J. Soc. Cosmet. Chem., 45, 43–52 (January/February 1994)

Photochemical studies on trans-3-methylbutyl 4-methoxycinnamate[1]

A. SCHRADER, J. JAKUPOVIC, and W. BALTES, *Creachem GmbH, Cosmetic Industry Research Institute, Holzminden (A.S.), and Institute for Organic Chemistry (J.J.) and Institute for Food Chemistry (W.B.), Technical University of Berlin, Berlin, Germany.*

Received July 29, 1993.

Synopsis

The sunscreen filter substance trans-3-methylbutyl 4-methoxycinnamate was irradiated with a defined solar simulator. Initial *in vivo* irradiation under in-use conditions caused discolorations in the UV absorber. Subsequent *in vitro* irradiation resulted in seven degradation products, the structures of which were determined by means of spectroscopic methods, especially nuclear magnetic resonance (NMR) spectroscopy.

INTRODUCTION

Products containing sunscreens have in recent years often been mentioned in connection with initiation of dermal photoreactions. Photochemical degradation products of the actives are frequently among the causal factors of such reactions (1).

The toxic potential of UV-induced degradation products from sunscreens is growing in significance due to increasing demand by dermatologists for use of sunscreen agents, even in non-sunscreen products.

MATERIALS AND METHODS

IRRADIATION APPARATUS

We used an optically filtered 300 W Xenon system with six emission spots with a diameter of 1 cm^2 each (Figure 1). In the method development phase, the six spots, supplied by a single light source, allow for absorption of a radiation dose gradient that facilitates determination of the dosage to be used for the irradiation object in a single procedure.

Correlation of the emission spectrum of the solar simulator to natural sunlight is indispensable for the measurement procedure (3,4). Figure 2 shows the spectral curve of sunlight on a summer day in the wavelength range of 288–320 nm (5).

[1] Taken in part from the Ph.D. thesis of A. Schrader. A part of this work has been submitted as a preliminary elsewhere.

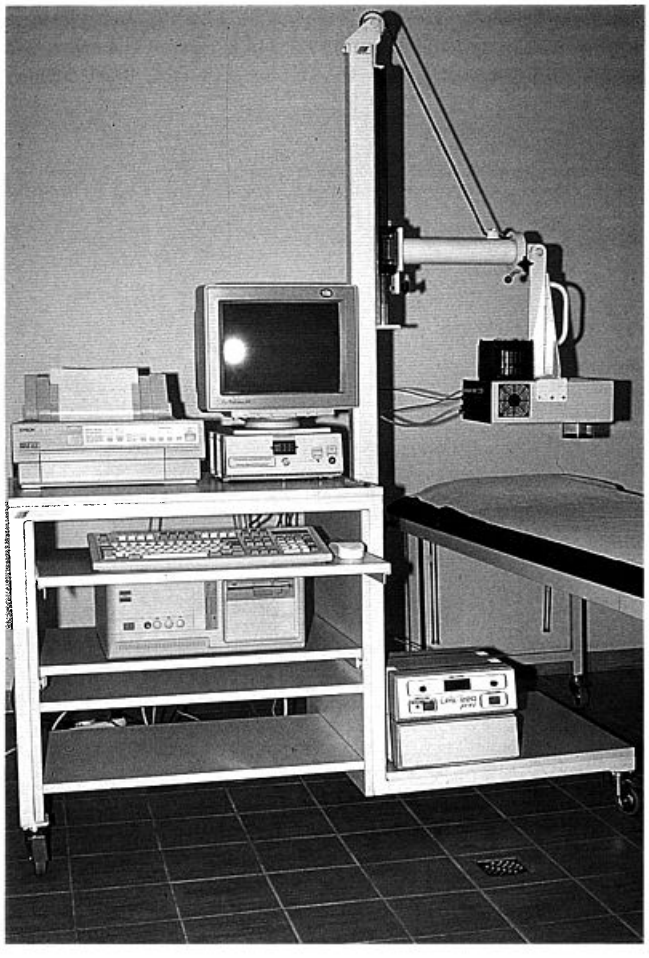

Figure 1. Overall view of the solar simulator system (2).

