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PHOTOLYSIS OF TESTOSTERONE, PROGESTERONE AND 17β-ESTRADIOL BY UVA LIGHT

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ABSTRACT: Wastewater treatment facilities and agricultural production are potential sources of steroid hormones in the environment. The fate of steroid hormones in the environment has not been extensively studied. Important degradation pathways must be identified to predict the long-term impact of steroid hormones and their metabolites. Chemical oxidation, biodegradation, and photodegradation have been identified as potential mechanisms for the transformation of these compounds. While some studies have investigated the photodegradation of steroid hormones under conditions relevant for engineered systems, few studies have examined the importance of photolysis on the transformation of steroid hormones under environmentally relevant conditions. Consequently, the objectives of this study were to determine the potential for direct and indirect (i.e., by humic acid and nitrate) photolysis of 17β-estradiol, testosterone and progesterone by light in the UVA range (305 to 410 nm) in a phosphate buffered media at pH 5.5. Photolysis of 17β-estradiol, testosterone, and progesterone was observed. The photolysis was modeled using pseudo first-order reaction kinetics and half-lives were found to be in the range from 3 to 6 hours, depending on the type of hormone and solution composition. Both testosterone and progesterone were directly photolyzed. In the presence of humic acid (HA), testosterone was photolyzed at increased rates, while HA did not influence the photolysis of progesterone. 17β-estradiol was only photolyzed (indirectly) in the presence of HA. This study indicates that photolysis of hormones might be an important removal mechanism in natural systems and that organic matter can influence the photodegradation potential.
KEY TERMS: Hormones; Photolysis; Photosensitizers; Organic Matter; Nitrate; Water

INTRODUCTION

Steroid hormones are given to livestock to increase muscle mass and regulate hormonal cycles (Andersson and Skakkebaek, 1999, Ternes et al., 1999). In addition, humans ingest steroid hormones as hormone replacement therapies or for contraception. Generally, these hormones are excreted in urine or feces, either unchanged or as steroid metabolites (Andreozzi et al., 2003). These hormones can enter the environment through runoff from manure lagoons (Shore and Shemesh, 2003) or following the application of manure to agricultural fields, through leaching into groundwater from concentrated animal feed lots (Fine et al., 2003), and from human sources such as waste water treatment plants (Belfroid et al., 1999).

There has been a large increase in concern regarding the presence of steroid hormones in natural systems due to recent studies showing adverse impacts of steroid hormones in the environment (de Voogt et al., 2003, Jobling and Tyler, 2003, Kolpin et al., 2002, Tyler et al., 1998), including detrimental reproductive effects in animals (Ankley et al., 2003). Environmental concentrations of steroid hormones are present in the μg/L range and lower (Snyder et al., 1999); hormones have been shown to have biological effects at these concentrations (Baronti et al., 2000).

Chemical oxidation, biodegradation, and photodegradation have been identified as potential mechanisms for the transformation of steroid hormones. Some studies have investigated photodegradation of steroid hormones under conditions relevant for engineered systems. For example, one study found that photolysis in the presence of TiO_2 (photocatalyst) resulted in a 98% removal of 17β-estradiol after 3.5 hours of reaction with light in the UVC range (Coleman et al., 2000,

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Zhang et al., 2007). However, few studies have examined the importance of photolysis under environmentally relevant conditions (i.e., by UVA light and in the presence of potential natural photosensitizers such as organic matter and nitrate).

Photodegradation of organic molecules, including steroid hormones, may occur as a result of direct or indirect photolysis. Direct photolysis occurs when photons with certain energy are absorbed by bonds in organic molecules. This excites the bonds and causes chemical transformations such as fragmentation, isomerization, or electron transfer. Indirect photolysis occurs when photons are absorbed by intermediate molecules, commonly called "photosensitizers," to form radicals that may react with and chemically transform organic molecules in the same matrix (Schwarzenbach et al., 2002, Vione et al., 2006).

Compounds subject to photodegradation can form many different metabolites through processes of isomerization, oxidation, fragmentation, and electrophilic addition.

