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Original Communication

Reactions of cosmetic UV filters with skin proteins: model studies of esters with primary amines

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ABSTRACT

Cosmetic UV filter substances are known triggers for contact or photocontact allergies. UV filters contain reactive carbonyl groups, which are possible reaction partners for free amino acids or proteins from human skin, through which they can act as haptens. Prior screening using high performance thin layer chromatography (HPTLC) amino phase showed that commonly used UV filters with responsive ester groups were able to bind covalently to the amino side chains of the plate after heating and/or UV irradiation. The aim of the study presented here was to investigate the underlying reaction mechanisms and to assign possible reaction products for the UV filter substances octocrylene (OCR), ethylhexyl methoxycinnamate (EHMC), ethylhexyl salicylate (EHS), octyldimethyl-p-aminobenzoic acid (OD-PABA), and ethylhexyl triazone (EHT), using two primary amines, namely ethanolamine and butylamine, as reaction partners. Heating of the reaction batches completely transformed OCR into its corresponding benzophenone imines, while for EHS, EHT, and EHMC, ester aminolysis mainly yielded their respective amides. In the case of EHMC, a Michael-type addition reaction also occurred, which resulted in addition of the primary amines to the conjugated double bond. Further UV irradiation of the reaction batches slightly affected the product distribution of OCR and of EHMC, but not of EHS and EHT. The observed reactions generally had great influences on the absorption spectra. For EHS, a significant bathochromic shift and an increased absorbance were observed, while for EHMC, and especially for OCR, UVA+B efficiency was clearly lost. In contrast, for OD-PABA, no reaction products could be generated under the conditions used.

KEYWORDS: UV filters, UV irradiance, protein binding, ester-amine adducts, mass spectrometry, NMR, OCR, EHMC, UV absorbance

INTRODUCTION

Various case reports, patch test and photopatch test data published in recent years suggest that synthetic UV filter substances are often the cause of allergic and photoallergic contact reactions of the skin [1-8]. This certainly is, among other things, attributed to the increasing use of UV filters in a variety of cosmetic products, including specific sun protection products and also in many daily body care products such as hand and face cream, hair spray and make-up products. These products advertise UV protection to prevent early signs of skin aging triggered by daily sun exposure [9, 10].

UV filters are important cosmetic ingredients. However, their extended usage with increased skin contact time challenges their behavior and stability, including photostability, and hence their protection capability [11-13]. As a result, their protection capability could be quite different on the skin than in a comparable *in vitro* test [14].

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Indeed, reactions of UV filters and their photodegradation products with skin proteins are to be expected. The formation of protein adducts is associated with a certain hapten activity and the incidence of contact allergic skin reactions [15-17]. Several publications have focused on the identification of reactive groups and elucidation of possible underlying reaction mechanisms [18-20]. However, the reaction potential of cosmetic UV filter substances with proteins has not been extensively examined [21-23].

Recently, we developed a fast and simple screening method using an HPTLC amino plate as a protein model layer to get an initial evaluation of the reactivity of different UV filter substances towards amino groups of skin constituents [24]. The tested UV filters were either ketones or esters, and they revealed a different degree of reactivity after heating or irradiation. To further examine the underlying reaction mechanisms, we applied two primary amines as reaction partners for the common UV filters butylmethoxy-dibenzoylmethane (BM-DBM), benzophenone-3 (BP-3), 3-benzylidene camphor (3-BC), 4-methylbenzylidene camphor (4-MBC), and hydroxy-4-methoxybenzophenone-5-sulfonic acid (HMBS), all of which provide reactive keto or diketo groups. Different reaction products and conversion rates could be identified, which depended on the UV filter skeletons and the reaction conditions [25].

Therefore, the aim of the present study was to extend these studies to UV filter substances containing ester groups, such as ethylhexyl methoxycinnamate (EHMC), octocrylene (OCR), ethylhexylsalicylate (EHS), octyldimethyl-p-aminobenzoic acid (OD-PABA), and ethylhexyl triazone (EHT). Using butylamine and ethanolamine as reaction partners and simple models for amino acids or proteins of the skin, reaction products formed under the conditions of UV irradiation and/or under slight heating were isolated and identified. Finally, the UV spectra of reaction batches were recorded to determine the effects of reaction products on the UVA and UVB absorbance.

MATERIALS AND METHODS

Ethylhexyl methoxycinnamate (EHMC, Eusolex 2292), octyldimethyl-p-aminobenzoic acid (OD-PABA, Eusolex 6007), octocrylene (OCR, Eusolex OCR) and methanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). Toluene-4-sulfonic acid monohydrate (~99%) and ethanolamine (≥99%) were obtained from Fluka (Neu-Ulm, Germany). Acetonitrile (HPLC grade) was purchased from Carl Roth (Karlsruhe, Germany). 2-Ethylhexyl salicylate (EHS), butylamine (≥99.5%), ammonium formate (≥99%), dimethyl sulfoxide-d_6 (DMSO-d_6, 99.96 atom% D), D_2O (99.99 atom % D), acetone-d_6 (99.9 atom% D) and deutero-chloroform ($CDCl_3$, 99.8 atom% D) were purchased from Aldrich (Steinheim, Germany). Ethylhexyl triazone (EHT, Uvinul T 150) was kindly provided by BASF (Ludwigshafen, Germany).

High-performance liquid chromatography (HPLC)

HPLC measurements were carried out on a 1100 liquid chromatograph (Agilent, Waldbronn, Germany), using a quaternary HPLC pump (G 1311A), a degasser (G 1315A), an autosampler (G 1313A), a column oven set to 30 °C (G 1316A), and a diode array detector (G 1315B) with DAD detection wavelength of 275 nm, 313 nm, and 360 nm (spectral bandwidth (SBW) 8 mm). The reference wavelength was 500 nm (SBW 8 mm). For data processing, the HP ChemStation software (rev. A.04.02) was used. The stationary phase was a Eurospher 100-5 C 18 HPLC column, 250 mm x 3 mm (Knauer, Berlin, Germany). The mobile phase (0.5 mL/min) consisted of acetonitrile (A) and 10 mM ammonium formate buffer set to pH 4.0 (B). For EHT, isocratic elution (90% A / 10% B) was used. For the other UV filters the gradient was % B (t(min)): 40 (0)-40 (4)-25 (9)-25 (13)-10 (17)-24 (40)-26 (40). The injection volume was 10 µL.

