (B) in DI water (filled circles), MRW (open circles), deoxygenated MRW (squares), MRW with sodium azide (triangles), MRW with isopropanol (diamonds), and MRW with sorbic acid (hexagons).

Deoxygenation of the MRW solution slowed the reaction of daidzein substantially ($k_{deoxygenated}/k_{MRW} = 0.37$), so it was slightly slower than a solution of daidzein in DI water ($k_{DI}/k_{MRW} = 0.46$). Sorbic acid slowed the loss of daidzein in MRW to a rate even slower ($k_{sorbic\ acid}/k_{MRW} = 0.02$) than the rate in DI water.

Sorbic acid is known to quench ^{3}NOM, so these results suggest that ^{3}NOM may play a role in the indirect photolysis of genistein and daidzein. To a lesser extent, sorbic acid may also be a quencher of $^1O_2$ ($k < 5 \times 10^7$ M^{-1} s^{-1}), but given the minor effects of the other quenchers on genistein, it seems most likely that $^1O_2$ and ·OH are not important and that ^{3}NOM is largely responsible for the observed reaction enhancement in MRW.[36,37] In the case of daidzein, it seems that $^1O_2$ may play a role, because deoxygenating the MRW slows the reaction to the rate at which it proceeds in DI water. It is unclear why azide did not quench the singlet oxygenation, but oxygen may play an additional role (see below). Adding sorbic acid, however, to either genistein or daidzein in MRW slows the reaction down to an even slower rate than that which occurs in DI water.

To further explore this issue, genistein and daidzein were exposed to sunlight in deoxygenated DI water, or in DI water in the presence of sorbic acid, both at pH 8.5. The screening of sunlight by sorbic acid accounts for a 6% decrease in rate constant (data not shown). The results in Figure 4 demonstrate that sorbic acid quenches the direct photolysis of genistein and daidzein in DI water. Thus, this explains why the sorbic acid slows the reaction in MRW to a rate slower than that in DI water: the sorbic acid is quenching direct photolysis as well as any reaction with ^{3}NOM. This indicates that the direct photolysis of these two phytoestrogens proceeds through a triplet excited state. The deoxygenated experiments also showed a slower rate of transformation for both genistein and daidzein, indicating either possible self-sensitization of singlet oxygen generation by genistein or daidzein or that oxygen is somehow otherwise involved in the direct photolysis reaction.

To investigate whether genistein and daidzein sensitize the formation of singlet oxygen, FFA was exposed to simulated sunlight in the presence of DI water with and without genistein or daidzein and MRW with and without genistein or daidzein. FFA in the solutions containing genistein or daidzein was removed at the same rate as in the solutions without genistein or daidzein (Figure 5). These results indicate that these

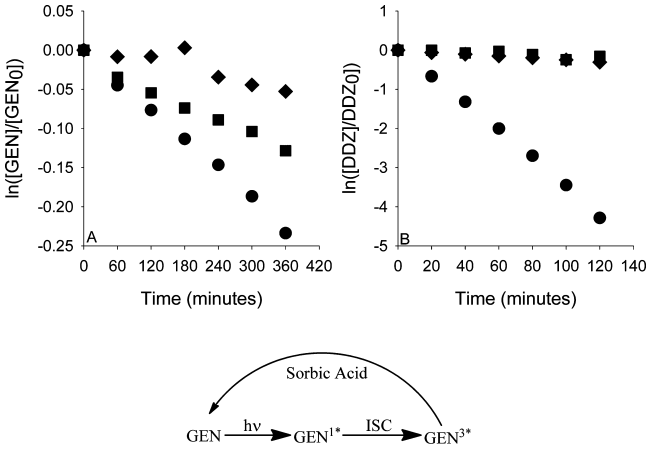

Figure 4. Photolysis of genistein (A) and daidzein (B) in DI water with (diamonds) and without (circles) sorbic acid present and in deoxygenated DI water (squares). The schematic shows the quenching effect of sorbic acid on triplet genistein (which would be equivalent for daidzein). While the quenching effect of sorbic acid suggests a role for the triplet excited state in direct photolysis, the slowing of the rate upon deoxygenation also indicates a role for oxygen in the direct photolysis process.

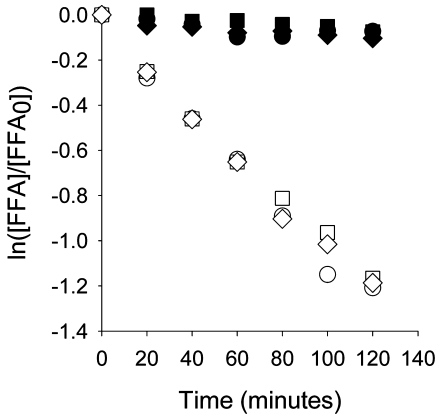

Figure 5. Degradation of furfuryl alcohol in the absence (circles) or presence of genistein (diamonds) or daidzein (squares), in DI water (solid), or MRW (outlined).

phytoestrogens do not serve as singlet oxygen sensitizers. Either the triplet states do not have sufficient energy to generate singlet oxygen or they are too short-lived to produce singlet oxygen (i.e., once formed via intersystem crossing, a chemical bond is broken, completing the direct photolysis step). The latter explanation is consistent with the dramatic quenching effect of sorbic acid on the direct photolysis rate. The slowing of the reaction in DI water in the absence of oxygen (meaning oxygen is not acting as a triplet quencher) and the lack of production of singlet oxygen by irradiation of genistein and daidzein suggests that either additional reactive oxygen species are produced during the photolysis of these phytoestrogens or that oxygen is involved in the direct photolysis reaction of these compounds.

