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Investigation of the Sunscreen Octocrylene's Interaction with Amino Acid Analogs in the Presence of UV Radiation

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ABSTRACT

Octocrylene is an organic UV filter, commonly used in sunscreens and cosmetics, which can give rise to both contact and photocontact allergy. Our aim was to investigate octocrylene's interaction with amino acid analogs in the presence of UV radiation to better understand the reason for octocrylene's photoallergenic capacity. The amino acid analogs were photolysed in presence and absence of octocrylene for 1 h in cyclohexane. The rate of degradation was considerably slower for all amino acid analogs when octocrylene was present in the mixture. Benzylamine, the lysine analog, did react with octocrylene during the photolysis and the corresponding amide was formed in an acylation reaction. By varying the benzylamine concentration and keeping the octocrylene concentration fixed the reaction rate was shown to be independent of the amine concentration. The same type of acylation reaction took place when octocrylene alone was photolysed in ethanol in which the ethyl ester was formed from octocrylene and ethanol. Our results suggest that octocrylene's ability to cause photocontact allergy could be due to its photoinduced reactivity toward primary amines and alcohols.

INTRODUCTION

The recommended way of self-protection from the sun's harmful radiation is by use of sunscreens containing UV filters as active ingredients. Today UV filters are not only added to sun blocks but also they are frequently added to everyday cosmetics and skin care products to protect the skin from UV exposure. A large area of the body is therefore repeatedly exposed to different UV filters. In spite of the common skin exposure to these substances there is little published literature about the interaction of skin proteins with electronically excited UV filters.

Octocrylene (Fig. 1) is an organic UV filter that belongs to the cinnamate family and provides protection against UVB as well as short UVA wavelengths (1). It is considered to be highly photostable and has also been suggested to stabilize other UV filters, such as 4-*tert*-butyl-4′-methoxydibenzoylmethane, from photodegradation (2–4). These attractive properties have contributed to its widespread use in sunscreens and cosmetic preparations during the last 10 years and it is approved by EU (5) and FDA (6) in concentrations up to 10%.

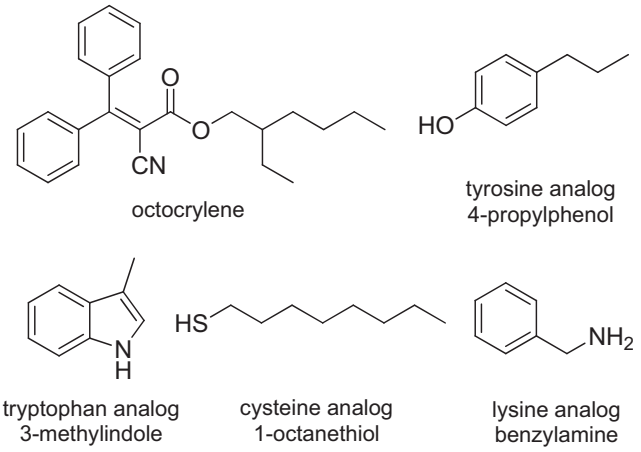

Figure 1. Chemical structures of compounds studied in this article.

The first cases of contact allergy and photocontact allergy from octocrylene were reported as late as 2003 by Carrotte-Lefebvere *et al.* (7). Since then, the number of reports on allergic reactions caused by octocrylene has increased substantially and octocrylene has been suggested to be the single organic UV filter that causes most allergic reactions (8). Contact allergy is caused by a wide range of chemicals upon skin contact. Its clinical manifestation, allergic contact dermatitis, is developed upon repeated contact with the allergen. The compounds that cause contact allergy are called haptens and in order for them to be recognized by the immune system and cause an allergic reaction they have to react with a biomacromolecule (usually considered to be proteins) in the skin, thus forming an immunogenic hapten–protein complex (9). Photoallergic contact dermatitis arises when a compound subsequent to absorption of light, usually UV radiation, forms a hapten or an immunogenic complex that causes an allergic reaction (10,11).

The primary aim of this study was to investigate the reactivity of octocrylene in its excited state toward different amino acid

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analogs, to better understand why octocrylene causes photocontact allergy. We also aimed at studying octocrylene's ability to protect amino acid analogs from photodegradation, *i.e.* to mimic its protective ability of proteins.

MATERIALS AND METHODS

Chemicals. Reagents were obtained from commercial suppliers and used without further purification. Benzylamine was obtained from Fluka, Sigma–Aldrich Chemie (Steinheim, Germany) and benzophenone from Merck–Schuchardt (Hohenbrunn, Germany). 2-Ethylhexyl-2-cyano-3,3-diphenylacrylate (octocrylene), 3-methylindole, 4-propylphenol and 1-octanethiol were purchased from Sigma–Aldrich Chemie. Argon (99.99%) and synthetic air (78% N_2, 22% O_2) were used as received from AGA (Sweden).

Instrumentation and modes of analyses. Column chromatographic separations were performed using Merck silica gel Geduran Si 60 (0.063–0.200 mm) and Sigma–Aldrich hexane mixture of isomers (bp 68–70°C). Photolysis reactions were performed in a falling film photoreactor, according to Professor de Meijere (12) with forced liquid circulation, purchased from NORMAG Labor- und Prozesstechnik. The photoreactor was equipped with a medium pressure mercury UV lamp (700 W, Heraeus, TQ 718, Z4 doped) as irradiation source. The total radiant power of the lamp in the wavelength interval 200–600 nm is 389 W. Both the lamp and the reaction mixture were cooled with water, which kept the temperature of the reaction mixture at 20–25°C during the experiments. Argon or synthetic air was continuously bubbled through the sample and allowed to pass through the reaction zone. For a more detailed description of the photoreactor equipment see Supporting Information.

Photolysis experiments were also performed in a Solarlux^{TM} solar simulation system from EYE Lighting International/Iwasaki Electric that generates similar spectral distribution as the sun. Light source: EYE Solarlux^{TM} 150R lamp with AM1.5 spectral filter. Solar spectral match is class B according to IEC standards; spectral match 0.2 and 0.8 in the intervals 300–350 and 350–400 nm, respectively. The distance between the lamps and the quartz glass reaction vessel (4 cm^2) was 40 cm and the irradiation power controller was set on 11. This set up gives an intensity of 1033 W m^{-2}, which corresponds to approximately one sun. The temperature of the reaction mixture was 43–45°C during the experiment. A more detailed description of the solar simulation system is available on the homepage of the EYE Lighting International/Iwasaki Electric of North America (13).

