

Is a low content in atranol/chloroatranol safe in oak moss-sensitized individuals?

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Background: Chloroatranol and atranol are degradation products of chloroatranorin and atranorin, respectively, and have been identified as important contact allergens in oak moss absolute (*Evernia prunastri*).

Objectives: To investigate whether chemically modified extracts of oak moss produce positive patch test reactions in previously sensitized subjects.

Methods: A sample of oak moss was treated by a polymer-based method to reduce the content of the two main allergens, namely atranol and chloroatranol, from 3.4% to less than 75 p.p.m. and from 1.8% to less than 25 p.p.m., respectively. Fourteen subjects with positive reactions to oak moss from Trolab and/or Chemotechnique were patch tested to this sample, diluted 1% in petrolatum.

Results: The chemically modified sample reacted negatively in six but gave still positive reactions in eight subjects, with the same intensity as the commercially available oak moss patch test materials.

Conclusions: Polymer-based treatment of oak moss extract reduces the allergenic elicitation potential in previously sensitized individuals only to a minor extent. The residual concentrations of atranol and chloroatranol being less than 75 p.p.m. and 25 p.p.m., respectively, are unsafe for the consumer.

Key words: atranol; chloroatranol; contact allergy; elicitation; fragrances; oak moss; sensitization.
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Conflicts of interest: The authors have declared no conflicts.

Accepted for publication 3 September 2008

Oak moss absolute, a popular natural extract derived from the lichen *Evernia prunastri*, is widely used in perfumery because of its woody aroma and fixative properties (1, 2). It has been identified as a major cause of allergic reactions, accounting for at least 20% of the reactions observed with the Fragrance Mix I (1, 3, 4). For this reason, oak moss absolute is part of the diagnostic screening test for fragrance contact allergy (1). Chloroatranol and atranol have been identified as the main allergens in oak moss absolute by a bioassay-guided chemical fractionation procedure. Both substances are not found to be significantly present in the natural form of oak moss. However, during the production of the fragrance material, they can be formed by decomposition of depsides present in the lichen such as chloroatranorin and atranorin, which are non-volatile and odourless

substances (5). The content of atranol and chloroatranol in cosmetic products depends upon the oak moss absolute used, upon the degradation of atranorin and chloroatranorin in the product matrix, and, finally, upon the matrix effect in the determination of these molecules (6).

It has been found that oak moss absolute contains approximately 2.1% (w/w) of atranol and 0.9% (w/w) of chloroatranol (5). For this reason, exposure seems to be greater to atranol than to chloroatranol in perfumes. Indeed, quantitative exposure to chloroatranol and atranol has been studied in some popular perfumes, eaux de parfum and eaux de toilette available on the European market. Thirty-one products were analysed by liquid chromatography–electrospray ionization tandem mass spectrometry (LC-ESI-MS-MS) for their contents of atranol and

chloroatranol. The two substances were found in 87% ($n = 27$) of the products investigated, atranol in a median concentration in perfumes of 0.5 p.p.m. and chloroatranol of 0.25 p.p.m., with a maximum of 190 p.p.m. and 53 p.p.m., respectively (6, 7). In 2007, Rastogi et al. (6, 8) observed a significant decrease in the frequency of presence of chloroatranol in the products analysed in comparison with the above-mentioned study.

The sensitizing capacity of chloroatranol and atranol, as well as of oak moss absolute, has been studied in the local lymph node assay, and all proved to be strong sensitizers. It was shown that less chloroatranol was required than atranol to induce sensitization (9). Moreover, it was also shown that chloroatranol was more likely to produce a patch test response than atranol when tested in equimolar concentrations in sensitized individuals. The elicitation capacity of chloroatranol relative to atranol was 217%, i.e. a factor of 2.2 based on molar concentrations (7). The difference in exposure to atranol and chloroatranol may, therefore, counterbalance the difference in elicitation capacity with regard to clinical importance.

The Scientific Committee on Consumer Products (SCCP), an independent advisory committee to the European Commission, recommended in 2004 that chloroatranol and atranol should not be present in any cosmetic product because of their potency as allergens and considered untreated oak moss to be unsafe for the consumer (9). Therefore, an important challenge today for the cosmetic industry is to remove selectively the oak moss absolute allergy-causing components in a fashion that will not affect most of the other components of the extract neither its olfactive properties.

