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

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ABSTRACT

As most UV filter substances approved for usage in sunscreens have reactive carbonyl groups, the possibility of their reaction with amino groups of proteins or free amino acids of the human skin cannot be precluded. An initial screening on high performance thin layer chromatography (HPTLC) amino plates showed that benzophenones and dibenzoylmethanes were strongly bound to the amino phase after heating and/or UV irradiation, while camphor derivatives were less reactive. To understand the underlying mechanisms and to identify reaction products, the reactions of benzophenone-3 (BP-3), dibenzoylmethane (DBM), 4-t-butyl-4'-methoxydibenzoylmethane (BM-DBM), hydroxymethylbenzoyl sulfonic acid (HMBS), 3-benzylidene camphor (3-BC), and 4-methylbenzylidene camphor (4-MBC) in the presence of butyl amine or ethanolamine as protein models were studied. Heating the reaction batches transformed BP-3 and HMBS into benzophenone imines with high yields, while DBM and BM-DBM afforded enamines and, due to α-clevages, acetophenone and benzamide derivatives. An additional UV irradiation of the reaction batches affected the product distribution in the cases of BM-DBM and DBM, but not for BP-3 and HMBS. The amine reactions generally had great influence on the UV absorption spectra. For both BP-3 and HMBS, a significant bathochromic shift together with increased absorbance was observed, thus an increased UVA protection, while the dibenzoylmethanes clearly lost UVA efficiency. According to the slight binding to the HPTLC amino layer, 3-BC and 4-MBC did not yield any reaction product with butylamine or ethanolamine.

KEYWORDS: UV filters, UV irradiance, protein binding, ketone-amine-adducts, mass spectrometry, NMR, UV absorbance

INTRODUCTION

Moderate exposure to direct sunlight has various positive effects on skin [1-5] and the human psyche [6-9], mostly associated with the generation of vitamin D_3. Besides these positive effects, different undesirable consequences such as sunburn, premature skin aging or wrinkle formation are described and attributed to extensive exposure to sunlight [10-13]. Of particular importance are serious long-term effects of extensive sun exposure, like skin cancer or irreversible eye damages, which become manifest only after several years [14-18]. To avoid such negative consequences at an early stage, there are currently 27 UV filter substances permitted for usage in cosmetic products in the European Union [19]; only a balanced combination of several UV filters offers a broad protection against UVA and UVB radiation by a sunscreen [20-22]. Therefore in 2006, the European Commission issued a recommendation on the efficacy of sunscreen products, including that the level of UVA protection provided by a product should be at least 1/3 ratio of its sun protection factor (SPF) [23]. However, the usage

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of these UV filters is not limited to special sunscreen products. A variety of daily care products like face creams, lipsticks etc. often include two or more UV filters to claim a particular UV and anti-aging protection [24, 25].

Although most of the UV filter substances are not known to be common contact allergens, there are various case reports and patch test study results over the last few years, which suspect UV filter substances becoming more and more responsible as triggers for allergies [26-32]. This is certainly a result of the steadily increasing usage of UV filters in daily care products, leading to a long contact time of UV filters with the skin. The formation of protein adducts is seen as one important step in the incidence of allergic skin reactions [33, 34]. Therefore, the identification of typical reactive groups responsible for the reaction of a substance with proteins and the underlying reaction processes were the subject of several publications [35-39]. Thus, it could be confirmed that both UV radiation as well as heating can initiate or accelerate a reaction with proteins due to the formation of reactive groups. However, UV filter substances as possible reaction partners of proteins have hardly been considered previously [40-42].

To get a first evaluation of the overall reactivity of different UV filter substances towards proteins, we developed a fast and simple screening method using an HPTLC amino plate as protein model system [43]. The screening results showed that the studied UV filters significantly differ in their reaction potential and their response to different reaction initiators such as heating or UV irradiation.

The aim of the present study was to further explore the underlying reaction processes for the UV filters benzophenone-3 (BP-3), hydroxyl-methylbenzoyl sulfonic acid (HMBS), 3-benz-ylidene camphor (3-BC), 4-methylbenzylidene camphor (4-MBC), 4-t-butyl-4'-methoxydibenz-oylmethane (BM-DBM), and the unsubstituted dibenzoylmethane (DBM), all providing keto or diketo groups. As reaction partners and simple models for amino acids or proteins, two primary amines, ethanolamine and butylamine, were selected. The obtained reaction products formed under different conditions were identified and examined for their influence on the UV spectra of the UV filters.

MATERIALS AND METHODS

4-*t*-Butyl-4'-methoxydibenzoylmethane (BM-DBM, Eusolex 9020), 1,3-diphenylpropan-1,3-dion (≥98%) (dibenzoylmethane, DBM), benzophenone-3 (BP-3, Eusolex 4360) and methanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). 3-Benzylidene camphor (3-BC) was obtained from Chemos GmbH (Regenstauf, Germany). Hydroxymethylbenzoyl sulfonic acid (HMBS, Uvinul MS 40), ethanolamine (≥99%) and toluene-4-sulfonic acid monohydrate (~99%) were obtained from Fluka (Neu-Ulm, Germany). Acetonitrile (HPLC grade) and dimethyl sulfoxide (DMSO) (HPLC grade) were purchased from Carl Roth (Karlsruhe, Germany). Butyl amine (≥99.5%), dimethyl sulfoxide-d_6, ammonium formate (≥99.0%) and deutero-chloroform ($CDCl_3$) were purchased from Aldrich (Steinheim, Germany). 4-Methylbenzylidene camphor (4-MBC) was synthesized according to a previously published method [43].

High-performance liquid chromatography (HPLC)

HPLC analyses were performed on an 1100 liquid chromatograph (Agilent, Waldbronn, Germany), consisting of a degasser (G 1315A), a quaternary HPLC pump (G 1311A), an autosampler (G 1313A), a column oven (G 1316A) set to 30 °C, and a diode array detector (G 1315B). DAD detection wavelengths were 275 nm, 313 nm and 360 nm (spectral bandwidth (SBW) 8 nm), while the reference wavelength was 500 nm (SBW 8 nm). Data processing was performed by Agilent ChemStation software (rev. A.04.02). As stationary phase, a Eurospher 100-5 C 18 HPLC column, 250 mm x 3 mm (Knauer, Berlin, Germany) was used. The mobile phase (0.5 mL/min) consisted of 10 mM ammonium formate buffer pH 4.0 (A) and acetonitrile (B). Gradient % A (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)

The LC/MS system consisted of an identical Agilent 1100 chromatograph as described above,

coupled to a G1956B MSD single-quadrupole mass spectrometer (Agilent) equipped with an electrospray ionization (ESI) interface, operated under the following conditions: capillary voltage 4 kV, skimmer voltage 35 V, source temperature 100 °C, nebulizer gas pressure 20 psig, drying gas temperature 300 °C, drying gas flow rate 10 L/min^{-1}, fragmentor voltage 80 V, gain 1, threshold 100, step size 0.1. Data processing for MS measurements was carried out with ChemStation software (Agilent). Mass spectra were recorded in the positive and negative (for HMBS) full scan mode (*m/z* 50-1000). Column and gradient were as described under section HPLC.