GC/MS analyses were performed using electron impact ionization (70 eV) on a Hewlett-Packard model 5973 mass spectrometer (scanned *m/z* 50–500), connected to a gas chromatograph (Hewlett-Packard model 6890). The GC was equipped with an on-column inlet and an HP-5MSI fused silica capillary column (30 × 0.25 mm, 0.25 μm film thickness). Helium was used as carrier gas, the flow rate was 1.2 mL min^{-1}, and the injection volume was 1 μL. The column temperature was 100°C at injection and raised to 200°C at a rate of 5°C min^{-1}, then raised from 200 to 270°C at a rate of 15°C min^{-1}, and finally held at 270°C for 20 min.

HPLC/MS analyses were performed using electrospray ionization on a Hewlett-Packard 1100 HPLC/MS. The system included a vacuum degasser, a binary pump, an autoinjector, a column thermostat, a diode array detector and a single quadrupole mass spectrometer. The electrospray interface was used with the following spray chamber settings: nebuliser pressure, 35 psig; capillary voltage, 3000 V; drying gas temperature, 350°C; and drying gas flow rate, 12 L min^{-1}. For mass spectral analysis, the mass spectrometer was used in the scan mode detecting ions with *m/z* ranging from 50 to 1000. Mass spectral analysis was performed in positive ionization mode with fragmentor voltage of 70 V. A Zorbax SB-C18 column (150 × 2.1 mm, 5 μm particles; Agilent Technologies) was used and the column temperature was set to 40°C. Mobile phase A consisted of 0.1% formic acid in milli-Q water, and mobile phase B consisted of 0.1% formic acid in acetonitrile. Aliquots of 5 μL were injected onto the column and eluted with a gradient flow of 0.40 mL min^{-1}. A linear gradient from 10% to 100% B in 20 min was followed by 10 min of isocratic eluation at 100% B. The column was equilibrated with 10% B for 10 min between each run.

Preparative HPLC was performed using a Gilson pump model 305, a Gilson UV/VIS detector model 119 and a Zorbax Semi-Preparative column (250 × 9.4 mm, 5 μm particles; Agilent Technologies), the flow rate was 13.36 mL min^{-1} and the compounds were monitored at 230 nm. Aliquots of 100 μL were injected onto the column and eluted with acetonitrile/water 9:1.

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3CN on a JEOL eclipse + 400 MHz spectrometer at 400 and 100 MHz, respectively. Chemical shifts (δ) are reported in ppm with the solvent residual peaks as internal standard: $CHCl_3$ δ 7.26 (^{1}H NMR), $CDCl_3$ δ 77.0 (^{13}C NMR), CH_3CN δ 1.94 (^{1}H NMR) and CD_3CN δ 118.2 (^{13}C NMR). ^{1}H and ^{13}C NMR spectra were assigned using ^{13}C distortionless enhancement by polarization transfer (DEPT), ^{1}H-^{1}H correlation spectroscopy (COSY), ^{1}H-^{1}H total correlation spectroscopy (TOCSY), ^{1}H-^{13}C heteronuclear multiple-quantum correlation spectroscopy (HMQC) and ^{1}H-^{13}C heteronuclear multiple-bond correlation spectroscopy (HMBC).

Photolysis of octocrylene in EtOH. Photolysis was performed on 370 mL of a 10 mM solution of octocrylene in EtOH, continuously saturated with air by bubbling with synthetic air through a sintered glass frit in the reaction mixture reservoir. The mixture was illuminated for 6 h and samples of approximately 4 mL were withdrawn from the photoreactor at 0.25, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 h and analyzed with HPLC/MS. Each sample was diluted with acetonitrile (1:20) before analysis with HPLC/MS. Octocrylene and ethyl 2-cyano-3,3-diphenyl acrylate were quantified with HPLC/UV at 280 nm by using standard curves (see Supporting information).

Photolysis of octocrylene in EtOH in the absence of oxygen. Photolysis was performed on 370 mL of a 10 mM solution of octocrylene in EtOH. Instead of synthetic air, argon was bubbled through the mixture during the experiment. The mixture was illuminated for 6 h and samples of approximately 4 mL were withdrawn from the photoreactor at 0.00, 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 h and analyzed with HPLC/MS. Each sample was diluted with acetonitrile (1:20) before analysis with HPLC/MS. Ethyl 2-cyano-3,3-diphenyl acrylate was quantified with HPLC/UV at 280 nm by using a standard curve (see Supporting information).

Fractionation of the mixture obtained from 6 h photolysis of octocrylene in EtOH. The reaction mixture from the 6 h photolysis experiment of octocrylene in EtOH was concentrated under reduced pressure and fractionated by semipreparative HPLC. The main compound formed in the photolysis experiment was ethyl 2-cyano-3,3-diphenyl acrylate. It was isolated as a white solid and the obtained NMR spectra were in accordance with reference (14). Three other, much smaller fractions, contained compounds that were possible to identify: 3,3-diphenylacrylonitrile, benzophenone and 2-ethylhexyl 2-ethoxy-4-phenylquinoline-3-carboxylate. 3,3-Diphenylacrylonitrile was isolated and the obtained ^{1}H NMR was in agreement with the literature (14,15). The formation of benzophenone was verified with a standard. Characterization data for 2-ethylhexyl 2-ethoxy-4-phenylquinoline-3-carboxylate: ^{1}H NMR ($CDCl_3$) δ: 7.87 (d, 1H, J = 8.4 Hz, H_9), 7.63 (dd, 1H, J_1 = 8.4 Hz, J_2 = 6.9 Hz, H_8), 7.52–7.43 (m, 4H, H_6 and H_{24}-H_{25}), 7.41–7.36 (m, 2H, H_{23}), 7.29 (dd, 1H, J_1 = 8.4 Hz, J_2 = 6.9 Hz, H_7), 4.61 (q, 2H, J = 7.0 Hz, H_{11}), 3.98–3.90 (m, 2H, H_{14}), 1.44 (t, 3H, J = 7.0 Hz, H_{12}), 1.41–1.34 (m, 1H, H_{15}), 1.29–1.13 (m, 8H, H_{16}-H_{18} and H_{20}), 0.87 (t, 3H, J = 7.1 Hz, H_{19}), 0.78 (t, 3H, J = 7.4 Hz, H_{21}). ^{13}C NMR ($CDCl_3$) δ: 166.9 (C_{13}), 157.8 (C_2), 148.1 (C_4), 146.7 (C_{10}), 135.4 (C_{12}), 130.2 (C_8), 129.4 (C_{23}), 128.6 (C_{25}), 128.4 (C_{24}), 127.5 (C_9), 126.7 (C_6), 124.4 (C_7), 123.8 (C_5), 119.6 (C_3), 67.5 (C_{14}), 62.4 (C_{11}), 38.6 (C_{15}), 30.2 (C_{16}), 28.9 (C_{17}), 23.5 (C_{20}), 23.0 (C_{18}), 14.6 (C_{12}), 14.2 (C_{19}), 10.9 (C_{21}). High resolution mass spectrum (ESI) *m/z* calculated for $C_{26}H_{31}NO_3$ + H, 406.2382; found, 406.2377.

