Mammalian cell toxicity of the photoproducts of octyldimethyl para-aminobenzoic acid

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ABSTRACT

Octyldimethyl para-aminobenzoic acid (OD-PABA) is an organic ultraviolet filter chemical (UVFC), UVFCs are UV-absorbing compounds that are found in many cosmetic products, including lip balms and sunscreens, to protect the skin from sun damage.[1] When exposed to UV light, OD-PABA produces photoproducts;[2] we are examining the cellular toxicities of these photoproducts to determine what levels can be deemed safe for human exposure. In conducting this research, we placed a prepared solution of OD-PABA in a solar simulator to create the effect of sunlight exposure, which in turn generated the photoproducts. We then used high performance liquid chromatography (HPLC) to separate the photoproducts. Individual photoproducts were then tested for their cellular toxicities. We are currently focusing on three photoproducts which we call "PII," "PIII," and "PIV" for products II, III and IV, respectively, which are the second, third and fourth products that elute from the HPLC. So far, we have tested a range of concentrations of PIV from 0.001 mM to 2 mM, and our data show that concentrations of 0.25 mM and higher are significantly toxic to mammalian cells. Ongoing research is focused on characterizing PII and determining the relationship between the concentrations of PII and PIII and cellular toxicity. These methods can also be applied to other photoproducts of OD-PABA to determine their respective cellular toxicities.

INTRODUCTION

- Organic UV filter chemicals (UVFCs) are compounds used to protect skin from ultraviolet radiation.[1] With an increased concern over the harmful effects of exposure to UV radiation over the years, these compounds have been added to many personal care products such as sunscreens and cosmetics, as well as plastics and textiles to shield them from UV radiation.[3]
- The quantities of UVFC added to these products have been increasing,[3] so there is great interest in researching any harmful effects these filters may cause to organisms as well as the environment.
- These compounds have been found in samples collected from surface waters, fish, soil, sludge and humans who have taken them in through skin contact or bioaccumulation.[3] In one study, 75% of breast milk samples had traces of UVFCs with a possibility of mother-infant transfer.[3]
- Problems with UVFCs: They can be absorbed through the skin, are possible endocrine disruptors, and some are photounstable, making them less effective in protection from UV radiation.[1] There is also no current wastewater treatment process capable of completely eliminating traces of UVFCs.[4]
- One such photounstable[2,5] UVFC, and the focus of our research, is octyldimethyl para-aminobenzoic acid (OD-PABA):

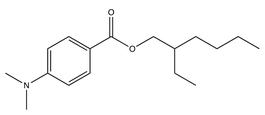


Figure 1. Structure of OD-PABA

- OD-PABA has been found to exhibit both antiestrogenic and antiandrogenic activity.[3]
- OD-PABA has been found in concentrations in the tens of µg/L in water samples collected from shower wastes and swimming pools.[5]

RESEARCH GOALS

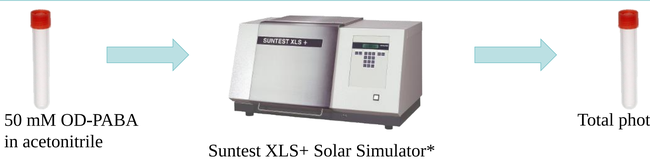
Our main goals for this research are to isolate and identify the photoproducts of OD-PABA through photolysis and NMR analysis of the photoproducts isolated through HPLC, and to determine their cellular toxicity through neutral red cellular visibility assays with mouse fibroblast cells (NIH/3T3 line).

METHODS/MATERIALS

General procedure used to carry out our research goals:

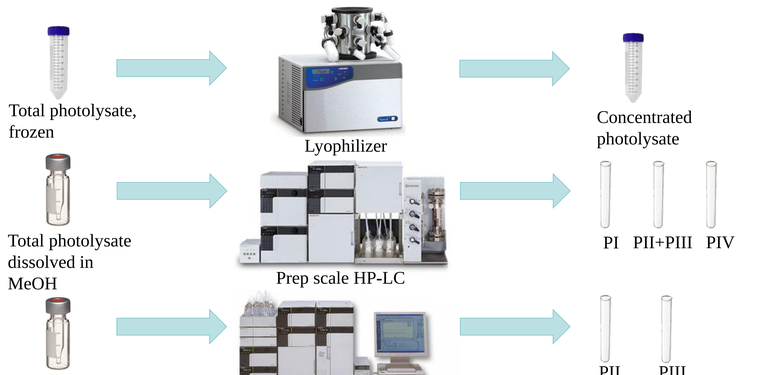
1. Solution preparation of OD-PABA in acetonitrile and photolysis of the solution using the Suntest XLS+ Solar Simulator
2. Isolation of PI, PII+PIII and PIV using a prep-scale HPLC
3. Isolation of PII and PIII using a semi-prep-scale HPLC
4. Product formation via LC-MS
5. Characterization of photoproducts through NMR analysis
6. Determination of cellular toxicity via cellular toxicity assays

SOLUTION PREPARATION AND PHOTOLYSIS OF OD-PABA



*90-min photolysis run in preparation of PIV, 5-hr photolysis run in preparation of PII and PIII, both run at 500 W/m^2

