

Lavender oil lacks natural protection against autoxidation, forming strong contact allergens on air exposure

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Background: Lavender oil is an essential oil frequently used as a fragrance ingredient and in traditional herbal medicine. We have previously studied the effect of air oxidation on the skin sensitizing potency of the monoterpenes linalyl acetate, linalool and β -caryophyllene, the main constituents of lavender oil.

Objective: The aim of this study was to investigate if the autoxidation observed for the single synthetic terpenes, resulting in strong contact allergens, will take place also in lavender oil.

Methods: Lavender oil was exposed to air and the autoxidation was followed by chemical analysis. The sensitizing potency before and after air exposure was investigated in mice using the local lymph node assay. Patients with patch test reactions to oxidized linalool were tested to investigate if air-exposed lavender oil could elicit dermatitis in these individuals.

Results: The terpenes oxidized in air-exposed lavender oil at the same rates as the pure compounds exposed to air, and the same oxidation products were identified. The sensitizing potency of lavender oil increased accordingly on air exposure. Patch testing showed positive reactions to air-exposed lavender oil and also to oxidized linalyl acetate in patients with contact allergy to oxidized linalool.

Conclusion: This study shows that lavender oil lacks natural protection against autoxidation, and that air-exposed lavender oil can be an important source of exposure to allergenic hydroperoxides.

Key words: allergic contact dermatitis; autoxidation; essential oils; fragrances; gas chromatographic analysis; hydroperoxide; lavender oil; linalool; linalyl acetate; LLNA; patch testing. © Blackwell Munksgaard, 2008.

Accepted for publication 5 April 2008

Lavender oil is the essential oil obtained from steam distillation of freshly cut flowering tops of lavender (*Lavendula angustifolia* or *Lavendula officinalis*) (1). The oil is almost colourless with a sweet, floral, herbaceous odour and has since ancient times been a very common fragrance ingredient (2). It has also been used for centuries in traditional herbal medicine, based on its antibacterial, antifungal, smooth muscle relaxing, sedative, and antidepressive effects (3). Lavender oil is still widely used in aromatherapy because of its pleasant scent or as an antimicrobial agent (3). However, today most often synthetic substances are used to give lavender scent to cosmetics,

perfumes, soaps, etc. Linalool (Fig. 1), one of the main components in lavender oil, is the most commonly used fragrance chemical in the world.

Essential oils are complex mixtures of different compounds. The oil composition depends on which species and what parts of the plant are used for distillation. The origin of the plant, the time of harvest and the distillation process can also influence the composition of the oil. Numerous lavender oils with different chemical compositions are present on the market, but the main constituents of the oils are linalyl acetate and linalool (Fig. 1). β -Caryophyllene is another component of the oil (Fig. 1).