Spectroscopy

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

^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity Inova-300 spectrometer (Varian, Darmstadt, Germany) at 300 MHz (^{1}H) and 75 MHz (^{13}C). The samples were dissolved in $CDCl_3$, or DMSO-d_6. The signal assignments were based on chemical shifts related to tetramethylsilane (TMS) and H-H and C-H correlation data; s = singlet, d = dublet, t = triplet, q = quartet, and m = multiplet.

Thermal reaction of UV filters with amines

The respective UV filter (0.5 mmol) was weighed into a 10-mL screw-capped glass tube (SCHOTT AG, Mainz, Germany) and suspended in acetonitrile (5 mL). Toluene-4-sulfonic acid monohydrate (1 mg, 5 µmol) and 1 mL of butyl amine (10 mmol) or 0.8 mL of ethanolamine (10 mmol) were added. After heating the mixture for 3 h at 40 or 80 °C, the reaction was stopped by cooling the tubes under running tap water. Additionally, a reaction batch was stored in the dark at ambient temperature.

To determine if the amount of amine has an impact on the reaction, different amounts of ethanolamine were used (equimolar, 2.5-fold, and 5-fold excess for BP-3 and HMBS, and 5-fold, 10-fold, and 20-fold excess for BM-DBM and DBM).

Photoreaction of UV filters in the presence of amines

The respective UV filter (2 mmol) was weighed into a 50-mL quartz beaker (diameter 38 mm, Th. Geyer, Renningen, Germany) and suspended in acetonitrile (20 mL). Toluene-4-sulfonic acid monohydrate (3.8 mg, 20 µmol) and either butyl amine or ethanolamine (40 mmol) were added, and the beaker was covered by a teflon cap and irradiated for 3 h. To maintain a constant temperature (20 °C or 60 °C), the beaker was placed in a chamber of quartz glass, which was flushed with water provided by a chiller (Model RML 6, Lauda, Germany). Irradiation was performed by a modified sun simulator SOL 500 with a metal halide lamp (430 W) (Dr. Höhnle, Gräfelfing, Germany). The modification consisted of the replacement of the front filter glass by an aluminum plate with two gaps (each 16 cm^2) to hold WG 295 glass filters (Schott, Mainz, Germany). The irradiation intensity was 0.55 mW/cm^2 in the UVB and 12.5 mW/cm^2 in the UVA range. The corresponding light doses for 3 hours of irradiation were 1410 kJ/m^2 (2.3 kJ/quartz beaker). The solutions were stirred with a magnetic stirrer, Variomag Micro (Thermo Scientific). To distinguish between the effect of UV radiation or heat on the reaction, a second identical batch was prepared in another quartz beaker, which was completely covered by aluminum foil and placed aside the irradiated sample.

Isolation of reaction products

The reaction solutions were evaporated at a temperature of 35 °C to dryness in a LABCONCO (Kansas City, USA) CentriVap concentrator equipped with a CentriVap cold trap. For HMBS and BP-3, the obtained residues could be directly used for NMR spectroscopy. For the DBM batches, the residues were taken up in 5 mL methanol, and 1 mL of the methanolic solution was subjected to preparative HPLC (five injections). The HPLC system consisted of a Kronlab (Sinsheim, Germany) HD 2-200 HPLC pump, a Variable Wavelength Monitor (Knauer,

Berlin, Germany), a C-R3A Chromatopac integrator (Shimadzu), and a YMC (Dinslaken, Germany) HPLC column (ODS-A, RP 18, 5 µm, 20 mm x 25 cm). The eluent was acetonitrile/water (60/40) for 14 min followed by flushing the column with pure acetonitrile for 3 min. The flow rate was 8 mL/min, the detection wavelength 275 nm.

Reaction products isolated from batches at 80 °C for three hours

BP-3

2-[(Z)-[(2-Hydroxyethyl)imino](phenyl)methyl]-5-methoxyphenol. 133 mg (98% yield) of pure **1a** were obtained as yellow, viscous liquid. UV/Vis (methanol) λ_{max} (nm) (log ε) 302 (4.11), 384 (3.87). IR (ATR) ν (cm^{-1}): 3380-3050 (m), 3075 (w), 2940-2850 (w), 2860 (w), 1589 (s), 1535 (m), 1485 (m), 1464 (m), 1343 (w), 1261 (w), 1232 (s), 1113 (m), 1076 (w), 1031 (w), 972 (w), 831 (m), 772 (m), 698 (m). LC-MS (ESI^+) (t_R = 6.07) *m/z* (relative intensity) = 543 ($2MH^+$, 10), 272 (MH^+, 100), 106 (55). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm) 16.14 (NH, bs, 1H), 7.51 (m, 3H, ^{3}J = 2.88 Hz), 7.27 (m, 2H, ^{3}J = 3.45 Hz), 6.62 (d, 1H, ^{3}J = 9.2 Hz), 6.32 (d, 1H, ^{3}J = 2.5 Hz), 6.07 (dd, 1H, ^{3}J = 9.0, 2.3 Hz), 3.82 (t, 2H, ^{3}J = 5.4 Hz), 3.78 (s, 3H), 3.42 (t, 2H, ^{3}J = 5.37 Hz). ^{13}C-NMR ($CDCl_3$, 300 MHz) δ (ppm) 174.6, 172.3, 164.9, 133.2, 132.5, 129.5, 129.4, 128.7, 127.7, 105.8, 102.2, 61.6, 55.2, 50.9.

2-[(Z)-(Butylimino)(phenyl)methyl]-5-methoxyphenol. 139 mg (98% yield) of pure **1b** were obtained as yellow, very viscous liquid. UV/Vis (methanol) λ_{max} (nm) (log ε) 302 (4.12), 385 (3.94). IR (ATR) ν (cm^{-1}): 3338 (w), 3077 (w), 2953 (m), 2931 (m), 2864 (w), 1595 (s), 1535 (m), 1490 (w), 1434 (w), 1343 (w), 1268 (m), 1209 (m), 1165 (m), 1113 (m), 1076 (w), 1031 (m), 968 (w), 842 (w), 804 (w), 775 (w), 708 (m). LC-MS (ESI^+) (t_R = 14.72) *m/z* (relative intensity) = 589 ($2MH^+$, 10), 284 (MH^+, 100), 106 (50). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.50 (m, 3H), 7.26 (m, 2H), 6.65 (dd, 1H, ^{3}J = 9.1, 2.4 Hz), 6.37 (d, 1H, ^{3}J = 2.4 Hz), 6.11 (dd, 1H, ^{3}J = 9.1, 2.4 Hz), 3.78 (s, 3H), 3.27 (t, 2H, ^{3}J = 6.8 Hz), 1.61 (m, 2H), 1.39 (m, 2H), 0.88 (t, 3H, ^{3}J = 7.3 Hz). ^{13}C-NMR ($CDCl_3$, 300 MHz) δ (ppm) 173.4, 173.1, 165.0, 133.2, 132.9, 129.7, 129.6, 128.9, 127.8, 105.8, 102.5, 55.5, 48.3, 32.7, 20.5, 13.9.