HPLC-electrospray ionization mass spectrometry (LC/ESI-MS)

LC/MS measurements were performed on an identical Agilent 1100 chromatograph as described above, coupled with an MSD single-quadrupole mass spectrometer (G1956B, Agilent) equipped with an electrospray ionization (ESI) interface. Mass spectra were generally recorded in the ESI positive full scan mode (*m/z* 50-1200), and in the

case of OCR reactions, additionally in the ESI negative full scan mode (*m/z* 50-800) using the following settings: capillary voltage 4 kV, skimmer voltage 35 V, nebulizer gas pressure 20 psig, source temperature 100 °C, drying gas temperature 300 °C, drying gas flow rate 10 L/min, fragmentator voltage 80 V, gain 1, threshold 100, and step size 0.1. For data processing, ChemStation software (Agilent) was used.

Spectroscopy

Infrared (IR) spectra were recorded on a Dura Sampler SMART ATR installed at the Avatar 320 FT-IR-Spectrometer (Thermo Nicolet, Madison, USA). The samples were applied on a diamond crystal and were recorded between 4000 and 500 cm^{-1}. A minimum of 32 scans was signal-averaged with a resolution of 2 cm^{-1}.

UV spectra were measured using a Perkin-Elmer Lambda 2 (Überlingen, Germany). ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz (^{1}H) and 75 MHz (^{13}C) on a Varian Unity Inova-300 spectrometer (Varian, Darmstadt, Germany). The samples were dissolved in $CDCl_3$, DMSO-d_6, D_2O, or acetone-d_6. The signal assignments were made based on chemical shifts related to tetra-methylsilane (TMS) and H-H and C-H correlation data; s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet.

Thermal reaction of UV filters with amines

Each UV filter (0.5 mmol) was weighed into a 10 mL screw-capped glass tube (Schott, Mainz, Germany) and suspended in acetonitrile (5 mL), except for EHT, which was suspended in 5 mL acetonitrile/toluene (80/20). Butylamine (1 mL, 10 mmol) or 0.6 mL of ethanolamine (10 mmol) and toluene-4-sulfonic acid monohydrate (1 mg, 5 µmol) were added. The mixture was heated for 3 h at 40 or 80 °C. Afterwards, the reaction was stopped by cooling the tubes under running tap water. As controls, reaction batches were stored in the dark, at an ambient temperature of approximately 20 °C.

To determine the impact of the quantity of amine on the reaction, different amounts of ethanolamine were used (equimolar, 3- and 5-fold excess for OCR, 5-, 10- and 20-fold excess for EHS and EHMC, and 10-, 20- and 30-fold excess for EHT).

Photoreaction of UV filters in the presence of amines

Each UV filter (2 mmol) was weighed into a 50 mL quartz beaker with a diameter of 38 mm (Th. Geyer, Renningen, Germany) and suspended in acetonitrile (20 mL), except for EHT, where acetonitrile/toluene (80/20, 20 mL) was used. Either butylamine or ethanolamine (40 mmol) and toluene-4-sulfonic acid monohydrate (3.8 mg, 20 µmol) were added. The beaker was tightly closed by a teflon cap and irradiated for 3 h. To maintain a consistent temperature (20 °C or 60 °C), the beaker was placed inside a quartz glass flow chamber, which was connected to a chiller (Model RML 6, Lauda, Germany). For irradiation, a modified sun simulator SOL 500 with a 430 W metal halide lamp (Dr. Höhnle, Gräfelfing, Germany) was used. The modification involved the front filter glass being replaced by an aluminum plate with two 16 cm^2 gaps to hold two WG 295 glass filters (Schott, Mainz, Germany). The irradiation intensities were 12.5 mW/cm^2 in the UVA and 0.55 mW/cm^2 in the UVB range. For 3 hours of irradiation, the corresponding light doses were 1410 kJ/m^2 (2.3 kJ/quartz beaker). The solutions were stirred continuously using a Variomag Micro stirrer (Thermo Scientific). To distinguish between the effects of heat or UV radiation on the reaction, a second batch was prepared in another quartz beaker in the same manner, but was completely covered by aluminum foil and placed aside the irradiated sample.

Isolation of the reaction products

The reaction solutions were evaporated to dryness in a Labconco (Kansas City, USA) CentriVap concentrator equipped with a CentriVap cold trap at a temperature of 35 °C. For OCR, the obtained residues could be directly used for NMR spectroscopy. For the reaction batches of the other UV filters, the residues were dissolved in 5 mL methanol. To isolate the reaction products, 1 mL of the methanolic solution (five injections) was subjected to a preparative Kronlab HPLC system (Sinsheim, Germany) consisting of a HD 2-200 HPLC pump, a C-R3A Chromatopac Integrator

(Shimadzu), and a Variable Wavelength Monitor (Knauer, Berlin, Germany). For separation, a YMC (Dinslaken, Germany) HPLC column (ODS-A, RP 18, 5 µm, 20 mm x 25 cm) was used. For the first 14 min elution was performed with acetonitrile/water (60/40), followed by a 3 min flushing of the column with pure acetonitrile. The detection wavelength was 275 nm and the flow rate 8 mL/min. The respective fractions were collected, the solvent was evaporated and the residue dried over phosphorus pentoxide. The purity of the products was examined by HPLC/DAD.

