Supporting Material for:

Photolysis and cellular toxicities of the organic ultraviolet filter chemical octyl methoxycinnamate and its photoproducts

Hannah V. Stein^a, Courtney J. Berg^b, Jessica N. Maung^a, Lauren E. O'Connor^a, Alexandra E. Pagano^a, Laura A. MacManus-Spencer^{a,*}, and Margot G. Paulick^{a,*}

^a Departments of Chemistry and ^b Biology, Union College, 807 Union St. Schenectady, NY 12308, USA ^{*} To whom correspondence should be addressed.

Email: paulickm@union.edu, macmanul@union.edu

This Supporting Material contains experimental details about the photolysis of commercial sunscreen products, GC-MS analysis of the photolysis extracts and standards, quantification of OMC photolysis products by HPLC, Figures S1-S17, and Table S1-S2.

Sunscreen Photolysis. Two commercial sunscreen products were photolyzed: Coppertone Water Babies Pure & Simple SPF 50 (Broad Spectrum) (7.5% OMC; also containing 5% octisalate and 14.5% zinc oxide) and Banana Boat Kids SPF 50+ (UVB) (7.5% OMC; also containing 15% homosalate, 5% octisalate, and 2.4% titanium dioxide). For the one-hour photolysis experiment, the Coppertone (0.027 g) and Banana Boat (0.030 g) sunscreens were spread evenly on separate glass microscope slides (at a thickness of 2 mg/cm²) and were photolyzed for one hour at 500 W/m² in a Suntest XLS+ solar simulator with Solar Standard Filter H (Atlas Materials Testing) at 25 (\pm 2) °C. For the eight-hour photolysis experiment, the Coppertone (0.313 g) and Banana Boat (0.301 g) sunscreens were spread evenly on separate glass microscope slides and were photolyzed for eight hours at 500 W/m² in a Suntest XLS+ solar simulator with Solar Standard Filter H (Atlas Materials Testing) at 25 (\pm 2) °C. The slides were retrieved and were extracted with 30 mL of LC-MS/Optima grade methanol for 30 min in a sonicating bath at 30 °C. The methanol extract was filtered through a 0.2-µm filter, and an aliquot was placed in an autosampler vial for analysis by GC-MS and LC-MS.

GC-MS Analysis. Each sunscreen photolysis extract, as well as authentic standards of 2-ethylhexanol (2-EH, 100 μ M) and 4-methoxybenzaldehyde (4-MBA, 100 μ M), was injected (5 μ L) through a split/splitless inlet liner held at 250 °C onto a 30-m cross-linked 5% phenyl methyl silicone bonded-phase column in an HP Agilent 6890N GC by pulsed splitless injection (24.00 psi injection pulse pressure until 0.98 min; 50.0 mL/min purge flow to split vent at 1.00 min). The following temperature program was used to separate analytes: 100 °C held from 0 – 1 min, ramped to 250 °C at 10 °C /min, then held at 250 °C for the remainder of the run. Mass spectra were obtained with an HP Agilent 5973 inert Mass Selective Detector held at 280 °C in scan mode (50 – 550 m/z).

Quantification of OMC Photolysis Products. A 50 mM stock solution of *trans*-OMC was prepared in acetonitrile in a 10 mL amber volumetric flask. A photolysis sample was made by dilution of the stock