HMBS

4-Hydroxy-5[(Z)-[(2-hydroxyethyl)imino](phenyl)methyl]-2-methoxybenzenesulfonic acid. 170 mg (97% yield) of pure **2a** were obtained as light yellow, fine powder. UV/Vis (methanol) λ_{max} (nm) (log ε) 303 (4.05), 384 (3.85). IR (ATR) ν (cm^{-1}): 3450-3250 (m), 2930 (w), 2871 (w), 1578 (s), 1534 (m), 1491 (w), 1430 (w), 1415 (w), 1306 (w), 1219 (s), 1176 (s), 1078 (s), 1014 (s), 834 (w), 779 (w), 749 (w), 687 (m), 601 (m). LC-MS (ESI^-) (t_R = 2.56) *m/z* (relative intensity) = 350 (M^-, 100). ^{1}H-NMR (DMSO-*d6*, 300 MHz) δ (ppm) 7.58 (m, 3H), 7.32 (m, 2H), 7.13 (s, 1H), 6.20 (s, 1H), 3.72 (s, 3H), 3.38 (t, 2H, ^{3}J = 5.4 Hz), 3.55 (t, 2H, ^{3}J = 5.4 Hz); ^{13}C-NMR (DMSO-*d6*, 300 MHz) δ (ppm) 174.5, 174.0, 162.2, 133.0, 132.4, 130.3, 129.5, 128.3, 125.5, 109.7, 101.9, 60.9, 56.0, 51.1.

5[(Z)-(Butylimino)(phenyl)methyl]-4-hydroxy-2-methoxybenzenesulfonic acid. 178 mg (98% yield) of pure **2b** were obtained as yellow, fine powder. UV/Vis (methanol) λ_{max} (nm) (log ε) 303 (4.08), 383 (3.89). IR (ATR) ν (cm^{-1}): 3450-3420 (w), 2957 (m), 2925 (m), 2871 (w), 1578 (s), 1520 (m), 1486 (w), 1472 (w), 1420 (w), 1225 (s), 1171 (s), 1078 (s), 1013 (s), 921 (w), 825 (w), 774 (w), 745 (w), 686 (w), 604 (w). LC-MS (ESI^-) (hR_F = 2.96) *m/z* (relative intensity) = 362 (M^-, 100). ^{1}H-NMR (DMSO-*d6*, 300 MHz) δ (ppm) 7.59 (m, 3H), 7.32 (m, 2H), 7.13 (s, 1H), 6.20 (s, 1H), 3.73 (s, 3H), 3.21 (t, 2H, ^{3}J = 6.7 Hz), 1.51 (m, 2H), 1.30 (m, 2H), 0.84 (t, 3H, ^{3}J = 7.3 Hz); ^{13}C-NMR (DMSO-*d6*, 300 MHz) δ (ppm) 174.1, 174.0, 162.2, 132.9, 132.2, 130.4, 129.6, 128.1, 125.5, 109.6, 102.0, 56.1, 47.8, 29.7, 20.2, 14.1.

DBM

***N*-(2-Hydroxyethyl)benzamide.** 45 mg (54 mol%) of pure **3a** were obtained as light-brown, very viscous liquid. UV/Vis (isopropanol) λ_{max} (nm) (log ε) 226 (4.10). IR (ATR) ν (cm^{-1}) 3380-3100 (s), 3057 (w), 2938 (w), 2872 (w), 2360 (w), 1632 (s), 1536 (s), 1491 (m), 1425 (w), 1306 (m), 1217 (w), 1068 (m), 801 (w), 712 (w), 690 (w). LC-MS (ESI^+) (t_R = 2.72) *m/z* (relative intensity) = 353 ($2MNa^+$, 10), 166 (MH^+, 100), 106 (5). ^{1}H-NMR (DMSO-*d6*, 300 MHz) δ (ppm) 8.41 (t, 1H, ^{3}J = 5.0 Hz), 7.81 (m, 2H), 7.47 (m, 3H), 4.83 (s, 1H), 3.50 (m, 2H), 3.32 (m, 2H).

***N*-Butylbenzamide.** 23 mg (26 mol%) of pure **3b** were obtained as dark-brown, viscous liquid. UV/Vis (isopropanol) λ_{max} (nm) (log ε) 225 (4.08). IR (ATR) ν (cm^{-1}) 3350-3250 (m), 3061 (w), 2957 (m), 2935 (m), 2868 (m), 2364 (w), 1636 (s), 1532 (s), 1494 (w), 1464 (w), 1310 (m), 1146 (w), 1080 (w), 1028 (w), 849 (w), 805 (w), 694 (m); LC-MS (ESI^+) (t_R = 4.47) *m/z* (relative intensity) = 355 ($2MH^+$, 10), 178 (MH^+, 100), 106 (55). ^{1}H-NMR (DMSO-*d6*, 300 MHz) δ (ppm) 7.75 (m, 2H), 7.44 (m, 3H), 6.20 (2, 1H), 3.40 (q, 2H, ^{3}J = 13.1, 6.3 Hz), 1.59 (m, 2H), 1.42 (m, 2H), 0.95 (t, 3H, ^{3}J = 7.3 Hz); ^{13}C-NMR ($CDCl_3$, 300 MHz) δ (ppm) 167.8, 135.1, 131.5, 128.8, 127.1, 40.1, 32.0, 20.4, 14.0.

(2*Z*)-3-[(2-Hydroxyethyl)amino]-1,3-diphenyl-prop-2-en-1-one. 49 mg (36 mol%) of pure **9a** were obtained as brown, very viscous liquid. UV/Vis (isopropanol) λ_{max} (nm) (log ε) 244 (3.99), 252 (3.97), 342 (4.42). IR (ATR) ν (cm^{-1}) 3390-3180 (s), 3054 (w), 2928 (w), 2876 (w), 2364 (w), 1591 (s), 1569 (s), 1480 (m), 1435 (w), 1324 (m), 1295 (m), 1228 (w), 1153 (w), 1065 (m), 1030 (w), 887 (w), 746 (w), 694 (w); LC-MS (ESI^+) (t_R = 6.27) *m/z* (relative intensity) = 268 (MH^+, 100), 106 (50). ^{1}H-NMR (DMSO-*d6*, 300 MHz) δ (ppm) 11.46 (s, 1H), 7.88 (m, 2H), 7.43 (m, 3H), 7.40 (m, 2H), 7.39 (m, 3H), 5.78 (s, 1H), 3.74 (t, 2H, ^{3}J = 5.3 Hz), 3.38 (q, 2H, ^{3}J = 11,2, 5.6 Hz); ^{13}C-NMR ($CDCl_3$) δ (ppm) 188.8, 167.6, 140.5, 135.7, 131.1, 129.8, 128.8, 128.5, 128.1, 127.4, 94.3, 62.3, 47.2.