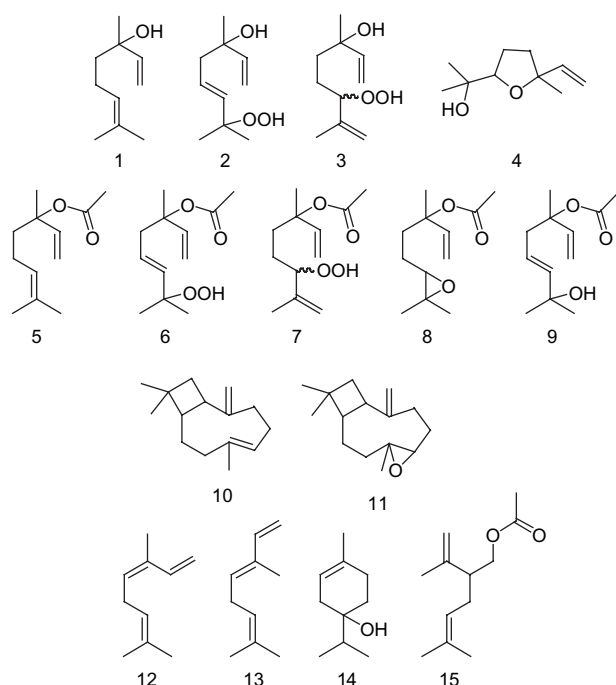


Fig. 1. Structures of linalool **1** and oxidation products; linalool hydroperoxides **2,3** and linalool oxide **4**, linalyl acetate **5** and oxidation products; linalyl acetate hydroperoxides **6,7**, 1-[2-(3,3-dimethyloxiran-2-yl)ethyl]-1-methylprop-2-en-1-yl acetate **8**, 5-hydroxy-1,5-dimethyl-1-vinylhex-3-en-1-yl acetate **9** and β-caryophyllene **10** and its oxidation product caryophyllene oxide **11**. Compounds **12–15** were identified in not air-exposed lavender oil; *cis*-ocimene **12**, *trans*-ocimene **13**, terpinen-4-ol **14** and lavandulol acetate **15**.

Normally, a contact allergen is an electrophilic or radical reactive compound that can penetrate the skin barrier and react with macromolecules (proteins) in the skin to form complexes that trigger the immune system. The chemical structure of linalool does not show any of these features, and consequently it does not possess any or very weak contact allergenic activity (4, 5). As an ester, linalyl acetate possesses an electrophilic centre. It has been shown to be a weak sensitizer (6), which is in accordance with the literature, as aliphatic esters previously have been shown to be weak or non-sensitizing (7). However, both linalool and linalyl acetate oxidize immediately when exposed to air and several oxidation products have been identified (4–6). As expected, the autoxidation greatly influenced the skin sensitizing capacity of linalool and linalyl acetate and the primary oxidation products, that is, the hydroperoxides, were found to be the strongest allergens in both cases (4, 6). A patch test study showed that a relatively high percentage (1.3%) of consecutive dermatitis patients gave positive reactions to oxidized linalool 1% petrolatum (pet.) (8).

The autoxidation of β-caryophyllene (Fig. 1) has also been studied (9). β-Caryophyllene oxidizes

rapidly when exposed to air, but the autoxidation was found to affect the sensitizing capacity to a lesser extent compared with linalool and linalyl acetate. This was explained by the fact that only a moderately sensitizing epoxide was detected in the oxidation mixture, indicating that the hydroperoxides formed were immediately decomposed to less sensitizing secondary oxidation products (9).

The aim of the present study was to investigate if the autoxidation observed for the single synthetic terpenes resulting in strong contact allergens, will take place also in lavender oil. Lavender oil was exposed to air and the autoxidation was followed by chemical analysis. The sensitizing potency before and after air exposure was investigated in mice using the local lymph node assay (LLNA). Patients with patch test reactions to oxidized linalool were patch tested to investigate if air-exposed lavender oil could elicit dermatitis in these individuals.

Materials and Methods

Chemicals

Two batches of lavender oil 40/42 (Robert Bontoux, Clos d'Aguzon, France) were used. Gas chromatographic (GC) analyses showed linalyl acetate and linalool to be the major constituents of the non-oxidized lavender oils: 50% and 36%, respectively, in batch 1 and 48% and 39%, respectively, in batch 2. The concentration of β-caryophyllene was 2% in batch 1 and 3% in batch 2. The analyses also showed the presence of *cis*-ocimene, *trans*-ocimene, terpinene-4-ol and lavandulol acetate in concentrations <2% of each (Fig. 2). These compounds were not further investigated.

Linalool (3,7-dimethyl-1,6-octadien-3-ol), 97% and linalyl acetate (3,7-dimethyl-1,6-octadien-3-yl acetate) 97% were purchased from Sigma-Aldrich (Schnelldorf, Germany). β-Caryophyllene (trans-(1R,9S)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene) 98.6%, linalool oxide (2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-ol) 97.8% and caryophyllene oxide ((1R,4R,6R,10S)-4,12,12-trimethyl-9-methylene-5-oxatricyclo-[8.2.0.0^{4,6}]dodecane) 99.8% were purchased from Fluka (Buchs, Switzerland). Acetone was purchased from Merck (Darmstadt, Germany) and olive oil from Apoteket AB (Goteborg, Sweden). White non-stabilized pet. was purchased from Kebo Lab (Albertslund, Denmark).

Instrumentation and mode of analysis

The instrumentation used and the mode of analysis were the same as previously described (6).

