

Autoxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens

MARIA SKÖLD, LINA HAGVALL AND ANN-THERESE KARLBERG

Department of Chemistry, Dermatochemistry and Skin Allergy, Göteborg University, SE-412 96 Göteborg, Sweden

Background: Fragrances are among the most common causes of allergic contact dermatitis. We have in previous studies shown that linalool, present in lavender oil, autoxidizes on air exposure, forming allergenic oxidation products. Oxidized linalool was found to be a frequent cause of contact allergy in a patch test study on consecutive dermatitis patients. Linalyl acetate, the main component of lavender oil is commonly used as a fragrance chemical in scented products. Because of structural similarities, linalyl acetate should also be susceptible to oxidation on air exposure, forming similar oxidation products as linalool.

Objective: The aim of the present study was to investigate the autoxidation of linalyl acetate and the influence of oxidation on its sensitizing potency.

Methods: Analyses were performed using gas chromatography, nuclear magnetic resonance spectrometry and mass spectrometry. Sensitizing potencies of compounds were determined using the local lymph node assay (LLNA) in mice.

Results: Analyses showed that the content of linalyl acetate decreased over time on air exposure and other compounds were formed. Hydroperoxides, an epoxide and an alcohol were identified as oxidation products from linalyl acetate. In the LLNA, linalyl acetate of high purity showed a weak sensitizing potency (EC3 25%). Autoxidation increased the sensitizing potency of linalyl acetate, and a 10 weeks oxidized sample gave an EC3 value of 3.6%. As for linalool, the hydroperoxides were shown to be the oxidation products with the highest sensitizing potency.

Conclusion: It is concluded that autoxidation of the weakly allergenic linalyl acetate leads to formation of allergenic oxidation products.

Key words: allergic contact dermatitis; autoxidation; fragrance; gas chromatographic analysis; hydroperoxide; linalool; linalyl acetate; local lymph node assay. © Blackwell Munksgaard, 2007.

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Linalyl acetate is a frequent fragrance ingredient used in decorative cosmetics, fine fragrances, shampoos, soaps, and other toiletries. Linalyl acetate is also commonly used in non-cosmetic products such as household cleaners and detergents (1). In a study where deodorants on the European market were analyzed for their content of different fragrance materials, linalyl acetate was found in 51 of 73 products (2). In another study, occupational and domestic products were analyzed and linalyl acetate was shown to be present in 16 of 59 products (3). When 400 cosmetic products in the USA were analyzed, linalyl acetate was found to be the third most common fragrance material,

found in 78% of the products (4). Linalyl acetate **1** and linalool **2** (Fig. 1) are the main constituents of lavender oil (5). Lavender oil is widely used in aroma therapy, another source of dermal exposure to linalyl acetate. Linalyl acetate also occurs naturally in clary sage (6).

The toxicological and dermatological properties of linalyl acetate have been extensively reviewed (1). Its skin sensitizing properties have been investigated in a number of human maximization tests. Linalyl acetate in concentrations of 10% or 20% were used and resulted in reactions in totally 3 out of 131 subjects. When retested, no sensitization reactions were produced. In

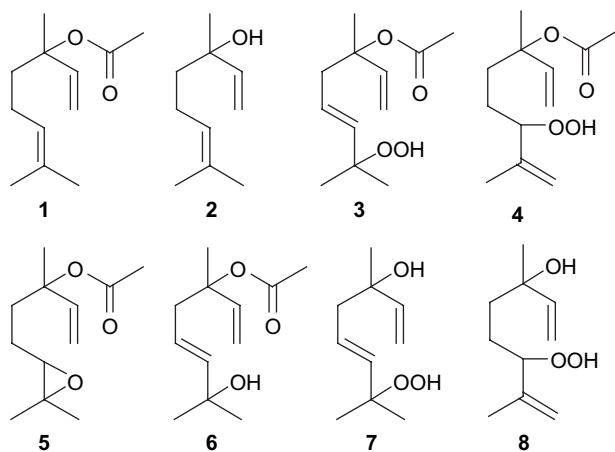


Fig. 1. Structures of linalyl acetate **1**, linalool **2**, oxidation products identified in air-exposed linalyl acetate: 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-yl acetate **3**, 6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-yl acetate **4**, 6,7-epoxy-3,7-dimethyl-1-octene-3-yl acetate **5**, 7-hydroxy-3,7-dimethylocta-1,5-diene-3-yl acetate **6**, and the previously described linalool hydroperoxides **7–8** (7, 8).

patch-test studies, on dermatitis patients, only a few reactions to linalyl acetate were observed (1).

