Appendix S1.

SUPPLEMENTARY MATERIAL AND METHODS

Instrumentation and mode of analysis

Analysis of the composition of the lavender oil was performed using a Hewlett Packard (HP) 6890 gas chromatograph equipped with an on-column injector and a flame ionization detector, using a 30-m fused silica column (HP-5; ID 0.25 mm, 0.25 µm film thickness) and nitrogen as carrier gas. The column temperature was 35°C at injection, held isothermally for 2 min, raised to 185°C at a rate of 5°C /min, and finally held at 185°C for 5 min. The detector temperature was 250°C and 1,2,3,5-tetramethylbenzene was used as internal standard. This analysis revealed a content of 48% of linalyl acetate, 39% of linalool and 3% of β-caryophyllene.

Analysis of oxidation mixtures was performed on a reversed-phase high performance liquid chromatography (RP-HPLC), Agilent 1260 Infinity using a diode array detector (DAD SL G1315C, Agilent 1260 Infinity; Agilent Technologies, Santa Clara, CA, USA) and a ZORBAX Eclipse Plus C8 (4,6×150 mm; Agilent Technologies, Santa Clara, CA, USA) column. The sample rack was kept at a constant temperature of 20°C and the column oven temperature was set to 65°C. Mobile phase: starting at 70% water and 30% acetonitrile at 0 min, a linear gradient to 100% acetonitrile at 15 min was used. A mobile phase consisting of 100% acetonitrile was kept for 5 min further, giving a total program length of 20 min. The flow rate was 1.0 ml/min and the detector wavelength 195 nm. Quantification was performed using external calibration curves of linalyl acetate, linalool, linalyl acetate hydroperoxides and linalool hydroperoxides.

Analysis of the patch test preparations was performed to determine the stability of the hydroperoxides according to previous experience (12). Linalyl acetate, linalool, linalyl acetate hydroperoxides and linalool hydroperoxides were extracted using solid phase extraction (SPE) on an ISOLUTE silica SPE column (500 mg, 6 ml, Biotage AB, Uppsala, Sweden). Twenty mg of the patch test preparation was dissolved in hexane (1 ml) and the column was activated with hexane (5 ml) after the test material solution was applied. The petrolatum was rinsed from the column using hexane (5 ml). The analytes were eluted with ethyl acetate (5 ml) and collected in 1-ml fractions. The fractions were evaporated and dissolved in acetonitrile (1.5 ml) after HPLC analysis was carried out, as described above. Degassing of solvents used in SPE was performed using a Branson 2200 ultrasonic water bath.

Synthesis

Linalyl acetate hydroperoxides and linalool hydroperoxides were synthesized as described previously (8, 9).