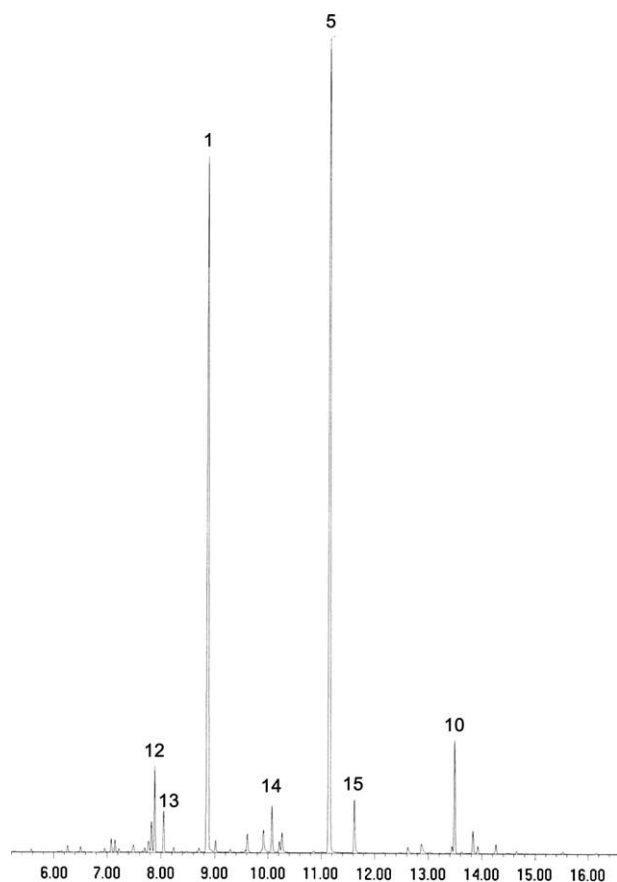


Fig. 2. Gas chromatogram of lavender oil not exposed to air. The major components linalool **1** and linalyl acetate **5** are the most abundant in the oil, followed by β -caryophyllene **10**. Some of the minor components were identified as *cis*-ocimene **12**, *trans*-ocimene **13**, terpinen-4-ol **14** and lavandulol acetate **15**. Numbering according to the chemical structures in Fig. 1.

Preparation of a synthetic mix

A synthetic mix was prepared, where linalyl acetate, linalool and β -caryophyllene were present in the same ratios as in lavender oil, but without any other compounds present. The single components were purified by distillation under reduced pressure before mixing. The concentrations of the main components in the mix were linalyl acetate 56.4%, linalool 40.2%, and β -caryophyllene 3.3%.

Air exposure and isolation of oxidation products

Samples of lavender oil, synthetic mix, linalyl acetate, and linalool were exposed to air in Erlenmeyer flasks, as previously described (6, 10). Samples of oxidized lavender oil were taken on a regular basis to be analysed with GC for quantification of linalyl acetate, linalool, and β -caryophyllene, respectively. In the synthetic mix, these compounds were quantified at 10 weeks

only. Oxidized linalyl acetate and oxidized linalool to be used in patch testing were analysed continuously for quantification of linalyl acetate and linalool, respectively.

To isolate oxidation products in the air-exposed lavender oil, the samples to be analysed were first fractionated using flash chromatography on silica gel columns using mixtures of *iso*-hexane and ethyl acetate for elution, starting with 10% ethyl acetate in hexane, whereafter the proportion of ethyl acetate was increased. Isolated fractions/compounds were characterized with nuclear magnetic resonance (NMR) spectrometry and mass spectrometry (MS).

Quantification of hydroperoxides of linalyl acetate and linalool using high-performance liquid chromatography

An analytical silica column, Purospher® STAR (46 × 250 mm, 5 μ m particles; Merck) was used. The temperature of the column and the sample rack in the autosampler was set to 20°C. Mobile phase: 0 min: 25% *tert*-butyl methyl ether and 75% *n*-hexane; 0–25 min: linear gradient reaching 60% *tert*-butyl methyl ether and 40% *n*-hexane; 25–30 min: 60% *tert*-butyl methyl ether and 40% *n*-hexane, isocratic elution. The flow rate was 1 ml/min. Blank subtractions of the gradient were performed. Pure reference compounds were used to make external calibration curves from which the concentrations of linalyl acetate hydroperoxides and linalool hydroperoxides in air-exposed lavender oil could be determined.