Reaction products isolated from the respective batches after 3 hours at 80 °C

EHS

***N*-(2-Hydroxyethyl)-2-methoxybenzamide.** 90 mg (99 mol%) of pure **1a** was obtained as reddish-brown, highly viscous oil. UV/Vis (methanol) λ_{max} (nm) (log ε) 315 (3.60). IR (ATR) ν (cm^{-1}): 3355-3270 (m), 3055 (w), 2932 (m), 2862 (m), 1609 (s), 1554 (2), 1448 (m), 1322 (m), 1243 (w), 1144 (w), 1070 (m), 1030 (m), 853 (w), 766 (w), 700 (w). LC-MS (ESI+) (t_R = 2.77) *m/z* (relative intensity) = 182 (MH^+, 100), 147 (22), 121 (10), 106 (21). ^{1}H NMR (D_2O, 300 MHz) δ (ppm) 7.82 (m, 1H), 7.31 (m, 1H), 6.77 (m, 1H), 6.65 (m, 1H), 3.77 (t, 2H, ^{3}J = 5.6 Hz), 3.55 (t, 2H, ^{3}J = 5.6 Hz). ^{13}C NMR (D_2O, 300 MHz) δ (ppm) 171.2, 168.8, 134.0, 129.7, 122.2, 118.4, 114.2, 60.7, 41.3.

***N*-Butyl-2-methoxybenzamide.** 87 mg (90 mol%) of pure **1b** was obtained as yellow-brown viscous oil. UV/Vis (methanol) λ_{max} (nm) (log ε) 317 (3.63). IR (ATR) ν (cm^{-1}): 3505-3270 (m), 3055 (w), 2956 (s), 2929 (m), 2870 (m), 1633 (s), 1597 (s), 1538 (s), 1498 (m), 1456 (m), 1365 (w), 1306 (m), 1227 (m), 1144 (w), 1034 (w), 865 (w), 754 (m), 699 (w). LC-MS (ESI+) (t_R = 4.69) *m/z* (relative intensity) = 194 (MH^+, 100), 147 (22), 121 (3), 106 (32). ^{1}H NMR (acetone-d_6, 300 MHz) δ (ppm) 7.82 (m, 1H), 7.37 (m, 1H), 6.89 (m, 1H), 6.81 (1H, m), 3.41 (2H, t, ^{3}J = 7.0 Hz), 1.61 (2H, m), 1.39 (2H, m), 0.93 (3H, t, ^{3}J = 7.3). ^{13}C NMR (acetone-d_6, 300 MHz) δ (ppm) 170.4, 162.7, 134.4, 127.6, 118.8, 118.6, 115.9, 39.6, 32.2, 20.7, 14.0.

EHMC

(2E)-*N*-(2-Hydroxyethyl)-3-(4-methoxyphenyl)-prop-2-enamide. 21.6 mg (20 mol%) of pure **2a** was obtained as yellow, very viscous liquid. UV/Vis (methanol) λ_{max} (nm) (log ε) 291 (4.27), 224 (4.21). IR (ATR) ν (cm^{-1}): 3320-3270 (s), 2930 (m), 2865 (m), 2355 (w), 1649 (m), 1595 (s), 1551 (m), 1508 (s), 1247 (m), 1225 (m), 1171 (m), 1062 (w), 1030 (w), 976 (w), 824 (m). LC-MS (ESI+) (t_R = 3.21) *m/z* (relative intensity) = 465 ($2MNa^+$, 35), 244 (MNa^+, 5), 222 (MH^+, 100), 161 (20%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 8.09 (t, 1H, ^{3}J = 5.5 Hz), 7.51 (m, 2H), 7.36 (d, 1H, ^{3}J = 15.8 Hz), 6.96 (m, 2H), 6.51 (d, 1H, ^{3}J = 15.8 Hz), 3.77 (s, 3H), 3.46 (t, 2H, ^{3}J = 5.9 Hz), 3.24 (m, 2H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ (ppm) 166.5, 161.0, 139.3, 129.9, 128.1, 120.2, 115.1, 60.6, 56.0, 42.3.

(2E)-*N*-Butyl-3-(4-methoxyphenyl)-prop-2-enamide. 8.2 mg (7 mol%) of pure **2b** was obtained as light-brown, very viscous liquid. UV/Vis (methanol) λ_{max} (nm) (log ε) 290 (4.29), 225 (3.99). IR (ATR) ν (cm^{-1}): 3290-3210 (m), 3071 (w), 2952 (m), 2930 (m), 2865 (w), 2344 (w), 1649 (m), 1595 (s), 1551 (m), 1508 (s), 1453 (w), 1301 (w), 1286 (w), 1247 (m), 1225 (m), 1171 (m), 1029 (w), 976 (w), 824 (w). LC-MS (ESI+) (t_R = 5.43) *m/z* (relative intensity) = 467 ($2MH^+$, 20), 234 (MH^+, 100), 161 (3%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.57 (d, 1H, ^{3}J = 15.5 Hz), 7.44 (m, 2H), 6.68 (m, 2H), 5.55 (t, 1H, ^{3}J = 6.1 Hz), 6.25 (d, 1H, ^{3}J = 15.5 Hz), 3.83 (m, 3H), 3.39 (m, 2H), 1.55 (m, 2H), 1.40 (m, 2H), 0.95 (t, 3H, ^{3}J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 300 MHz) δ (ppm) 166.4, 161.1, 140.6, 129.5, 127.9, 118.7, 114.5, 55.6, 39.7, 32.1, 20.4, 14.0.

***N*-(2-Hydroxyethyl)-3-[(2-hydroxyethyl)-amino]-3-(4-methoxyphenyl)propanamide.** 14.4 mg (10 mol%) of pure **4a** was obtained as light-brown, very viscous liquid. UV/Vis (methanol) λ_{max} (nm) (log ε) 226 (4.17), 201 (4.37). IR (ATR) ν (cm^{-1}): 3390-3280 (s), 2953 (m), 2920 (m), 2863 (w), 1716 (w), 1632 (m), 1594 (s), 1545 (m), 1508 (s), 1453 (w), 1306 (w), 1247 (m), 1225 (m), 1170 (m), 1057 (w), 1029 (w), 975 (w), 823 (w). LC-MS (ESI+) (t_R = 4.12) *m/z* (relative intensity) = 283 (MH^+, 100), 222 (5%).