Linalool is a monoterpenic alcohol and not allergenic in itself (7). We have shown that when exposed to air, linalool is autoxidized and allergenic oxidation products are formed (7, 8). It has also been shown in a multi-centre study on 1511 consecutive dermatitis patients that oxidation products of linalool are frequent sensitizers (9). Of the tested patients, 1.7% reacted to the oxidation mixture and/or to the main allergens, the linalool hydroperoxides.

Autoxidation is a free radical chain reaction where the hydrogen atom abstraction forming peroxy radicals shows selectivity for positions forming stable radicals. Similar to linalool, the only positions in linalyl acetate available for formation of stable radicals are the allylic positions of the double bond (C6C7). Thus, it could be expected that hydroperoxides corresponding to those identified in autoxidized linalool (Fig. 1) are formed on air exposure of linalyl acetate. Linalyl acetate is a non-conjugated ester of linalool and differs structurally from linalool only by the esterified hydroxyl group. As non-conjugated esters are very weak sensitizers it could be expected that the allergenic activity of linalyl acetate is increased after air exposure because of the formation of allergenic hydroperoxides.

The aim of this study was to investigate the autoxidation of linalyl acetate and to identify the oxidation products formed on air exposure. We also wanted to evaluate the effect of autoxidation on the sensitizing capacity of linalyl acetate.

Materials and Methods

Chemicals

Linalyl acetate (3,7-dimethyl-1,6-octadien-3-yl acetate) **1** (Fig. 1) (97%) was purchased from Sigma-Aldrich Chemie (Germany) and purified by distillation. Acetone was purchased from Merck (Germany) and olive oil from Apoteket AB (Sweden).

Instrumentation and mode of analysis

Chromatographic separations were performed using Scharlau silica gel 60 (230–400 mesh ASTM). Thin layer chromatography plates (Merck, 60 F₂₅₄ silica gel) were, after development, sprayed with a visualizing reagent containing anisaldehyde, sulfuric acid and acetic acid in ethanol. Gas chromatographic (GC) analyses were performed on a Hewlett Packard (HP) 6890 gas chromatograph equipped with an on-column injector and a flame ionisation detector, using a 30 m fused silica column (HP-5; ID 0.25 mm, 0.25 μ m film thickness) and nitrogen as carrier gas. The column temperature was 35° Celsius at injection, held isothermally for 2 min, raised to 185° Celsius at a rate of 5° Celsius/min, and finally held at 185° Celsius for 5 min. The detector temperature was 250° Celsius and 1,2,3,5-tetramethylbenzene was used as internal standard. Electron impact mass spectral data (70 eV) were obtained on an HP mass selective detector 5973 (scanned m/z : 50–500), connected to a GC HP 6890. High performance liquid chromatography (HPLC) analyses were conducted on a Merck LaChrom 2000 instrument with a diode array detector. ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were recorded on a JEOL eclipse+ 400 MHz spectrometer using CDCl₃ as solvent. Chemical shifts (δ) are reported in ppm relative to CHCl₃ at δ 7.25 and δ 77.0 for ¹H and ¹³C spectra, respectively. Photooxidation was performed using a Rayonet reactor equipped with 16 RPR 350 nm lamps.

