

Selected oxidized fragrance terpenes are common contact allergens

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Terpenes are widely used fragrance compounds in fine fragrances, but also in domestic and occupational products. Terpenes oxidize easily due to autoxidation on air exposure. Previous studies have shown that limonene, linalool and caryophyllene are not allergenic themselves but readily form allergenic products on air-exposure. This study aimed to determine the frequency and characteristics of allergic reactions to selected oxidized fragrance terpenes other than limonene. In total 1511 consecutive dermatitis patients in 6 European dermatology centres were patch tested with oxidized fragrance terpenes and some oxidation fractions and compounds. Oxidized linalool and its hydroperoxide fraction were found to be common contact allergens. Of the patients tested, 1.3% showed a positive reaction to oxidized linalool and 1.1% to the hydroperoxide fraction. About 0.5% of the patients reacted to oxidized caryophyllene whereas 1 patient reacted to oxidized myrcene. Of the patients reacting to the oxidized terpenes, 58% had fragrance-related contact allergy and/or a positive history for adverse reaction to fragrances. Autoxidation of fragrance terpenes contributes greatly to fragrance allergy, which emphasizes the need of testing with compounds that patients are actually exposed to and not only with the ingredients originally applied in commercial formulations.

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Allergenic fragrance chemicals are ubiquitous in our environment, not only in fine fragrances but also in a wide range of cosmetic, household and industrial products. Commercial perfumes may contain several hundred fragrance compounds. Fragrance mix, 8% petrolatum (pet.) (FM), included in the standard series, is the main tool used for the diagnosis of fragrance allergy. The FM contains 8 common allergens (eugenol, isoeugenol, cinnamal, cinnamic alcohol, geraniol, hydroxycitronellal, amylcinnamal and oak moss absolute) and is believed to detect 50%–70% of the fragrance allergies (1). Therefore, new markers of fragrance allergy are required.

Terpenes (mainly mono- and sesquiterpenes) are common fragrance compounds of natural origin containing double bonds and are therefore prone to oxidation on air exposure

(autoxidation) (2). *R*-(+)-Limonene (*d*-limonene) is obtained from the peels of citrus fruits and is one of the most commonly used fragrance chemicals in scented products (3, 4) (Fig. 1). Oxidative degradation of *R*-(+)-limonene was shown to take place at room temperature, turning the non-allergenic compound into a complex oxidation mixture with sensitizing capacity (5). Patch test studies on several thousand consecutive patients showed the oxidized limonene to be a common cause of contact allergy in dermatitis patients in Europe (6, 7). This is in contrast to earlier studies using pure limonene or limonene of unknown quality, which revealed very few cases (8–10).

Other terpenes than limonene used as fragrance chemicals have the same capability of formation of allergenic autoxidation products. We decided

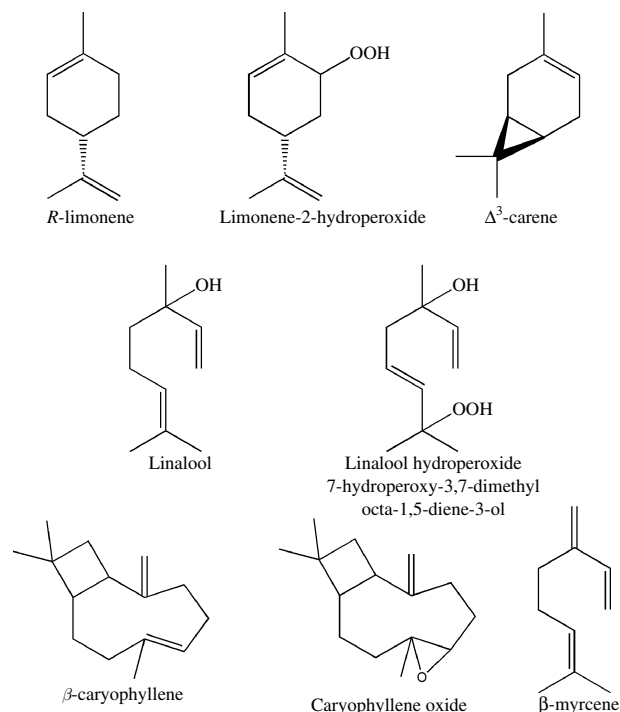


Fig. 1. Chemical structures of the compounds investigated and/or discussed in this paper.