IRRADIATIONS AND ANALYSES

The UV-B sunscreen agent (10%) was dissolved in propan-2-ol for purposes of irradiation. For specimen irradiation, 100 µl of the UV filter solution was applied to human skin limited by a silicone-fixed glass ring (Ø2.5 cm).

Following a sunscreen agent contact time of 20 minutes, the object was irradiated by a dose of 600 mJ/cm^2 according to 10 MED. The distance to the skin was 3–5 mm. The skin field was subsequently extracted with 500 µl of propan-2-ol. To obtain larger specimen volumes, six "back extracts" per test person were combined in a single solution.

For *in vitro* irradiation a 100-ml sunscreen filter solution was placed in a quartz bowl and irradiated with direct vertical light at the same object-to-source distance as for the *in vivo* trial. The irradiation dose was 16.4 J/cm^2. The irradiation dosages for *in vitro* and *in vivo* studies are not comparable. Fractionation of the mixture was done by means of analytical TLC.

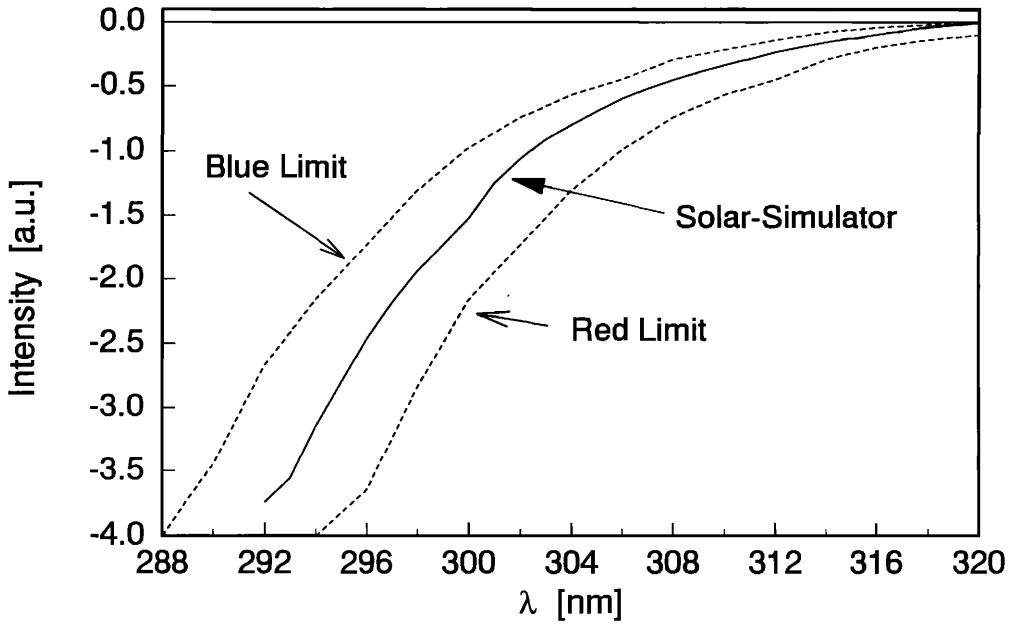

Figure 2. Emission spectrum of the solar simulator in the UV-B range.