(2*Z*)-3-(Butylamino)-1,3-diphenylprop-2-en-1-one. 50 mg (36 mol%) of pure **9b** were obtained as orange, very viscous liquid. UV/Vis (isopropanol) λ_{max} (nm) (log ε) 243 (4.09), 349 (4.39). IR (ATR) ν (cm^{-1}) 3065 (m), 2953 (m), 2924 (m), 2872 (m), 2660 (s), 1588 (s), 1573 (s), 1477 (m), 1328 (m), 1298 (m), 1217 (m), 1143 (s), 1054 (s), 1034 (s), 1002 (s), 927 (s), 742 (m), 690 (m), 616 (s); LC-MS (ESI^+) (t_R = 14.51) *m/z* (relative intensity) = 280 (MH^+, 100), 106 (10). ^{1}H-NMR (DMSO-*d6*, 500 MHz) δ (ppm) 11.43 (s, 1H), 7.88 (m, 2H, ^{3}J = 7.8 Hz), 7.44 (m, 3H), 7.40 (m, 1H), 7.42 (m, 2H), 7.38 (m, 2H), 5.74 (s, 1H), 3.21 (q, 2H, ^{13}J = 13.0, 6.6 Hz), 1.56 (m, 2H), 1.37 (m, 2H), 0.87 (t, 3H, ^{3}J = 7.2 Hz); ^{13}C-NMR ($CDCl_3$, 500 MHz) δ (ppm) 188.8, 167.6, 140.5, 135.7, 131.1, 129.8, 128.8, 128.4, 127.9, 127.3, 93.4, 44.7, 33.1, 20.2, 13.9.

1-Phenylethanone (acetophenone). 30 mg (59 mol%) and 15 mg (25 mol%) of pure **5** were obtained as colourless viscous liquid from the reaction with ethanolamine and butylamine, respectively. UV/Vis (isopropanol) λ_{max} (nm) (log ε) 240 (4.1), 319 (1.7). LC-MS (ESI^+) (t_R = 1.70) *m/z* (relative intensity) = 241 ($2MH^+$, 3), 121 (MH^+, 100), 106 (6). IR (ATR) ν (cm^{-1}) 3600 (w), 3352 (w), 3090-2870 (m), 1685 (s), 1601 (s), 1588 (m), 1451 (s), 1432 (m), 1362 (s), 1315 (m), 1270 (s), 1182 (m), 1080 (m), 967 (m), 762 (s), 691 (s), 589 (s). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.92 (m, 2H), 7.53-7.36 (m, 3H), 2.55 (s, 3H); ^{13}C-NMR ($CDCl_3$, 300 MHz) δ (ppm) 196.1, 136.7, 131.8, 128.5, 128.4, 24.9.

By-products assigned by mass spectrometry:

2-{[(1*E*)-1-Phenylethylidene]amino}ethanol (12a). LC-MS (ESI^+) (t_R = 4.71) *m/z* (relative intensity) = 327 ($2MH^+$, 4), 164 (MH^+, 100), 148 (5).

***N*-[(1*E*)-1-Phenylethylidene]butan-1-amine (12b).** LC-MS (ESI^+) (t_R = 5.96) *m/z* (relative intensity) = 351 ($2MH^+$, 3), 176 (MH^+, 100), 106 (50).

BM-DBM

***N*-(2-Hydroxyethyl)-4-methoxybenzamide (4a).** LC-MS (ESI^+) (t_R = 1.56), *m/z* (relative intensity) = 391 ($2MH^+$, 14), 196 (MH^+, 100), 135 (5), 106 (3).

***N*-Butyl-4-methoxybenzamide (4b).** LC-MS (ESI^+) (t_R = 4.46), *m/z* (relative intensity) = 415 ($2MH^+$, 17), 208 (MH^+, 100), 135 (4), 106 (3).

1-(4-*tert*-Butylphenyl)ethanone (6). LC-MS (ESI^+) (t_R = 2.11), *m/z* (relative intensity) = 353 ($2MH^+$, 81), 177 (MH^+, 100), 106 (2).

4-*tert*-Butyl-*N*-(2-hydroxyethyl)benzamide (7a). LC-MS (ESI^+) (t_R = 17.86), *m/z* (relative intensity) = 443 ($2MH^+$, 48), 222 (MH^+, 100), 106 (2).

***N*-Butyl-4-*tert*-butylbenzamide (7b).** LC-MS (ESI^+) (t_R = 8.27), *m/z* (relative intensity) = 467 ($2MH^+$, 35), 234 (MH^+, 100), 106 (2).

1-(4-Methoxyphenyl)ethanone (8). LC-MS (ESI^+) (t_R = 1.96), *m/z* (relative intensity) = 301 ($2MH^+$, 75), 151 (MH^+, 100), 106 (4).

(2*Z*)-3-[(2-Hydroxyethyl)amino]-1-(4-*tert*-butyl-phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (10a). LC-MS (ESI^+) (t_R = 4.62), *m/z* (relative intensity) = 707 ($2MH^+$, 46), 354 (MH^+, 100).

(2*Z*)-3-(2-Butylamino)-1-(4-*tert*-butylphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (10b). LC-MS (ESI^+) (t_R = 20.66), *m/z* (relative intensity) = 731 ($2MH^+$, 43), 366 (MH^+, 100).

(2*Z*)-3-[(2-Hydroxyethyl)amino]-3-(4-tert-butylphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (11a). LC-MS (ESI^+) (t_R = 5.41), *m/z* (relative intensity) = 707 ($2MH^+$, 76), 354 (MH^+, 100).

2*Z*)-3-(2-Butylamino)-3-(4-*tert*-butylphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (11b). LC-MS (ESI^+) (t_R = 21.82), *m/z* (relative intensity) = 731 ($2MH^+$, 82), 366 (MH^+, 100).

2-{[(1*E*)-1-(4-*tert*-Butylphenyl)ethylidene]amino} ethanol (13a). LC-MS (ESI^+) (t_R = 20.58), *m/z* (relative intensity) = 439 ($2MH^+$, 20), 220 (MH^+, 100).