to investigate whether contact allergy to the oxidation products of selected fragrance terpenes constitutes a common problem. Linalool (Fig. 1) is one of the most frequently used fragrance compounds in both fine fragrances and scented household products in Europe. This monoterpene is used as a fragrance chemical as such, but is also a common ingredient in essential oils, e.g. in lavender oil. In studies on the chemical composition of deodorants, it was shown that 97% of them contained linalool in concentrations up to 0.2%, whereas 61% of the so called household chemicals (soaps, surface cleaners, fabric conditioners, laundry detergents for hand wash and dish wash) investigated, contained linalool in concentrations up to 0.04% (4, 11). Furthermore, linalool was found to be present in 91% of analysed cosmetic products on the Dutch market (12).

Other compounds chosen in this study were the sesquiterpene β -caryophyllene and the monoterpene β -myrcene (Fig. 1). Both of them are present in several essential oils, e.g. caryophyllene is found in clove oil (13) and myrcene in oil of hops (14). These are also common fragrance chemicals. Caryophyllene was detected in 33% of the investigated cosmetic products on the Dutch market (12) and in 45% of the investigated deodorants on the European market (3). Myrcene was also a frequently identified compound in deodorants (3). Experimental studies show that linalool (15, 16) and caryophyllene (17), as well as myrcene

(unpublished data) easily autoxidize on air exposure. The pure compounds were found to be non-sensitizers in animal experiments. However, their oxidation mixtures were all sensitizing (15–17, unpublished data). Linalool hydroperoxides were strong sensitizers while the other main oxidation products in the oxidation mixture of linalool were moderate sensitizers or without sensitizing capacity (15). In autoxidized caryophyllene only one oxidation product was detected, caryophyllene oxide, which had a low-sensitizing potential (17).

The aim of the present study was to screen among consecutively tested dermatitis patients in Europe for contact allergy to autoxidized linalool, caryophyllene and myrcene as well as to some isolated allergenic oxidation products. Furthermore, we also wished to investigate the role of involvement of hands, as well as adverse reaction to fragrances in contact allergy to oxidized terpenes. Therefore, in a targeted study, patients with known and/or suspected fragrance allergy and patients with hand eczema without known adverse reaction to fragrances were included.

Materials and Methods

Test materials

Linalool (3,7-dimethyl-1,6-octadien-3-ol) 97% was obtained from Lancaster Synthesis (Eastgate, Lancashire, UK) and Sigma Aldrich Chemie (Germany), β -caryophyllene [*trans*-(1R,9S)-4,11,11-trimethyl-8-methylenebicyclo(7.2.0) undec-4-ene] of technical quality from Firmenich (Switzerland) (according to our analysis, 94% pure), caryophyllene oxide [(1R,4R,6R,10S)-4,12,12-trimethyl-9-methylene-5-oxatricyclo (8.2.0.0^{4,6}) dodecane] 99.8%, from Fluka Chemie (Switzerland) and β -myrcene (7-methyl-3-methyleneocta-1,6-diene) of technical quality from Sigma Aldrich (Germany) Chemie (92% according to our analysis). To mimic the oxidation that takes place during handling and storage, linalool, β -caryophyllene and β -myrcene were air exposed in Erlenmeyer flasks, covered with aluminium foil to prevent contamination. They were stirred for 1 h, 4 times a day, as previously described (5). The oxidation/degradation processes were followed using chemical analyses. A difference in oxidation rates were found: myrcene = caryophyllene > linalool (15, 17, unpublished data). Oxidized samples of linalool (air-exposed for 45 weeks), of β -caryophyllene (air-exposed for 10 weeks) and of β -myrcene (air-exposed for 12 weeks) were stored under nitrogen at -20°C , until test preparations were made. A fraction

containing hydroperoxides was isolated from air-exposed linalool using flash chromatography. The oxidation mixtures and the isolated fraction, used for patch testing, were tested according to the local lymph node assay (LLNA) in mice and were shown to be sensitizing (15, 17).