***N*-Butyl-3-(butylamino)-3-(4-methoxyphenyl)-propanamide.** 6.3 mg (4 mol%) of pure **4b** was

obtained as bright yellow, fine powder. UV/Vis (methanol) λ_{max} (nm) (log ε) 226 (4.23), 201 (4.43). IR (ATR) ν (cm^{-1}): 3299 (w), 2952 (m), 2919 (m), 2865 (w), 1714 (m), 1627 (m), 1594 (m), 1540 (w), 1508 (s), 1453 (w), 1440 (w), 1247 (m), 1160 (m), 1030 (w), 824 (w). LC-MS (ESI+) (t_R = 10.21) *m/z* (relative intensity) = 307 (MH^+, 100), 234 (5%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.21 (m, 2H), 6.88 (m, 2H), 3.79 (2, 3H), 3.89 (m, 1H), 3.22 (m, 2H), 2.48 (m, 2H), 2.43 (m, 2H), 1.42 (m, 4H), 1.31 (m, 4H), 0.89 (m, 6H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ (ppm) 171.7, 159.1, 135.2, 127.8, 114.2, 59.7, 55.5, 47.0, 44.4, 39.1, 32.5, 31.9, 20.7, 20.4, 14.2, 14.0.

By-products (identified by mass spectrometry): 2-Ethylhexyl 3-[(2-hydroxyethyl)amino]-3-(4-methoxyphenyl)propionate (3a). LC-MS (ESI+) (t_R = 5.16) *m/z* (relative intensity) = 352 (MH^+, 100).

2-Ethylhexyl 3-(butylamino)-3-(4-methoxyphenyl) propionate (3b). LC-MS (ESI+) (t_R = 16.20) *m/z* (relative intensity) = 364 (MH^+, 100).

OCR

2-[(Diphenylmethylene)imino]ethanol. 111 mg (98 mol%) of pure **5a** was obtained as yellow highly viscous oil. UV/Vis (methanol) λ_{max} (nm) (log ε) 245 (4.11). IR (ATR) ν (cm^{-1}): 3500-3250 (m), 3080-3010 (m), 2919 (m), 2872 (m), 1656 (s), 1620 (s), 1593 (m), 1573 (m), 1442 (s), 1314 (m), 1271 (s), 1068 (w), 1024 (w), 941 (w), 762 (w), 695 (s), 639 (w). LC-MS (ESI+) (t_R = 4.08) *m/z* (relative intensity) = 226 (MH^+, 100), 106 (4). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.61 (m, 4H), 7.30-7.48 (m, 6H), 3.84 (t, 2H, ^{3}J = 5.3 Hz), 3.49 (2H, t, ^{3}J = 5.3 Hz). ^{13}C NMR ($CDCl_3$, 300 MHz) δ (ppm) 169.9, 136.7, 130.2, 128.3, 127.7, 62.9, 55.5.

***N*-Butyl-1,1-diphenylmethanimine.** 115 mg (97 mol%) of pure **5b** was obtained as yellow highly viscous oil. UV/Vis (methanol) λ_{max} (nm) (log ε) 244 (4.12). IR (ATR) ν (cm^{-1}): 3500-3200 (w), 3080-3015 (w), 2961 (s), 2921 (s), 2859 (s), 1659 (m), 1617 (s), 1593 (m), 1576 (m), 1442 (m), 1311 (w), 1278 (m), 1070 (w), 1029 (w), 780 (m), 764 (m), 694 (s), 637 (w). LC-MS (ESI+) (t_R = 14.46) *m/z* (relative intensity) = 238 (MH^+, 100), 106 (3). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.59 (m, 4H), 7.31-7.49 (m, 6H), 3.37 (2H, t, ^{3}J = 7.0 Hz), 1.65 (2H, m), 1.34 (2H, m), 0.87 (3H, m). ^{13}C NMR ($CDCl_3$, 300 MHz) δ (ppm) 167.7, 137.1, 129.7, 128.4, 127.9, 53.6, 33.4, 20.6, 13.9.

By-products (identified by mass spectrometry): 2-Ethylhexyl cyanoacetate (6). LC-MS (ESI-) (t_R = 4.19) *m/z* (relative intensity) = 196 ($[M-H]^-$, 100).

2-Cyano-*N*-(2-hydroxyethyl)acetamide(7a).C-MS (ESI-) (t_R = 3.12) *m/z* (relative intensity) = 127 ($[M-H]^-$, 100).

***N*-Butyl-2-cyanoacetamide(7b)**. LC-MS (ESI-) (t_R = 3.38) *m/z* (relative intensity) = 139 ($[M-H]^-$, 100).

By-products formed under additional UV irradiation (identified by mass spectrometry): 2-Hydroxyethyl-2-cyano-3,3-diphenyl-2-propenamide (8a). LC-MS (ESI+) (t_R = 2.86) *m/z* (relative intensity) = 293 (MH^+, 100), 315 (MNa^+, 5).

2-Butyl-2-cyano-3,3-diphenyl-2-propenamide (8b). LC-MS (ESI+) (t_R = 12.68) *m/z* (relative intensity) = 305 (MH^+, 100), 327 (MNa^+, 6), 631 ($2MNa^+$, 30).