solution to 1 mM in LC-MS-grade water in a 10 mL amber volumetric flask. This solution was placed in a 10 mL sealed quartz vial and was photolyzed for one hour in a Suntest XLS+ solar simulator (Atlas Materials Testing) with Solar Standard H (Atlas Materials Testing). The irradiance was set to 500 W/m², and the temperature was kept constant at 25 (\pm 2) °C. An aliquot of the photolysate was placed in an autosampler vial for analysis by HPLC. Standard aqueous solutions of *trans*-OMC (1 – 1000 μ M), *cis*-OMC (1 – 1000 μ M), 4-MBA (0.1 – 100 μ M), dimer 2 (0.1 – 100 μ M), and dimer 3C (0.1 – 100 μ M) were analyzed by HPLC alongside the photolysate aliquot to quantify the concentration of *trans*-OMC and each photoproduct in the photolysate. Standards of trans-OMC, cis-OMC and 4-MBA, along with the photolysate, were analyzed using a Phenomenex Synergi Hydro-RP C18 column ($150 \times 4.2 \text{ mm}, 4 \mu \text{m}$). The injection volume was 50 µL, and the flow rate was 1 mL/min. The elution was a binary gradient using LC-MS/Optima grade methanol and water: 0 - 0.5 min: 85: 15 methanol: water, 0.5 - 2 min: ramp to 100: 0 methanol: water, 2 - 7 min: hold at 100: 0 methanol: water. The diode array detector was set to detect analytes at wavelengths of 230, 280, 290, and 310 nm. Standards of dimer 2 and dimer 3C, along with the photolysate, were analyzed using a Phenomenex Kinetex C18 column (250×4.6 mm, 4 µm). The injection volume was 50 µL, and the flow rate was 1 mL/min. The elution was a binary gradient using LC-MS/Optima grade acetonitrile and water: 1 - 10 min: 50: 50 acetonitrile: water, 10 - 20 min: ramp to 95: 5 acetonitrile: water, and 20 - 40 min: hold at 95: 5 acetonitrile: water. The diode array detector was set to detect at 230 nm.



241.1

130.1 161.1 195.0 223.0 291.2 330.3 368.4 401.3 4 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450

0.2-0.1-

0-

161.1 130.1



 69.2
 535.3
 603.3

 475
 500
 525
 550
 575
 600
 625

 Counts vs. Mass-to-Charge (m/z)

639.4 660.6

650

709.2 741.3

839.4 871.5

675 700 725 750 775 800 825 850 875 900 925 950 975 1000

929.5

451.2



Figure S1. LC-MS chromatogram of OMC photolysate after 5 h of UV exposure and mass spectra of cyclodimers 1-4. (a) Extracted ion chromatogram (EIC) for m/z = 581, corresponding to the cyclodimer [M+H⁺] ion. (b) Mass spectrum for dimer 1. (c) Mass spectrum for dimer 2. (d) Mass spectrum for dimer 3. (e) Mass spectrum for dimer 4.



Figure S2. Structures of the 15 possible truxinate (head-to-head) and truxillate (head-to-tail) cyclodimers formed upon OMC dimerization. For the truxinates, the ω and μ cyclodimers result from a *cis* OMC + *cis* OMC interaction, the β and δ cyclodimers result from a *trans* OMC + *trans* OMC interaction, and the *neo* and ξ cyclodimers result from a *trans* OMC + *cis* OMC interaction. For the truxillates, the *peri* cyclodimer results from a *cis* OMC + *cis* OMC interaction, the *epi* and γ cyclodimers result from a *trans* OMC + *cis* OMC interaction. For the truxillates, the *peri* cyclodimer results from a *cis* OMC + *cis* OMC interaction, the *epi* and γ cyclodimers result from a *trans* OMC + *cis* OMC + *cis* OMC interaction, the *cis* OMC + *cis* OMC + *cis* OMC interaction, the *cis* OMC + *cis* OMC interaction, the *cis* OMC + *cis* OMC + *cis* OMC interaction.



Figure S3. HPLC-UV chromatogram for dimer 3, which resolves into three separate cyclodimers, 3A, 3B, and 3C. HPLC separation was achieved on an Agilent 1100 Series HPLC system, using a Phenomenex Kinetex C18 column ($250 \times 4.6 \text{ mm}$, 4 µm) maintained at 35 °C. The mobile phase was composed of water containing 2 mM ammonium acetate (A) and acetonitrile containing 2 mM ammonium acetate (B). Separation was achieved using the following method: 1 - 10 min: 50% B, 10 - 20 min: ramp to 95% B, and 20 – 40 min: hold at 95% B. The sample injection volume was 40 µL, and the flow rate was 1.0 mL/min. The wavelength monitored was 230 nm.