Photolysis of octocrylene in EtOH using the solar simulation system. Photolysis was performed on a 10 mM solution of octocrylene in EtOH in a 1.5 mL quartz cuvette. The mixture was illuminated for 30 h and samples of approximately 0.1 mL were withdrawn from the reaction vessel at 0.00, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 24 and 30 h and analyzed with HPLC/MS. The samples were diluted with acetonitrile (1:20) before analysis with HPLC/MS using selected ion monitoring (SIM) to detect ions with the *m/z* of 278.0 (see Supporting Information). To verify that ethyl 2-cyano-3,3-diphenyl acrylate was formed the 30 h sample was spiked with a synthetic standard (see Supporting Information).

General procedure for photolysis of amino acid analogs. Photolysis was performed on 370 mL of 10 mM cyclohexane solutions of amino acid analogs, either containing octocrylene (10 mM) or devoid of octcorylene. The mixtures were illuminated for 1 h and samples of approximately 4 mL were withdrawn from the photoreactor at 0.00, 0.25, 0.50, 0.75 and 1.0 h and analyzed with HPLC/MS or GC/MS.

Analysis of photolysis of 4-propylphenol, 3-methylindole and benzylamine. The samples from the photolysis experiments with 4-propylphenol, 3-methylindole and benzylamine (with and without octocrylene) were diluted with acetonitrile (1:20) before analysis with HPLC/MS. 4-Propylphenol, 3-methylindole and octocrylene were quantified with HPLC/UV at 280 nm by using standard curves and benzylamine was quantified with the HPLC/MS total ion count (TIC) by using a standard curve (see Supporting Information).

Analysis of photolysis of 1-octanethiol. The samples from the photolysis experiments with 1-octanethiol (with and without octocrylene) were diluted with either acetonitrile (1:20) before analysis with HPLC/MS, or n-hexane (1:20) before analysis with GC/MS. 1-Octanethiol was quantified with the GC/MS TIC and octocrylene with HPLC/UV at 280 nm, by standard curves (see Supporting Information).

Purification of N-benzyl-2-cyano-3,3-diphenylacrylamide. The reaction mixture from the photolysis experiment of octocrylene and benzylamine was purified using column chromatography on silica gel (5% ethyl acetate in hexanes), which afforded the product as a pale yellow solid. Characterization data for *N*-benzyl-2-cyano-3,3-diphenylacrylamide: ^{1}H NMR (CD_3CN) δ: 7.50–7.20 (m, 11H, H_8-H_9 and H_{12}-H_{13} and H_{17}-H_{18} and H_7/H_{11}), 7.21–7.16 (m, 2H, H_7/H_{11}), 7.06–7.01 (m, 2H, H_{16}), 5.98 (bs, 1H, H_1), 4.35 (d, 2H, J = 5.6 Hz, H_{14}). ^{13}C NMR (CD_3CN) δ: 164.5 (C_5), 161.9 (C_2), 138.4 (C_6/C_{10}), 138.1 (C_6/C_{10}), 136.9 (C_{15}), 131.1 (C_9/C_{13}), 130.5 (C_9/C_{13}), 130.1 (C_7/C_{11}), 129.6 (C_{17}), 128.9 ($C_7/C_8/C_{11}/C_{12}$), 128.7 ($C_7/C_8/C_{11}/C_{12}$), 128.6 ($C_7/C_8/C_{11}/C_{12}$), 128.1 (C_{16}), 127.9 (C_{18}), 117.5 (C_4), 107.3 (C_3), 44.4 (C_{14}). High resolution mass spectrum (ESI) *m/z* calculated for $C_{23}H_{18}N_2O$ + H, 339.1497; found, 339.1501.

Photolysis of octocrylene and benzylamine to measure the formation of N-benzyl-2-cyano-3,3-diphenylacrylamide. Photolysis was performed on 370 mL solutions of octocrylene (10 mM) in cyclohexane with varying concentrations of benzylamine (0.25, 0.50, 1.0, 2.0 and 4.0 equivalents). The solutions were air saturated by continuous bubbling with synthetic air through a sintered glass frit in the reaction mixture reservoir. Two experiments were performed for each benzylamine concentration. The mixtures were illuminated for 3 h and samples of approximately 4 mL were withdrawn from the photoreactor at 0.00, 0.25, 0.50, 1.0, 2.0 and 3.0 h. The samples were diluted with acetonitrile (1:20) before analysis with HPLC/MS, and *N*-benzyl-2-cyano-3,3-diphenylacrylamide was quantified with HPLC/UV at 280 nm by a standard curve (see Supporting Information).

Photolysis of octocrylene and benzylamine in the absence of oxygen. Photolysis was performed on a 370 mL solution containing 10 mM of octocrylene and 2.5 mM of benzylamine in cyclohexane. The solution was continuously bubbled with argon instead of synthetic air. The mixture was illuminated for 3 h and samples of approximately 4 mL were withdrawn from the photoreactor at 0.00, 0.25, 0.50, 1.0, 2.0 and 3.0 h. The samples were diluted with acetonitrile (1:20) before analysis with HPLC/MS using SIM to detect ions with the *m/z* of 339.1 (see Supporting Information). *N*-Benzyl-2-cyano-3,3-diphenylacrylamide was quantified with HPLC/MS by a standard curve (see Supporting Information).

Photolysis of octocrylene and benzylamine using the solar simulation system. Photolysis was performed on a solution containing 10 mM of octocrylene and 2.5 mM of benzylamine in cyclohexane in a 1.5 mL quartz cuvette. The mixture was illuminated for 30 h and samples of approximately 0.1 mL were withdrawn from the reaction vessel at 0.00, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 24 and 30 h and analyzed with HPLC/MS. The samples were diluted with acetonitrile (1:20) before analysis with HPLC/MS using SIM to detect ions with the *m/z* of 339.1 (see Supporting Information). To verify that *N*-benzyl-2-cyano-3,3-diphenylacrylamide was formed the 24 h sample was spiked with a synthetic standard (see Supporting Information).

RESULTS AND DISCUSSION

There are several studies showing that the UV-filter octocrylene is a very photostable compound (2–4,16). Indeed, also in our photolysis of octocrylene in cyclohexane, only minute amounts of degradation products could be detected. Furthermore, no decrease in octocrylene's concentration could be seen with our method, even after 6 h (results not shown). However, when ethanol was used as solvent in the photolysis of octocrylene, a detectable amount of octocrylene did react with the solvent during the photolysis (Fig. 2).