To improve the skin tolerance to atranol and chloroatranol, Ehret et al. (10) described the treatment of oak moss absolute with amino acids such as lysine and leucine, which lowered the content of allergenic constituents. The resulting oak moss absolute was tested in comparative studies on guinea-pigs and healthy volunteers. The results of the guinea-pig maximization test and human repeated insult patch test indicated that, in comparison with the commercial test sample, the allergenicity of the new oak moss absolute was considerably reduced. However, appropriate clinical studies on individuals with characterized contact allergy to oak moss have never been performed, to our knowledge, and are still required to demonstrate the dose–response characteristics of elicitation reactions with oak moss-treated preparations.

An elegant and efficient procedure reported in the literature for the removal of allergenic components of some essential oils was based on their binding to an insoluble polymer support through a mild coupling reaction (11, 12). As an example, the use of aminoethyl-polystyrene in ethanol was described to allow the removal of allergenic α -methylene- γ -butyrolactones from costus oil by Michael addition of the polymer nucleophilic ends with the electrophilic conjugated system of the lactones. In the course of our studies on allergenic natural extracts, we adapted this methodology to oak moss in order to remove sensitizing molecules such as aldehydes atranol and chloroatranol. The current clinical study was undertaken to investigate whether this chemically modified oak moss absolute, treated by a polymer-based method and containing less amounts of chloroatranol and atranol, still produced positive patch test reactions in previously sensitized subjects.

Materials and Methods

A sample of oak moss absolute was treated by a polymer-based method to reduce the content in atranol and chloroatranol, the two main allergens present in oak moss, at the Laboratory of Dermatochemistry, University Louis Pasteur, Strasbourg, France. The natural extract was treated with a polystyrene derivative having a specific reactivity towards aldehydic chemical functions. After treatment, the polymer containing the aldehydes bound was filtered-off, the solvent of the filtrate was removed under reduced pressure, and the remaining product was the polymer-treated oak moss absolute (sample A). It was possible to recover afterwards the polymer-bound aldehydes through chemical treatment. Atranol and chloroatranol contents were decreased (sample A) from 3.4% to less than 75 p.p.m. and from 1.8% to less than 25 p.p.m., respectively (high-performance liquid chromatography–ultraviolet measurements).

From February 2004 until November 2006, 14 patients, 4 males and 10 females (age range 24–76 years), known to be sensitized to oak moss absolute, were investigated at the Contact Allergy Unit in Leuven. They were retested to oak moss absolute from Trolab and Chemotechnique and patch tested to sample A, diluted in a 1% concentration in petrolatum. The patches were applied to the upper back using either Finn Chamber[®] with filter discs affixed with Scanpor[®] tape or Van Der Bend patch test chambers (Van Der Bend, Brielle, the Netherlands) on Micropore[™] (3M Health Care, Borken, Germany) and fixed with Mefix[®] (Mölnlycke Health Care, Göteborg, Sweden).

The patch tested readings were performed according to the international guidelines by the International Contact Dermatitis Research Group (13) after 2 days and 4 days, except in six subjects who did not want to come back for the second reading, but who were asked to report on additional late positive reactions by telephone.

Results

Table 1 summarizes the patch test results. All 14 subjects had a positive reaction to oak moss absolute, from Trolab, Chemotechnique, or both. In six of them, negative reactions to sample A were observed: three had negative readings at D2 and D4 and the other three reacted negatively at D2 and did not report on additional late positives. Eight subjects reacted to sample A with the same intensity as the commercially available oak moss patch test materials.

Figures 1 and 2 illustrate the positive patch test reactions observed in patient number 3. In Fig. 2, extreme positive reactions with intense erythema, infiltration, and coalescing vesicles to oak moss absolute from Trolab and Chemotechnique are visible, whereas the treated oak moss sample A is negative. Additionally, the patient also reacted to other components of Fragrance Mix I, i.e. isoeugenol and eugenol.

Discussion

According to the EU Cosmetics Directive, the maximum authorized concentration of oak moss absolute in cosmetic products is 0.1% (14). An oak moss sample was found to contain approximately 2.1% atranol and 0.9% chloroatranol (5).

Considering this as representative of atranol and chloroatranol in different oak moss absolutes samples, cosmetic products may contain up to 0.0021% (21 p.p.m.) atranol and 0.0009% (9 p.p.m.) chloroatranol. With regard to elicitation, chloroatranol was shown to cause reactions at the p.p.m. level (0.0005%, i.e. 5 p.p.m.) by repeated open exposure and at the p.p.b. level on patch testing (50% reacted to an extreme low concentration at 0.000015%, i.e. 150 p.p.b.) (15). Judged from this elicitation profile, chloroatranol is considered to be the most potent allergen present in consumer products today.

