

## Synthesis and Photoirradiation of Isomeric Ethylchrysenes by UVA Light Leading to Lipid Peroxidation

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**Abstract:** Polycyclic aromatic hydrocarbons (PAHs) are widespread genotoxic environmental pollutants. We have recently demonstrated that photoirradiation of PAHs leads to cytotoxicity, DNA damage, and induction of lipid peroxidation. In this paper we report the synthesis of all the six isomeric ethylchrysenes and the study of light-induced lipid peroxidation by these ethylchrysenes. 5-Ethylchrysene was synthesized by reaction of 5-keto-5,6,6a,7,8,9,10,10a-octahydrochrysene with  $\text{CH}_3\text{CH}_2\text{MgBr}$  followed by dehydration catalyzed by *p*-toluenesulfonic acid and dehydrogenation with DDQ in benzene. 1- and 4-Ethylchrysenes were similarly prepared by reaction of 1-keto-1,2,3,4,5,6-hexahydrochrysene and 4-keto-1,2,3,4-tetrahydrochrysenes, respectively with  $\text{CH}_3\text{CH}_2\text{MgBr}$  followed by dehydration and dehydrogenation. Direct acetylation of chrysene followed by Wolff-Kishner or Clemmensen reduction resulted in the formation of 2-, 3-, and 6-ethylchrysenes in 4%, 16%, and 43% yields, respectively. Photoirradiation of these compounds with 7 and 21  $\text{J}/\text{cm}^2$  UVA light in the presence of methyl linoleate all resulted in lipid peroxidation. For comparison, photoirradiation of 4-methylchrysene and 5-methylchrysene was similarly conducted. For irradiation at a UVA light dose of 21  $\text{J}/\text{cm}^2$ , the level of induced lipid peroxidation is in the order 4-methylchrysene = 5-methylchrysene = 5-ethylchrysene = 4-ethylchrysene = chrysene > 1-ethylchrysene = 2-ethylchrysene > 3-ethylchrysene > 6-ethylchrysene. Compared with chrysene, these results indicate that the ethyl group at C4 or C5 position either slightly enhances or has no effect on the light-induced lipid peroxidation, while at C1-, C2-, C3-, or C6 position reduces light-induced lipid peroxidation.

**Keywords:** Polycyclic aromatic hydrocarbons (PAHs), ethylchrysene, methylchrysene, photoirradiation, UVA light, lipid peroxidation,

### Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread genotoxic environmental pollutants [1-3]. It is established that PAHs require metabolic activation in order to exert biological activities, including carcinogenicity [1-5]. There are three metabolic activation pathways for PAHs leading to carcinogenicity *in vivo*, namely, metabolism into *bay*-region diolepoxides, radical-cation intermediates, and quinones [6-9].

It has also been reported that PAHs can be activated by light irradiation to produce phototoxicity [10, 11]. Since skin is the largest body organ in humans and is unavoidably exposed to light, it is of particular importance and

significance to investigate human health risks posed by exposure to the combination of PAHs and light. However, this activation pathway has received relatively much less attention [12]. We previously reported the photoirradiation of a series of parent and methyl-substituted PAHs with UVA light in the presence of methyl linoleate. It was found that 20 out of the 21 tested PAHs induce lipid peroxidation, forming lipid peroxides (methyl linoleate hydroperoxides). We also determined that the photoinduced lipid peroxidation by PAH is mediated by reactive oxygen species (ROS), specifically singlet oxygen and superoxide, which are generated during the photoirradiation of PAHs [13]. The study of the twelve isomeric methylbenz[*a*]anthracenes determined that the level of lipid peroxidation induced by

methylbenz[*a*]anthracenes does not correlate with energy gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). These results indicate that lipid peroxidation induced by the twelve methylbenz[*a*]anthracenes is not directly related to their excited-state properties [13]. Also, the relative levels of UVA-induced lipid peroxidation by the twelve methylbenz[*a*]anthracenes do not correlate with the relative carcinogenic activity or the tumor-initiation activity [13].

As a continuation of the study on a structure-activity basis, it is interesting to study whether or not ethylated PAHs concomitantly exposed to UVA light would also induce lipid peroxidation. We report in this paper the synthesis of all the six isomeric ethylchrysenes and photoirradiation of these compounds by UVA light in the presence of methyl linoleate. For comparison, photoirradiation of 4-methylchrysene and 5-methylchrysene was also studied. The results indicate that under experimental conditions, all the tested PAHs induce lipid peroxidation and the potency of lipid peroxidation depends on the position of the ethyl substitution.

## Materials and Methods

### Materials and General Procedures

Chrysene, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), sodium thiosulfate, acetic anhydride, and ethyl magnesium bromide were purchased from Aldrich Chemical Co. (Milwaukee, WI). 4-Methylchrysene and 5-methylchrysene were obtained from the National Cancer Institute's Chemical Repository. Xanthine oxidase (XOD) was purchased from Roche Applied Science (Indianapolis, IN). All other reagents were obtained through commercial sources and were the highest quality available. All solvents used were HPLC grade. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride prior to use.

### Instrumentation

A Waters Alliance HPLC system, consisting of a 2695 separation module and a 2996 photodiode array detector, was used for the separation and analysis of the lipid hydroperoxide products. The mass spectra were determined on JEOL-DX 300 mass spectrometer. The NMR spectra were obtained on Varian VXR-300 spectrometer with tetramethylene silane as an internal standard in CDCl<sub>3</sub>.