***N*-[(1*E*)-1-(4-*tert*-Butylphenyl)ethylidene]butan-1-amine (13b).** LC-MS (ESI^+) (t_R = 22.20), *m/z* (relative intensity) = 463 ($2MH^+$, 18), 232 (MH^+, 100).

2-{[(1*E*)-1-(4-Methoxyphenyl)ethylidene]amino} ethanol (14a). LC-MS (ESI^+) (t_R = 5.38), *m/z* (relative intensity) = 387 ($2MH^+$, 26), 194 (MH^+, 100).

***N*-[(1*E*)-1-(4-Methoxyphenyl)ethylidene]butan-1-amine (14b).** LC-MS (ESI^+) (t_R = 1.82), *m/z* (relative intensity) = 411 ($2MH^+$, 22), 206 (MH^+, 100).

RESULTS AND DISCUSSION

During the reactions of the selected UV filters (Table 1) with butylamine or ethanolamine, different temperatures (20, 40, 60, and 80 °C) were selected. The higher temperatures should enforce the reactions and increase the yield of products, while the lower temperatures should assure that reactions also took place at moderate terms, which can be reached on the skin surface in direct summer midday sunlight within 15–20 min [44]. Comparing the two primary amines, it generally was observed that ethanolamine was clearly more reactive than butylamine (Figure 1), which is difficult to explain, but might be attributable to the inductive effect of the hydroxyl group.

BP-3 and HMBS showed the highest reaction rates with both amines (Figure 2). Already 60 min at 80 °C led to a nearly complete conversion with ethanolamine (Figure 2A). With butylamine, the same conversion was achieved only after 2 hours (Figure 2B). In the presence of ethanolamine, the same results were also obtained after 3 h at 40 °C and even at room temperature (Figure 1B), while in the presence of butylamine at room temperature a conversion of only about 40% (Figure 1A) was obtained. As compared to benzophenones, the reaction rates of the dibenzoylmethanes were clearly lower (Figure 2). Additionally, there was no apparent spontaneous conversion at room temperature, but heating to 40 °C significantly increased the turnover in the presence of both ethanolamine and butylamine (Figure 1A/B).

As should be expected, the amount of amine used for the reaction strongly influenced the reaction rates; the more amine, the faster the reaction. After 1 hour heating at 40 °C in the presence of equimolar amounts of ethanolamine, nearly 30% of BP-3 was transformed (Figure 3) . With a 2.5-fold or a 5-fold excess of ethanolamine, the reaction conversion doubled or even tripled. For HMBS, the results were nearly the same (Figure 3). In case of the dibenzoylmethanes, however, an excess of amines only had a minor effect on the conversions (Figure 3), which is in agreement with the generally lower reactivity.

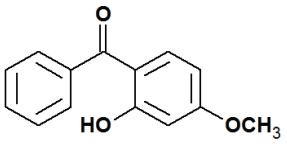
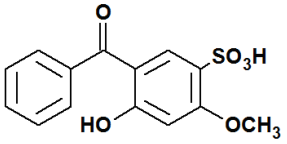
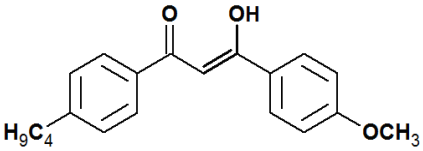
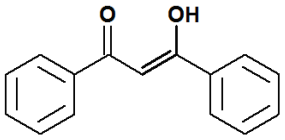
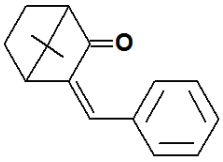
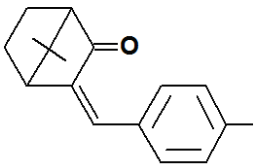
An additional UV irradiation of the reaction batches had no influence on the reactions of BP-3 and HMBS (Figure 1). For the dibenzoylmethanes, additional radiation led to a slightly increased conversion of up to 5% and 6% for DBM and BM-DBM, respectively, both at room temperature and at 60 °C, when the difference was more pronounced at ambient temperature (Figure 1).

In contrast to the highly reactive benzophenones, the two camphor derivatives 3-BC and 4-MBC (both also ketones) did not afford detectable reaction products under the used conditions with both amines. HPLC analyses resulted in recoveries of >98% of the UV filters in any case. The differences in the reaction behaviour obviously depend on sterical hindrance or on the cyclic keto group. During the former HPTLC screening, 3-BC and 4-MBC also showed only a slight binding to the amino phase [43].

Reaction products

The reaction of BP-3 and HMBS with both amines only led to the respective imines **1a/b** and **2a/b** (Figure 4). Any by-products could not be detected, and an additional UV irradiation had also no influence on the reactions of both BP-3 and HMBS.

Table 1. UV filter substances under study.

Name (shortcut)	Chemical structure
Benzophenone-3 (BP-3)	
Hydroxymethylbenzoyl sulfonic acid (HMBS)	
4-t-Butyl-4'-methoxydibenzoylmethane (BM-DBM)	
Dibenzoylmethane (DBM)	
3-Benzylidene camphor (3-BC)	
4-Methylbenzylidene camphor (4-MBC)	

BM-DBM, however, afforded a multiplicity of reaction products (Figure 5), which were assigned by LC-MS. The benzamides with both methoxy (**4a/b**) and *t*-butyl substituents (**8a/b**), the corresponding acetophenone derivatives (**6** and **7**), and the constitution isomers of the enamines **10a/b** and **11a/b** were detected. In the presence of an amine excess, two imines (**13a/b** and **14a/b)** were additionally identified, resulting from a further reaction of products **6** and **7** with the amines.

For an easier preparative isolation of the different reaction products and clear confirmation by NMR spectroscopy, the unsubstituted DBM was chosen. The two amides **3a/b**, the cleavage product acetophenone (**5**), and the enamines **9a/b** unequivocally could be identified (Figure 5).

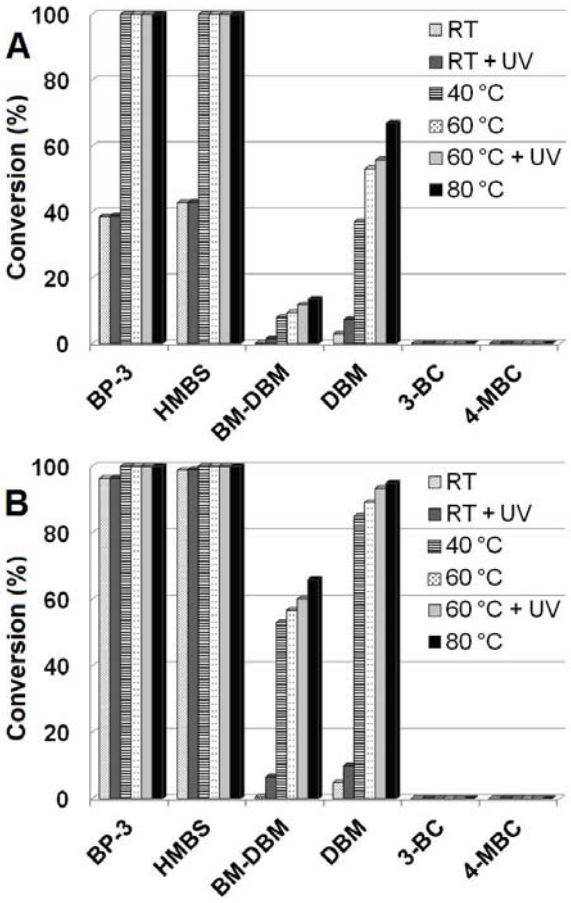


Figure 1. Conversion rates for the reactions of the studied UV filter substances with butylamine (A) or ethanolamine (B) after 3 hours under different conditions; RT (room temperature), UV (UV irradiation).