Air exposure procedure

Linalyl acetate was air exposed in an Erlenmeyer flask, covered with aluminum foil to prevent contamination. It was stirred for 1 hr, 4 times a day, as previously described (10). A daylight fluorescent lamp (Philips, 18 W) was used to mimic normal daylight, and the flask was exposed to light 12 hr a day. Samples were taken from the flask after 10, 16, 24, and 28 weeks. The samples were analyzed with GC to determine the decreasing concentrations of linalyl acetate with time on air exposure. The oxidized samples were chromatographed on silica gel columns in order to isolate and identify oxidation products formed. Mixtures

of *iso*-hexane and ethyl acetate were used for elution, starting with 20% ethyl acetate, whereafter the proportion of ethyl acetate was increased. Isolated fractions/compounds were characterized with NMR and GC/mass spectrometry (MS).

Synthesis

7-Hydroperoxy-3,7-dimethylocta-1,5-diene-3-yl acetate (3) and 6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-yl acetate (4). Linalyl acetate (0.8 g, 4.1 mmol) was added to a CHCl_3 solution of tetrabutylammonium salt of Bengal rose, prepared according to a procedure described by Bäckström et al. (11). The solution was irradiated for 6 hr using a Rayonet reactor and a constant flow of oxygen. The solvent was removed under reduced pressure and the crude product was purified by chromatography on a silica gel column (10:90 ethyl acetate:hexane). This resulted in a colourless oil containing a mixture of **3** and the two diastereomers of **4**, in 58% yield (0.5 g).

3: ^1H NMR δ 8.18 (1H, s), 5.93 (1H, m), 5.60 (2H, m), 5.12 (2H, m), 2.57 (2H, m), 1.97 (3H, s), 1.49 (3H, s), 1.30 (6H, s); ^{13}C NMR δ 170.05, 141.37, 137.53, 125.47, 113.54, 82.32, 81.99, 42.21, 24.31, 24.28, 23.73, 22.07.

4: ^1H NMR δ 7.67 (1H, s), 5.90 (1H, m), 5.12 (2H, m), 4.98 (2H, 2×s), 4.25 (1H, m), 1.98 (3H, s), 1.80 (4H, m), 1.69 (3H, s), 1.50 (3H, s); ^{13}C NMR δ 170.05, 143.21, 141.22, (114.62, 114.61), (113.49, 113.44), 89.27, (82.59, 82.52), 35.61, (24.83, 24.78), (23.63, 23.59), 22.07, 17.04.

6,7-Epoxy-3,7-dimethyl-1-octene-3-yl acetate (5). *m*-chloroperbenzoic acid 77% (4.9 g, 19.9 mmol) was added to a solution of linalyl acetate (3.5 g, 18.1 mmol) in CH_2Cl_2 (100 ml) at 0° Celsius. The reaction mixture was stirred for 1 hr and then 20 ml of NaOH (2 M) was added. The organic phase was dried over MgSO_4 and evaporated. The crude product was purified by flash chromatography on silica gel with 10% ethyl acetate in *iso*-hexane which gave **5** (3.65 g) as a 1:1 mixture of diastereomers in 95% yield. ^1H NMR δ 5.94 and 5.89 (1H, 2×dd, J = 18.7, 11.0), 5.12 (2H, m), 2.67 (1H, dt, J = 6.4, 1.83), 1.97 and 1.96 (3H, 2×s), 1.52 and 1.50 (3H, 2×s), 1.61–1.39 (4H, m), 1.26 (3H, s), 1.22 (3H, s); ^{13}C NMR δ 169.79, 141.41 (141.22), 113.47 (113.40), 82.42 (82.29), 63.91, 58.30, 36.24, 24.78, 23.63 (23.55), 23.38, 22.07, 18.52. EIMS m/z 152.1(22), 137.1(17), 119.1(20), 109.1(45), 96.05(19), 95.1(23), 93.1(21), 91.1(28), 82.1(28), 81.1(100), 79.1(79), 77.1(24), 71.1(36), 69.1(22), 68.1(71), 67.1(83), 55.1(49), 53.1(32) and 152.1(21), 119.1(24), 109.1(43), 96.05(19), 95.1(23), 93.1(21), 91.1(34), 82.1(28), 81.1(100),

79.1(84), 77.1(28), 71.1(36), 69.1(23), 68.1(71), 67.1(83), 65.1(16), 55.1(44), 53.1(36).