Figure S4. Expanded ¹H NMR spectrum for dimer 2.



Figure S5. Expanded ¹³C NMR spectrum for dimer 2.



Figure S6. Homonuclear correlation spectroscopy (COSY) spectrum for dimer 2.



Figure S7. Heteronuclear single quantum correlation (HSQC) spectrum for dimer 2.



Figure S8. Expanded ¹H NMR spectrum for dimer 3C.



Figure S9. Expanded ¹³C NMR spectrum for dimer 3C.



Figure S10. Expanded NOESY spectrum for dimer 3C.



Figure S11. Homonuclear correlation spectroscopy (COSY) spectrum for dimer 3C.



Figure S12. Heteronuclear single quantum correlation (HSQC) spectrum for dimer 3C.



Figure S13. Overlaid GC-MS chromatograms of authentic standards of 2-EH and 4-MBA with methanol extracts of photolyzed Coppertone and Banana Boat sunscreens.



Figure S14. Mass spectra extracted from GC-MS chromatograms of photolyzed sunscreen products (8 h photolysis) and authentic standards of photolysis products. (a) Mass spectrum of 2-EH from an authentic standard. (b) Mass spectrum of 4-MBA from an authentic standard. (c) Mass spectrum of 2-EH from an extract of photolyzed Banana Boat sunscreen. (d) Mass spectrum of 4-MBA from an extract of photolyzed Coppertone sunscreen. (f) Mass spectrum of 4-MBA from an extract of photolyzed Coppertone sunscreen.



Figure S15. LC-MS chromatograms of extracts from photolyzed sunscreen products (8 h photolysis). (a) Extracted ion chromatogram (EIC) for m/z = 581, corresponding to the cyclodimer [M+H⁺] ion, for Coppertone sunscreen extract. (b) Extracted ion chromatogram (EIC) for m/z = 581, corresponding to the cyclodimer [M+H⁺] ion, for Banana Boat sunscreen extract.



Figure S16. LC-MS chromatograms of extracts from complete OMC photolysate (1 h photolysis). (a) Extracted ion chromatogram (EIC) for m/z = 581, corresponding to the cyclodimer [M+H⁺] ion, for a 10 µM solution of OMC in water. (b) Extracted ion chromatogram (EIC) for m/z = 581, corresponding to the cyclodimer [M+H⁺] ion, for a 100 µM solution of OMC in water.



Figure S17. LC-MS chromatograms of extracts from photolyzed sunscreen products (1 h photolysis). (a) Extracted ion chromatogram (EIC) for m/z = 581, corresponding to the cyclodimer [M+H⁺] ion, for Coppertone sunscreen extract. (b) Extracted ion chromatogram (EIC) for m/z = 581, corresponding to the cyclodimer [M+H⁺] ion, for Banana Boat sunscreen extract.

Table S1.	Concentrations	of trans-OMC,	, cis-OMC,	4-MBA,	dimer 2	, and	dimer	3C in	complete
OMC pho	otolysate using ar	n initial concent	ration of <i>tra</i>	ins-OMC	of 1 mM	I (1 h j	photoly	sis).	

Photolysis product	[Product] in complete OMC photolysate (µM)
trans-OMC	40
cis-OMC	16
4-MBA	7
dimer 2	13
dimer 3C	6

Table S2. Ratios of dimers 2 and 3C relative to remaining *trans*-OMC for varying initial concentrations of OMC $(1 \text{ h photolysis})^{a}$.

initial [OMC]	Dimer 2/trans-OMC ratio	Dimer 3C/ <i>trans</i> -OMC ratio	(Dimers 2+3C)/trans- OMC ratio
10 µM	1.71	0.89	2.61
100 µM	3.00	1.22	4.22
1 mM	0.70	0.28	0.97

 a 50 μ L of OMC complete photolysate were injected onto an Agilent 1100 Series HPLC and analyzed as described in the Experimental Section. Ratios were determined using the HPLC peak areas of the indicated compounds.