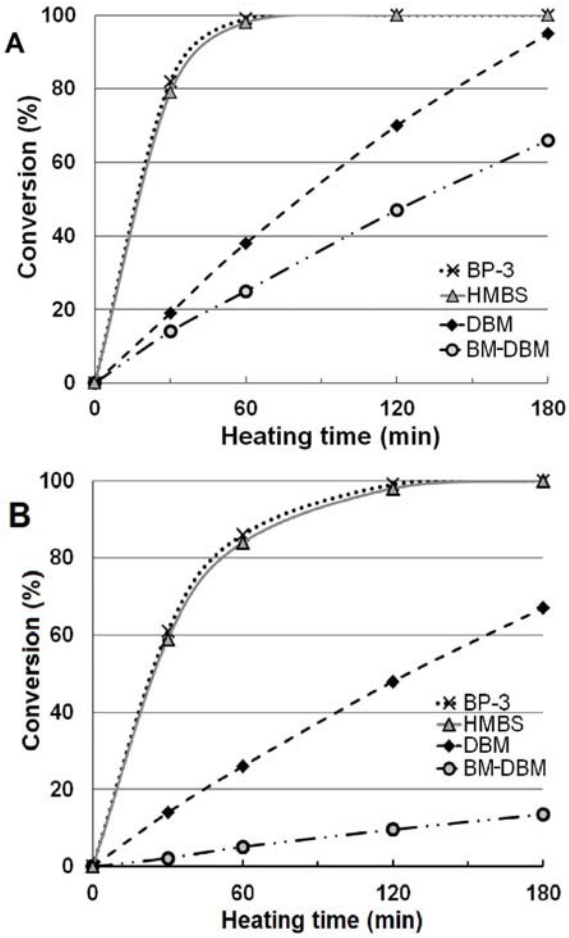


Figure 2. Reactions rates of the studied UV filter substances in the presence of ethanolamine (A) or butylamine (B) at 80 °C.

The additional imine by-products **12a/b** were assigned by LC-MS.

As for the imine formation of BP-3 and HMBS, the formation of the dibenzoylmethane enamines generally was depending on temperature and the reaction time. Accordingly, after 3 hours at room temperature, enamines were not detectable (Figure 6). Heating at 60 °C yielded the respective enamines as main products, while an additional UV irradiation had no significant influence on their amount. Contrarily, both temperature and irradiation affected the formation of the amide products (Figure 6). At 20 °C, irradiation of the ethanolamine reaction batch yielded 10 and 7 mol% amides from DBM and BM-DBM, respectively. Also at 60 °C, the additional UV irradiation resulted in slightly increased amide formation (Figure 1).

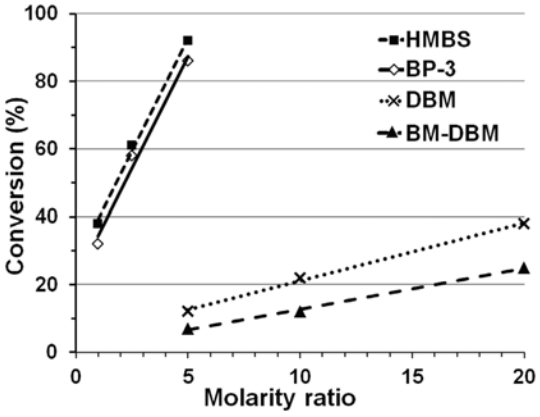
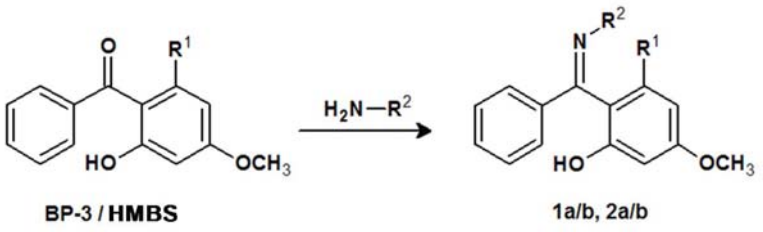
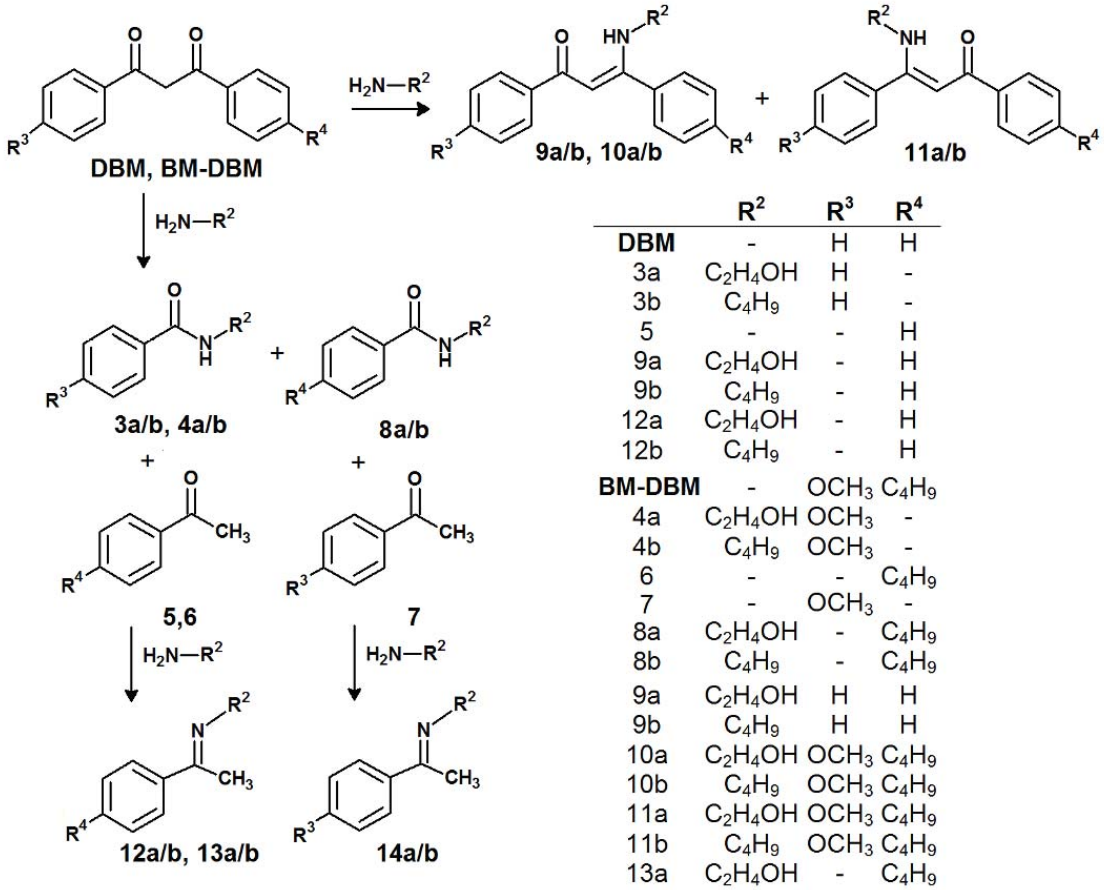