Photodegradation of these hormones can occur through both direct and indirect photolysis. Direct photolysis occurs when a photon of light with certain energy is absorbed by bonds in an organic molecule. This excites the bond and causes transformations such as fragmentation, isomerization, or electron transfer. Indirect photolysis occurs when a photon of light interacts with electrons of molecules of the matrix such as nitrates or dissolved organic matter. This interaction will cause the formation of radicals that will attack the organic species in solution causing degradation (Schwarzenbach et al., 2002, Vione et al., 2006). A matrix-matrix attack can cause quenching of the amount of photons put into solution and therefore decrease the rate of photolysis. These photolysis reactions can occur at light in UVA, UVB, and UVC ranges (Coleman et al., 2004) in natural systems, which could cause potential degradation of the substances in soils and waters exposed to sunlight. The effectiveness of photolysis can be measured through determination of quantum yield.

Calculation of quantum yields of solutions allows for characterization of degradation. The quantum yield describes the number of molecules that were able to degrade per amount of photons that the light source produced at certain wavelengths that attacked the solution over a specific amount of time. This quantity depends on the overall irradiance of the lamps used. Newer lamps should produce higher quantum yields because the excited gas inside is able to produce a higher amount of photons. Older lamps can produce similar "false" quantum yields due to irradiance from the lamp's filament instead of irradiance from the excited gases. The measurement of irradiance of the lamps used for photodegradation can be taken with a specialized UV light meter that measures irradiance over a total spectrum or measured by chemical actinometry.

A chemical actinometer is a compound whose rate of degradation can be used to determine the amount of irradiance coming from a light source.(Schwarzenbach et al., 2002) A good actinometer is one that has a similar absorption spectrum to the analyte that's being measured (similar electronic structure)(Dulin and Mill, 1982). The rates of degradation of the chemical and the compound should be similar to accurately measure the total amount of photons in solution.

Photolysis reactions can occur at light in UVA, UVB, and UVC ranges (Coleman et al., 2004) . Because some of these wavelengths are present in natural sunlight, organic molecules (including steroid hormones) in soils and waters exposed to light may be able to degrade by photolysis. The objectives of this paper are thus to 1) determine the potential for direct photolysis of 17β-estradiol, testosterone, and progesterone by UVA light, 2) elucidate the impact of photosensitizers (i.e., nitrate and humic acid) on the photolysis of these compounds, and 3) establish the corresponding photolysis rate constants.

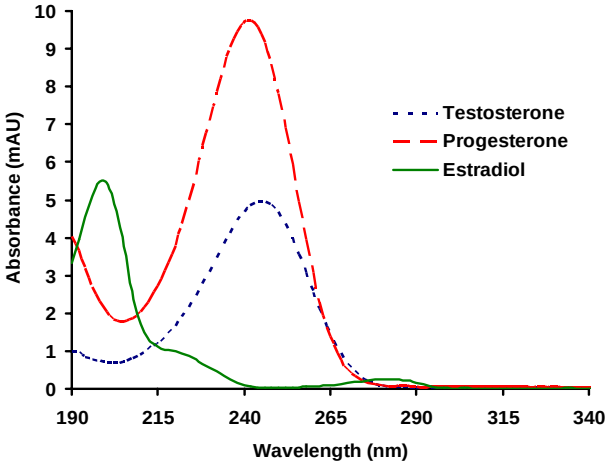

Figure 1. UV absorption spectra for 17β-estradiol, progesterone, and testosterone

EXPERIMENTAL METHODS

Chemicals

17β-estradiol was obtained from Calbiochem (La Jolla, CA), testosterone was obtained from Pfaltz and Bauer (Waterbury, CT), and progesterone was obtained from Acros Organics (New Jersey, USA). Potassium phosphate monobasic, KH_2PO_4, and potassium nitrate as well as HPLC grade acetonitrile and methanol were obtained from Fisher Scientific (Fair Lawn, NJ). Deionized water was produced by a Millipore Milli-Q Gradient System (Billerica, MA). Elliot soil Humic Acid (1S102H) was obtained from the International Humic Substances Society.