ISOLATION AND PURIFICATION OF PRODUCTS



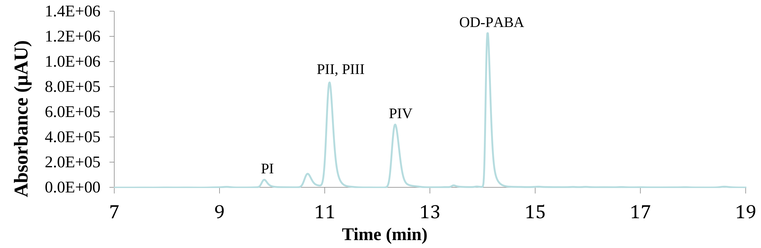
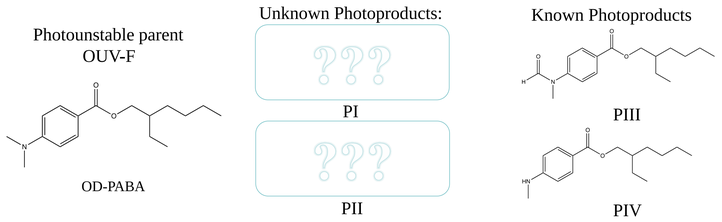
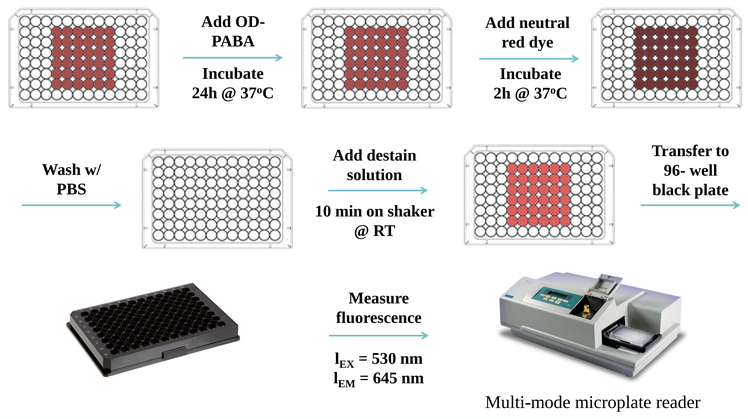


Figure 2. Chromatogram run with Phenomenex C18 column (150 mm x 21.2 mm x 4 µm), UV detection at 270 and 290 nm, gradient method with water and methanol after a 90-min photolysis of OD-PABA. Peaks shown represent the four photoproducts as well as the parent UVFC. A semi-prep-scale HPLC is used to further separate the PII and PIII peaks to isolate these two photoproducts.

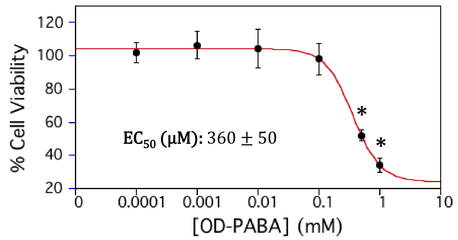


CELLULAR TOXICITY ASSAY

- We used the mouse fibroblast line NIH/3T3 to analyze the toxicity of OD-PABA and its photoproducts using the neutral red dye.[6,7]
- Neutral red dye is taken up by viable cells. The amount of neutral red taken up by cells is determined through its measured fluorescence.
- Greater florescence translates to higher cell viability. We can assess cellular toxicity through cell viability, with lower levels of florescence corresponding to higher cellular toxicity.

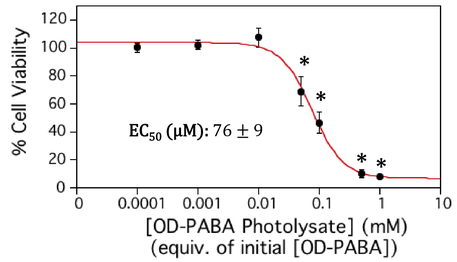


TOXICITY RESULTS OF OD-PABA, ITS TOTAL PHOTOLYSATE AND ITS PHOTOPRODUCTS TO MAMMALIAN CELLS



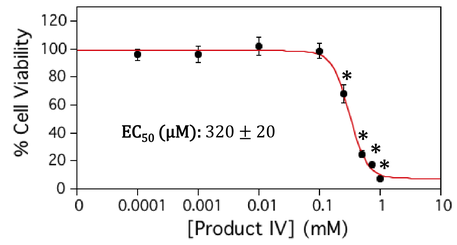
OD-PABA

Figure 3. Toxicity data for OD-PABA



OD-PABA Total Photolysate

Figure 4. Toxicity data for OD-PABA photolysate



Photoproduct IV

Figure 5. Toxicity data for OD-PABA Photoproduct IV

*p < 0.01 when compared to negative control cells incubated with 0.5% DMSO in DMEM, n=24-30

CONCLUSIONS

- EC_{50} values indicate micromolar concentrations at which 50% of the cells die. We use these values to define significant cellular toxicity.
- OD-PABA, its total photolysate and photoproduct IV demonstrate significant cellular toxicity as compared to the control at values indicated by asterisks in Figures 3, 4 and 5.
- OD-PABA's total photolysate exhibits significant cellular toxicity at concentrations lower than OD-PABA and photoproduct IV.
- These data suggests that an additive effect of the parent UVFC and the photoproducts' individual toxicities in the photolysate could account for the increased toxicity at lower concentrations.

FUTURE DIRECTIONS

Continuing research will focus on the characterization of photoproduct PII through NMR analysis, as well as cellular toxicity assays on photoproducts PII and PIII to determine their effects on mammalian cells.

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ACKNOWLEDGEMENTS

I would like to acknowledge all of the support I have received for this project from the Union College Chemistry Department, everyone in the Paulick and LAMS labs, the Union College Student Research Grant, and the Union College Scholar's Program.