In the present paper, the term 'oxidation mixture of linalool' and 'oxidation mixture of caryophyllene' are used to describe the patch test material applied, containing all possible allergens formed after oxidation. 'Oxidized linalool' and 'oxidized caryophyllene' are terms used generally.

Chemical analysis

The terpenes used for patch testing were analysed using gas chromatography and high performance liquid chromatography, according to methods previously described (15, 16). The oxidized linalool was found to contain 30% linalool and 16% linalool hydroperoxide (7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol) (Fig. 1). The hydroperoxide fraction of linalool contained 83% of this hydroperoxide. It also consisted of another linalool hydroperoxide and minute amounts of a linalool aldehyde. Oxidized β -caryophyllene contained 25% β -caryophyllene (Fig. 1) and 35% caryophyllene oxide (Fig. 1) but no detectable amounts of hydroperoxides. Oxidized β -myrcene was found to contain 30% β -myrcene (Fig. 1). Details on the chemical analyses of the different oxidation mixtures are described elsewhere (15–17).

Irritation studies

Irritation tests were performed with the oxidized fragrance terpenes and with the specific oxidation compounds and fractions in different concentrations, in order to establish maximal non-irritant concentrations for the multicentre patch test study. 41 dermatitis patients in Odense and Leuven without suspected fragrance allergy participated. No considerable irritation was seen, except for 3 faint questionable reactions at the first reading only, to the linalool hydroperoxide fraction 0.5% pet.

The irritation of the oxidation mixture of linalool was further studied in another 40 consecutive dermatitis patients using 1%, 2% and 3% in pet. The 3% concentration gave 4 questionable reactions. Thus, the concentrations stated below were chosen for the multi-centre study.

Patch test materials

Standard allergens used for patch testing were in Malmö obtained from Chemotechnique AB (Malmö, Sweden), in Dortmund, Gentofte,

Leuven and London from Trolab-Hermal (Reinbek, Germany). The standard series used in Odense was TRUE Test™ (Mekos A/S, Hillerød, Denmark), supplemented by test substances from Hermal; specifically, the FM 8% pet. was routinely used in the diagnosis of fragrance allergy.

The terpenes were stored under nitrogen at -20°C . The preparations in non-stabilized white petrolatum (Kebo Laboratory A/S, Albertslund, Denmark) for patch testing were made every 2–3 months from the same batch and filled into 5 ml syringes at the Department of Dermatochemistry and Skin Allergy in Gothenburg. The syringes were sent to the test centres by airmail and kept in the refrigerator between the tests.

Patch test materials of approximately 20 μl were applied in small (8 mm) Finn Chambers® on Scanpor® tape (Epitest, Tuusula, Finland). Readings were taken twice, on days 2 and 4 (exceptionally on day 3) in Leuven, London and Dortmund, and on days 3 and 6/7 in the other centres, and scored according to the ICDRG standard recommendation (18).

Multicentre study

Consecutive dermatitis patients were tested at the participating dermatology clinics of Copenhagen, Dortmund, Leuven, London, Malmö and Odense during 2002–03.

A questionnaire about clinical data (i.e. localization of symptoms, history of atopy and occupation) was filled out before testing. The patients' history of adverse reactions to fragrances in the past was included and categorized according to the following questions: (a) certain: has reacted with an itching dermatitis to at least one fine perfume or after shave and also to other scented products; (b) probable: has reacted to one or more scented products, but no certain product has been identified as the cause of a clinical reaction; (c) questionable: has reacted to various cosmetics with or without fragrances, i.e. materials other than fragrance constituents may be the cause of reaction; (d) none: has never reacted to scented materials [modified after (19)].

The following test concentrations were used: 2.0% (w/w) oxidation mixture of linalool, 0.5% (w/w) linalool hydroperoxide fraction, 3.0% (w/w) oxidation mixture of β -caryophyllene, 3.9% (w/w) caryophyllene oxide and 3.0% (w/w) oxidized β -myrcene.