Synthesis

7-Hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol and 6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-ol (linalool hydroperoxides; Fig. 1). The photo-oxidation of linalool was performed as previously described (4) and yielded the linalool hydroperoxides in an approximate ratio of 5:3.

7-Hydroperoxy-3,7-dimethylocta-1,5-diene-3-yl acetate and 6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-yl acetate (linalyl acetate hydroperoxides; Fig. 1). The photo-oxidation of linalyl acetate was performed as previously described (6) and yielded the linalyl acetate hydroperoxides in an approximate ratio of 5:3.

Sensitization experiments in mice

The sensitizing potency of the chemicals was investigated using the LLNA (11) as previously described (4). All animal procedures were approved by the local ethics committee. Each

compound was tested in three or five different concentrations, using mice in groups of four or three, respectively.

Patch testing

Based on previous experience (12), non-stabilized pet. was used for all patch test preparations. Patch test materials were stored at -20°C until used. Small Finn Chambers[®] (8 mm, Epitest Ltd Oy, Tuusula, Finland) on Scanpor[®] tape (Norgesplaster A/S, Vennesla, Norway) were used for the study. The patches were applied on the upper back for 2D. Readings were performed according to the International Contact Dermatitis Research Group recommendation (13) on D3 and D7. The patch testing was performed at the Department of Dermatology, Sahlgrenska University Hospital, Göteborg, Sweden.

Initially, 22 patients with no personal history of fragrance sensitivity were selected among the consecutive dermatitis patients and patch tested to evaluate the irritative effect of the test preparations of oxidized lavender oil 2%, 4% and 6% pet. and oxidized linalyl acetate 2%, 4% and 6% pet. Informed consent was obtained. No irritation was seen to the tested concentrations. As in parallel patch test studies, we found a test concentration of 4% pet. of oxidized linalool to be suitable for screening, a concentration of 4% was chosen for further testing of oxidized linalyl acetate and lavender oil.

In a following study, performed in May 2006 and June 2007, individuals with known positive patch test reactions (++ or +++) to oxidized linalool were selected from 1985 patients with dermatitis, patch tested between 2004 and 2007. A letter was sent to nine individuals who met the above-mentioned criteria. The response rate was nine of nine (100%) individuals, of whom five agreed to further contact. Two individuals declined participation and the remaining three were included in the study. Exclusion criteria were ultraviolet therapy or sunlight to the back within 4 weeks of testing and ongoing medication with cytostatic drugs or oral corticosteroids. The mean age was 49.3 years. The group consisted of two males and one female. These patients were tested with oxidized linalool 4%, 2%, 1%, and 0.5% pet., mixture of linalool hydroperoxides 1%, 0.75%, 0.5%, 0.25%, 0.12 % and 0.06% pet., oxidized lavender oil 4% pet., and oxidized linalyl acetate 4% pet. The test concentrations of linalool hydroperoxides were chosen from prior experience (14). New patch test materials were made within 2 weeks before testing. The study was approved by the local ethics committee.

Results

Air oxidation

The content of all three main components of lavender oil decreased over time on air exposure. Lavender oil from batch 1 showed a decrease in the concentration of linalyl acetate from 48% to 39%, of linalool from 39% to 30%, and of β -caryophyllene from 2% to 0.3% after 10 weeks of air exposure (Fig. 3). Lavender oil from batch 2 showed a decrease in the concentration of linalyl acetate from 50% to 41%, of linalool from 36% to 28%, and of β -caryophyllene from 3% to 0.3% after 10 weeks of air exposure. After further 35 weeks of air exposure, the content in batch 2 decreased to 9% linalyl acetate, 5% linalool and 0.1% β -caryophyllene (Fig. 3).