Experimental Approach

Each treatment was made in 10 mM KH_2PO_4 buffer solution. Humic acid stock solutions were prepared by dissolving humic acid in 0.1 M NaOH. Stock solutions of 17β-estradiol, testosterone, and progesterone were prepared by dissolving each hormone in methanol. Nitrate solutions were prepared at a concentration of 10 mg/L potassium nitrate in KH_2PO_4 buffer. Humic acid (HA) solutions were prepared by adding HA from the stock solution to the KH_2PO_4 buffer to achieve a concentration of 5 mg/L. These concentrations were chosen to be consistent with a study by Rosenfeldt and Linden (2004) and to simulate environmentally relevant concentrations (Rosenfeldt and Linden, 2004). Each solution was stirred for 1 hour to ensure complete mixing. Samples were pH adjusted to 5.5. All solutions were filtered through a 0.22 μM MAGNA nylon filter (GE Osmonics, Minnetonka, MN) before hormones were added to prevent the presence of particulate matter. All solutions were wrapped in aluminum foil and stored at 2 °C to prevent photodegradation. Each hormone was added into solutions of deionized water (controls), phosphate buffer, nitrate (P-buffered), humic acid (P-buffered), and humic acid with nitrate (P-buffered) to a concentration of 1 mg/L.

Photolysis of Endocrine Disruptors and Analysis

Degradation was conducted in a Rayonet RPR-200 photochemical reactor chamber with Hg lamps (Rayonet model RPR 3500) of maximum spectral density of 0.05 mW/cm^2/nm and a range of irradiance of 305-410 nm (UVA Range). The lamps have an output of quasi-monochromatic light at 350 nm (all lamp data are based on information provided by Rayonet). The measured total UVA irradiance was 128.75 W/m^2. The reactor was equipped with a RMR-500 merry go-round that spun at 5 rpm to ensure equal distribution of light intensity for each sample (Chu, 1999). Samples containing a starting volume of 8 mL were contained in borosilicate glass culture tubes. At each time point, the tubes were inverted three times to ensure proper mixing before sampling occurred. Samples containing 200 μL of solution were taken out of each culture tube and put into HPLC autosampler vials for analysis.

Steroid hormones and their degradation products were analyzed using an Agilent 1200 Series High Performance Liquid Chromatograph (HPLC) equipped with a Diode Array Detector. HPLC analysis was performed using a Zorbax Eclipse XCD-C18 column (4.6x150 mm; 5 μM particle diameter). Injection volume was 20 μL and the column temperature was held constant at 35 °C. For 17β-estradiol and testosterone, an isocratic method of 60% water and 40% acetonitrile was pumped at a flow rate of 1 mL/min with an overall run time of 15 minutes. For progesterone, the mobile phase was kept constant at 60% water and 40% acetonitrile for 9 minutes and then ramped to 100% acetonitrile over five minutes and held at 100% acetonitrile for five minutes. The solvent ratio was returned to initial settings over one minute and was held for an additional five minutes before the next sample injection. The overall run including conditioning was 25 minutes. Testosterone was measured at a wavelength of 244 nm, progesterone was measured at 245 nm, and 17β-estradiol was measured at 220 nm (Figure 1).

RESULTS

Photolysis of 17β-estradiol, testosterone, and progesterone was observed in the presence of UVA light (Figures 2, 3, and 4). Direct photolysis was observed for testosterone and progesterone in both DI water (control) and phosphate buffered solutions (Table 1). Nitrate did not influence the degradation rate of testosterone (Figure 2). The higher photolysis rate constant for progesterone in the presence of nitrate was determined to be statistically insignificant (Table 1 and Figure 4). Direct photolysis was not observed for 17β-estradiol and indirect photolysis was not observed in the presence of nitrate (Figure 3).

Table 1. Photolysis rate constants of testosterone, progesterone and 17β-estradiol and the corresponding half-lives ($t_{1/2}$).

	Testosterone		**Progesterone**		**17β-Estradiol**	
	k (h^{-1})	**$t_{1/2}$ (h)**	**k (h^{-1})**	**$t_{1/2}$ (h)**	**k (h^{-1})**	**$t_{1/2}$ (h)**
DI Water	0.158 ± 0.007	4.387	0.126 ± 0.008	5.501	—	—
Phosphate Buffer	0.199 ± 0.005	3.483	0.124 ± 0.011	5.590	—	—
+ Nitrate	0.194 ± 0.002	3.573	0.130 ± 0.008	5.331	—	—
+ Humic Acid	0.231 ± 0.002	3.001	0.126 ± 0.005	5.501	0.155 ± 0.006	4.472
+ Humic Acid and Nitrate	0.230 ± 0.002	3.013	0.117 ± 0.002	5.924	0.208 ± 0.009	3.332

Indirect photolysis was observed in the presence of humic acid and humic acid with nitrate for testosterone and 17β-estradiol (Figure 2 and 3). The degradation of 17β-estradiol was 32% faster in treatments with the concurrent presence of humic acid and nitrate compared to treatments with only humic acid (Figure 3).