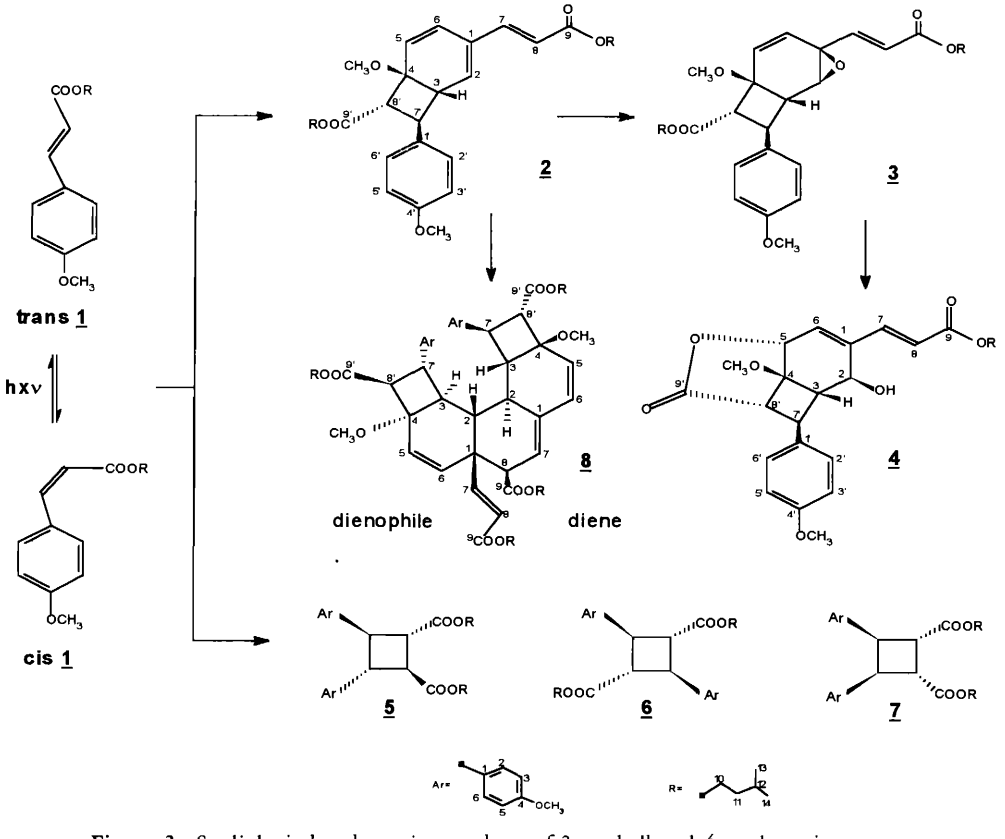

Figure 3. Sunlight-induced reaction products of 3-methylbutyl 4-methoxycinnamate.

Table I
NMR Data of 2

	^{1}H-NMR δ [ppm]	NOE	Coupling constants J [Hz]	^{13}C-NMR δ [ppm]		COLOC
H-2	6.34 (brd)	H-7 (5%) H-7′ (2%)	6.5	C-1	130.2 (s)	H-5; H-8; H-3
H-3	3.10 (ddd)	H-8′ (5%) H-2′/6′ (3%) H-2 (7%)	9.5; 6.5; 1.5	C-2	134.6 (d)	
H-5	5.82 (brd)		10.5	C-3	46.1 (d)	H-7′; H-8′
H-6	6.66 (dd)		10.5; 1.5	C-4	73.0 (s)	H-2; H-3; H-8′; 4-OMe
H-7	7.24 (d)		16	C-5	124.8 (d)	
H-8	6.08 (d)		16	C-6	126.2 (d)	H-2
H-10	4.20 (t)		7	C-7	143.6 (d)	H-2; H-3; H-2′
H-11	1.60–1.50 (m)			C-8	116.6 (d)	
H-12	1.72 (m)			C-9	167.2 (s)	H-7
H-13	0.94 (d)		7	C-10	63.2 (t)	
H-14	0.94 (d)		7	C-11	37.3 (t)	
4-OMe	3.16 (s)	H-8′ (2%) H-5 (4%)		C-1′	133.1 (s)	
H-2′/6′	7.21 (d)		8	C-2′	127.9 (d)	H-7′
	} AA′ BB′ system			C-3′	114.0 (d)	
H-3′/5′	6.87 (d)		8	C-4′	158.7 (s)	H-2′; 4′-OMe
H-7′	3.25 (dd)	H-2′/6′ (3%)		C-5′	114.0 (d)	
		H-2′/6′ (3%) H-3 (3%)	10; 9.5	C-6′	127.9 (d)	
H-10′	4.15 (m)			C-7′	41.5 (d)	
H-11′	1.60–1.50 (m)			C-8′	59.1 (d)	H-7′
H-12′	1.65 (m)			C-9′	63.5 (t)	H-8′
H-13′	0.90 (d)		7	C-11′	37.4 (t)	
H-14′	0.90 (d)		7	C-12′	25.1 (d)	
4′-OMe	3.80 (s)			C-13′	22.4 (q)	
				C-14′	22.4 (q)	
				4′-OMe	55.3 (q)	

The UV spectroscopic data were determined densitometrically with the aid of the (Camag) HPTLC. The ^{1}H NMR (400 MHz) and the ^{13}C NMR (400 MHz) spectra were recorded using the Bruker AC 400 instrument.