Sensitization experiments in mice

The sensitizing potency of the chemicals was investigated using the local lymph node assay (LLNA) (12) as previously described (7). All animal procedures were approved by the local ethics committee. Each compound was tested in 3 or 4 different concentrations, using mice in groups of four or three, respectively. Results are expressed as mean d.p.m./lymph node for each experimental group and as stimulation index (SI), i.e. test group/control group ratio. Test materials that at 1 or more concentrations caused an SI greater than 3 were considered to be positive in the LLNA. EC3 values (the estimated concentration required to induce an SI of 3) were calculated by linear interpolation and used to compare the sensitizing capacity of the different test materials (13). The sensitizing potency of the test substances was classified according to the following: $\text{EC3} < 0.1\%$, extreme; $\text{EC3} \geq 0.1 - < 1\%$, strong; $\text{EC3} \geq 1 - < 10\%$, moderate; $\text{EC3} \geq 10 - < 100\%$, weak (14). The hydroperoxides **3** and **4** were synthesized by photooxidation and tested as a mixture (in an approximate ratio of 1:1).

Results

Air oxidation of linalyl acetate

Samples taken from air-exposed linalyl acetate after 10, 16, 24, and 28 weeks were analyzed with GC to determine the remaining amount of linalyl acetate. The analyses showed that 74% of the linalyl acetate remained after 10 weeks, 55% after 16 weeks, 38% after 24 weeks, and 24% after 28 weeks (Fig. 2).

Oxidation products in autoxidized linalyl acetate

Air-exposed linalyl acetate was subjected to flash chromatography in order to isolate oxidation products. The chromatographic and spectral properties of the isolated products were compared with those of synthesized potential oxidation products. The following oxidation products were isolated and identified: 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-yl acetate **3**, 6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-yl acetate **4**, 7-epoxy-3,7-dimethyl-1-octene-3-yl acetate **5**, and 7-hydroxy-3,7-dimethylocta-1,5-diene-3-yl acetate **6** (Fig. 1). The alcohol **6** was identified without a reference compound. The NMR and mass spectral data agree with literature values (15).

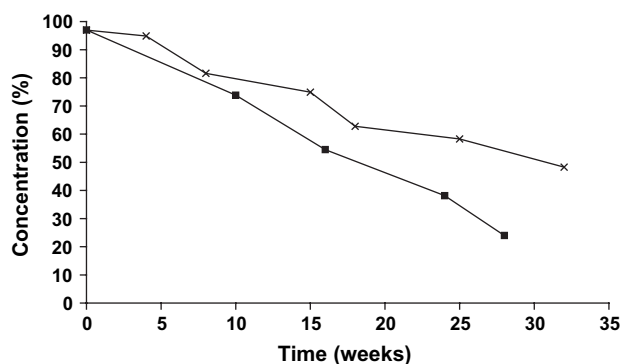


Fig. 2. Quantification of linalyl acetate (■), performed with gas chromatography using on column technique. In air-exposed linalyl acetate 74% of the compound was left after 10 weeks, 55% after 16 weeks, 38% after 24 weeks, and 24% after 28 weeks. The oxidation rate for linalyl acetate was approximately the same as for the previously studied linalool (×).

Skin sensitizing potency

The sensitizing potency of linalyl acetate, linalyl acetate air-exposed for 10 weeks, the linalyl acetate hydroperoxides, and the linalyl acetate epoxide was determined in the LLNA. The results are presented in Table 1 and Fig. 3. Molarity of the air-exposed sample was calculated using the molecular weight of linalyl acetate. As alcohols analogous to **6** previously have been shown to

be non-sensitizers (7, 16), **6** was not tested in the LLNA.

Discussion

In the present study, the autooxidation of linalyl acetate was investigated. It was found that linalyl acetate oxidizes on air exposure, in a manner similar to the previously studied linalool (7), forming allergenic hydroperoxides. This was also expected from a chemical point of view, because the chemical structure of linalyl acetate is closely related to that of linalool, containing the same active sites for hydroperoxide formation. An epoxide was identified as a secondary oxidation product, which was not found in the oxidation mixture of linalool. This epoxide was shown to be a non-sensitizer, presumably because of rapid conversion in the skin to the non-sensitizing cyclic compounds seen in the oxidation of linalool (7). Both linalyl acetate and linalool are commonly used as fragrance chemicals. They are also present in high concentrations in lavender oil, which is widely used both as it is and as a fragrance in cosmetic products. Thus, there is a high exposure of these oxidized materials. This is reflected in the high frequency of patients positive to oxidized linalool in patch testing (9).