Figure 3. Conversion rates of the studied UV filter substances in the presence of ethanolamine at different molar ratios after 1 hour at 40 °C (BP-3 and HMBS) and at 80 °C (BM-DBM and DBM).



	R^1	R^2
BP-3	H	-
1a	H	C_2H_4O
1b	H	C_4H_9
HMBS	SO_3H	-
2a	SO_3H	C_2H_4O
2b	SO_3H	C_4H_9

Figure 4. Reaction products of BP-3 and HMBS with butylamine and ethanolamine.



	R^2	R^3	R^4
DBM	-	H	H
3a	C_2H_4OH	H	-
3b	C_4H_9	H	-
5	-	-	H
9a	C_2H_4OH	-	H
9b	C_4H_9	-	H
12a	C_2H_4OH	-	H
12b	C_4H_9	-	H
BM-DBM	-	OCH_3	C_4H_9
4a	C_2H_4OH	OCH_3	-
4b	C_4H_9	OCH_3	-
6	-	-	C_4H_9
7	-	OCH_3	-
8a	C_2H_4OH	-	C_4H_9
8b	C_4H_9	-	C_4H_9
9a	C_2H_4OH	H	H
9b	C_4H_9	H	H
10a	C_2H_4OH	OCH_3	C_4H_9
10b	C_4H_9	OCH_3	C_4H_9
11a	C_2H_4OH	OCH_3	C_4H_9
11b	C_4H_9	OCH_3	C_4H_9
13a	C_2H_4OH	-	C_4H_9
13b	C_4H_9	-	C_4H_9
14a	C_2H_4OH	OCH_3	-
14b	C_4H_9	OCH_3	-

Figure 5. Reaction products of DBM and BM-DBM with butylamine and ethanolamine.

Influence of amine reactions on the UV spectra

For BP-3 and HMBS, the bonded amines participate in the resonance delocalisation process, which resulted in strong bathochromic shifts (Figure 7). A strong increase of absorbance in the UVA range was calculated for both the butylamine and ethanolamine reaction batches, which was at the expense of UVB absorption, but nevertheless resulted in an increased absorption of approximately 10% for the whole UVA + B range (Table 2).

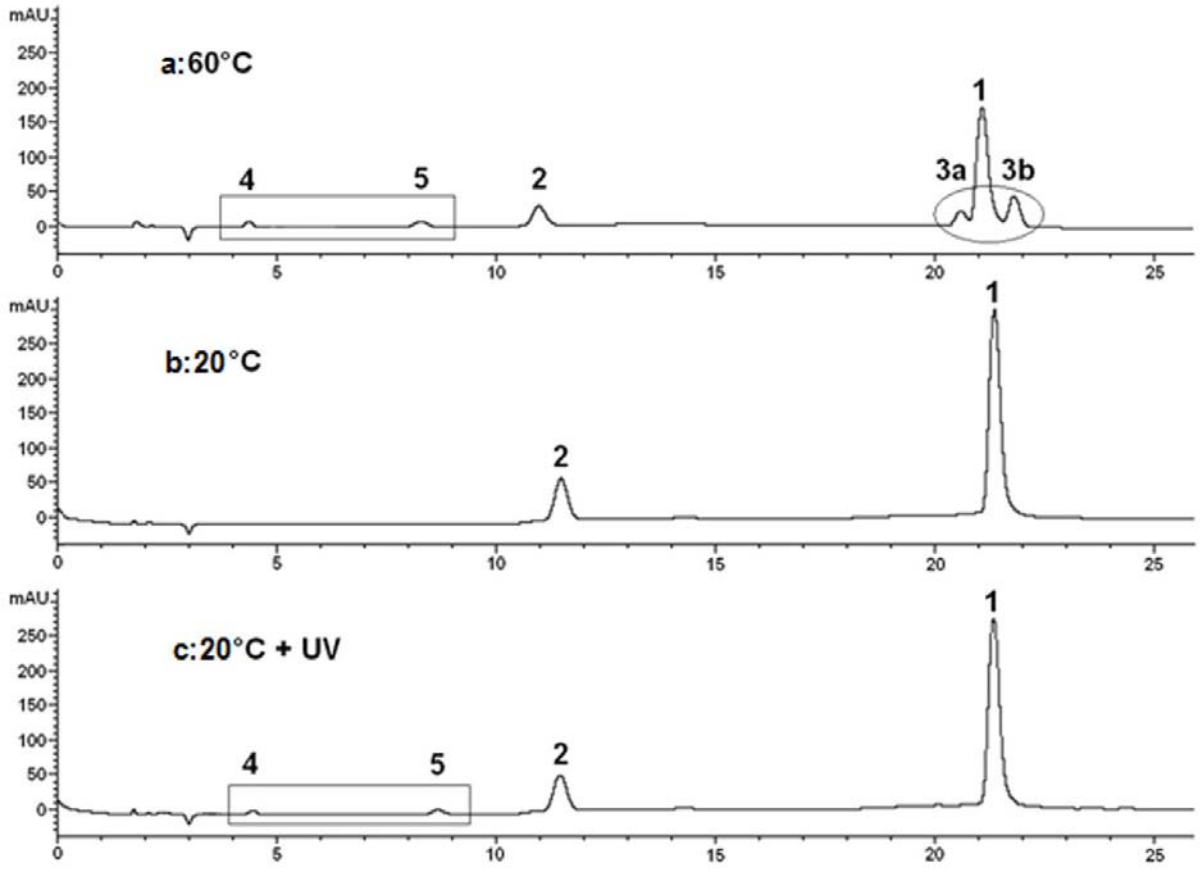


Figure 6. HPLC chromatograms of reaction batches of BM-DBM with butylamine after (a) heating for 3 hours at 60 °C under light protection, (b) 3 hours storage in the dark at 20 °C, and (c) UV irradiation at 20 °C. Detection wavelength: 250 nm; measured concentrations: 0.7 mmol/L.
1: BM-DBM keto-enol form
2: BM-DBM diketo form
3a /3b: enamines **10b** and **11b**
4: 4-Methoxybenzamide (**4b**)
5. 4-*t*-Butylbenzamide (**8b**)
Contrary to previous assumptions [45, 46] that the diketo form of BM-DBM arises only under irradiation, it was already detectable in untreated standard solutions at the appropriate wavelength.