The three components of the synthetic mix also autoxidized with time and the content of linalyl acetate, linalool and β -caryophyllene decreased. After 10 weeks of air exposure, the sample of the synthetic mix showed a decrease in the concentration of linalyl acetate from 56% to 44.9%, of linalool from 40% to 32% and of β -caryophyllene from 3.3% to 0.37%.

Oxidation products in autoxidized lavender oil

After 10 weeks of air exposure, oxidized lavender oil (sample 1) contained 3.3% of linalyl acetate hydroperoxides and 0.48% of linalool hydroperoxides (Fig. 4). The following oxidation products were identified but not quantified: linalool oxide (2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-ol), 1-[2-(3,3-dimethyloxiran-2-yl)ethyl]-1-methylprop-2-en-1-yl acetate, 5-hydroxy-1,5-dimethyl-1-vinylhex-3-en-1-yl acetate, and caryophyllene oxide ((1R,4R,

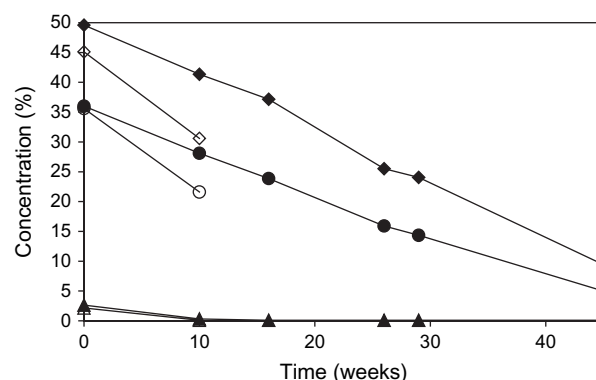


Fig. 3. Autoxidation of lavender oil on air exposure. Concentrations of linalyl acetate ($\diamond$), linalool ($\circ$), and β -caryophyllene ($\triangle$) in sample 1 (10 weeks of air oxidation). Concentrations of linalyl acetate ($\blacklozenge$), linalool ($\bullet$), and β -caryophyllene ($\blacktriangle$) in sample 2 (45 weeks of air oxidation). The quantifications were performed with gas chromatography using on column technique.

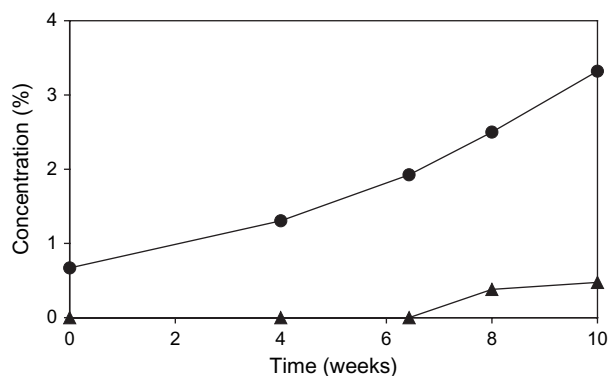


Fig. 4. Accumulation of linalyl acetate hydroperoxides (●) and linalool hydroperoxides (▲) in lavender oil sample 1 on air exposure. The quantifications were performed with high-performance liquid chromatography.

6R,10S)-4,12,12-trimethyl-9-methylene-5-oxatricyclo-[8.2.0.0(4,6)] dodecane) (Fig. 1).

Skin sensitizing potency

The sensitizing potencies of lavender oil (not exposed to air), of lavender oil (exposed to air for 10 and 45 weeks) and of the synthetic mix (exposed to air for 10 weeks) were investigated using the LLNA (Figs 5 and 6). It was shown that the sensitizing potency increased in all cases on air exposure, and that the increase of the sensitizing potency of the synthetic mix was comparable with that of the samples of lavender oil. The results are presented in detail in Table 1.