The dominating reaction that took place was a transesterification giving the ethyl 2-cyano-3,3-diphenyl acrylate (Fig. 3). The formation rate of this adduct was measured by HPLC/MS using a standard curve (Fig. 4). Three other compounds were identified: 3,3-diphenylacrylonitrile, benzophenone and 2-ethylhexyl 2-ethoxy-4-phenylquinoline-3-carboxylate (Fig. 3). However, only trace amounts of these three compounds were detected. Noteworthy, both 3,3-diphenylacrylonitrile and benzophenone were also seen, in approximately the same amounts, in the photolysis experiment of octocrylene in cyclohexane. The formation of 2-ethylhexyl 2-ethoxy-4-phenylquinoline-3-carboxylate, on the other hand, is specific for the photolysis of octocrylene in ethanol, just as the formation of ethyl 2-cyano-3,3-diphenyl acrylate, since in both cases the UV filter has reacted with the solvent. Ethanol can primarily be seen as an analog for the amino acid serine, but also for the amino acid threonine, because both contain a hydroxyl group in the side chain. To verify that the transesterification reaction is not promoted by the small amounts of UVC radiation that the lamp in the falling film photoreactor emits, a photolysis experiment was performed in a solar simulator with similar spectral distribution and intensity as the sun. The formation of ethyl 2-cyano-3,3-diphenyl acrylate was indeed seen in this experiment (see Supporting Information), which confirms that the transesterification is promoted by radiation of the wavelengths within the solar spectrum.

A number of studies have shown that octocrylene is able to protect other UV filters, such as 4-*tert*-butyl-4′-methoxydibenzoylmethane, from photodegradation (2–4). However, to the best of our knowledge, this is the first time that octocrylene has been shown to stabilize the groups of protein side chains from UV-induced degradation. Amino acid

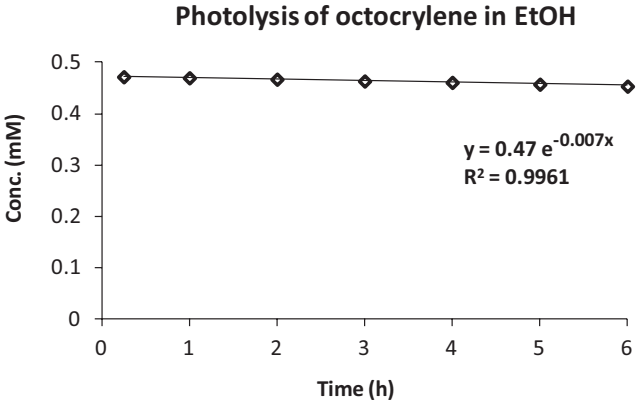

Figure 2. Photolysis of octocrylene in ethanol, showing a slight depletion of octocrylene in concentration with time.

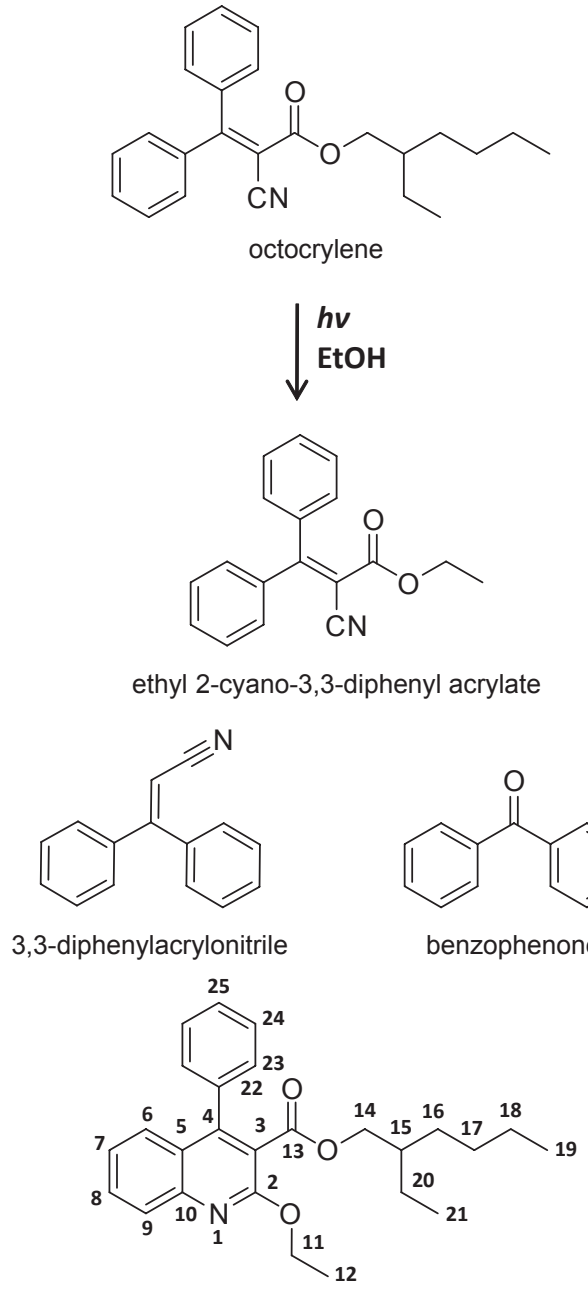

Figure 3. Schematic picture over the degradation products formed when octocrylene (10 mM) was photolysed in ethanol for 6 h. The major adduct was ethyl 2-cyano-3,3-diphenyl acrylate. The other three compounds (3,3-diphenylacrylonitrile, benzophenone and 2-ethylhexyl 2-ethoxy-4-phenylquinoline-3-carboxylate) were formed in very small amounts.

analogs containing the same functional group were used instead of the natural amino acids themselves, because of better solubility in organic solvents. In total four different amino acid analogs were used (Fig. 1): 4-Propylphenol was used as an analog for tyrosine, 3-methylindole was used as an analog for tryptophan, 1-octanethiol was used as an analog for cysteine and benzylamine was used as an analog for lysine. Tryptophan and tyrosine are the two amino acids most prone

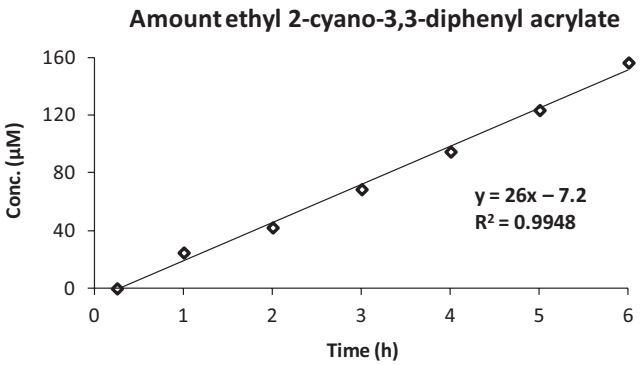

Figure 4. Formation of ethyl 2-cyano-3,3-diphenyl acrylate, in concentration with time, in the 6 h photolysis of octocrylene in ethanol.