Because the dibenzoylmethanes not only reacted to enamines, but also suffered bond breakages of the 1,3-diketo group, the spectral changes correlated with the conversion rate, and especially with the formation of amides (Figure 7). The isolated enamines **9a**/**9b** showed only a small bathochromic shift and a slightly reduced absorbance (log ε = 4.46 at 359 nm). Thus, the effect was not as distinct as in the case of the benzophenones. Obviously, the enamines provide a nearly identical chromophore as the keto-enol forms of dibenzoylmethanes. The amides **3a**/**3b**, however, completely lost the dibenzoylmethane chromophore, leading to a strong spectrum change. The absorption maximum was located at 225 nm and 226 nm, respectively, and the absorption coefficient decreased considerably to log ε 4.07 and 4.10 for the butylamine and ethanolamine derivatives, respectively. Therefore, the spectra of the reaction batches of both DBM and BM-DBM with ethanolamine showed a strong decrease in absorbance over the whole UVA + B range by 84 and 59%, respectively. According to the lower conversion rates, the decrease in absorbance was also lower for the reaction batches with butylamine (Table 2).

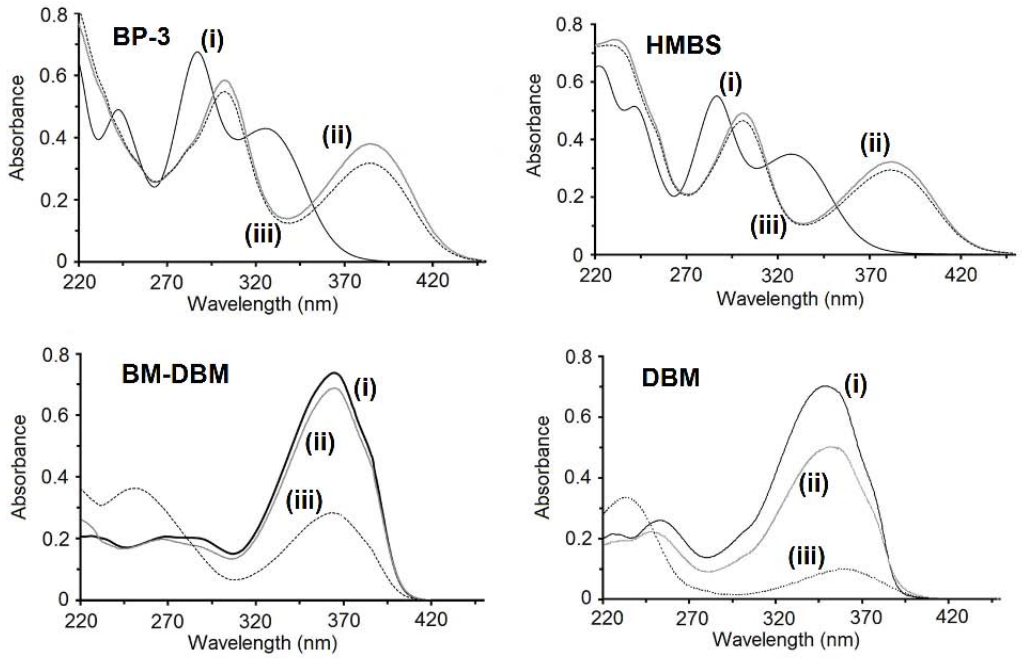


Figure 7. UV spectra of the respective UV filter standard solutions (i) and of reaction batches with butylamine (ii) and ethanolamine (iii) after 3 hours heating at 80 °C. Measured concentrations: about 5 mg/L.

Table 2. UV absorbance characteristics of the pure UV filter substances and the reactions mixtures with ethanolamine (EA) or butylamine (BA) after heating for 3 hours at 80 °C, measured at concentrations of 5 mg/L and calculated as area under the curve (AUC).

	UV-A range		UV-B range		UV-A and UV-B	
	AUC	**Percentage change**	**AUC**	**Percentage change**	**AUC**	**Percentage change**
BP-3	13.3		20.6		34.0	
+ EA	17.8	+ 34%	17.9	- 13%	35.8	+ 5%
+ BA	20.8	+ 56%	18.9	- 8%	39.7	+ 17%
HMBS	9.1		12.5		21.6	
+ EA	12.1	+ 18%	24.7	-11%	23.2	+ 7%
+ BA	13.0	+ 43%	11.6	- 7%	24.7	+ 14%
DBM	83.8		228.1		111.9	
+ EA	12.1	- 82%	2.2	- 89%	14.3	- 84%
+ BA	67.1	- 20%	19.7	- 30%	86.8	- 22%
BM-DBM	39.0		8.1		46.8	
+ EA	14.8	- 62%	4.3	- 47%	19.0	- 59%
+ BA	36.3	- 7%	7.2	- 11%	43.2	- 8%

CONCLUSION

The present study shows that the UV filter substances BP-3, HMBS, DBM, and BM-DBM (all with a functional carbonyl group) indeed were able to react with primary amines. Under the influence of heat and/or UV irradiation, the generation of different reaction products like imines, amides, and enamines could be detected, which are also to be expected in the presence of skin proteins. With a molar excess of amine

(corresponding to the conditions after application on the skin), the reaction rates increased significantly.

Reactions with primary amines clearly affect the UV spectra. In the case of DBM and BM-DBM, reactions are associated with a significant decrease of absorption strength and a loss of the UVA protection. On the contrary, for BP-3 and HMBS the conversions lead to bathochromic shifts and hence to approved UVA protection.

The observation that the camphor derivatives 3-BC and 4-MBC did not form detectable reaction products with amines under the conditions used reflects the results of our previous HPTLC screenings.

Further studies with proteins and skin models will have to show, if the results obtained in this study are transferable to more complex skin model systems. In addition, experiments with further UV filters, e. g. with ester structures, will show, whether the already developed fast screening actually allows direct conclusions about the reactivity of sunscreen filter substances with proteins. Ideally, the screening is also suitable for a first assessment of other cosmetic ingredients having moieties reactive towards proteins.

CONFLICT OF INTEREST STATEMENT

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

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