Patch testing

In the initial irritation study, one of the patients who showed positive reactions to oxidized linalool

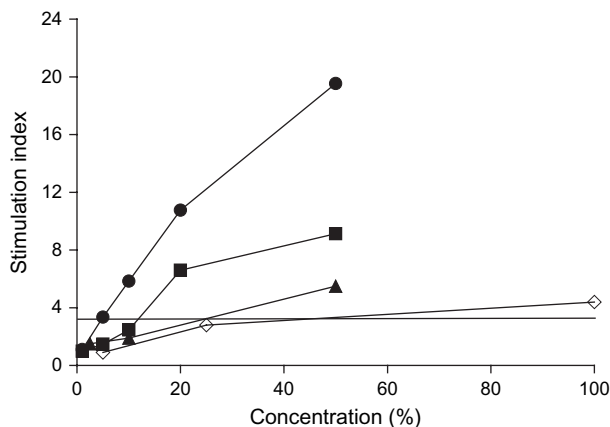


Fig. 5. Dose-response curves from the local lymph node assay for lavender oil (◇), air-exposed lavender oil, sample 1 (10 weeks) (■) and air-exposed lavender oil sample 2 [10 weeks (▲) and 45 weeks (●)]. The sensitizing potency increases on air exposure.

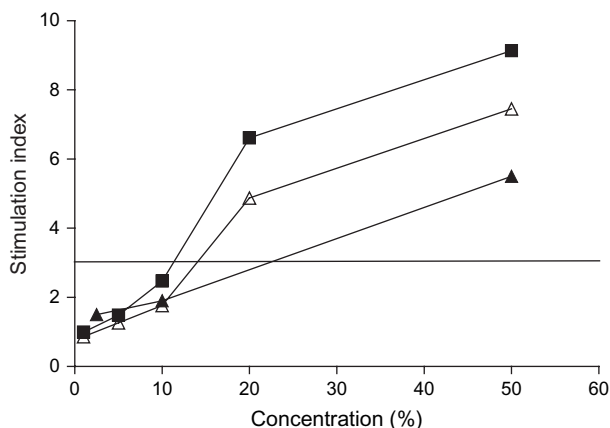


Fig. 6. Dose-response curve from the local lymph node assay for air-exposed synthetic mix (10 weeks) (△) compared with air-exposed lavender oil sample 1 (10 weeks) (■) and air-exposed lavender oil sample 2 (10 weeks) (▲). The synthetic mix follows the same pattern of autoxidation as the lavender oil samples, which is reflected in the dose-response curve.

2% pet. in the standard series, also reacted to oxidized lavender oil and oxidized linalyl acetate (Table 2).

In the study on three individuals with known positive reactions to oxidized linalool, all three reacted to oxidized linalool and to oxidized lavender oil. Two individuals reacted to the linalool hydroperoxide mixture and one to oxidized linalyl acetate (Table 2).

Discussion

In the present study, the autoxidation of lavender oil was investigated. It was found that the main components of lavender oil (linalyl acetate, linalool and β -caryophyllene) autoxidize on air exposure, forming the same oxidation products found in previous studies of the pure synthetic terpenes. Experimental sensitization studies showed that air exposure of lavender oil increased the sensitizing potency. Patch test results showed a connection between positive reactions to oxidized linalool and oxidized lavender oil. It could be concluded that lavender oil itself is not protected from autoxidation.

It has been speculated that products extracted from natural sources, that is, essential oils are protected from the autoxidation seen in synthetically produced terpenes because of antioxidant properties of the plants used as raw materials. We found no support for this with regard to lavender oil. Despite a possible presence of compounds with antioxidative effects, the terpenes were found to autoxidize at approximately the same rate in lavender oil, in the synthetic mix, and as separate pure compounds. Therefore, it is not surprising to find that terpenes that make up an essential oil will

Table 1. Local lymph node assay^a responses for pure lavender oil, lavender oil exposed to air for 10 and 45 weeks, and synthetic mix exposed to air for 10 weeks

Test material/concentration (%)	d.p.m./lymph node	SI	EC3 (%)
Lavender oil			
Control	699		36
5	615	0.9	
25	1934	2.8	
100	3047	4.4	
Air-exposed lavender oil sample 1 (10 weeks)			
Control	1179		11
1	1171	0.99	
5	1741	1.5	
10	2917	2.5	
20	7791	6.6	
50	10 770	9.1	
Air-exposed lavender oil sample 2 (10 weeks)			
Control	716		23
2.5	1058	1.5	
10	1326	1.9	
50	3947	5.5	
Air-exposed lavender oil sample 2 (45 weeks)			
Control	556		4.4
1	616	1.1	
5	1861	3.4	
10	3244	5.8	
20	5985	11	
50	10 854	20	
Air-exposed artificial mix (10 weeks)			
Control	1025		14
1	879	0.86	
5	1294	1.3	
10	1805	1.7	
20	4995	4.9	
50	7643	7.5	