Predicted Photolysis Half-lives. The half-life of direct photolysis of daidzein in sunlit summer and winter near-surface waters was estimated using eq 10[25]

$$t_{1/2} = \ln(2)\left[\sum_{\lambda} \varepsilon_{\lambda,H_2DDZ} \times L(\lambda) \times \Phi_{H_2DDZ} + \sum_{\lambda} \varepsilon_{\lambda,HDDZ^-} \times L(\lambda) \times \Phi_{HDDZ^-} + \sum_{\lambda} \varepsilon_{\lambda,DDZ^{2-}} \times L(\lambda) \times \Phi_{DDZ^{2-}}\right] \quad (10)$$

To estimate the half-life of genistein, the same equation was used, with an additional term in the denominator to account for the additional protonation state in genistein. For both equations, L was assumed to be at 40 ° N latitude, taken from Leifer (sunlight intensity averaged over 24 h).[25] For daidzein at pH 8.5, the half-life was 0.014 days (0.34 h) in summer and 0.046 days (1.1 h) in winter. For genistein at pH 8.5, the half-life was 1.48 days (35.5 h) in the summer and 4.91 days (117.8 h) in the winter. When samples were irradiated in natural sunlight in MRW at pH 8.7, a half-life of 10 h was observed for genistein and a half-life of 1.1 h was observed for daidzein (data not shown).

Assuming a steady state concentration of $^1O_2$ ($[^1O_2]_{ss}$) of 10^{-13} M, an environmentally relevant value,[38] and multiplying by the second order rate constants for genistein or daidzein determined at pH 8.5 gives pseudo-first order rate constants of 3.57×10^{-6} s^{-1} (half-life of 53.9 h) and 1.84×10^{-6} s^{-1} (half-life of 104.6 h), respectively. These half-lives cannot be directly compared to the half-lives of direct photolysis because they do not account for the variable sunlight intensity throughout a day. Loss of genistein is more likely to occur via direct photolysis than reaction with singlet oxygen under conditions with similar steady-state concentrations of singlet oxygen. Loss of daidzein occurs much more quickly via direct photolysis than reaction with singlet oxygen, but singlet oxygen may play a role (Figure 3B).

For hydroxyl radical typical values are on the order of 10^{-17} to 10^{-15} M.[38] The pseudofirst order rate constants would then be on the order of 10^{-8} s^{-1} to 10^{-6} s^{-1} (half-lives of 190–19,000 h). Under circumstances with high concentrations of hydroxyl radical, such as high-nitrate waters, reaction with hydroxyl radical could become an important loss process for genistein and daidzein. The contribution of ^{3}NOM to loss of genistein and daidzein is harder to address because NOM can act as an oxidant or as an antioxidant.[39] If ^{3}NOM were the most important loss process, we would expect to see a decreased rate of transformation in the presence of sorbic acid and an increased rate of transformation under deoxygenated conditions. The effects of sorbic acid or deoxygenation on daidzein, shown in Figures 3 and 4, suggest that while ^{3}NOM may have a limited role for daidzein, reaction with an oxygen-based species is the dominant indirect photolysis process. The effects of sorbic acid are complicated, though, by its quenching of both ^{3}NOM and direct photolysis. The picture is clearer for genistein. Genistein in pH 8.7 MRW exposed to natural sunlight had a half-life of about 10 h (data not shown), substantially faster than the predicted half-life of direct photolysis or reaction with singlet oxygen or hydroxyl radical. This reaffirms that reaction with ^{3}NOM is an important loss process for genistein in surface waters. It is possible that reactive oxygen species other than singlet oxygen are involved, given that deoxygenating samples of genistein or daidzein decreases the rate of transformation, sodium azide does not

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decrease the rates, and the transformation of FFA is not sensitized by genistein or daidzein. Another possibility is that reaction with oxygen is a necessary step in the direct photolysis process. The role of oxygen in the photolysis of these compounds needs to be investigated further.

The parameters developed in this study will help in estimating the concentration of genistein and daidzein in sunlit surface waters subject to inputs of genistein and daidzein. Estimating the concentrations will help assess the risk to aquatic life. Genistein may be lost at rates on the order of days to weeks, while daidzein may be lost at rates on the order of hours to days. Further research is needed to determine whether other loss processes, such as sorption to solids or biodegradation, are important for genistein and daidzein and to determine if the transformation products retain estrogenicity.

■ ASSOCIATED CONTENT

Supporting Information

Additional text and three figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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