In 2008, based on new experimental sensitization data on chemically treated and untreated oak moss absolute samples, the SCCP was of the opinion that treatment of oak moss absolute at a laboratory scale was able to reduce the levels of atranol and chloroatranol to less than 2 p.p.m. each. Therefore, the levels of these allergens in cosmetic products in which oak moss is used at 0.1% would be such that the risk of induction and elicitation of allergic reactions to them would be low. However, the SCCP still considers that any reduction in elicitation will need to be demonstrated by appropriate clinical testing of subjects previously sensitized (16).

In the clinical study presented here, a chemically modified oak moss absolute, by using a polystyrene derivative with specific reactivity towards aldehydic chemical functions, was used to investigate if it still produced positive patch test reactions in previously sensitized subjects. The chemical treatment with the polymer gave a quality of oak moss with a low content of atranol and chloroatranol. It was estimated that atranol and chloroatranol contents were decreased from 3.4% to

Table 1. Patch test results at D2 and D4

Patient	Oak moss Trolab		Oak moss Chemotechnique		Oak moss Sample A		Other
	D2	D4	D2	D4	D2	D4	
1	+	NR	–	NR	+	NR*	FM I
2	–	–	–	+	–	NR*	FM I
3	+++	NR	+++	NR	–	NR*	FM I
4	+	NR	+	NR	+	NR	FM I, colophonium
5	+	NR	+	NR	+	NR	FM I
6	+	NR	+	NR	–	NR*	FM I, colophonium
7	+	+	+	+	–	–	FM I
8	+	+	+	–	+	+	FM I
9	+	++	+	++	+	++	FM I
10	+	++	–	–	+	++	FM I
11	+	+	+	+	+	+	FM I
12	+	+	–	+	–	–	FM I
13	–	+	–	–	–	+	FM I, colophonium
14	–	++	–	++	–	–	FM I

+, doubtful reactions; FM I, fragrance mix I; NR, not read.

*Did not report on additional late positive reactions.



Fig. 1. Positive patch test reaction to Fragrance Mix I (subject 3).

less than 75 p.p.m. and from 1.8% to less than 25 p.p.m., respectively. The patch test results presented here showed that treated oak moss produced elicitation reactions in 8 of 14 oak moss previously sensitized subjects. Therefore, it must be considered that the residual concentrations of atranol and chloroatranol being less than 75 p.p.m. and 25 p.p.m., respectively, are still far too high to be safe regarding elicitation.

However, we did show in a previous study that methyl- β -orcinol carboxylate, a depside degradation product and the most important monoaryl derivative of oak moss from an olfactory stand point, was also eliciting a reaction in patients sensitized to oak moss included in the study, although to a lower extent compared with atranol and chloroatranol (5). In reality, freshly harvested oak moss has no scent. The moss contains various types of depsides, which are non-volatile and odourless such as atranorin and chloroatranorin (17). The characteristic oak moss fragrance is developed after cleavage of the depsides under treatment of the oak moss concrete with alcohols

to give volatile, scented monoaryl derivatives (18). As such, the transesterification and decarboxylation of atranorin and chloroatranorin during the ethanolic treatment of the moss gives not only atranol and chloroatranol but also methyl- β -orcinol carboxylate, which is the major responsible for the characteristic earthy like odour of oak moss products (5). The treated oak moss absolute used in this study was obtained after a polymer-based treatment that was only specific for the removal of aldehyde derivatives such as atranol and chloroatranol. Therefore, methyl- β -orcinol carboxylate was still present in the tested sample A. As this compound was shown to elicit allergy reactions to previously sensitized oak moss patients and as it was not removed by the chemical treatment of the moss, it might be also suggested that the methodology used for that treatment did not completely eliminate the eliciting potential of oak moss absolute, so could also be an extra factor to explain why some patients still elicited positive reactions to the treated oak moss.

Conclusions

We could show that polymer-based treatment of oak moss extract reduces the allergenic elicitation potential in previously sensitized individuals only to a minor extent. In view of more recent data and considering elicitation, the major sensitizers atranol and chloroatranol should be eliminated almost completely from oak moss absolute in order to be safe for consumers.

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Fig. 2. Positive patch test reactions to the ingredients in the same patient, the chemically modified sample A (OM010) being negative.

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