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Allergenic Oxidation Products from Fragrance Terpenes

Chemical Analysis and Determination of Sensitizing Potency

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ABSTRACT

The ubiquitous presence of fragrance compounds in consumer products has resulted in a high frequency of contact allergy to fragrances in the population. For prevention purposes and according to EU regulations, cosmetics must be labeled when containing the most prominent fragrance allergens in concentrations exceeding 0.001% in "stay-on" products and 0.01% in "rinse-off" products. Previous studies have shown that the most common fragrance terpenes oxidize upon contact with air, forming hydroperoxides which are strong skin sensitizers. Thus, cosmetics may contain allergens in form of oxidation products formed by air exposure of the fragrance terpenes. In this thesis allergenic oxidation products from fragrance terpenes have been investigated. The studies have included development of analytical methods and chemical analyses for detection of hydroperoxides in complex mixtures. Furthermore, structure-activity relationships (SARs) were investigated based on studies of oxidation products and sensitizing potencies from autoxidation of the monoterpenes α-terpinene and citronellol.

Monoterpene hydroperoxides are generally difficult to determine as they have weak chromophores, exhibit low thermostability, and fragment in a similar way as parent compounds and other oxidation products in mass spectrometry. For the first time, analytical methods for detection of the highly sensitizing hydroperoxides in complex mixtures are now available utilizing mass spectrometry in combination with either liquid chromatography or gas chromatography. Low detection limits were achieved using liquid chromatography/mass spectrometry, while a high peak capacity useful for separation of hydroperoxide isomers was obtained with gas chromatography/mass spectrometry. The methods presented are sensitive enough for detection of hydroperoxides at concentrations that people may come in contact with. Thus, the analytical methods open up the possibility of investigating the clinical relevance of products in the patients' environment through chemical analysis.

Fragrance compounds in essential oils are often stated to be protected from autoxidation due to the presence of natural antioxidants. This study showed that hydroperoxides were present in the investigated essential oils already upon arrival from the supplier and that the hydroperoxide concentrations steadily increased with time and air exposure. The hydroperoxides were formed to the same extent in the essential oils compared to what is seen at air exposure of the corresponding fragrance terpenes. Thus, no significant protection against autoxidation by antioxidant activity could be observed. Neither was the rate of autoxidation substantially affected by the experimental conditions; stirring and intensity of daylight did not have any considerable effects.

Autoxidation studies of α-terpinene and citronellol were performed to gain knowledge regarding oxidation products and sensitizing potencies, and the results were compared to previously studied fragrance terpenes. It was shown that minor structural differences have a major impact on the dominant oxidation pathway and the stability of the oxidation products. For both compounds the sensitizing potency (as examined by the murine local lymph node assay) after air exposure was largely enhanced, around ten-fold. This shows the importance of testing with oxidized material in clinical studies and not only with the pure terpenes. In the risk assessment of fragrance chemicals, the possibility of autoxidation should be considered.

To summarize, the studies in this thesis have contributed to a method toolbox of complementary analytical methods with focus on mass spectrometry for detection and quantification of fragrance hydroperoxides. In addition, fragrance terpenes as constituents of essential oils form hydroperoxides, showing that the essential oils are not natural protectors for the formation of oxidation products but rather stabilize the hydroperoxides formed. Furthermore, this thesis shows that minor structural differences between fragrance terpenes can have a major impact on the dominant autoxidation pathway and oxidation products formed. Increased understanding of how fragrance terpenes can be activated to skin sensitizers by autoxidation is necessary to obtain valid SARs for this type of compounds. In the risk assessment, their susceptibility to autoxidation as well as the sensitizing potency of formed oxidation products should be considered.

Keywords: Autoxidation, Contact allergy, Essential oils, Fragrances, Hydroperoxides, Ionization techniques, Local lymph node assay, Mass spectrometry, Mouse, Prehapten, Sensitization, Skin, Structure-activity relationships, Terpenes, TMS derivatives