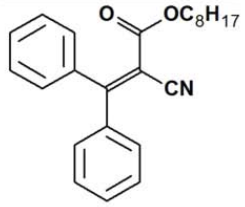
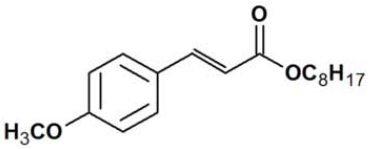
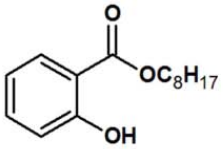
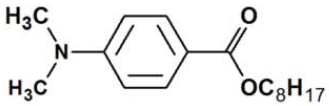
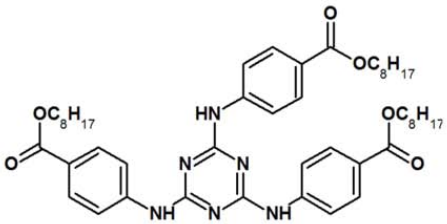
EHT

4-[[4,6-Bis[[4-(2-ethylhexoxy-oxomethyl)-phenyl] amino]-1,3,5-triazin-2-yl]amino]benzoic acid 2-hydroxyethyl amide. 20.3 mg (5 mol%) of pure **9** was obtained as colorless powder. UV/Vis (methanol) λ_{max} (nm) (log ε) 313 (5.02). IR (ATR) ν (cm^{-1}): 3500-3200 (s), 2958 (m), 2924 (s), 2855 (m), 2360 (s), 2341 (m), 1693 (w), 1609 (m), 1490 (m), 1414 (m), 1310 (w), 1278 (m), 1248 (w), 1177 (w), 1111 (w), 851 (w), 768 (w), 668 (w). LC-MS (ESI+) (t_R = 17.91) *m/z* (relative intensity) = 754 (MH^+, 100), 308 (10). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.02 (t, 1H, ^{3}J = 5.51 Hz), 7.81 (m, 2H), 7.74-7.66 (m, 10H), 4.25 (m, 4H), 3.89 (t, 2H, ^{3}J = 5.4 Hz), 3.66 (t, 2H, ^{3}J = 5.4 Hz), 1.73 (m, 2H), 1.45-1.20 (m, 16H), 0.96 (m, 6H), 0.90 (m, 6H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ (ppm) 167.8, 166.3, 164.2, 150.5, 142.4, 130.7, 129.8, 128.0, 125.2, 119.9, 119.4, 67.8, 62.6, 42.9, 38.9, 30.6, 28.9, 24.0, 23.1, 14.0, 11.1.

RESULTS AND DISCUSSION

The reactions of the UV filter substances that were analyzed (Table 1) with the amino acid

Table 1. UV filter substances under study.

Name (shortcut)	Structure
Octocrylene (OCR)	
Ethylhexyl methoxycinnamate (EHMC)	
Ethylhexyl salicylate (EHS)	
Octyl dimethyl PABA (OD-PABA)	
Ethylhexyl triazone (EHT)	

models butylamine and ethanolamine were conducted at different temperature levels: 20, 40, 60, and 80 °C. The moderate temperatures were chosen to reflect standard environmental conditions such as natural warming of the skin in direct summer sunlight within 20 min [26]. The higher temperatures should accelerate the reactions and increase the yield of the products to be isolated and elucidated. To observe the influence of UV radiation on the reactions, the batches at 20 °C and 60 °C were additionally irradiated, and the results compared to the non-irradiated samples. As already observed during the experiments with ketones [25], ethanolamine was significantly more reactive towards esters than butylamine (Figure 1).

Among the selected UV filters, OCR and EHS showed the highest reaction rates with both butylamine and ethanolamine (Figure 2), which is in good agreement with the results obtained during the previous amino HPTLC screening [24]. After only 10 min at 80 °C, OCR completely reacted with ethanolamine (Figure 2B), while with butylamine the conversion was complete within 20 min (Figure 2A). Even at room temperature, a high conversion of >70% or >50% was observed in the presence of ethanolamine and butylamine, respectively, after a reaction time of 3 h (Figure 1). EHS showed the same tendency as OCR, but an overall lower reactivity. At room temperature, 35% and 12% of the initial EHS reacted with ethanolamine and butylamine, respectively, within 3 h. As for

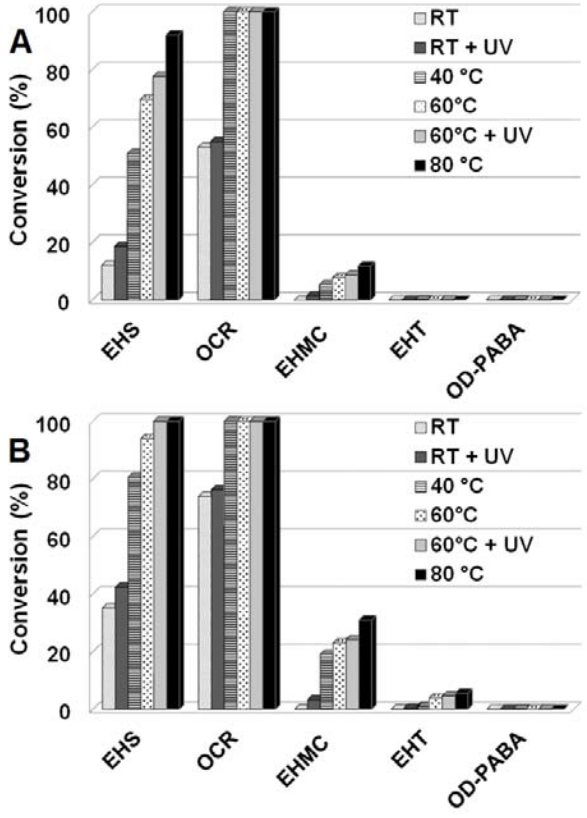


Figure 1. Conversion rates of the studied UV filters in the presence of a 20-molar excess of butylamine (A) or ethanolamine (B) under different conditions after 3 h; UV (UV irradiation), RT (room temperature).