Retest study

In May 2003, retesting was performed with the oxidized terpenes in Malmö in 3 out of the 5

patients who had shown positive reactions to the oxidation mixture of linalool and/or the linalool hydroperoxide fraction. Non-oxidized linalool was distilled and tested additionally in the concentrations 20%, 6%, 2%, 0.6% and 0.2% (w/w) in non-stabilized white petrolatum.

Targeted study

A two step targeted study was performed in Odense and Leuven including patients with fragrance hypersensitivity without hand eczema, and patients with hand eczema but without adverse reaction to fragrances. The patients with fragrance allergy ($n = 21$) were partly recruited for retesting among previously tested individuals who had shown positive reactions to fragrance allergy markers and own perfumes, and partly chosen from consecutive dermatitis patients who had a certain history of adverse reactions to fragrances (questionnaire under *multicentre study*). Only 8 patients in this group reacted to FM and/or Myroxylon pereirae, whereas 8 patients showed positive patch and/or use test reactions to their own perfumes tested. The hand dermatitis patients without a certain or probable history of adverse reaction to fragrances ($n = 66$) were selected from consecutive dermatitis patients. The participants were tested with the following compounds or mixtures of compounds diluted in non-stabilized petrolatum: non-oxidized linalool 20%, linalool hydroperoxide fraction 0.5%, oxidation mixture of linalool 1%, caryophyllene oxide 3.9% and the oxidation mixture of caryophyllene 3%.

Statistics

Chi-square test was used for the analysis of concomitant positive reactions.

The study was approved by the local ethics committee, and all participants were included after informed consent.

Results

Out of the 1511 consecutively tested dermatitis patients in the 6 clinics, 31 (2.1%) showed positive patch test reactions to one or more of the oxidized fragrance terpene materials. Details on the patch test reactions and variations between the different test centres are given in Table 1. 25 patients (1.7%) reacted positively to the oxidation mixture and/or to the hydroperoxide fraction of linalool. Of these patients, 20/25 (80%) reacted to the oxidation mixture, while the hydroperoxide fraction gave positive results in 16/25 (64%) cases. Reactions only to the

oxidation mixture of linalool were observed in 9/25 (36%) and only to the hydroperoxide fraction in 5/25 (20%) of the cases. Testing with oxidation mixture of caryophyllene gave positive reactions in 8/1511 patients (0.5%), of whom 2 also reacted to caryophyllene oxide. Only 1 out of the 1511 patients presented with a positive reaction to oxidized myrcene (Table 1). In total, we observed 47 reactions in 31 patients reacting to the oxidized fragrance terpene test materials. Of the 47 reactions, 15 were ++ and 32 were + reactions. No +++ reactions were seen. Questionable and follicular reactions were observed in 65 out of 1511 patients, with a wide geographical variation. In some centres, no such reactions were recorded, in others, 18% of the patient files contained data about questionable and/or follicular reactions.

Concomitant reactions to FM, colophonium and Myroxylon pereirae (balsam of Peru) were common in patients reacting to the oxidized terpene patch test materials (Table 2). Of these patients ($n = 31$), 39% showed positive reactions to FM, 39% to colophonium and 21% to Myroxylon pereirae. In the total study population tested ($n = 1511$), the respective frequencies of positive reactions were 6.9% (FM), 3.8% (colophonium) and 4.6% (Myroxylon pereirae). In the study, 12 of the 31 oxidized fragrance terpene-positive subjects reacted to FM compared with 93 out of 1480 subjects negative to oxidized fragrance terpenes ($P < 0.001$). Similarly, significant correlations were observed also to colophonium and Myroxylon pereirae ($P < 0.001$) (Table 2). The frequency of positive reactions to standard patch test materials varied greatly between the participating test centres. Contact allergy was observed to FM in 4.0%–12% of the patients tested, to colophonium in 1.9%–6.8% and to Myroxylon pereirae in 2.2%–5.5%.