^aThe sensitization experiments were performed as described in Materials and Methods. The Stimulation index (SI) corresponds to the increase in thymidine incorporation of treated groups relative to vehicle-treated controls. EC3 (the estimated concentration required to induce an SI of 3) values were calculated using linear interpolation. The sensitizing potency of the test substances was classified according to the following: EC3 < 0.1%, extreme; EC3 ≥ 0.1 to <1%, strong; EC3 ≥ 1 to <10%, moderate; EC3 ≥ 10 to <100%, weak (13).

react the same way independent of whether they are in pure form or present in an essential oil.

Already in the 1950s, turpentine and some essential oils for pharmaceutical use were investi-

gated in Sweden and reported to oxidize when exposed to air (15, 16). The degree of decomposition of the oils was measured by their peroxide content, which was determined using an iodometric method. It was shown that lavender oil was among the most unstable of the essential oils studied. However, no conclusions were drawn from these results and the consequences of the decomposition were not investigated further.

The same oxidation products were detected in the samples of air-exposed lavender oil, as in the autoxidation studies of pure terpenes (4, 6). The hydroperoxides, which are considered primarily responsible for the sensitizing potency of oxidized terpenes, were found to be formed in amounts corresponding to those from the oxidation of the pure terpenes (4). This indicates that the autoxidation follows the same mechanism in the oil as in the pure terpenes. As the hydroperoxides accumulate to the same extent in the oil as in the oxidation mixtures of the pure terpenes, it can be assumed that they are equally stable in the oil as in the oxidation mixtures of pure terpenes, again indicating that no antioxidants or scavenging compounds are present in the oil. When comparing the sensitizing potency of autoxidized lavender oil with that of the autoxidized synthetic mix, no difference in the sensitizing potencies was observed (Fig. 6). Furthermore, the sensitizing potency of lavender oil was affected by autoxidation to approximately the same extent as the pure terpenes, indicating that the linalool and linalyl acetate hydroperoxides are the main contributors to the allergenic activity of air-exposed lavender oil (Fig. 7). Caryophyllene, the third major constituent of lavender oil, was present in much lower concentrations compared with linalool and linalyl acetate. We have previously shown that its sensitizing potency is affected only marginally by autoxidation because of the instability of the hydroperoxides formed (9). The sensitizing capacity of other constituents of the oil was not studied because they were present in only small amounts.

Table 2. Patch test results in four patients tested with pure and oxidized linalool, lavender oil and linalyl acetate. Three of the four patients were also tested with linalool hydroperoxides^a

Patients	Linalool oxidized 45 weeks				Linalool hydroperoxides						Lavender oil oxidized 45 weeks	Linalyl acetate oxidized 45 weeks
	4	2	1	0.5	1	0.75	0.5	0.25	0.12	0.06	4	4
1 (first study), female 63 years	NT	+	NT	NT	NT	NT	NT	NT	NT	NT	+	+
2, female 26 years	+	+	neg	neg	++	NT	++	+	neg	neg	+	+
3, male 65 years	++	++	+	neg	neg	neg	neg	neg	neg	neg	++	neg
4, male 56 years	+++	+++	++	+	++	+	+	+	neg	neg	+++	? ^b

+++ , vesicles, papules, erythema and infiltrate; ++ , papules, erythema and infiltrate; + , erythema and infiltrate; ? , doubtful reaction; neg , no positive reaction; NT , not tested.

^aReactions graded according to International Contact Dermatitis Research Group (ICDRG) guidelines (15).

^bPatient 4 was also tested with oxidized linalyl acetate 6% in petrolatum, which gave a positive reaction.

To prevent autoxidation, the fragrance industry adds antioxidants to linalool as well as natural extracts containing high levels of linalool (A. M. Api pers. comm.). The preventive effect of antioxidants is hard to control and depends on the purity of the fragrance ingredient, as has previously been shown (17).