### Light Source

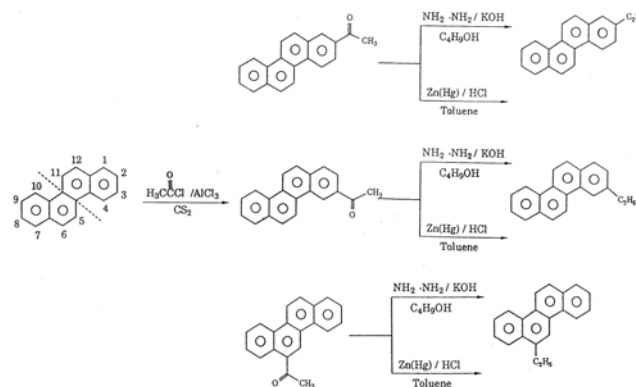
The UVA light box was custom made using 4 UVA lamps (National Biologics, Twinsburg, OH) [13]. The irradiance of the light box was determined using an Optronics OL754 Spectroradiometer (Optronics Laboratories, Orlando, FL), and the light dose was routinely measured using a Solar Light PMA-2110 UVA detector (Solar Light Inc., Philadelphia, PA). The UVA intensity is 5.1 mW/cm<sup>2</sup> at the distance of 13 cm. The maximum

emission of the UVA light box was 350-352 nm with the following spectral distribution: UVA (315-400 nm), 98.93%; UVB (280-315 nm), 1.07%; UVC (250-280 nm), <0.0001%.

### Synthesis of Ethylchrysenes

#### Synthesis of 2-, 3-, and 6-ethylchrysenes (Figure 1)

In a 250 ml round bottom flask, chrysene (4.0 g, 17.5 mmol), dissolved in 100 ml carbon disulfide, was slowly added with 30 ml acetyl chloride, 5g aluminum trichloride, and 10 ml of carbon disulfide under ice-water temperature. The reaction mixture was stirred at ambient temperature for 16 hrs, and was quenched by diluted hydrochloric acid, followed by extraction with ethyl acetate and sodium bicarbonate. The organic layer was collected, solvent removed, and the residue was chromatographed on a silica gel column. Elution with 5% ethyl acetate in hexane sequentially gave 6-, 3-, and 2-acetylchrysene in 2.01 g (43%), 762 mg (16%), and 193 mg (4%), respectively as white solids. 6-Acetylchrysene: mp. 141-143°C; mass (70 eV) *m/z* 270 (M<sup>+</sup>); <sup>1</sup>H-NMR (ppm): 2.9 (s, 3, CH<sub>3</sub>), 7.6-7.7 (m, 4, H<sub>2,3,8,9</sub>), 7.9-8.0 (dd, 2, H<sub>1,11</sub>), 8.6-8.8 (m, 4, H<sub>4,11,7,10</sub>), and 9.1 ppm (s, 1, H<sub>5</sub>); 3-Acetylchrysene: mp. 159-161°C; mass (70 eV) *m/z* 270 (M<sup>+</sup>); <sup>1</sup>H-NMR (ppm): 2.8 (s, 3, CH<sub>3</sub>), 7.6-7.8 (m, 2, H<sub>8,9</sub>), 7.9-8.1 (dd, 4, H<sub>1,6,7,12</sub>), 8.7-8.9 (m, 3, H<sub>5,10,11</sub>), and 9.4 ppm (s, 1, H<sub>4</sub>); 2-Acetylchrysene: mp. 252-254°C; mass (70 eV) *m/z* 270 (M<sup>+</sup>); <sup>1</sup>H-NMR (ppm): 2.7 (s, 3, CH<sub>3</sub>), 7.6-7.8 (m, 2, H<sub>8,9</sub>), 7.9-8.1 (m, 3, H<sub>3,6,12</sub>), 8.2 (d, 1, H<sub>7</sub>), 8.5 (s, 1, H<sub>1</sub>), and 8.7-8.9 ppm (m, 4, H<sub>4,5,10,11</sub>).



**Figure 1:** Synthesis of 2-, 3-, and 6-ethylchrysenes

6-Acetylchrysene was reduced to 6-ethylchrysene by Wolff-Kishner reaction. Briefly, 6-acetylchrysene (4.0 g, 14.8 mmol) in 80 ml *n*-butanol was reduced by anhydrous hydrazine (8 ml) by heating to reflux for 24 hrs. After cooling to ambient temperature, 16 g KOH was added and the reaction continued for an additional 24 hrs under reflux. The reaction mixture was poured into ice-water, and the precipitate was filtered, dried, and crystallized in acetone and *n*-butane yielding 6-ethylchrysene as a white solid. Yield 2.5 g (68%): mp. 124-125°C (ref [14], mp. 124-125°C); mass (70 eV) *m/z* 256 (M<sup>+</sup>); <sup>1</sup>H-NMR (ppm): 1.4 (t, 3,

$J = 7.5$  Hz,  $\text{CH}_3$ ), 2.9 (q, 2,  $J = 7.6$  Hz,  $\text{CH}_2$ ), 7.5-8.0 (m, 7,  $\text{H}_{1,2,3,7,8,9,12}$ ), 8.6-8.8 (m, 3,  $\text{H}_{4,10,11}$ ), and 8.5 ppm (s, 1,  $\text{H}_5$ ).

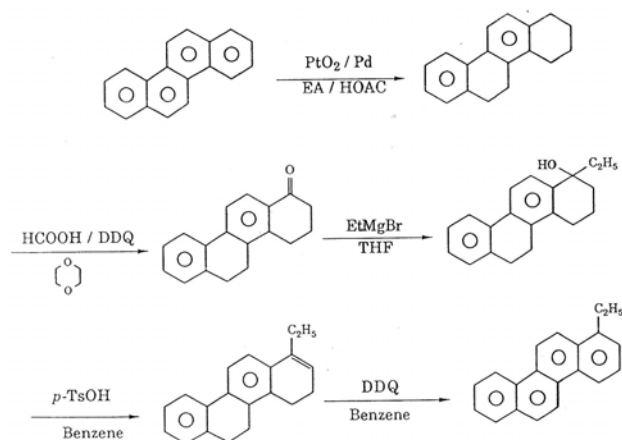
3-Ethylchrysene was similarly synthesized by Wolff-Kishner reduction of 3-acetylchrysene. 3-Acetylchrysene (2.0 g, 7.4 mmol) in 80 ml *n*-butanol was reduced by anhydrous hydrazine (5 ml) followed by treatment with potassium hydroxide (10 g). After work up and crystallization, 3-ethylchrysene was obtained as a white solid in 1.03 g (84%): mp. 114-115 °C (ref [15], mp. 113-114°C); mass (70 eV)  $m/z$  256 ( $\text{M}^+$ );  $^1\text{H-NMR}$  (ppm): 1.4 (t, 3,  $J = 7.4$  Hz,  $\text{CH}_3$ ), 2.9 (q, 2,  $J = 7.7$  Hz,  $\text{CH}_2$ ), 7.6-7.8 (m, 2,  $\text{H}_{8,9}$ ), 7.9-8.1 (m, 4,  $\text{H}_{1,6,7,12}$ ), 8.6 (s, 1,  $\text{H}_4$ ), and 8.7-8.9 ppm (m, 3,  $\text{H}_{5,10,11}$ ).