Fragrance-related contact allergy was considered in patients who showed positive reactions to own perfumes or perfumed products, and/or to known perfume allergens (e.g. FM, Myroxylon pereirae, ingredients of FM, hydroxyisohexyl 3-cyclohexene carboxaldehyde and essential oils) and/or had certain or probable history for adverse reaction to fragrances (according to answers in the questionnaire). Such a correlation was detected in 18 out of 31 patients (58%). In 5 out of these 18 cases, no positive reaction to FM and/or Myroxylon pereirae was observed. Contact allergy to colophonium was recorded in 2 of these 5 cases.

Atopy (defined as hay fever/asthma and/or a positive history of eczema in the knee/elbow folds as a child) was present in 9 of 31 patients showing

Table 1. Positive patch test reactions to oxidized terpenes and oxidation fractions in 1511 consecutive dermatitis patients at 6 dermatological centres

	Dortmund <i>n</i> = 223	Gentofte <i>n</i> = 230	Leuven <i>n</i> = 200	London <i>n</i> = 302	Malmö <i>n</i> = 294	Odense <i>n</i> = 262	Total <i>n</i> = 1511
Oxidation mixture of linalool 2.0% petrolatum (pet.)	4 (1.8%)	1 (0.4%)	1 (0.5%)	4 (1.3%)	3 (1%)	7 (2.7%)	20 (1.3%)
Linalool hydroperoxide 0.5% pet.	3 (1.3%)	0	2 (1%)	2 (0.7%)	4 (1.4%)	5 (1.9%)	16 (1.1%)
Oxidation mixture of caryophyllene 3.0% pet.	2 (0.9%)	0	1 (0.5%)	2 (0.7%)	0	3 (1.1%)	8 (0.5%)
Caryophyllene oxide 3.9% pet.	1 (0.4%)	0	0	0	0	1 (0.4%)	2 (0.1%)
Oxidized myrcene 3.0% pet.	0	0	1 (0.5%)	0	0	0	1 (0.1%)
Total number of patients with positive reactions	5 (2.2%)	1 (0.4%)	3 (1.5%)	5 (1.7%)	5 (1.7%)	12 (4.6%)	31 (2.1%)

positive reactions to oxidized terpenes (29%). No typical occupations were recorded among the patients, most of them (20 out of 31 patients) working in administrative job and/or engaged in house keeping work. The most common localizations affected the legs and feet (42%), hands (23%) and face (23%).

In the retest study, contact allergy to oxidized linalool was reconfirmed in the 3 patients, who had previously shown allergic patch test reactions to oxidized linalool (oxidation mixture of linalool 2% pet. and/or linalool hydroperoxide 0.5% pet.). No allergic reactions to non-oxidized linalool (20%) were observed.

In the two-step-targeted study, there were 66 patients with hand eczema and no history for adverse reaction to fragrances. None of these had positive reactions to the oxidized terpenes, and 3 had positive reactions to FM. Among the 21 patients with fragrance hypersensitivity and no hand eczema, there were 3 positive reactions to the oxidized terpenes observed in 2 of the patients. Linalool hydroperoxide gave 2 positive reactions, whereas the oxidation mixture of caryophyllene gave 1 reaction. Linalool hydroperoxide was the only positive patch test reaction in 1 case (besides the previously positive use test to the patient's own perfume). In the other case, a positive reaction to FM was observed together with reactions to linalool hydroperoxides and the oxidation mixture of caryophyllene. Positive and questionable reactions to the oxidized terpenes

tested in the targeted study are summarized in Table 3.

Discussion

In our current study, we investigated contact allergy to some commonly used fragrance terpenes (linalool, β -caryophyllene and β -myrcene) that had been air-exposed and consequently autoxidized. A total of 2.1% of consecutively tested eczema patients reacted positively to at least one of the oxidized fragrance terpene materials. Most (1.7%) reacted to the oxidation mixture of linalool and/or the hydroperoxide fraction of oxidized linalool. The frequency of positive reactions to oxidized linalool proved this oxidized terpene to be a common cause of contact allergy in routinely tested dermatitis patients. Reactions to oxidized caryophyllene were observed less frequently, and only 1 reaction to oxidized myrcene was reported.