Photolysis rate constants and half lives for each system were calculated using a pseudo first order degradation model (Table 1). No metabolites were observed in the studies of progesterone and 17β-estradiol with the applied HPLC method. However, photolysis of testosterone resulted in at least two degradation products and their UV absorption spectra are shown in Figure 5.

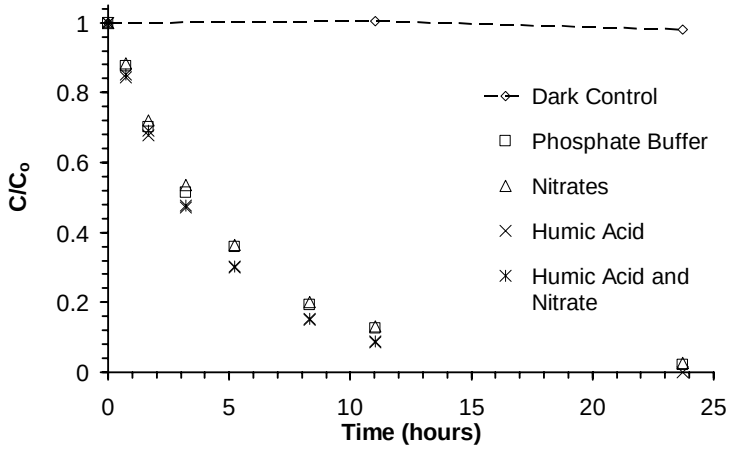

Figure 2. Photolysis of testosterone as a function of time.

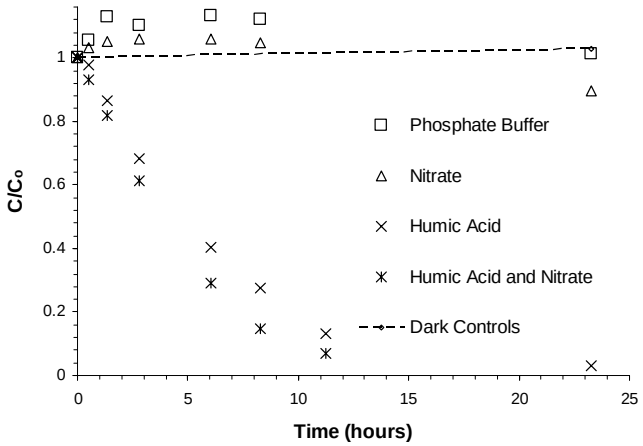

Figure 3. Photolysis of 17β-estradiol as a function of time

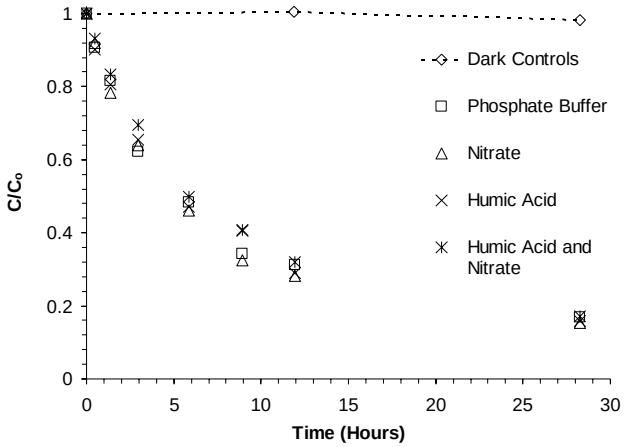

Figure 4. Photolysis of progesterone as a function of time.