As described for the synthesis of 6- and 3-ethylchrysenes, Wolff-Kishner reduction of 2-acetylchrysene (50 mg) yielded 2-ethylchrysene as white solid in 34 mg (72% yield): mp. 237-238°C (ref [16] mp. 236-237°C); mass (70 eV)  $m/z$  256 ( $\text{M}^+$ );  $^1\text{H-NMR}$  (ppm): 1.4 (t, 3,  $J = 8.0$  Hz,  $\text{CH}_3$ ), 2.9 (q, 2,  $J = 7.7$  Hz,  $\text{CH}_2$ ), 7.5-7.7 (m, 3,  $\text{H}_{2,8,9}$ ), 7.8 (s, 1,  $\text{H}_1$ ), 8.6 (t, 3,  $\text{H}_{6,7,12}$ ), 8.9 (d, 3,  $J = 8.7$  Hz,  $\text{H}_{5,10,11}$ ), and 8.7 ppm (d, 1,  $J = 8.0$  Hz,  $\text{H}_4$ ).

These three ethylchrysenes were also prepared from 2-, 3-, and 6-acetylchrysene by Clemmensen reduction in 80, 62, and 61% yield, respectively.

#### Synthesis of 1-Ethylchrysene (Figure 2)

Following the previously published procedure [17], chrysene (500 mg) dissolved in ethyl acetate (15 ml) was hydrogenated with 45 psi of hydrogen gas at ambient temperature for 72 hrs with shaking and the reaction was catalyzed by platinum oxide (75 mg) and palladium on charcoal (96 mg). After workup and column chromatography of the residue, elution with *n*-hexane gave 1,2,3,4,5,6-hexahydrochrysene in 40% yield. Following our previously developed method [18], oxidation of 1,2,3,4,5,6-hexahydrochrysene (250 mg, 1.07 mmol) with DDQ (727 mg, 3.2 mmol) in 88% formic acid at ambient temperature for 24 hrs followed by chromatography with 5% ethyl acetate in hexane gave 1-keto-1,2,3,4,5,6-hexahydrochrysene as white solid (145 mg, 40% yield).



**Figure 2:** Synthesis of 1-ethylchrysene.

In a 250 ml round bottom flask, 1-keto-1,2,3,4,5,6-hexahydrochrysene (241 mg, 1.0 mmol) dissolved in 100 ml of freshly distilled THF under nitrogen was slowly added with 1.3 ml of ethyl magnesium bromide ( $\text{EtMgBr}$ ). The resulting solution was stirred at ambient temperature for 8 hrs, before the reaction was quenched by aqueous ammonium chloride. The reaction mixture was extracted with 150 ml ethyl acetate. The organic layer was collected, solvent removed, and the resulting residue chromatographed with a silica gel column. Elution with 5% ethyl acetate in hexane gave 1-ethyl-1-hydroxy-1,2,3,4,5,6-hexahydrochrysene as white solid: 178 mg, 67% yield; mp. 118-120°C, mass (70 eV)  $m/z$  278 ( $\text{M}^+$ );  $^1\text{H-NMR}$  (ppm): 0.9 (t, 3,  $J = 7.3$  Hz,  $\text{CH}_3$ ), 1.8-2.1 (m, 4,  $\text{H}_{2,3}$ ), 1.92 (q, 2,  $J = 8$  Hz,  $\text{CH}_2$ ), 2.7-2.9 (m, 6,  $\text{H}_{4,5,6}$ ), and 7.2-7.7 ppm (m, 6, aromatic).

1-Ethyl-1-hydroxy-1,2,3,4,5,6-hexahydrochrysene (120 mg) underwent acid-catalyzed dehydration with *p*-toluenesulfonic acid (15 mg) under reflux for 2 hrs. After workup and column chromatography, elution with 5% ethyl acetate in hexane gave 1-ethyl-3,4,5,6-tetrahydrochrysene as white solid: 108 mg, 92% yield; mp. 102-103°C, mass (70 eV)  $m/z$  260 ( $\text{M}^+$ );  $^1\text{H-NMR}$  (ppm): 1.2 (t, 3,  $J = 7.0$  Hz,  $\text{CH}_3$ ), 2.3 (m, 2,  $\text{H}_3$ ), 2.5 (q, 2,  $J = 7.5$  Hz,  $\text{CH}_2$ ), 2.8 (t, 2,  $J = 7.9$  Hz,  $\text{H}_4$ ), 7.2-7.3 (m, 4,  $\text{H}_{7,8,9,12}$ ), 5.8 (t, 1,  $J = 4.2$  Hz,  $\text{H}_2$ ), and 7.6-7.7 ppm (dd, 2,  $J = 8.4$  Hz,  $J = 7.5$  Hz,  $\text{H}_{10,11}$ ).