to undergo photochemical reactions (17), whereas cysteine and lysine are the two amino acids most frequently invoked in the immunogenic hapten–protein complex formation (*via* an electrophilic–nucleophilic interaction; 18). The degradation of amino acid analogs, in presence and absence of octocrylene, can be described by the following equations:

$$A + h\nu \rightarrow A^* \quad (1)$$

$$A^* \rightarrow I \rightarrow DP \quad (2)$$

$$Oct + h\nu \rightarrow Oct^* \quad (3)$$

$$A^* + Oct \rightarrow A + Oct^* \quad (4)$$

The amino acid analog in its ground state (A) is promoted to its excited state (A*) upon light absorption, see Eq. (1). The excited amino acid analog (A*) can either return to its ground state *via* emission and internal conversion, or form degradation products (DP) *via* one or several reactive intermediates (I), see Eq. (2). Octocrylene (Oct) protects the amino acid analog from decomposition in two ways: firstly by absorbing some of the incident light, described by Eq. (3), thus shielding the amino acid analog from the harmful radiation, and secondly by quenching the excited state of the amino acid analog according to Eq. (4) (4,19). The degradation rate decreases because less amino acid analog is being excited, but also because the quenching of the amino acid analog's excited state by octocrylene competes with the photochemical degradation.

An exponential decay, in concentration with time, was observed for all amino acid analogs upon irradiation and excellent curve fitting to a first-order type expression (Fig. 5) was obtained. Photodegradation was considerably slower for all four amino acid analogs in the presence of octocrylene (Fig. 5), and no degradation of octocrylene itself could be detected within the time span of the experiment (see Supporting Information).

In the presence of octocrylene, the photodegradation of the amino acid analogs still occur by apparent first-order kinetics. The half-life for each amino acid analog, in presence and absence of octocrylene, was therefore estimated using the exponential factor obtained from these first-order curve fittings. In the absence of octocrylene, as expected, the tyrosine ($t_{1/2}$ = 0.21 h) and tryptophan ($t_{1/2}$ = 0.24 h) analogs were degraded much faster than the analogs of cysteine

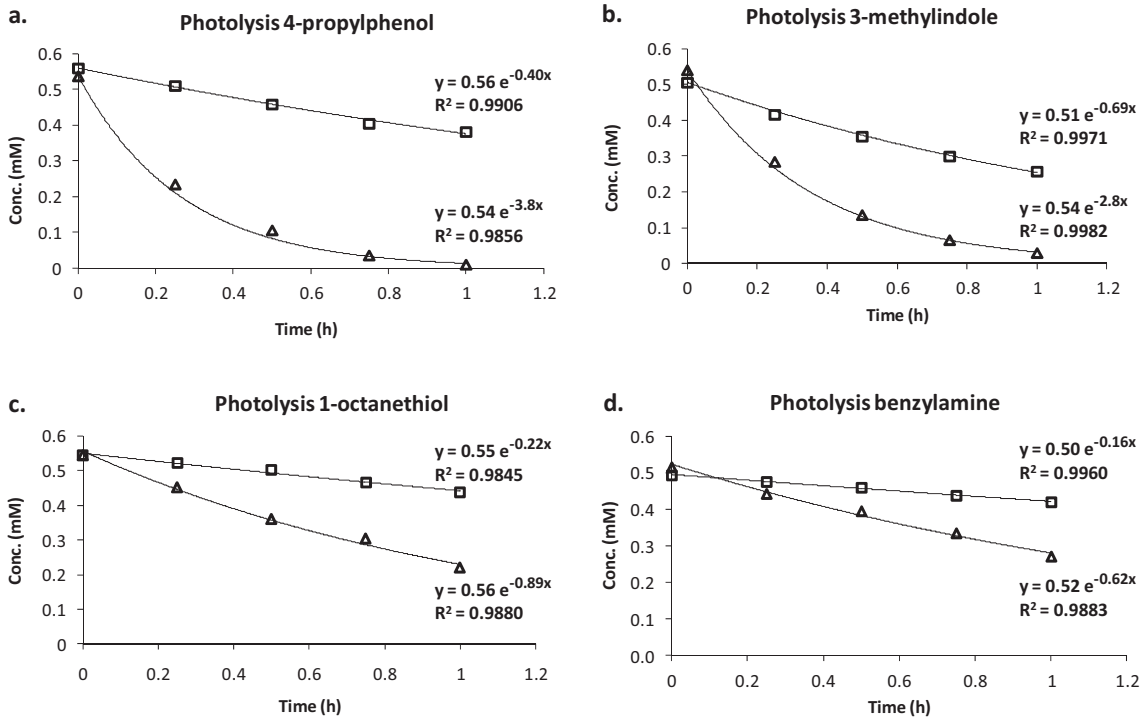

Figure 5. Results from photolysis of amino acid analogs with and without octocrylene. Experiments without octocrylene are indicated with the symbol Δ, and experiments with octocrylene in the mixture are indicated with the symbol □. (a) Concentration of the tyrosine analog 4-propylphenol with time. (b) Concentration of the tryptophan analog 3-methylindole with time. (c) Concentration of the cysteine analog 1-octanethiol with time. (d) Concentration of the lysine analog benzylamine with time.

Table 1. Half-lives* for the amino acid analogs in the presence and absence of octocrylene.

Compound	Octocrylene	$t_{1/2}$ (h)
4-Propylphenol†	No	0.21 ± 0.02
4-Propylphenol†	Yes	1.7 ± 0.1
3-Methylindole†	No	0.24 ± 0.01
3-Methylindole†	Yes	0.85 ± 0.1
1-Octanethiol‡	No	0.78
1-Octanethiol‡	Yes	3.2
Benzylamine‡	No	1.1
Benzylamine‡	Yes	4.3

*$t_{1/2} = \frac{ln2}{k}$, for first-order reactions, where $[A] = [A]_0 e^{-kt}$; †$t_{1/2}$ is based on two experiments; ‡$t_{1/2}$ is based on one experiment.

($t_{1/2}$ = 0.78 h) and lysine ($t_{1/2}$ = 1.1 h), see Table 1. The half-lives for the cysteine and lysine analogs were based on one experiment, whereas the half-lives for the tyrosine and tryptophan analogs were based on two experiments. It is noteworthy that the degradation of 4-propylphenol was approximately eight times slower when octocrylene was present, whereas the degradation of 3-methylindole, 1-octanethiol and benzylamine was about four times slower (Table 1). No significant difference in spectral overlap between the absorption spectra of the different amino acid analogs and that of octocrylene could be seen (results not shown). Therefore, octocrylene's ability to provide better protection for the tyrosine analog than for the other analogs is not known. Possibly, the excited state of 4-propylphenol is more efficiently quenched by octocrylene than that of 3-methylindole.