RESULTS

The ^{1}H NMR spectrum of the crude mixture from irradiation shows, besides the signals for the parent material, those of the corresponding cis derivatives as the main reaction product. The trans/cis ratio is 25:1, easily determined by integrating the olefinic signals of each of the isomers. The signals of the rest of the reaction products are also already detectable. Subsequent separation revealed photochemical products identified using NMR spectroscopy (Figure 3).

Photochemical product 2 represents the main component of the reaction product. The

Table II
NMR Data of 3

	^{1}H-NMR δ [ppm]	Coupling constants J [Hz]
H-2	3.545 (d)	2
H-3	2.99 (brd)	8
H-5	5.78 (dd)	10.5; 1
H-6	6.38 (d)	10.5
H-7	6.84 (d′)	16
H-8	6.15 (d)	16
H-10	4.20 (t)	7
H-11	1.60–	
H-12	1.50 (m)	
H-13	0.935 (d)	7
H-14	0.935 (d)	7
4-OMe	3.22 (s)	
H-2′/6′	7.215 } AA′BB′-System	
H-3′/5′	6.88	
H-7′	3.20 (m)	
H-8′	3.20 (m)	
H-10′	4.13 (t)	7
H-11′	1.55 (m)	
H-12′	1.65 (m)	
H-13′	0.91 (d)	7
H-14′	0.91 (d)	7
4′-OMe	3.80 (s)	

following structural features of product 2 are recognizable by their characteristic signals in the ^{1}H NMR spectrum:

- One 1,4 disubstituted aromatic (AA′BB′ system)
- One each cis and trans double bonds (10 Hz/16 Hz)
- One each aromatic and aliphatic methoxyl groups
- Two 3-methylbutyl esters
- One trisubstituted double bond (H-2)
- Three up-field shifted signals that by spin decoupling completed the sequence 2-3-7′-8′

The allylic couplings of H-2 to H-6 and H-7 facilitate the linkage of the sequences. The ^{1}H/^{13}C hetero correlated COLOC spectrum (long range correlation through two or three bonds) allows for a definitive statement. The NOE experiments also summarized in Table I established the stereochemistry. The cycloaddition takes place between the trans double bond of a cinnamic acid ester and the 3,4 double bond of the aromatic ring of a second cinnamic acid ester. A similar reaction between 4-cyanoanisole and trans-cinnamonitrile in acetonitrile solution to give bicyclo-[4.2.0.] derivatives was recently reported (6).

When compound 2 is exposed to atmospheric oxygen, it spontaneously oxidizes to form the epoxide 3. The structure of 3, which we were not able to isolate in pure form, was determined on the basis of the ^{1}H NMR spectrum of a 3:1 mixture with 2. The ^{1}H

Table III
NMR Data of 4

	^{1}H-NMR δ [ppm]	NOE	Coupling constants J [Hz]	^{13}C-NMR δ [ppm]	
H-2	4.46 (brdd)	H-3 (7%) H-7′ (6%) H-8 (20%)	5.5; 2.5	C-1	136.7 (s)
H-3	3.28 (dd)	H-2′/6′ (5%) H-2 (5%)	9; 2.5	C-2	63.2 (d)
H-5	5.14 (d)	H-6 (8%) 4-OMe (5%)	3.5	C-3	47.6 (d)
H-6	6.41 (d)		3.5	C-4	75.1 (s)
H-7	7.35 (d)		16	C-5	77.8 (d)
H-8	6.23 (d)		16	C-6	132.1 (d)
H-10	4.21 (t)		7	C-7	143.1 (d)
H-11	1.56 (dt)		7; 7	C-8	121.5 (d)
H-12	1.71 (tqq)		7; 7; 7	C-9	166.3 (s)
H-13	0.93 (d)		7	C-10	63.5 (t)
H-14	0.93 (d)		7	C-11	37.4 (t)
H-2′/6′	7.18 } AA′BB′-System			C-12	25.0 (d)
H-3′/5′	6.89			C-13	22.5 (q)
H-7′	2.66 (dd)	H-2′/6′ (5%) H-2 (4%)	9; 7	C-14	22.5 (q)
H-8′	3.21 (d)	H-2′/6′ (5%)	7	C-1′	131.7 (s)
4-OMe	3.40 (s)	H-5 (10%) H-8′ (5%)		C-2′/6′	127.7 (d)
4′-OMe	3.81 (s)			C-3′/5′	114.4 (d)
2-OH	1.84 (d)		5.5	C-4′	159.1 (s)
				C-7′	36.4 (d)
				C-8′	48.1 (d)
				C-9′	175.1 (s)
				4-OMe	51.8
				4′-OMe	55.4