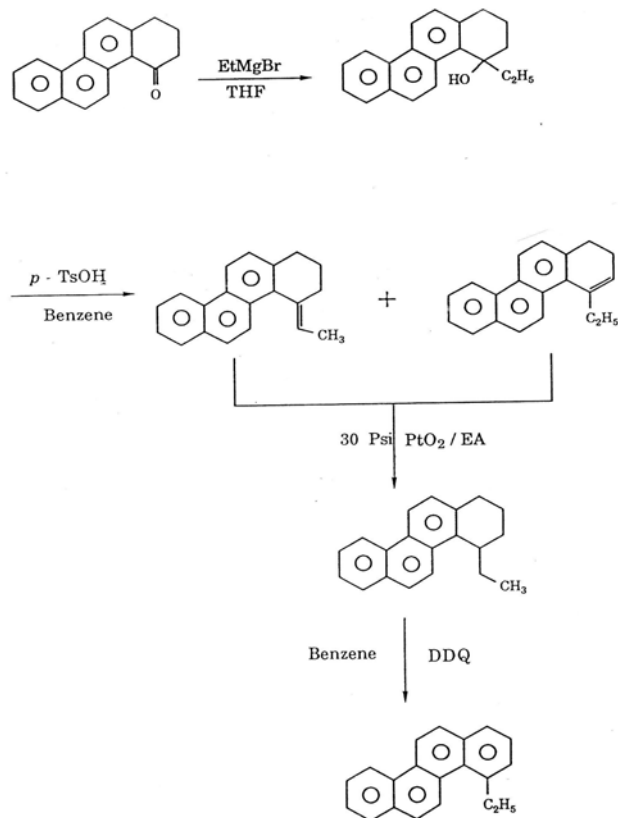
1-Ethyl-3,4,5,6-tetrahydrochrysene (100 mg) in benzene was dehydrogenated by reacting with DDQ (174 mg) at ambient temperature for 24 hrs. Chromatography of the resulting reaction residue and elution with hexane gave 1-ethylchrysene as white solid (103 mg, 91% yield): mp. 181-183 °C (ref [19], mp. 183-184°C), mass (70 eV)  $m/z$  256 ( $\text{M}^+$ );  $^1\text{H-NMR}$  (ppm): 1.5 (t, 3,  $J = 7.7$  Hz,  $\text{CH}_3$ ), (q, 2,  $\text{CH}_2$ ), and 7.54-8.82 ppm (m, 11, aromatic).

#### Synthesis of 4-Ethylchrysene (Figure 3)

4-Keto-1,2,3,4-tetrahydrochrysene (403 mg, 1.64 mmol) dissolved in 100 ml freshly distilled THF under nitrogen was slowly added 2 ml of ethyl magnesium bromide. The resulting solution was stirred at ambient temperature for 15 hrs, and then the reaction was quenched by aqueous ammonium chloride. The reaction mixture was extracted with 200 ml ethyl acetate. The organic layer was collected, solvent removed, and the resulting residue chromatographed on a silica gel column. Elution with 5% ethyl acetate in hexane gave 4-ethyl-4-hydroxyl-1,2,3,4-tetrahydrochrysene (345 mg, 75% yield) as a white solid: mp. 131-133 °C; mass (70 eV)  $m/z$  276 ( $\text{M}^+$ );  $^1\text{H-NMR}$  (ppm): 7.94 (dd, 1,  $\text{H}_8$ ), 8.02 (dd, 1,  $\text{H}_9$ ), 8.19 (d, 1,  $\text{H}_4$ ), 8.36 (d, 1,  $\text{H}_3$ ), 8.39 (d, 1,  $\text{H}_5$ ), 8.49 (d, 1,  $\text{H}_7$ ), 8.77 (d, 1,  $\text{H}_2$ ), 8.94 (s, 1,  $\text{H}_6$ ), 9.12 (d, 1,  $\text{H}_{12}$ ), 9.32 (d, 1,  $\text{H}_{10}$ ) and 9.57 ppm (d, 1,  $\text{H}_{11}$ );  $J_{2,3} = 8.3$  Hz,  $J_{4,5} = 9.2$  Hz,  $J_{7,8} = 8.1$  Hz,  $J_{8,9} = 6.9$  Hz,  $J_{9,10} = 8.6$  Hz, and  $J_{11,12} = 9.6$  Hz.

4-Ethyl-4-hydroxy-1,2,3,4-tetrahydrochrysene (154 mg, 0.6 mmol) in 100 ml toluene was dehydrated with *p*-toluenesulfonic acid monohydrate (15 mg) under refluxing temperature for 6 hrs. The reaction mixture in

ethyl acetate was washed with sodium bicarbonate solution. The organic layer was collected and solvent removed. The residue was chromatographed on a silica gel column. Elution with ethyl acetate in hexane (1/5; v/v) gave a mixture of 4-ethyl-1,2-dihydrochrysene and 4-(1'-methylmethylene)-1,2,3,4-tetrahydrochrysene in 135 mg (94% yield). This mixture dissolved in ethyl acetate (15 ml) was catalytically hydrogenated by PtO<sub>2</sub> (80 mg) at 30 psi at ambient temperature for 24 hrs. Upon filtration and solvent removal, the residue was chromatographed on a silica gel column. Elution with *n*-hexane gave 4-ethyl-1,2,3,4-tetrahydrochrysene as white solid (82 mg, 51% yield): mass (70 eV) *m/z* 260 (M<sup>+</sup>); <sup>1</sup>H-NMR (ppm): 1.15 (t, 3, J = 14 Hz, CH<sub>3</sub>), 3.01 (m, 2, CH<sub>2</sub>), 1.5-2.2 (m, 6, H<sub>1,2,3</sub>), 7.5-8.0 (m, 5, H<sub>5,6,7,8,9</sub>), 3.4 (d, 1, J = 11 Hz, H<sub>4</sub>), 7.3 (d, 1, J = 8.0 Hz, H<sub>12</sub>), and 8.4-8.7 ppm (dd, 2, J = 8.2 and 8.6 Hz, H<sub>10,11</sub>).