Interestingly, in the photolysis experiment of benzylamine and octocrylene a reaction product was formed between the amine and octocrylene. This reaction product corresponds to the amide formed when benzylamine is attacking the carbonyl carbon of octocrylene, see Fig. 6a. A photolysis experiment of octocrylene and benzylamine in the solar simulator confirmed that UV radiation emitted by the sun can promote the formation of *N*-benzyl-2-cyano-3,3-diphenylacrylamide (see Supporting Information).

We have previously shown that benzylamine reacts with octocrylene without activation by UV light (20). However, the reaction product formed between benzylamine and octocrylene in the absence of light is different from the one formed in the photoreactor, see Fig. 6a. In the dark reaction, benzylamine makes a Michael attack on the *β*-carbon of octocrylene, resulting in a retro-aldol condensation reaction, see Fig 6b. In contrast, when UV light is present, benzylamine preferably attacks the carbonyl carbon of octocrylene, instead of the *β*-carbon. To better understand the mechanism behind the UV-induced reaction between octocrylene and benzylamine a series of photolysis experiments were performed with different benzylamine concentrations. The octocrylene concentration was maintained at 10 mM, whereas benzylamine was varied from 0.25 to 4 equivalents. No change in the formation rate of *N*-benzyl-2-cyano-3,3-diphenylacrylamide could be seen when different concentrations of benzylamine was used, see Fig. 7.

The corresponding reaction took place in the photolysis of octocrylene in ethanol where the alcohol reacted with octocrylene in the same way as the amine did, producing the ethyl ester instead of the benzyl amide (compare Figs. 3 and 6). The formation rate of ethyl 2-cyano-3,3-diphenyl acrylate and

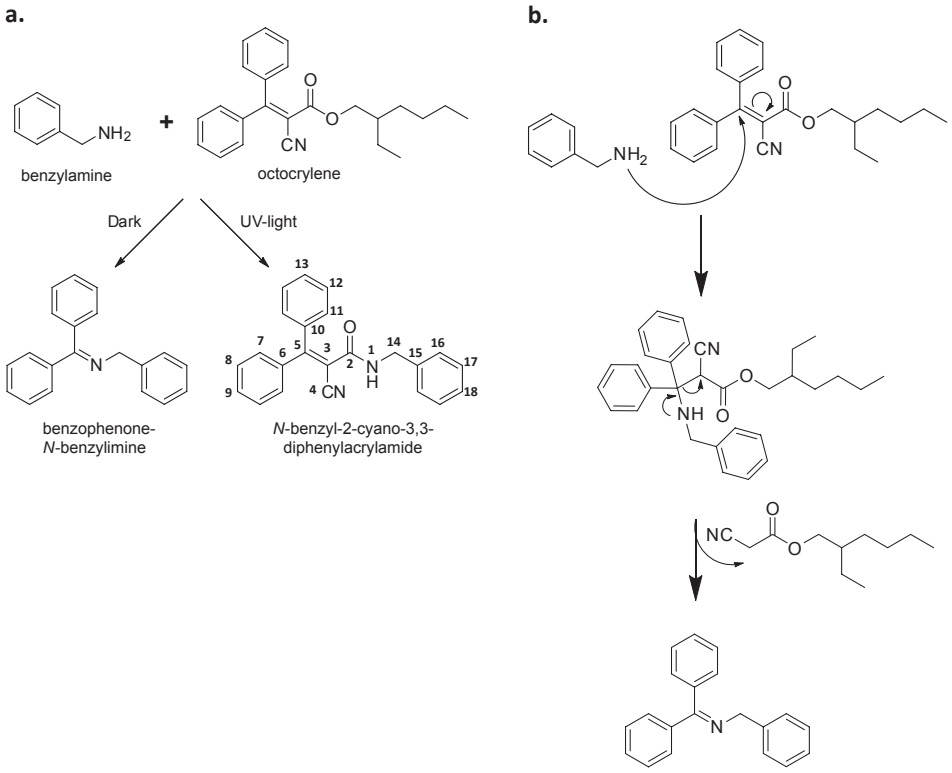

Figure 6. (a) Structures of the different products formed between benzylamine and octocrylene with and without UV light. (b) Mechanism for the formation of the product formed between benzylamine and octocrylene in absence of UV light.

N-benzyl-2-cyano-3,3-diphenylacrylamide are in the same range (compare Figs. 4 and 7), which further supports a mechanism that is independent on the concentration of the nucleophile, *i.e.* the alcohol or amine. These results indicate that it is the excited state formation of octocrylene that governs the rate of the reaction. The singlet excited state lifetime is usually too short for bimolecular photochemical reactions to occur. Thus, the state most likely involved is the triplet excited state of octocrylene. To further explore this hypothesis the photolysis of octocrylene and benzylamine in cyclohexane, as well as the photolysis of octocrylene in ethanol were repeated using inert conditions instead of an oxygen-containing atmosphere. Oxygen is a triplet quencher that reduces the lifetime of energy matched triplet states of other compounds, such as octocrylene (21). If the formation rate of the acylation product depends on the concentration of triplet excited octocrylene, then the presence of oxygen should decrease the formation rate by lowering the concentration of the triplet excited state. Indeed, the formation rate of ethyl 2-cyano-3,3-diphenyl acrylate was clearly enhanced in the absence of oxygen compared to the experiment when an oxygen-containing atmosphere was used, see Fig. 8. Although not as pronounced as for the ester formation, an increase was also seen in the formation rate of *N*-benzyl-2-cyano-3,3-diphenylacrylamide in the absence of oxygen, see Fig. 8. These experiments support the theory that UV radiation promotes octocrylene to its triplet excited state, which is much more reactive toward nucleophiles than the ground state species.

Octocrylene causes both contact allergy and photocontact allergy; most patients, about 60–80%, are photocontact allergic, whereas 20–40% of the octocrylene-positive patients are contact allergic (8,20,22,23). We have previously shown that octocrylene's ability to cause contact allergy (without UV radiation) is probably due to its reactivity toward the amino acid lysine (20). In that study, we showed that octocrylene, in the absence of light, reacted with benzylamine *via* a retro-aldol condensation reaction. In this study, octocrylene in the presence of light reacts with benzylamine *via* a different reaction pathway, which outcompetes the former reaction. The increased reactivity of excited state octocrylene toward benzylamine, compared with ground state octocrylene, could explain the higher incidence of photocontact allergy, compared with contact allergy, to octocrylene. Further, the difference in product outcome for the ground state reaction and the excited state reaction could explain why patients that are photocontact allergic to octocrylene do not necessarily display contact allergy as well. The two reaction pathways lead to different hapten–protein complexes, *i.e.* one hapten–protein complex that is involved in contact allergy and is formed in absence of UV light and another hapten–protein complex that is formed in the presence of light and is responsible for the photocontact allergy (Fig. 6). Furthermore, Duracher *et al.* have shown that UV radiation increases the skin absorption (dose of compound in dermis and epidermis without stratum corneum) of octocrylene more than three times, probably because of octocrylene's hydrophobic nature (LogP 6.9; 16). Another possible explanation for the higher incidence of photocontact allergy compared with contact allergy could therefore be the elevated dose of octocrylene in viable epidermis in combination with octocrylene's change in reaction pathway.