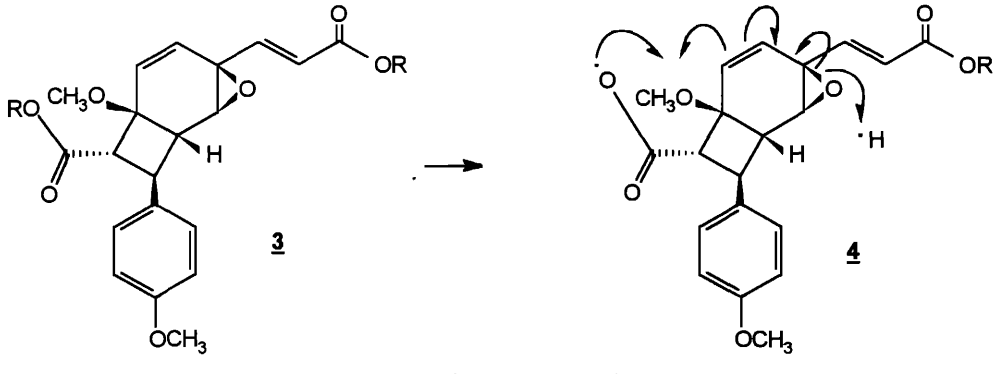

Figure 4. Formation of 4.

Table IV
NMR Data of 5, 6, and 7

	5 ^{1}H-NMR δ [ppm]	6 ^{1}H-NMR δ [ppm]	7 ^{1}H-NMR δ [ppm]	5, 6, 7 Coupling constants J [Hz]	5 ^{13}C-NMR δ [ppm]	
H-2/6	7.20	7.25	6.84		C-1	133.3 (s)
	} AA'BB' system	} AA'BB' system	} AA'BB' system		C-2/6	127.9 (d)
H-3/5	6.845	6.865	6.66		C-3/5	113.9 (d)
H-7	3.61	3.87	4.28		C-4	158.6 (s)
	} AA'BB' system	} A_2B_2 system	} AA'BB' system		C-7	46.9 (d)
H-8	3.35	3.16	4.28		C-8	45.0 (d)
H-10	4.15 (t)	4.16 (t)	4.16 (t)	7	C-9	172.7 (s)
H-11	1.51 (dt)	1.51 (dt)	1.75–	7; 7	C-10	63.6 (t)
H-12	1.64 (tqq)	1.63 (tqq)	1.50 (m)	7; 7; 7	C-11	37.3 (t)
H-13	0.90 (d)	0.89 (d)	0.92 (d)	7	C-12	25.0 (d)
H-14	0.90 (d)	0.89 (d)	0.92 (d)	7	C-13	22.5 (q)
OMe	3.79 (s)	3.80 (s)	3.71 (s)		C-14	22.5 (q)
					-O-Me	55.3 (q)

NMR data are similar to those of compound 2, with the exception of the chemical shift of H-2. Table II lists the ^{1}H NMR data.