DISCUSSION

The rapid photolysis of steroid hormones observed in this study illustrates the potential importance of UVA light in removing these compounds from waters exposed to sunlight. Previous studies have primarily focused on the use of UVB (280-315 nm) and UVC (200-280 nm) light for degradation of endocrine disrupting compounds. (Lin and Reinhard, 2005) showed that 17β-estradiol was transformed in DI water at pH 7 and river water (containing 4.6 mg/L dissolved organic carbon and 22.7 mg/L nitrate; pH 7.5) when exposed to light in the range 290-700 nm with observed photolysis rate constants of 0.02 and 0.35 h^{-1}, respectively. These rate constants are comparable to those observed in this study in the presence of humic acid. In contrast to our study, photolysis was observed in pure DI water, which is most likely due to the use of light in the UVB range. The overall higher rates observed by Lin et al. (2005) are probably also a result of the approximately 6 times higher irradiance intensity as well as the higher pH in their study (Lin and Reinhard, 2005, Zhang et al., 2007). In contrast to our study, Coleman et al. (2004) reported a (direct) photolysis rate constant for the removal of 17β-estradiol in distilled water of 0.66 h^{-1} using a 125 W high pressure Hg lamp (UVA light) (Coleman et al., 2004). We are currently investigating the impact of UVA light on 17β-estradiol in long-term experiments (> 5 d) to verify that direct photolysis is not taking place under the given conditions.

To the best of our knowledge, this is one of the first reports on photolysis of testosterone and progesterone under environmentally relevant conditions. Thus, it was not possible to compare the observed half lives in this study with literature values. However, the half lives observed for progesterone and testosterone were within the same order of magnitude of values previously reported for estrogens (Lin and Reinhard, 2005).

The presence of humic acid (5 mg/L) had a significant impact on the photolysis rate of testosterone and 17β-estradiol indicating indirect photolysis. In a parallel study, we found that the photolysis rate constant of testosterone increased as a function of humic acid concentration; however, at humic acid concentrations above 50 mg/L no further increase of the kinetic rate constant was observed. At high concentrations (> 50 mg/L) of humic acids, the rate of photolysis was probably inhibited due to the ability of humic acids to quench photons that come into solution. Conversely, lower concentrations of humic acid most likely resulted in enhanced degradation due to formation of organic radicals which chemically reacted with both testosterone and 17β-estradiol (Figure 2 and 3). These observations are in agreement with previously reported data showing increased photolysis of estrone and 17β-estradiol with increasing humic acid concentration (from 0 to 10 mg HA/L) in the concurrent presence of a TiO_2 catalyst by primarily UVC light (Zhang et al., 2007).

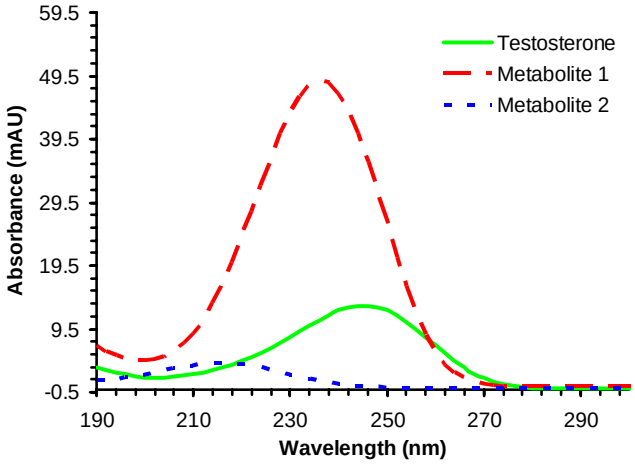

Figure 5. UV Absorbance spectra of Testosterone and two degradation products after 24 hrs of reaction time.

Photolysis of testosterone resulted in the formation of at least two different metabolites in all of the treatments (Figure 5). No metabolite peaks were observed during photolysis of progesterone and 17β-estradiol. The lack of identifiable metabolites could be due to adsorption of metabolites onto the HPLC column or production of metabolites that did not contain chromophores and thus were not detectable by a UV diode array detector. Current work in our laboratory is focused on identifying the degradation products formed in this study using advanced mass spectrometry.

In conclusion, our studies show the potential for photolysis of estrogens, androgens and progestogens in waters exposed to UVA light and that the rate of transformation can be significantly influenced by the presence of dissolved organic matter. Thus, when considering possible degradation pathways of steroid hormones, it is important to appreciate the impact of sunlight.

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