Table 1. LLNA^a responses for pure linalyl acetate, linalyl acetate air-exposed for 10 weeks, linalyl acetate hydroperoxides **3** and **4**, and linalyl acetate epoxide **5**

Test material/concentration	DPM/lymph node	SI	EC3		Classification
			(%)	(M)	
Linalyl acetate (distilled)			25	1.27	Weak
Control	747				
10%	903	1.2			
30%	2646	3.5			
100%	4843	6.5			
Air-exposed linalyl acetate (10 weeks) ^b			3.6	0.18	Moderate
Control	519				
0.5%	610	1.2			
10%	3564	6.9			
40%	7390	14.2			
Linalyl acetate hydroperoxides			3.6	0.16	Moderate
Control	707				
0.25%	1019	1.4			
1%	1132	1.6			
10%	4529	6.4			
Linalyl acetate epoxide			—	—	Non-sensitizing
Control	656				
1%	926	1.4			
10%	769	1.2			
40%	715	1.1			
60%	728	1.1			

LLNA, local lymph node assay; SI, stimulation index.

^aThe sensitization experiments were performed as described in Materials and Methods. The SIs correspond to the increase in thymidine incorporation of treated groups relative to vehicle-treated controls. EC3 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 – < 1%, strong; EC3 ≥ 1 – < 10%, moderate; EC3 ≥ 10 – < 100%, weak.

^bMolarity was calculated using the molecular weight of linalyl acetate.

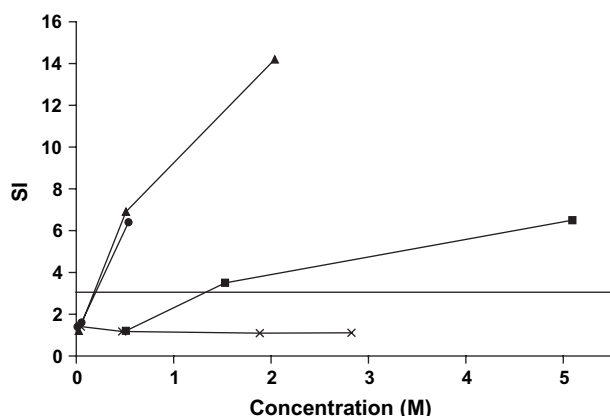


Fig. 3. Dose-response curves local lymph node assay (LLNA) for pure and air-exposed linalyl acetate together with identified oxidation products; distilled pure linalyl acetate (■), air-exposed linalyl acetate (10 weeks) (▲), linalyl acetate hydroperoxides **3** and **4** (●), and linalyl acetate epoxide **5** (×). SI, stimulation index.

Linalyl acetate was air-exposed according to previous experience from our investigation of the autoxidation of limonene (10) and linalool (7, 8) and the oxidation mixture obtained was flash chromatographed to isolate and identify oxidation products. The oxidation rate for linalyl acetate was approximately the same as for the previously studied linalool (7) (Fig. 2). The linalyl acetate hydroperoxides **3** and **4** (Fig. 1) were identified in autoxidized linalyl acetate, which is in agreement with the hydroperoxides **7** and **8** (Fig. 1), formed in autoxidized linalool and accords with our postulation. The alcohol **6** (Fig. 1), a secondary oxidation product from hydroperoxide **3**, was also identified in oxidized linalyl acetate.