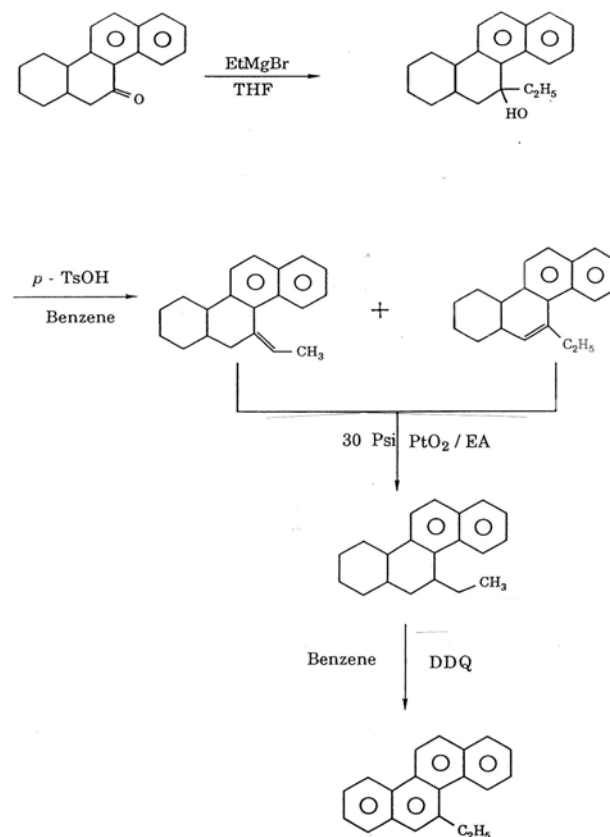
**Figure 3:** Synthesis of 4-ethylchrysene

4-Ethyl-1,2,3,4-tetrahydrochrysene (134 mg, 0.5 mmol) in 40 ml benzene was dehydrogenated by DDQ under refluxing for 24 hrs. The resulting solution was added 100 ml ethyl acetate and washed with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution. The organic layer was collected, dried, and the resulting residue chromatographed. Elution with *n*-hexane gave 4-ethylchrysene as white solid (70 mg, 53% yield): mp. 88-90 °C; mass (70 eV) *m/z* 256 (M<sup>+</sup>); <sup>1</sup>H-NMR (ppm):

1.6 (t, 3, J = 6.0 Hz, CH<sub>3</sub>), 3.6 (q, 2, J = 7.5 Hz, CH<sub>2</sub>), 7.6-7.9 (m, 8, H<sub>1,2,3,6,7,8,9,12</sub>), and 8.6-8.7 ppm (m, 3, H<sub>5,10,11</sub>).

#### Synthesis of 5-Ethylchrysene (Figure 4)

5-Ethylchrysene was similarly synthesized by reaction of 5-keto-5,6,6a,7,8,9,10,10a-octahydrochrysene with EtMgBr followed by *p*-toluenesulfonic acid catalyzed dehydration and dehydrogenation with DDQ in benzene. Upon chromatography of the resulting residue on silica gel column, elution with *n*-hexane gave 5-ethylchrysene as white solid: 28 mg (43% yield), mp. 87-88 °C (ref[20], mp. 91-92 °C); mass (70 eV) *m/z* 256 (M<sup>+</sup>); <sup>1</sup>H-NMR (ppm): 1.58 (t, 3, J = 7.0 Hz, CH<sub>3</sub>), 3.6 (q, 2, J = 7.0 Hz, CH<sub>2</sub>), 7.6-8.1 (m, 8, H<sub>1,2,3,6,7,8,9,12</sub>), and 8.7-8.8 ppm (m, 3, H<sub>4,10,11</sub>).



**Figure 4:** Synthesis of 5-Ethylchrysene.

#### Peroxidation of Methyl Linoleate Initiated by Photoirradiation of Chrysene, Ethylchrysenes, and Methylchrysenes with UVA Light

Experiments were conducted with a solution of 100 mM methyl linoleate and 0.2 mM substrate in methanol. Samples were placed in a UV-transparent cuvette and irradiated with 7 and 21 J/cm<sup>2</sup> of UVA light, respectively. After irradiation, the methyl linoleate hydroperoxide products were separated by HPLC using a Prodigy 5µm ODS column (4.6 x 250 mm, Phenomenex, Torrance, CA) eluted isocratically with 10% water in methanol (v/v) at 1

mL/min. The levels of lipid peroxidation were quantified by monitoring the HPLC peak areas at 235 nm [21, 22] followed by conversion to the concentration based on the molar extinction coefficient (at 235 nm) of the hydroperoxide as reported by Gibian and Vandenberg [23].

#### *Effect of $\text{NaN}_3$ on Peroxidation of Methyl Lineolate Initiated by Photoirradiation of 5-Ethylchrysene and 5-Methylchrysene*

$\text{NaN}_3$  is a single oxygen and free radical scavenger [13]. The effect of  $\text{NaN}_3$  on peroxidation of methyl lineolate was determined. 4-Ethylchrysene and 5-ethylchrysene were selected for the study. The experiments were similarly conducted as described above, with the exception that 50 mM  $\text{NaN}_3$  was present in the irradiation.

## Results

### *Synthesis of Isomeric Ethylchrysenes*

To study the induction of lipid peroxidation by PAHs on structure-activity bases, the six isomeric ethylchrysenes were synthesized. In our study, acetylation of chrysene generated 2-, 3-, and 6-acetylchrysenes in 4%, 16%, and 43% yields, respectively (Figure 1). The 6-carbon position is the most active site of chrysene in chemical reactions. Thus, acetylation of chrysene providing 6-acetylchrysene as the highest yield product is consistent with theoretical consideration. In our study, both Wolff-Kishner reduction and Clemmensen reduction were employed to reduce acetylchrysenes to the corresponding 6-, 3-, and 2-ethylchrysenes in good yields. Thus, direct acetylation of chrysene followed by Wolff-Kishner reduction or Clemmensen reduction is a convenient method to prepare 6-, 3-, and 2-acetylchrysenes at the same time.