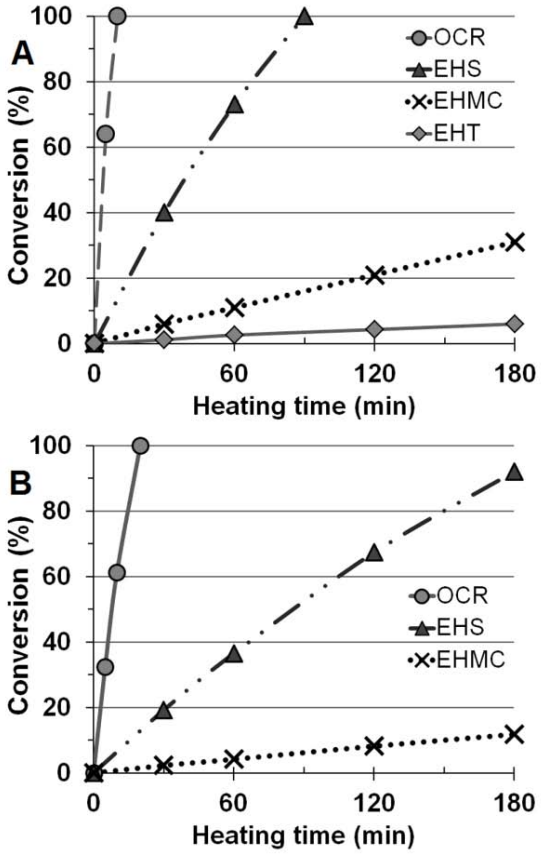


Figure 2. Reaction kinetics for the conversion of the studied UV filters in the presence of a 20-molar excess of ethanolamine (A) or butylamine (B) at 80 °C.

OCR, heating increased the conversion significantly (Figure 1). In the presence of ethanolamine, a complete conversion of EHS was achieved after 90 min, while it took >180 min with butylamine (Figure 2). Compared to OCR and EHS, reaction rates of EHMC were clearly lower (Figure 2). At room temperature, no spontaneous conversion could be observed, but even marginal heating to 40 °C or 60 °C increased the conversion in the presence of both butylamine and ethanolamine (Figure 1). The two p-aminobenzoates, EHT and OD-PABA, showed the lowest reactivity or generally no reactivity (Figure 1). For EHT, any reactivity was limited to the reactions with ethanolamine at high temperatures, only leading to a low conversion of up to 6%. In the presence of butylamine, EHT was completely recovered, even after 3 h at 80 °C. Reaction products of OD-PABA could not be identified under the conditions used, neither with butylamine nor with ethanolamine. HPLC analyses resulted in recoveries of >99% for OD-PABA. During the former HPTLC screening, OD-PABA seemed to show a moderate binding to the amino phase, but it was already suspected that the additional formation of two photodegradation products on the plate overestimated the determined binding rate [24]. Obviously, good resonance stabilization prevents the ester moieties of EHT and OB-PABA from nucleophilic attacks including aminolysis. Otherwise, an intermolecular self-aminolysis of at least OB-PABA, resulting in a polyamide, had to be expected in cosmetic formulations.

As expected, a higher amine/UV filter ratio accelerated the reactions and increased the conversions (Figure 3). After 60 min at 40 °C with an equimolar amount of ethanolamine, approximately 40% of the provided OCR was

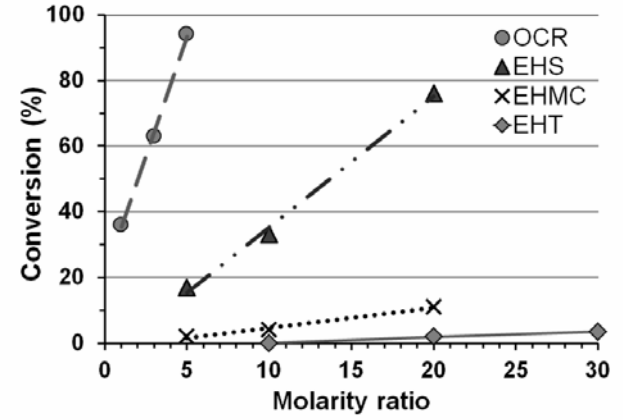


Figure 3. Conversion rates of the studied UV filters in the presence of ethanolamine at different molar ratios after 1 h at 40 °C (OCR) and at 80 °C (EHS, EHMC and EHT).

transformed. With 3-fold and 5-fold excess of ethanolamine, the conversion rate nearly doubled and tripled, respectively. For the other UV filter substances, an excess of amine increased the conversions, but due to the overall lower reactivity, the effect was less pronounced, especially for EHMC and EHT (Figure 3).

Additional UV irradiation of the reaction batches with EHS, EHMC, and EHT partly affected the reaction rates, but did not create new reaction products, except the respective Z-isomers of EHMC and **2a/b** due to the known photoisomerization [27]. This corresponds to the behavior of the ketones during the former study [25]. For OCR, however, two additional reaction products (**8a/b**) were formed under UV irradiation (Figure 4).

UV irradiation resulted in a slightly increased conversion of up to 8% for EHS, and 4% for OCR, both at room temperature and at 60 °C (Figure 1). Corresponding to the overall lower reactivity, the influence of UV irradiation on the conversion of EHMC and EHT was concurrently lower. At 20 °C, irradiation of the ethanolamine reaction batches only increased the conversion rates to 0.3% and 3% for EHT and EHMC, respectively (Figure 1). At 60 °C, the effect of additional UV irradiation was also insignificant. UV irradiation also could not activate OD-PABA in terms of reactions with primary amines.

Reaction products

The reaction of EHS with both ethanolamine and butylamine only generated the respective amides **1a/b** in high yields (Figure 4). Further by-products could not be detected, and an additional UV irradiation had an influence on the rate of conversion, but not on the kind of products.

EHMC reacted by both ester aminolysis and Michael-type addition. As main products, the amides **2a/b** and the products **4a/b**, which resulted from a twofold reaction with the primary amines, could be isolated. The pure Michael adducts **3a/b** could only be detected by LC/MS as by-products in relatively low amounts. The kinetic data suggests that the products **4a/b** were mainly formed from **2a/b** (data not shown)**.** The formation of the amines **3a/b** was solely dependent on temperature and reaction time. Additional UV irradiation did not significantly influence their yields (approximately the same peak areas were observed). On the contrary, both temperature and irradiation affected the formation of the amide products and the aminated amides. Irradiation of the ethanolamine reaction batch at 20 °C yielded 2 mol% of the amide **2a** and its Z-isomer (calculated as E-isomer) and 1 mol% of the aminated amide **4a,** while under dark conditions no conversion occurred. At 60 °C the additional irradiation led to an increased formation of **2a** and its Z-isomer (calculated as E-isomer) by about 8% as compared to the non-irradiated batch. This was partly at the expense of **4a**, which decreased by 5%.