The LLNA experiment showed linalyl acetate to be a weak sensitizer, which is in accordance with the literature, as aliphatic esters previously have been shown to be weak or non-sensitizers (17). As stipulated, autoxidation significantly increased the sensitizing capacity of linalyl acetate, and the 10 weeks oxidized sample gave an EC₃ value of 3.6%. The linalyl acetate hydroperoxides **3** and **4** were identified as sensitizers and gave the same EC₃ value as the oxidation mixture of linalyl acetate (EC₃ value of 3.6%). Because no other sensitizers were identified in the oxidation mixture we consider the hydroperoxides responsible for the sensitizing potency of air-exposed linalyl acetate.

The linalyl acetate epoxide **5** (Fig. 1) was isolated in the oxidation mixture of linalyl acetate. This is in contrast to autoxidized linalool, because instead of linalool epoxide two cyclic ethers, 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)-propan-2-ol and 2,2,6-trimethyl-6-vinyltetrahydro-2*H*-pyran-

3-ol, were identified in this oxidation mixture (Fig. 4). Their formation is explained by the attack of the hydroxyl group on the epoxide carbons, in the epoxide formed as a secondary oxidation product from the linalool hydroperoxide (7). In the case of linalyl acetate epoxide, the hydroxyl group is acetylated and consequently cannot attack the epoxide carbons. In the LLNA, linalyl acetate epoxide failed to induce a SI of 3 even at the highest concentration tested (60%) and was therefore considered a non-sensitizer. A most probable explanation is that the acetate group in this epoxide is hydrolysed by esterases in the skin, forming the corresponding linalool epoxide, which rapidly reacts to form the oxides as described above (Fig. 4). These compounds have previously been shown to be non-sensitizing (18).

Linalyl acetate is a frequently used fragrance chemical. There are only a few cases of contact allergy to linalyl acetate reported in the literature (1). In our studies on other frequently used fragrance terpenes, limonene (10), linalool (7, 8) and geraniol (16), we have shown that they all form allergenic oxidation products on air exposure leading to a marked increase in their sensitizing potential. For limonene and linalool patch testing of consecutive dermatitis patients showed that the oxidation mixtures are frequent causes of contact allergy in contrast to the non-oxidized compounds (9, 19, 20). Thus, it might also be the case that undiagnosed contact allergy to allergenic oxidation products of linalyl acetate is present in the

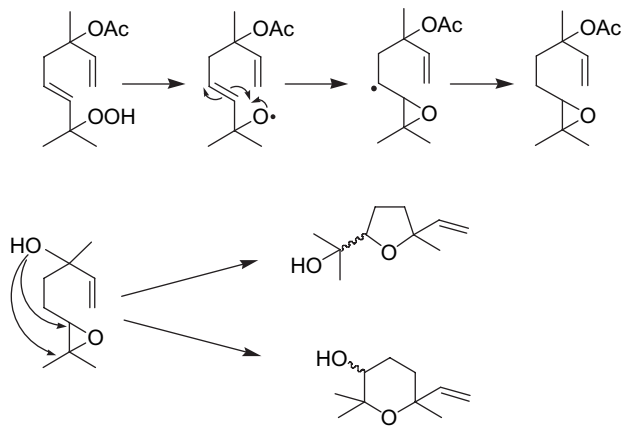


Fig. 4. In autoxidized linalyl acetate, an epoxide was isolated from the oxidation mixture. This compound can be formed as a secondary oxidation product from the linalyl acetate hydroperoxide. In autoxidized linalool, linalool oxides were formed as secondary oxidation products, but no epoxide could be isolated. The proposed mechanism suggested attack of the hydroxyl group on the corresponding linalool epoxide. The acetate group prevents the linalyl acetate epoxide from cyclization.

population. Patch testing with pure linalyl acetate would not be expected to give positive reactions in contrast to oxidized linalyl acetate which could show previously undetected cases of allergic contact dermatitis. The present study together with our previous investigations show the importance of careful handling and storage of fragrance compounds that are prone to autooxidation to prevent the formation of allergenic oxidation products leading to an increased risk of contact allergy in the population.

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Address:

Ann-Therese Karlberg
Department of Chemistry, Dermatochemistry and Skin Allergy
Göteborg University
SE-412 96 Göteborg
Sweden
Tel: +46 317724726
Fax: +46 317723840
e-mail: karlberg@chem.gu.se