Our strategy to synthesize 1-, 4-, and 5-ethylchrysenes is to employ the Grignard reaction involving the reaction of  $\text{EtMgBr}$  with a suitable keto starting material, followed by dehydration and hydrogenation (Figures 2-4). The keto compound for the synthesis of 1-ethylchrysene is 1-keto-1,2,3,4,5,6-hexahydrochrysene. This compound was conventionally prepared by catalytic hydrogenation of chrysene with a mixture of  $\text{PtO}_2$  and  $\text{Pd/C}$  to produce 1,2,3,4,5,6-hexahydrochrysene [17] which in turn was oxidized by DDQ in an acidic medium (88% formic acid) [18] (Figure 2).

The approaches for the synthesis of 1-, 4-, and 5-ethylchrysenes are similar; however, the product formation in the acid-catalyzed dehydration of the corresponding ethyl-hydroxy-hydrochrysenes is different (Figures 2-4). As shown in Figure 3, acid-catalyzed dehydration of 4-ethyl-4-hydroxy-1,2,3,4-tetrahydrochrysene yielded a mixture of 4-ethyl-1,2-dihydrochrysene and 4-(1'-methylmethylene)-1,2,3,4-tetrahydrochrysene. Similarly, acid-catalyzed dehydration of 5-ethyl-5-hydroxy-5,6,6a,7,8,9,10,10a-octahydrochrysene also formed two products, a mixture of 5-ethyl-6a,7,8,9,10,10a-

hexahydrochrysene and 5-(1'-methylmethylene)-5,6,6a,7,8,9,10,10a-octahydrochrysene (Figure 4). These results are different from the acid-catalyzed dehydration of 1-ethyl-1-hydroxy-1,2,3,4-tetrahydrochrysene which produced only one product, 1-ethyl-3,4-dihydrochrysene (Figure 2).

### *Photoirradiation of Ethylchrysenes and Methylchrysenes*

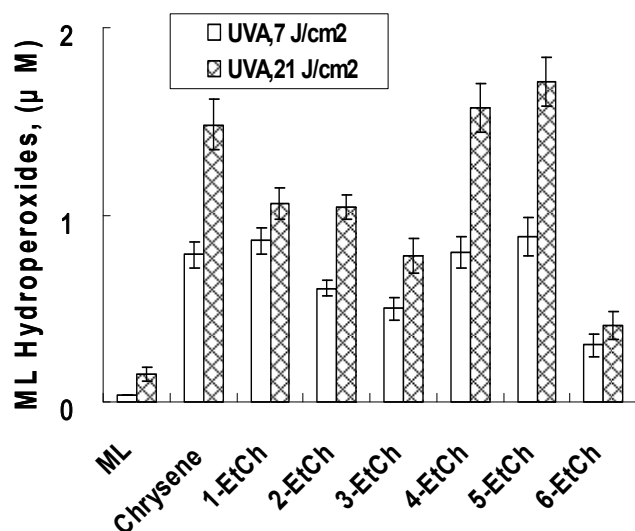
Photoirradiation of the six isomeric ethylchrysenes with UVA in the presence of a lipid, methyl linoleate, was studied. To compare the effect of the ethyl and methyl groups on lipid peroxidation, photoirradiation of 4-methylchrysene and 5-methylchrysene was similarly conducted. Each compound received two light doses, 7 and 21  $\text{J}/\text{cm}^2$ , respectively (Table 1). The results summarized in Table 1 and Figures 5 and 6 indicated that lipid peroxidation exhibited a dose and structural dependence. Higher light dose generate more peroxides as expected. The level of induced lipid peroxidation due to concomitant exposure to UVA irradiation (21  $\text{J}/\text{cm}^2$ ) is in the order 4-methylchrysene = 5-methylchrysene = 5-ethylchrysene = 4-ethylchrysene = chrysene > 1-ethylchrysene = 2-ethylchrysene > 3-ethylchrysene > 6-ethylchrysene (Statistically significant differences were identified when  $p \leq 0.05$ ). Thus, a methyl or an ethyl substitution at the same position does not affect the ability of the chrysene molecule to generate UVA light-induced lipid peroxidation.

**Table 1:** Induction of lipid peroxidation by chrysene, ethylchrysenes, and methylchrysenes with concomitant exposure to UVA light irradiation<sup>a</sup>

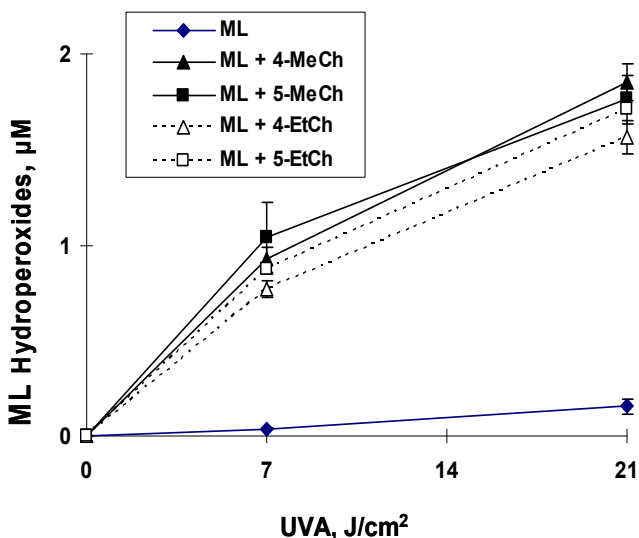
Compounds	7 $\text{J}/\text{cm}^2$	21 $\text{J}/\text{cm}^2$
	ML <sup>b</sup> Hydroperoxides, ( $\mu\text{M}$ )	
ML <sup>b</sup> (Control)	0.04 ± 0.003	0.15 ± 0.039
Chrysene (Ch)	0.79 ± 0.069	1.48 ± 0.132
1-EtCh	0.86 ± 0.07	1.06 ± 0.085
2-EtCh	0.61 ± 0.041	1.04 ± 0.063
3-EtCh	0.50 ± 0.062	0.78 ± 0.094
4-EtCh	0.80 ± 0.085	1.58 ± 0.130
5-EtCh	0.88 ± 0.103	1.71 ± 0.128
6-EtCh	0.30 ± 0.061	0.41 ± 0.710
4-MeCh	0.92 ± 0.066	1.85 ± 0.094
5-MeCh	1.04 ± 0.190	1.76 ± 0.128

<sup>a</sup>Data based on triplicate experiments; Values are means ± SD.