For OCR, the benzophenone imines **5a/b** were the only reaction products under dark conditions, in terms of conversion influenced by reaction temperature and time (Figure 2). With additional UV irradiation at 20 °C a small amount of the amide by-products **8a/b** was detectable. At higher temperatures, however, the formation of the imines **5a/b** predominated and the amides were not formed. Regarding the surprising formation of **5a/b**, a Michael-type addition of the primary amine must first be assumed, followed by elimination of ethylhexyl cyanoacetate (**6**), which could be identified by LC/MS. Regarding the reaction type, it is comparable to a retro-aldol cleavage. In the presence of an amine excess, the amides **7a/b** were additionally identified, resulting from a further reaction of product **6** with the amines.

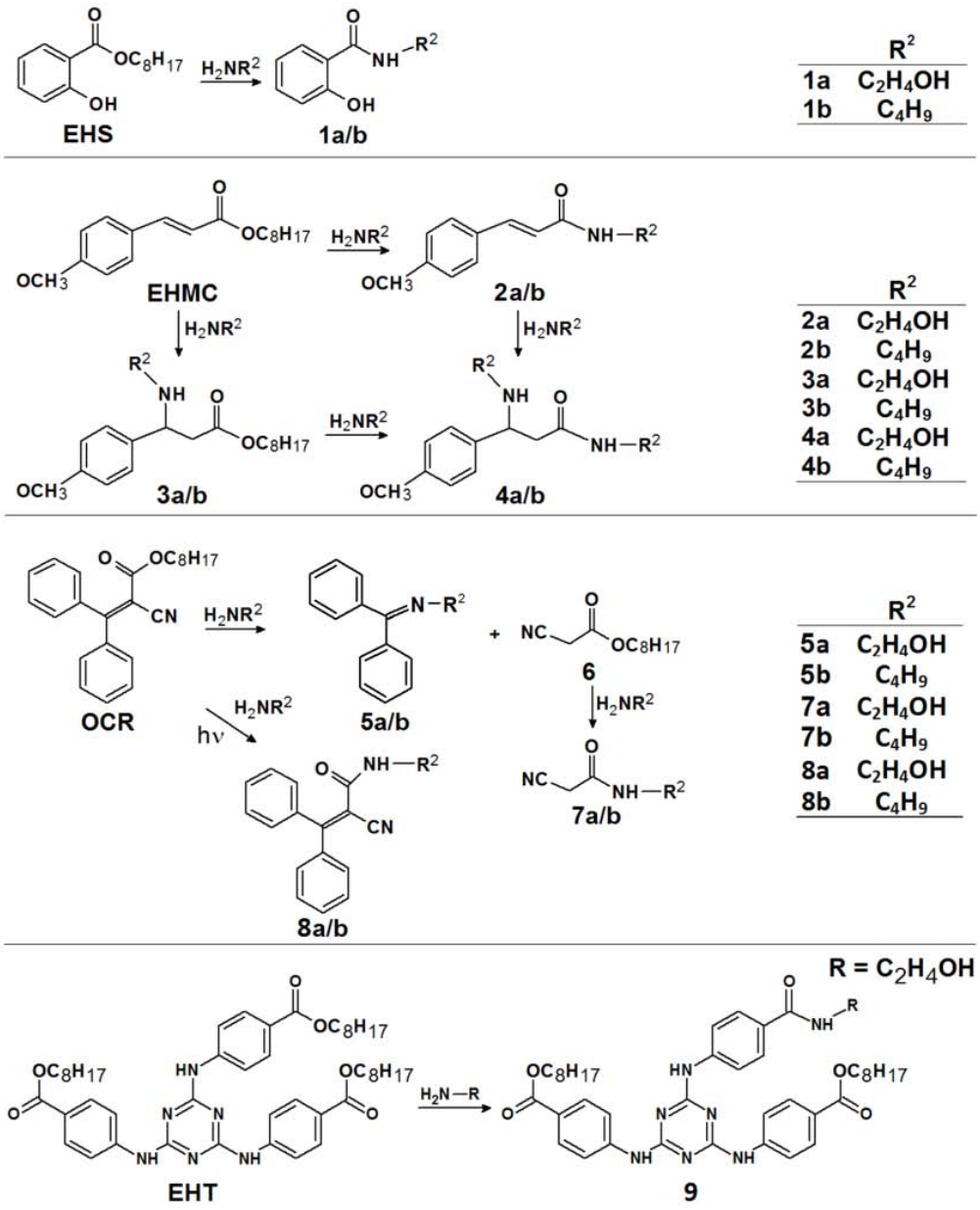


Figure 4. Overview of the reaction products of EHS, EHMC, OCR and EHT with butylamine and ethanolamine.

While there was no reaction between EHT and butylamine, in the presence of ethanolamine EHT was only transformed into the amide **9** (Figure 4), after only one ester group had reacted. For its formation, temperature and irradiation played a role. However, temperature had a much greater impact (Figure 1). Reactions at the two other available ester groups of EHT could not be observed, even in the presence of a high excess of ethanolamine. Evidently, the findings are in agreement with the high (photo) stability of EHT.