We observed four cases of positive patch test reactions to oxidized lavender oil in patients with contact allergy to oxidized linalool. Two of these patients also reacted to linalool hydroperoxides. This is in accordance with an earlier multicentre study where, of 25 patients reacting to either oxidized linalool or linalool hydroperoxides, 44% reacted to both preparations, 20% reacted only to the hydroperoxides, and 36% reacted only to oxidized linalool, indicating that other allergens may also be of importance (8). Furthermore, positive reactions to oxidized linalyl acetate were identified, indicating that linalyl acetate hydroperoxides could be important sensitizers (Table 2). The findings give a pattern of simultaneous patch test reactions to oxidized lavender oil, linalool, and linalyl acetate. They show that allergenic oxidation products present in air-exposed lavender oil can provoke an elicitation of contact dermatitis. The clinical data support the experimental findings. However, further studies on more patients are needed to make a conclusive statement.

There are several case reports in the literature on contact allergy to lavender oil (18–20). In a study from Japan, the number of cases of contact allergy to lavender oil over a 9-year period is

reported (21). The positivity rate increased from 1.1% in 1990 to 13.9% in 1998. The authors discuss that the increase is caused by an increased use of lavender oil in Japan during this period. To the best of our knowledge, no follow up has been published internationally. Aromatherapists are exposed to many essential oils, in high concentrations, handling them with bare, unprotected hands for massaging, etc. Some case reports of aromatherapists with multiple contact allergies to essential oils, including lavender oil have been published (22–25). In the published reports on contact allergy to lavender oil, linalool and linalyl acetate are mentioned as constituents of the oil, and possible allergens (19, 20, 23). We know from our studies that pure linalool and linalyl acetate show very weak sensitizing potencies. Also α -pinene, a minor constituent of lavender oil, present at <2%, is discussed as a possible allergen (24). However, α -pinene is a simple unsaturated hydrocarbon and should not itself be an allergen considering structure-activity relationships for contact allergens.

From our findings, we conclude that lavender oil lacks natural protection against autoxidation and that oxidized lavender oil, as well as the air-exposed synthetic terpenes linalool and linalyl acetate, are sources of exposure to allergenic hydroperoxides and can elicit allergic contact dermatitis (ACD).

Acknowledgements

The skilful technical assistance of Petri Karhunen is gratefully acknowledged. Financial support by the Research Institute for Fragrance Materials Inc. (RIFM), New York, USA and the Swedish Asthma and Allergy Association is acknowledged.

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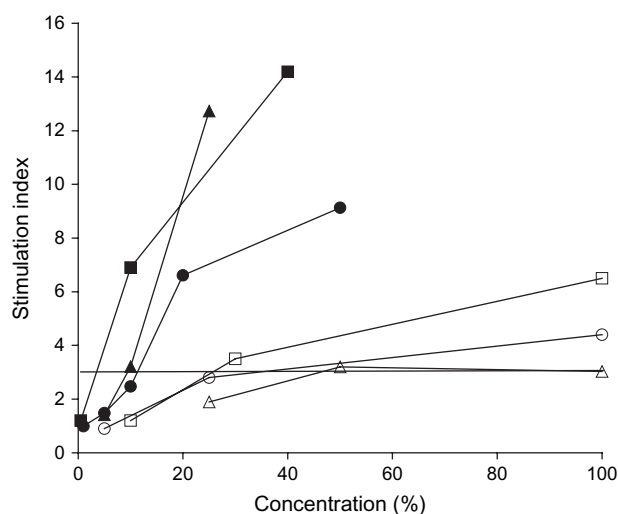


Fig. 7. Comparison between dose–response curves from the local lymph node assay for lavender oil and the major constituents of lavender oil, linalyl acetate and linalool, before and after air exposure. Lavender oil (not exposed to air) (○), pure linalyl acetate (□) and pure linalool (△). Air-exposed samples: lavender oil, sample 1 (10 weeks) (●), linalyl acetate (10 weeks) (■) and linalool (10 weeks) (▲).

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