The results of oxidized linalool are comparable with those of previous studies (6, 7) where 2.7% and 1.7% (respectively.) of consecutively tested patients reacted to oxidized *R*-(+)-limonene. Common contact allergens give rise to positive patch test reactions in greater than 1% in consecutively tested patients in several patch test studies. Fragrances are among the most common causes of contact allergy. The frequency of positive reactions to FM varies between 4% and 11%

Table 2. Positive reactions to FM, Myroxylon pereirae and colophonium (tested in the standard series) in 1511 consecutively tested patients with positive and negative test results to oxidized fragrance terpenes

Total test population (<i>n</i> = 1511)		
	Positive to oxidized linalool, caryophyllene, myrcene (31 patients)	Negative to oxidized linalool, caryophyllene, myrcene (1480 patients)
Fragrance mix	12/31 (39%) ^a	93/1480 (6%)
Myroxylon pereirae	6/31 (21%) ^a	63/1480 (4%)
Colophonium	12/31 (39%) ^a	46/1480 (3%)

^aStatistically significant concomitant reactions in subjects showing positive reactions to oxidized fragrance terpenes compared to those with negative reactions ($P < 0.001$)

Table 3. Questionable and positive patch test reactions to oxidized and non-oxidized terpenes, oxidation compounds and fractions in the targeted study of patients with hand eczema or fragrance hypersensitivity in Odense and Leuven

	Concentration	Fragrance hypersensitivity patients (<i>n</i> = 21)		Hand eczema patients (<i>n</i> = 66)	
		Questionable	Positive	Questionable	Positive
Linalool (pure)	20%	0	0	3	0
Linalool hydroperoxide	0.5%	1	2 (+ + and + + +)	6	0
Oxidation mixture of linalool	1%	1	0	2	0
Caryophyllene oxide	3.9%	2	0	1	0
Oxidation mixture of caryophyllene	3%	0	1 (+ +)	0	0

in patch-tested dermatitis patients (20), and 2.3% positive reactions to FM were observed in a general population in Denmark (21). Although commercial perfumes may contain several hundred fragrance compounds, the main diagnostic tool, the FM, investigates contact allergy to 8 common sensitizers. In a study on changes in the pattern of sensitization to standard allergens between 1986 and 1998, Johansen found that positive reactions to the FM increased from 4.1% to 10.9% in women (22). The FM is estimated to detect 50%–70% (1) of all cases of fragrance allergy. Therefore, new markers for fragrance contact allergy have to be identified and introduced for screening in dermatitis patients. In the last decade, several new candidates have emerged in different clinical studies, such as, e.g. hydroxyisohexyl 3-cyclohexene carboxaldehyde and farnesol (19, 23).

Causes of fragrance allergy can also be revealed by considering the stability of common fragrance chemicals at handling and storage. In previous studies (6, 7), the highly significant correlation between fragrance allergy and allergy to oxidized limonene in several test centres gave clinical support for oxidized limonene being a good fragrance allergy marker. Our current study proved that the same correlation holds true for other oxidized fragrance terpenes, especially linalool. Fragrance related contact allergy and/or adverse reaction to fragrances in the patients' history was observed in 58% of the patients giving positive reactions to oxidized fragrance terpenes. In this group of patients (*n* = 18) with high certainty of relevance of positive patch test reactions to oxidized terpenes, 5 patients (28%) did not react to FM and/or Myroxylon pereirae. Their allergy to a common fragrance material could have been missed if only the standard screening markers for fragrance allergy had been used. However, we observed significant concomitant patch test reactivity to the known fragrance allergy markers, i.e. FM and Myroxylon pereirae in patients allergic to oxidized terpenes and also a highly significant

correlation in reactivity to colophonium. Simultaneous reactions to FM, colophonium and Myroxylon pereirae in dermatitis patients are frequently registered in the literature (24–26). Also in previous patch test studies (7, 27) a high number of concomitant reactions to these patch test materials was observed in patients with positive reactions to oxidized limonene compared with those not reacting.