<sup>b</sup>ML designated methyl linoleate.



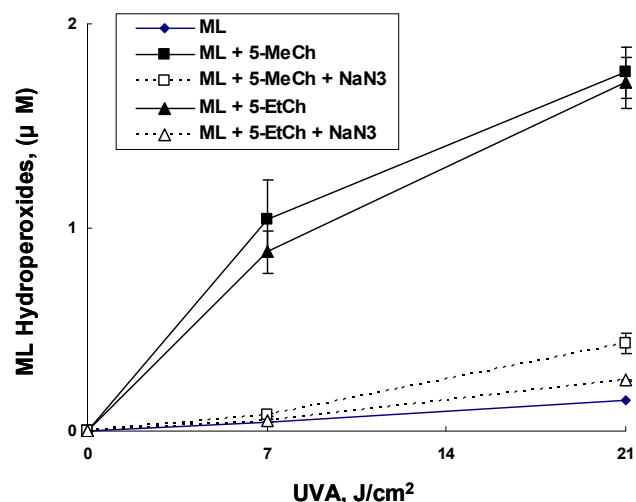
**Figure 5:** Peroxidation of methyl linoleate initiated by chrysene and all its six isomeric ethylchrysenes with 7 and 21 J/cm<sup>2</sup> of UVA light.



**Figure 6:** Peroxidation of methyl linoleate (ML) initiated by 4-ethylchrysene (4-EtCh), 5-ethylchrysene (5-EtCh), 4-methylchrysene (4-MeCh), and 5-methylchrysene (5-MeCh) with 7 and 21 J/cm<sup>2</sup> of UVA light.

#### Effect of NaN<sub>3</sub> on Peroxidation of Methyl Linoleate Initiated by Photoirradiation of 5-Ethylchrysene and 5-Methylchrysene

5-Ethylchrysene and 5-methylchrysene were irradiated with UVA light in the presence of methyl linoleate and NaN<sub>3</sub>, respectively. As shown in Figure 7, NaN<sub>3</sub> significantly inhibits lipid peroxidation initiated by these compounds ( $P \leq 0.05$ ).



**Figure 7:** Peroxidation of methyl linoleate (ML) initiated by 5-ethylchrysene (5-EtCh), and 5-methylchrysene (5-MeCh) in the presence or absence of sodium azide (NaN<sub>3</sub>).

#### Discussion

We previously determined that when irradiated with UVA light in the presence of methyl linoleate, 20 out of 21 PAHs initiated lipid peroxidation, forming the methyl linoleate hydroperoxide [13]. We also determined that the photoinduced lipid peroxidation is mediated by reactive oxygen species (ROS), specifically singlet oxygen and superoxide, which are generated during the photoirradiation of PAHs [13]. The results also indicate that there is no correlation between the level of lipid peroxidation induced and the tumorigenic potency of parent PAHs and the twelve isomeric methylbenz[*a*]anthracenes.

In this paper, we report the induction of lipid peroxidation by photoirradiation of the six isomeric ethylchrysenes with UVA. We found that the addition of an ethyl or a methyl group to the C4- or C5-position of chrysene either slightly enhances the level of induction or there is no apparent effect (Table 1) on the UVA-induced lipid peroxidation. In contrast, the addition of an ethyl group at the other positions (C1-, C2-, C3-, or C6-position) lowers the extent of lipid peroxidation. These results suggest that under our experimental conditions, an ethyl group located at the *bay*-region (the 4- and 5-positions) of chrysene can enhance lipid peroxidation or exerts no effect, while an ethyl group located at a non-*bay* region position exhibits an inhibitive effect.

5-Methylchrysene is a potent carcinogen while 4-methylchrysene is not [2]. The similar level of UVA-induced lipid peroxidation by these two compounds indicates that there is no correlation between the level of lipid peroxidation and the tumorigenic potency of the compounds studied. This is consistent with the previous finding from the twelve methylbenz[*a*]anthracenes [13]. The lack of correlation is understandable since the

mechanism leading to lipid peroxidation and the mechanism involving enzymatic metabolic activation leading to tumorigenicity are different.

Thus, our overall results indicate that photoirradiation of PAHs by UVA light generates reactive oxygen species (ROS), which induce lipid peroxidation. Lipid peroxidation can cause tumor induction in experimental animals [24, 25]. It has been well established that ROS can damage nucleic acids and proteins leading to aging, inflammation, cardiovascular diseases, cancer and other age-related diseases [26, 27]. Human body is unavoidably exposed to both sunlight and PAHs present in the environment or contaminated in the commercial products. As such, it warrants further investigation to determine the possible human health risks posed by exposure to the combination of PAHs and light, especially since coal tar, which contains PAHs, has been classified as a human carcinogen [28].