Influence of amine reactions on the UV spectra

As expected, the bonded amines participate in the resonance delocalization process of EHS, resulting in a significant bathochromic shift (Figure 5). For both amine reaction batches, a strong increase of absorbance in the UVA range of about 200% was

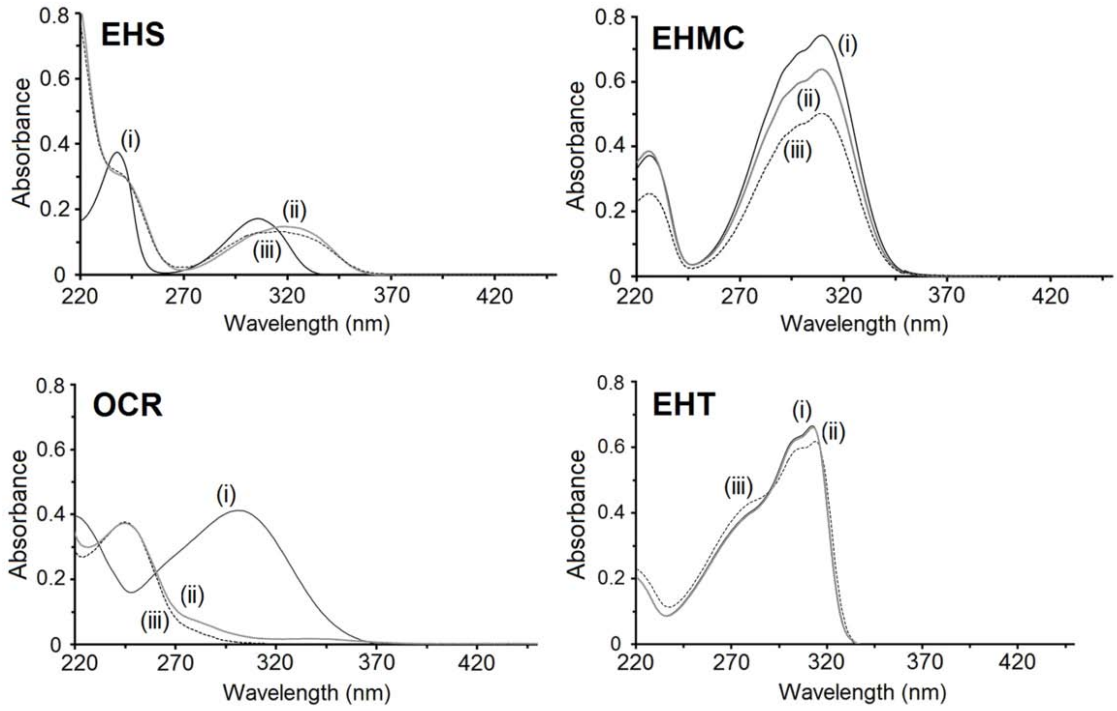


Figure 5. UV spectra of standard solutions of the studied UV filters (i) and of 20-molar reaction batches with butylamine (ii) and ethanolamine (iii) after 3 h at 80 °C; concentrations about 5 mg/L relating to the UV filter.

Table 2. UV absorbance characteristics of the studied UV filter substances and the reaction mixtures with an 20-molar excess of ethanolamine (EA) or butylamine (BA) after heating for 3 h at 80 °C, calculated as area under the curve (AUC), measured at concentrations of 5 mg/L.

		UVA range		UVB range		UVA and UVB	
		AUC	Percentage change	AUC	Percentage change	AUC	Percentage change
EHS		1.4		4.4		5.8	
	+ EA	3.8	+ 171%	3.5	- 20%	7.3	+ 26%
	+ BA	4.2	+ 200%	3.3	- 25%	7.5	+ 29%
EHMC		10.3		22.8		33.1	
	+ EA	7.0	- 32%	15.4	- 32%	22.4	- 32%
	+ BA	8.6	- 17%	19.8	- 13%	28.4	- 14%
OCR		6.0		9.5		15.4	
	+ EA	0.1	- 98%	0.3	- 97%	0.4	- 97%
	+ BA	0.7	- 88%	0.9	- 91%	1.6	- 90%
EHT		5.6		18.9		24.5	
	+ EA	5.3	- 5%	18.7	- 1%	24.0	- 2%
	+ BA	5.5	- 2%	18.9	± 0%	24.4	- 0.4%

observed, which was partly at the expense of UVB absorption. However, over the whole UVA+B range, an increase of the absorption strength of nearly 30% was calculated (Table 2).

In contrast to EHS, noticeable shifts of absorption maxima could not be detected for the reaction batches of EHMC with both amines. However, depending on the percentage of conversion, a significant decrease of the absorption strength over the whole UVA+B range could be calculated (Table 2).

Since for OCR, the benzophenone imines **5a/b** were the main reaction products resulting in loss of conjugation, the spectral changes of the reaction batches directly correlated with the percentage of conversion (data not shown). The absorption maxima of the benzophenone imines were at 245 nm; accordingly, the spectra of the reaction batches with both ethanolamine and butylamine showed a strong absorbance decrease over the whole UVA+B range by nearly 100%.

Due to the rather low conversion of EHT, the change in the UV spectrum of the reaction batches was insignificant. After the reaction with ethanolamine, there was only a marginal bathochromic shift of the spectrum to an adsorption maximum at 316 nm, and a small decrease of absorbance in the UVA+B range of about 2% (Table 2).

CONCLUSION

The study presented here shows that the UV filter substances EHS, OCR, EHMC and EHT (which all contain a functional ester group), but not OD-PABA, were able to react with primary amines. Under the influence of UV radiation and/or heat, different reaction products could be assigned. They primarily resulted from ester aminolysis and, in the cases of EHMC and OCR, from Michael-type additions, when OCR surprisingly lost the cyanoacetate moiety. The identified reactions generally are also conceivable in the presence of proteins. The reaction rates increased significantly after applying a molar excess of amines corresponding to the conditions for skin applications.

The time-dependent conversions led to a bathochromic shift of the respective UV spectra, especially for EHS, whereas for OCR a hypsochromic shift and a nearly complete loss of UVA+B protection were observed. For EHS, the amine reactions led to an improved UVA protection, while the conversions were associated with a decrease of the absorbance strength of EHMC and to some extent of EHT.

The results of this study and our previous study with common UV filters with keto or diketo groups confirm that the recently developed fast screening HPTLC method allows direct conclusions about the reactivity of sunscreen substances with protein structures, and consequently about their possible allergic potential. It is still unclear if the results obtained in this study are likely transferable to more complex skin model systems but further studies using proteins and skin analogs to confirm this are planned for the future.

CONFLICT OF INTEREST STATEMENT

The authors of this publication declare that there is no conflict of interest.

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