The ready oxidizability of the material is worthy of note because of the high reactivity of epoxides. The mechanism of the formation is not known. Usually, by exposure to the molecular oxygen, the double bond reacts to form a hydroperoxide. The stereochemistry of the epoxide ring in 3 is assumed to be opposite to that of the cis-annellated cyclobutane ring. Further evidence for the stereochemistry of 3 is supplied by lactone 4, which is formed as a degradation product of compound 3. In the ^{1}H NMR spectrum of 4 (Table III) only one set of signals for the side chain appears. The sequences established by the spin decoupling correspond to those of 2 and 3, differing only in the chemical shifts of signals for the six-membered ring. The presence of a γ-lactone ring is evident from the IR-spectrum (1789 cm^{-1}). Furthermore, H-2 couples with a D_2O exchangeable signal, indicating the position of the hydroxy group. The ^{13}C NMR spectrum (Table III) is in accordance with the structure.

The signals were assigned with aid of a hetero correlated spectrum. The formation of compound 4 could be easily explained in terms of a homolytic cleavage of an ester residue leading to a reactive carboxy radical, which subsequently would give 4 according to the scheme (Figure 4). An ionic mechanism under the experimental condition is rather unlikely.

In addition to these products, minimal amounts of three truxinic/truxillic acid derivatives were found (5–7). The NMR data are listed in Table IV; the structures are based on comparison with the values in the literature (7–10).

A further identified compound that occurred in minimum amounts only was 8. The ^{1}H NMR spectrum (Table V) shows two sets of signals that correspond to type-2 cyclobutane derivatives. Starting with the H-8 signal in each set, decoupling experiments led to two sequences that can then be linked by means of coupling between H-2 protons. A dimeric dimer is thus likely to be the structure. Long-range couplings between

Table V
NMR Data of 8

	Dienophile		Diene		NOE	
	^{1}H-NMR δ [ppm]	Coupling constants J [Hz]	^{1}H-NMR δ [ppm]	Coupling constants J [Hz]	Dienophile (dp)	Diene (di)
H-2	1.60 (dd)	11; 2	2.19 (brd)	11	H-8	
H-3	2.62 (brd)	9	2.59 (brd)	9	H-2′/6′	H-2′/6′
H-5	6.01 (brd)	10.5	5.50 (brd)	10		
H-6	6.31 (d)	10.5	6.54 (d)	10		
H-7	6.84 (d)	16	6.045 (dd)	2.5; 4.5		
H-8	5.83 (d)	16	3.06 (brd)	4.5		H-7; H-6 (dp)
H-10	4.23–4.03 (m)		4.23–4.03 (m)			
H-11	1.71–		1.71–			
H-12	1.44 (m)		1.44 (m)			
H-13	0.91 (d)	7	0.88 (d)	7		
H-14	0.91 (d)	7	0.88 (d)	7		
H-2′/6′	6.92 } AA′BB′-System		7.25 } AA′BB′-System			
H-3′/5′	6.76		6.87			
H-7′	3.09 (m[a])		2.96 (dd)	10; 9		H-2; H-2′/6′
H-8′	3.09 (m[a])		3.13 (d)	10		
H-10′	4.23–4.00 (m)		4.23–4.00 (m)			
H-11′	1.71–		1.71–			
H-12′	1.44 (m)		1.44 (m)			
H-13′	0.91 (d)	7	0.88 (d)	7		
H-14′	0.91 (d)	7	0.88 (d)	7		
4-OMe	3.01 (s)		2.72 (s)		H-5	H-8′; H-5
4′-OMe	3.81 (s[b])		3.76 (s[b])			

[a] Not first order, when C_6D_6 H-7′ 3.24 dd (J = 10 Hz; 9 Hz) and H-8′ 3.20 d (J = 10 Hz) are added.
[b] Possibly interchangeable.

olefinic and aliphatic protons complete the structure formed by a Diels-Alder addition of two molecules of 2, an endo product in respect to the side chain. In the mass spectra all compounds show a similar fragmentation pattern. The data are listed in Table VI. The main pathways are retro [2 + 2] reaction, Mc-Lafferty rearrangement, and homolytic cleavage of the ester residues.