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## References

- Connell, D. W.; Hawker, D. W.; Warne, M. J.; Vowles, P. P.: Polycyclic aromatic hydrocarbons (PAHs). In *Introduction into Environmental Chemistry* (McCombs, K., and Starkweather, A. W., eds), CRC Press LLC, Boca Raton, FL, 1997, pp. 205-217.
- Dipple, A.: Polycyclic Aromatic Hydrocarbons and Carcinogenesis, *American Chemical Society, Washington, DC, 1985*.
- Harvey, R. G.: Polycyclic Aromatic Hydrocarbons, *Wiley-VCH, New York, 1996*.
- IARC, Polynuclear aromatic compounds. Part I: Chemical, Environmental and Experimental Data, *International Agency for Research on Cancer, Lyon, 1983*.
- National Toxicology Program, P. H. S., US Department of Health and Human Services, 10th Report on Carcinogens. In *Integrated Laboratory Systems, Inc., Research Triangle Park, NC, 2002*, pp. III-201-204.
- Yang, S. K.; Silverman, B. D. (eds.): Polycyclic Aromatic Hydrocarbon Carcinogenesis: Structure-Activity Relationships, *CRC Press, Boca Raton, FL, 1988, Vols. I & II*.
- Lesko, S. A.: Chemical carcinogenesis: benzopyrene system, *Methods in Enzymology, 1984, 105, 539-550*.
- RamaKrishna, N. V. S.; Devanesan, P. D.; Rogan, E. G.; Cavalieri, E. L.; Jeong, H.; Jankowiak, R.; Small, G. J.: Mechanism of metabolic activation of the potent carcinogen 7,12-dimethylbenz[a]anthracene, *Chem. Res. Toxicol., 1992, 5, 220-226*.
- Penning, T. M.; Burczynski, M. E.; Hung, C. F.; McCoull, K. D.; Palackal, N. T.; Tsuruda, L. S.: Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active o-quinones, *Chem Res. Toxicol., 1999, 12, 1-18*.
- Yu, H.: Environmental Carcinogenic polycyclic aromatic hydrocarbons: photochemistry and phototoxicity. *J. Environ. Sci. Health. Part C, Environ. Carcinog. & Ecotox. Revs., 2002, C20, 149-83*.
- Arfsten, D. P.; Schaeffer, D. J.; Mulveny, D. C.: The effects of near ultraviolet radiation on the toxic effects of polycyclic aromatic hydrocarbons in animals and plants: A review. *Ecotoxicol. Environ. Safety, 1996, 33, 1-24*.
- Yu, H.; Xia, Q.; Yan, J.; Herreno-Saenz, D.; Wu, Y.-S.; Tang, I-W.; Fu, P. P.: Photoirradiation of polycyclic aromatic hydrocarbons with UVA light – a pathway leading to the generation of reactive oxygen species, lipid peroxidation, and DNA damage., *Int. J. Environ. Res. Public Health. 2006, 3(4), 348-354*.
- Xia, Q.; Chou, M.W.; Yin, J.J.; Howard, P.C.; Yu, H.; Fu, P.P.: Photoirradiation of representative polycyclic aromatic hydrocarbons and twelve isomeric methylbenz[a]anthracene with UVA light – formation of lipid peroxidation., *Toxicol. Ind. Health, 2006, 22, 147-156*.
- Newman, M. S.: A New synthesis of chrysene derivatives. *J. Am. Chem. Soc., 1938, 60(12):2947-2951*.
- Beckmann, P. A.; Buser, C. A.; Mallory, C. W.; Mallory, F. B.; Mosher, J.: Methyl reorientation in solid 3-ethylchrysene and 3-isopropylchrysene., *Solid State Nucl. Magn. Reson., 1998, 12(4):251-256*.
- Carruthers, W.: Friedel-Crafts acetylation of chrysene. *J. Chem. Soc., 1953, 3486-3489*.
- Fu, P. P.; R. G. Harvey.: Synthesis of the chrysene bay region anti-diolepoxide from chrysene, *J. Chem. Soc. Chem. Commun., 1978, 585-586*.
- Fu, P. P.; J. D. Clark; Huang, A. Y.: Synthesis of 10-oxo-7,8,9,10-tetrahydrobenzo[a]pyrene., *J. Chem. Res.(s), 1982, 5, 121*.
- Bachmann, W. E.; Struve, W. S.: The synthesis of methylchrysenes and related compounds, *J. Org. Chem., 1940, 5(4), 416-429*.
- Hofer, L. J. E.; Peebles, W. C.: X-ray diffraction patterns of solid aromatic hydrocarbons, *Anal. Chem., 1951, 23(5), 690-695*.
- Cherng, S.-H.; Xia, Q.; Blankenship, L. R.; Freeman, J. P.; Wamer, W. G.; Howard, P. C.; Fu, P. P.: Photodecomposition of retinyl palmitate in ethanol by UVA light - formation of photodecomposition products, reactive oxygen species, and lipid peroxides., *Chem. Res. Toxicol., 2005, 18, 129-138*.
- Tokita, M.; Morita, M.: Identification of new geometric isomers of methyl linoleate hydroperoxide and their chromatographic behavior, *Biosci. Biotechnol. Biochem., 2000, 64, 1044-1056*.
- Gibian, M. J.; Vandenberg, P.: Product yield in oxygenation of linoleate by soybean lipoxygenase: The value of the molar extinction coefficient in the

- spectrophotometric assay, *Anal. Biochem.*, **1987**, *163*, 343-349.
24. Nath, R. G.; Chung, F. L.: Detection of exocyclic 1,N2-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans., *Proc. Nat. Acad. Sci. USA*, **1994**, *91*, 7491-95.
  25. Wang, M. Y; Liehr, J. G.: Induction by estrogens of lipid peroxidation and lipid peroxide-derived malondialdehyde-DNA adducts in male Syrian hamsters: role of lipid peroxidation in estrogen-induced kidney carcinogenesis, *Carcinogenesis*, **1995**, *16*, 1941-45.
  26. Burkle, A.: Mechanism of Ageing, *Eye*, **2001**, *15* (part 3), 371-75.
  27. Loft, S.; Poulsen, H.E.: Cancer risk and oxidative DNA damage in man., *J. Mol. Medicine*, **1996**, *74*(6), 297-312.
  28. National Toxicology Program: Soots, Tars, and Mineral Oils. Eighth Report on Carcinogens, **1998**, pp 42-46 & 178-181.