Although colophonium is not considered as a fragrance allergen as such, patients allergic to colophonium are often reported to be allergic to other natural and fragrant substances as well (28). Colophonium is a natural complex material and similarly to the fragrance terpenes of this study, susceptible to air-oxidation, giving rise to the formation of several oxidation products with contact allergenic potential. 15-Hydroperoxyabietic acid, the oxidation product of the major resin acid, is considered to be the major allergenic culprit in sensitization to colophonium (29).

Already in the 50s, it was found that hydroperoxides of Δ^3 -carene (Fig. 1) were the major sensitizers in oil of turpentine (30). Δ^3 -Carene is the most easily oxidized terpene in turpentine, and its content varies depending on the type of oil of turpentine. Replacement of Δ^3 -carene-rich turpentine oils to those with low or negligible concentrations resulted in a sharp decline in the number of cases of contact allergy (31). Also hydroperoxides of fragrance terpenes have proved to be important haptens. Patch testing with the hydroperoxide fraction of *R*-(+)-limonene, containing, e.g. the limonene 2-hydroperoxide (Fig. 1), confirmed the experimental findings that the primary oxidation products, the hydroperoxides, are major allergens in the oxidation mixture of limonene (27, 32). Considerable amounts of stable hydroperoxides are also formed at autoxidation of linalool and sensitization studies according to the LLNA show the hydroperoxides to be the dominating sensitizers in the oxidation mixture (15). In the present study, the hydroperoxide fraction gave positive reactions in altogether 64% of the

patients allergic to oxidized linalool, which can be compared with the previous studies in patients allergic to oxidized *R*-(+)-limonene, where the hydroperoxides showed positive reactions in 59% of the patients allergic to the oxidation mixture (27). The fact that linalool is the most often used fragrance chemical in personal cosmetics and household cleaning products together with its ability to form highly allergenic stable hydroperoxides [EC3 value of 1.6% in the LLNA (15)] in considerable amounts, might explain the high frequency of positive test reaction (1.1%) to the hydroperoxide fraction tested.

Autooxidation of fragrance terpenes contributes to fragrance allergy to a great extent. In our current study, no positive reactions to non-oxidized linalool (tested up to 20% pet.) were seen when 3 patients with known allergy to oxidized linalool were retested. Previous studies showed (27) that non-oxidized limonene produced no positive reactions when 30 patients with known contact allergy to oxidized *R*-(+)-limonene were tested. Previous animal studies (16) and the lack of chemical substructures that indicate a possible contact allergenic effect argue for linalool itself being nonallergenic. Linalool has seldom been patch tested on patients with suspected allergic contact dermatitis. Since the formation of allergenic components from linalool was not considered previously, in the few articles reporting linalool contact allergy (33–35) the presence of oxidation products was not investigated. One publication from Japan (36) reports a high frequency of positive reactions to lavender oil 20% pet. in patients with suspected cosmetic contact dermatitis. Positive reactions to this patch test material were observed with a yearly increase from 0% to 13.9% during the 9-year period of the study. One can speculate that these results reflect the oxidation of the patch test material used and with the increase in the concentration of allergenic oxidation products, increasing amount of cases allergic to oxidized linalool became detected.

There also seems to be a relationship between compositae dermatitis and reactivity to terpenes, which are released from compositae plants. These terpenes released from the plants may, possibly through air oxidation, contribute to the airborne dermatitis pattern (37–39).

Caryophyllene is used less frequently compared with linalool, but it is a common fragrance chemical in deodorants (3). In the present study, only 0.5% of the patients reacted positively to oxidized caryophyllene. Besides its less frequent usage, the lower frequency of contact allergy might be explained by the fact that oxidized

caryophyllene showed a weak sensitizing effect in LLNA studies (17). The only oxidation product found in oxidized caryophyllene was caryophyllene oxide (Fig. 1), which proved to be a weak sensitizer in LLNA studies (17). Caryophyllene oxide gave positive reactions in only 2 out of 8 patients also reacting to the mixture of oxidized caryophyllene. Oxidized myrcene was shown to be a rare allergen.