DISCUSSION

UV irradiation of 3-methylbutyl 4-methoxycinnamate causes it to isomerize in the first reaction phase to a cis/trans ratio of 1:25. The photoproduct 2 from the trans compound results directly from [2 + 2] cycloaddition. 2, however, is unstable, and is thus oxidized to molecule 3 containing an epoxide group. The quantitative ratio of photoproduct 2 to 3 is 5:3 in freshly irradiated solutions and 1:3 after storage for a number of days. Repeated measurements confirm the instability of 2. The lactone 4 described as a degradation product of 3 within the reaction mechanism probably results from radical formation. Only traces of the tetrameric molecule 8 have been detected in the very small

Table VI
Mass Data of 2–8

	2	3	4	5	6	7	8
M^+	496 (1)	512 (4)	442 (8)	496 (3)	496 (6)	496 (6)	992 (1)
	248 (84)	264 (40)	424 (3)	409 (2)	409 (1.5)	409 (2.5)	744 (2)
	194 (27)	248 (56)	411 (2)	380 (3)	380 (3)	381 (9)	496 (3.5)
	178 (100)	194 (69)	383 (2.5)	248 (100)	248 (100)	248 (87)	494 (3)
	161 (96)	178 (100)	355 (5)	178 (77)	178 (89)	178 (100)	408 (4)
		161 (77)	178 (90)	161 (21)	161 (39)	161 (62)	380 (4)
			176 (77)				248 (22)
			161 (100)				178 (71)
							161 (100)

amounts of degradation products. The genesis of 8 could plausibly be explained either thermically or photochemically. The stereoisomeric truxinic/truxillic acid derivatives 5, 6, and 7 are compounds the basic structures of which are already known and which are also derived from [2 + 2] cycloaddition parallel to the bicyclic aromatic compounds.

We have as yet not succeeded in determining a structural connection between the yellow discoloration of the sunscreen filter substance and the reaction products detected resulting from *in vivo* as well as *in vitro* irradiation. The photochemical degradation products of 3-methylbutyl 4-methoxycinnamate are colorless as confirmed by HPTLC.

Due to extremely low yields of the photoproducts from *in vivo* irradiation, the NMR results obtained were not of a definitive nature in spite of a yellowing observed on the skin as well as multiple-field concentration. The prerequisites for high analytical yields are not met using precisely defined spotlights in *in vivo* trials, the lit field being too small. In spite of this, it must be assumed that the photochemical degradation products described above are present on the skin, although in minute traces only. It is improbable that cyclobutane systems will penetrate the skin due to their molecular size and conformation. An interesting area for future study would be the investigation of the toxicological potency of these sunscreen filter substance degradation products with the aim of achieving a further improvement in cosmetic product safety.

ACKNOWLEDGMENTS

We would like to thank Mr. Thomas Schuster for helpful discussion of several of the NMR spectra.

REFERENCES

(1) S. Schauder, Lichtfilterhaltige Hautpflegepräparate in Deutschland, in *Göttinger Liste* (BMV-Verlag, 1991).
(2) Photon Technology GmbH (PTI), Wedel/Holstein Hafenstraße 32, Germany.
(3) COLIPA, *The Recommendations of the COLIPA* "Sun Protection Measurement" *Task Force,* 1992 (unpublished).
(4) J. Lowe and A. Shaath, *Sunscreens—Development, Evaluation and Regulatory Aspects* (Dekker-Verlag 1990), p. 537.

(5) R. M. Sayre, C. Cole, W. Billhimer, J. Stanfield, and R. D. Ley, Spectral comparison of solar simulators and sunlight, *Photodermatol. Photoimmunol. Photomed.* 7, 159 (1990).
(6) N. Al-Jalal and A. Gilbert, Substituent effects on the photocycloaddition reactions of anisoles to acrylonitriles, *Travaux Chimiques des Pays-Bas,* **109**(1), 21 (1990).
(7) G. Montaudo, S. Caccamese, and V. Librando, Photodimers of cinnamic acid and related compounds. A stereochemical study by NMR, *Organ. Magnet. Reson.,* **6,** 534 (1974).
(8) A. Mustafa, Dimerisation reactions in sunlight, *Chem. Rev.,* 1 (1951).
(9) L. Paolillo, H. Ziffer, and O. Buchardt, A NMR-analysis of several photodimers containing cyclobutane rings, *J. Organ. Chem.,* **35**(1), 39 (1968).
(10) D. A. Ben-Efraim and B. S. Green, The use of mid-point or average NMR chemical shifts in stereochemical assignments, *Tetrahedron,* **30,** 2357 (1974).