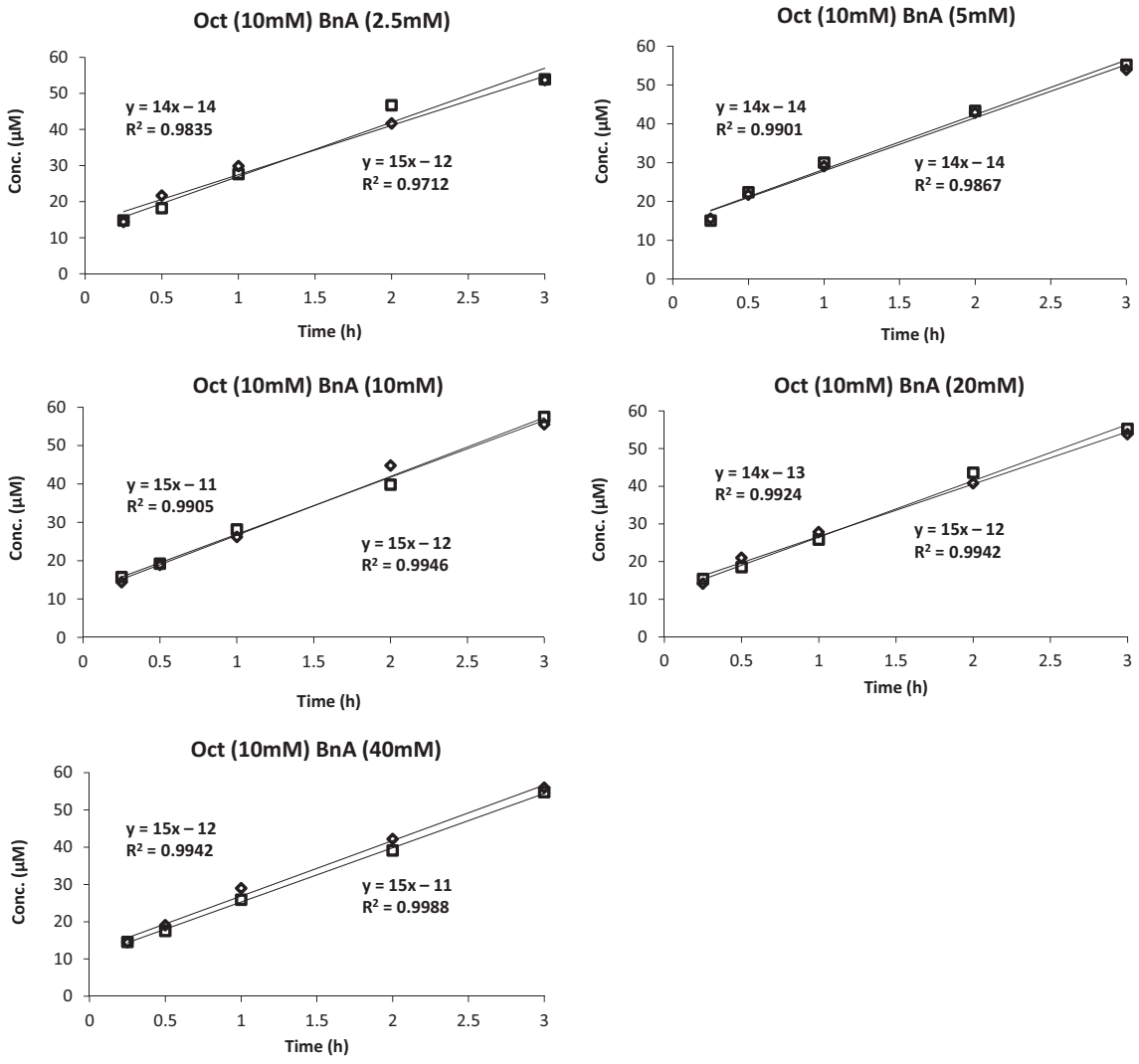

Figure 7. Formation of the UV-induced reaction product *N*-benzyl-2-cyano-3,3-diphenylacrylamide, in concentration with time, when the concentration of octocrylene (Oct) is 10 mM and the concentration of benzylamine (BnA) is varied from 2.5 to 40 mM. Two experiments were performed for each benzylamine concentration.

According to a number of clinical reports, many of the patients that suffer from photocontact allergy to octocrylene have a history of photocontact allergy to the nonsteroidal anti-inflammatory drug (NSAID) ketoprofen (20,23,24). Thus, there seems to be some kind of photocross-reactivity between ketoprofen and octocrylene. Ketoprofen appears to be a very special photocontact allergen that induces a number of other possible photocross allergies (20,23–27). Therefore, some clinics also test ketoprofen allergic patients for other potential cross-allergens, and in recent years octocrylene has become one of these compounds that are sometimes tested. Unfortunately, patients with adverse skin reactions to sunscreens, and no history of ketoprofen use, are generally not tested for photocontact allergy to ketoprofen. Hence, it is not known whether octocrylene can lead to photosensitization to ketoprofen, or if this apparent photocross-reactivity only goes one way. Nevertheless, none of the photoproducts that was identified in our photoreactivity experiments, with octocrylene and amino acid analogs of tyrosine, trypthophan, lysine and cysteine, could possibly be formed from ketoprofen (2-(3-benzoylphenyl)-propionic acid). The reaction product formed between an amine and ground state octocrylene corresponds to a Schiff base formation from benzophenone and the amine, see Fig. 6 (20). The major photodegradation product formed from ketoprofen, in neutral aqueous media, is 3-ethylbenzophenone (28,29). Therefore, one possible explanation for these apparent photocross-reactions to ketoprofen and octocrylene could be that benzophenone is the common denominator. However, if this theory was to be true, the ketoprofen photoallergic patients should react to octocrylene without UV radiation, which they usually do not (20,23). We therefore believe that octocrylene is able to induce sensitization *via* three different pathways: the first route is *via* octocrylene's ground state reactivity toward amines, the second possibility is through octocrylene's excited state reactivity toward amines and alcohols (that produces a different product outcome than the ground state reaction), and finally a third pathway that generates an immunogenic complex that can also be formed in the presence of ketoprofen and UV radiation. The first route induces contact allergy, whereas the other two induce