It is known that the frequency of positive reactions to fragrance materials varies according to region and the usage of products (19). Furthermore, the 6 centres in the present study are different in patient selection, which factor also contributes to the wide variations observed in frequency of contact allergy to FM, Myroxylon pereirae, colophonium and oxidized fragrance terpenes. Local habits in exposure to fragrance terpenes and differences in patch test reading between the centres can also influence the results (40). Scoring the patch test reactions includes some subjective judgement. Indeed, the large variation in the frequency of questionable reactions observed to the oxidized fragrance terpene test materials can only depend on the different accuracy in recording faint reactions in the participating dermatological clinics (41).

Allergic contact dermatitis from fragrance ingredients may present with localized or generalized symptoms. Facial, hand and axillary eczema (1) as well as dermatitis on the legs (42) have been frequently reported in fragrance-allergic patients. Rare localizations such as anogenital eczema and generalized symptoms have also been reported (1). Patients in our study reacting to oxidized fragrance terpenes presented most often with leg, hand and facial dermatitis. No special risk occupation was detected. This latter fact, as well as the localization of symptoms of patients allergic to oxidized fragrance terpenes, might be indicative for a ubiquitous allergen.

In the targeted study, patients with known or suspected fragrance allergy without hand eczema were selected in order to be able to evaluate the diagnostic role of oxidized fragrance terpenes in patients with suspected allergy to fine fragrances. All 21 patients had a certain history for adverse reaction to fragrances. The standard screening markers for fragrance allergy proved not to be sufficient diagnostic tools, as only 8 of the 21 patients showed positive reactions to the FM and/or Myroxylon pereirae. In 3 cases, contact allergy was only observed to own perfumes. One of the 2 cases of contact allergy to oxidized terpenes in this group was observed in an additional case of contact allergy to an own perfume, however, without positive reactions to standard

fragrance allergy markers. These results indicate that, although oxidized fragrance terpenes are not sufficient diagnostic tools in themselves for suspected allergy to fine fragrances, additional cases of fragrance allergy can be diagnosed with oxidized terpenes that would have been missed with using only standard fragrance allergy markers. The importance of patch testing with the formulated perfume itself in the diagnosis of fragrance allergy was previously also shown in the study of Johansen (43).

Hand eczema patients without known history for adverse reaction to fragrances were selected in order to study the diagnostic role of the oxidized terpenes in cases where sensitization to fragrances commonly used in household and industrial cleaning products could be suspected. No positive reactions to oxidized terpenes were recorded among hand eczema patients. Although patients with questionable and negative history for adverse reaction to fragrances (questionnaire in *Materials and methods*) were investigated, 3 reactions to FM (with negative history) were recorded in this group (4.5%). These data in a limited number of patients indicate that hand eczema alone, without a positive history for adverse reaction to fragrances, does not represent an increased risk for sensitization to oxidized fragrance terpenes. In this part of the study, only a 1% dilution of oxidation mixture of linalool was used (compared with 2% in the multicentre study), because the targeted study took place before the additional irritation studies with higher concentrations of the oxidation mixture of linalool were terminated. This fact might have contributed to false-negative reactions to this patch test material. Recent publications of Heydorn et al. (44, 45) studied the possible association between fragrance allergy and hand eczema. In a study including a large number of hand eczema patients, a panel of specific fragrance ingredients was tested. The fragrance chemicals represented allergens present in products to which hand exposure occurred. The most common patch test reactions observed to allergens not present in the FM were recorded to citral, hydroxyisohexyl 3-cyclohexene carboxaldehyde and an oxidized fragrance terpene, oxidized *S*-limonene (*l*-limonene). Further controlled exposure studies in a multiple dose design are needed to evaluate the role of oxidized linalool in hand eczema patients.

As a majority of cosmetic products and household chemicals is scented, the exposure to fragrance chemicals is immense in the population. In order to be able to decrease the frequency of contact allergy to fragrances, compounds with

low-allergenic potential and low content of sensitizing oxidation/degradation compounds should be used.

Our observations emphasize the need of testing with chemicals that patients actually come in contact with and not only ingredients that were originally applied in commercial formulations.

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