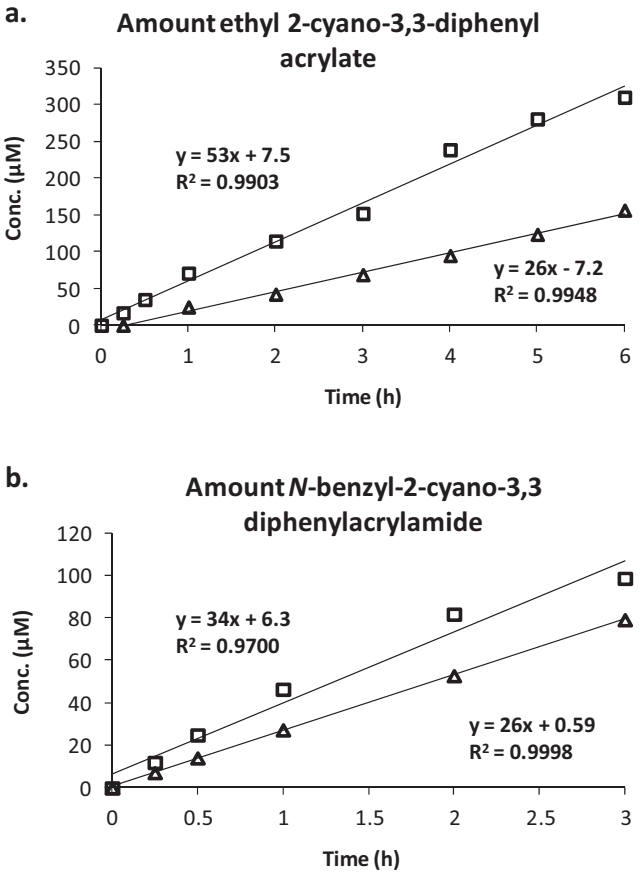

Figure 8. Results from experiments with an atmosphere of synthetic air are indicated with the symbol Δ, and results from experiments with an atmosphere of argon are indicated with the symbol □. (a) Formation of ethyl 2-cyano-3,3-diphenyl acrylate, in concentration with time, during the 6 h photolysis of octocrylene in ethanol. (b) Formation of the UV-induced reaction product *N*-benzyl-2-cyano-3,3-diphenylacrylamide, in concentration with time, during the 3 h photolysis of octocrylene and benzylamine.

photocontact allergy. If we are correct in our hypothesis that octocrylene can cause photocontact allergic reactions *via* the formation of two different immunogenic complexes, there should be one group of patients that are photoallergic to both octocrylene and ketoprofen, and one group that only reacts to octocrylene. To verify or discard this theory, more clinical studies are needed in which patients are tested simultaneously for photocontact allergy to both octocrylene and ketoprofen, regardless of their anamnesis. In addition, more photochemical studies of both octocrylene and ketoprofen are required to better understand the mechanism behind the adverse reactions they cause in presence of UV light.

CONCLUSIONS

In summary, octocrylene is able to protect analogs of the amino acids tyrosine, tryptophan, cysteine and lysine from photodegradation. We have also shown that octocrylene in its excited state reacts with the primary alcohol ethanol and the amine benzylamine *via* a mechanism with a monomolecular rate limiting step. In the skin, the excited state octocrylene will probably react with protein residues of either the amino acid serine (a primary alcohol), or the amino acid lysine (a primary amine). Although less likely, excited state octocrylene may also react with residues of proline and arginine, both containing an amine moiety or threonine that comprises a hydroxyl functional group. The ability of octocrylene in its excited state to form hapten–protein complexes could explain the observed incidence of photocontact allergy to octocrylene. Further, we have demonstrated that there is a difference in product outcome for the excited state and the ground state reaction of octocrylene toward benzylamine. This dissimilarity between the hapten–protein complexes formed by octocrylene may explain that photocontact allergic patients do not usually present a positive reaction in the absence of UV light. The apparent photocross-reactivity between the nonsteroidal anti-inflammatory drug (NSAID) ketoprofen and octocrylene cannot be explained by any of the detected products in our photolysis experiments with octocrylene. Therefore, more studies, both chemical and clinical, are needed to fully understand the correlation between the allergic reactions caused by octocrylene and ketoprofen.

Octocrylene's change in reactivity in its excited state, compared with its ground state, is of great importance when it comes to understanding why some compounds cause photocontact allergy. This knowledge is also essential in the development of stable and non allergenic UV filters to improve the photoprotection of both sunscreens and cosmetic formulations.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1.1. The radiant power (Φ) of the photoreactor at 200–600 nm and 700 W.

Table S2.1. Table of the values used to produce the standard curve for ethyl 2-cyano-3,3-diphenyl acrylate.

Table S2.2. Table of the values used to produce the standard curve for 4-propylphenol.

Table S2.3. Table of the values used to produce the standard curve for 3-methylindole.

Table S2.4. Table of the values used to produce the standard curve for 1-octanethiol.

Table S2.5. Table of the values used to produce the standard curve for benzylamine.

Table S2.6. Table of the values used to produce the standard curve for *N*-benzyl-2-cyano-3,3-diphenylacrylamide, based on the measured peak areas in the UV-chromatogram at 280 nm.

Table S2.7. Table of the values used to produce the standard curve for octocrylene.

Table S2.8. Table of the values used to produce the standard curve for *N*-benzyl-2-cyano-3,3-diphenylacrylamide, based on the measured peak heights in the MS TIC when using selected ion monitoring.

Figure S2.1. Standard curve for ethyl 2-cyano-3,3-diphenyl acrylate with the obtained k and R^2 values.

Figure S2.2. Standard curve for 4-propylphenol with the obtained k and R^2 values.

Figure S2.3. Standard curve for 3-methylindole with the obtained k and R^2 values.

Figure S2.4. Standard curve for 1-octanethiol with the obtained k and R^2 values.

Figure S2.5. Standard curve for benzylamine with the obtained k and R^2 values.

Figure S2.6. Standard curve for *N*-benzyl-2-cyano-3,3-diphenylacrylamide with the obtained k and R^2 values, based on the measured peak areas in the UV-chromatogram at 280 nm.

Figure S2.7. Standard curve for octocrylene with the obtained k and R^2 values.

Figure S2.8. Standard curve for *N*-benzyl-2-cyano-3,3-diphenylacrylamide with the obtained k and R^2 values, based on the measured peak heights in the MS TIC when using selected ion monitoring.

Figure S3.1. Concentration of octocrylene with time during the photolysis experiments of octocrylene and the different amino acid analogs in cyclohexane.

Figure S4.1. To verify that ethyl 2-cyano-3,3-diphenyl acrylate was formed in the photolysis experiment in the solar simulator the 30 h sample was spiked with a synthetic standard.

Figure S4.2. To verify that *N*-benzyl-2-cyano-3,3-diphenylacrylamide was formed in the photolysis experiment in the solar simulator the 24 h sample was spiked with a synthetic standard.

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