

# Patch testing with serial dilutions and thin-layer chromatograms of oak moss absolutes containing high and low levels of atranol and chloroatranol

Martin Mowitz, Erik Zimerson, Cecilia Svedman and Magnus Bruze

Department of Occupational and Environmental Dermatology, Skåne University Hospital, Lund University, SE-20502 Malmö, Sweden

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## Summary

**Background.** Oak moss absolute (*Evernia prunastri* extract) contains a large number of substances, among them the potent allergens atranol and chloroatranol. Since 2008, their content in oak moss absolute has been restricted by the International Fragrance Association to a maximum level of 100 ppm each.

**Objectives.** To compare the elicitation capacities of a traditional (sample A) and a treated (sample B) oak moss absolute containing, in total, 27 000 and 66 ppm of atranol and chloroatranol, respectively, and to investigate reactions to components of oak moss absolute separated by thin-layer chromatography (TLC).

**Methods.** Fifteen oak moss-allergic subjects were patch tested with serial dilutions and TLC strips of samples A and B.

**Results.** Fifteen subjects reacted to sample A at concentrations  $\leq 2.0\%$ , and 2 subjects reacted to sample B at 2.0% but not to lower concentrations. Among 13 subjects reacting to the TLC strip of sample A, 11 reacted to spots with retardation factor values corresponding to those of atranol and/or chloroatranol, and 11 reacted to other areas on the TLC strip. Only one subject reacted to the TLC strip of sample B.

**Conclusions.** The patch test reactivity of sample B was significantly lower than that of sample A. The TLC patch tests indicate the presence of sensitizers other than atranol and chloroatranol in oak moss absolute.

**Key words:** atranol; chloroatranol; *Evernia prunastri* extract; oak moss absolute

Oak moss absolute [International Nomenclature of Cosmetic Ingredients (INCI) name: *Evernia prunastri* extract] is an extract derived from the lichen *E. prunastri*. It is used in fragrance compositions for its woody aroma

and fixative properties. Oak moss absolute is one of the components of fragrance mix I (FM I), which has been used to diagnose fragrance contact allergy since the late 1970s (1). Oak moss absolute has been reported as the most common allergen when patients positive to FM I are patch tested with its individual constituents (2).

Atranol and chloroatranol have been identified as the main allergens in oak moss absolute (3). These substances are not present in the crude lichen, but are formed during the production of the oak moss extracts (3–5). However, oak moss absolute contains a large number of substances, including other known allergens, and it is not fully clear to what extent an allergic reaction to oak moss absolute can be explained by contact allergy to atranol and/or chloroatranol.

*Correspondence:* Martin Mowitz, Department of Occupational and Environmental Dermatology, Skåne University Hospital, 205 02 Malmö, Sweden. Tel: +46 40337410; Fax: +46 40336213. E-mail: martin.mowitz@med.lu.se

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**Table 1.** Patch test reactions to serial dilutions of untreated (sample A) and treated (sample B) samples of oak moss absolute as well as individual test substances; the strongest reaction on either D3/4 or D7 is given

| Test preparation     | Subject no. |     |    |     |    |     |     |     |     |     |     |     |     |    |    |
|----------------------|-------------|-----|----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|
|                      | 1           | 2   | 3  | 4   | 5  | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14 | 15 |
| <b>Sample A</b>      |             |     |    |     |    |     |     |     |     |     |     |     |     |    |    |
| 2.0% ac              | ++          | ++  | ++ | NT  | NT | ++  | NT  | ++  | ++  | ++  | +   | +++ | +   | ++ | +  |
| 0.63% ac             | +++         | ++  | +  | +++ | NT | ++  | NT  | ++  | (+) | ++  | +   | +++ | -   | ++ | -  |
| 0.20% ac             | ++          | ++  | +  | ++  | +  | (+) | ++  | +   | +   | +   | +   | ++  | -   | ++ | -  |
| 0.063% ac            | +           | ++  | -  | ++  | +  | -   | ++  | +   | -   | -   | (+) | +   | -   | +  | -  |
| 0.020% ac            | +           | ++  | -  | (+) | +  | -   | -   | (+) | -   | -   | -   | +   | -   | +  | -  |
| 0.0063% ac           | -           | +   | -  | -   | -  | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| 0.0020% ac           | -           | (+) | -  | -   | +  | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| 0.00063% ac          | -           | -   | -  | -   | -  | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| <b>Sample B</b>      |             |     |    |     |    |     |     |     |     |     |     |     |     |    |    |
| 6.3% ac              | NT          | NT  | +  | (+) | +  | ++  | ++  | -   | (+) | NT  | -   | NT  | -   | -  | -  |
| 2.0% ac              | -           | +   | -  | -   | -  | -   | (+) | -   | -   | +   | -   | -   | -   | -  | -  |
| 0.63% ac             | -           | (+) | -  | -   | -  | -   | -   | -   | -   | (+) | -   | -   | -   | -  | -  |
| 0.20% ac             | -           | -   | -  | -   | -  | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| Evermic acid         | -           | -   | -  | -   | -  | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| 0.1% pet             | -           | -   | -  | -   | +  | -   | +   | (+) | -   | -   | -   | -   | -   | -  | -  |
| Usnic acid           | -           | -   | -  | -   | -  | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| 0.1% pet             | -           | -   | -  | -   | -  | -   | -   | -   | -   | -   | -   | -   | -   | ++ | -  |
| <b>Atranorin</b>     |             |     |    |     |    |     |     |     |     |     |     |     |     |    |    |
| 0.1% pet             | +           | -   | -  | -   | +  | -   | NT  | +   | -   | -   | -   | -   | -   | -  | -  |
| 0.032% ac            | +           | NT  | NT | NT  | NT | NT  | ++  | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |
| 0.010% ac            | (+)         | NT  | NT | NT  | NT | NT  | +   | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |
| 0.0032% ac           | (+)         | NT  | NT | NT  | NT | NT  | +   | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |
| 0.0010% ac           | -           | NT  | NT | NT  | NT | NT  | ?   | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |
| <b>Atranol</b>       |             |     |    |     |    |     |     |     |     |     |     |     |     |    |    |
| 0.050% ac            | +           | -   | -  | NT  | +  | +   | NT  | +   | -   | -   | (+) | -   | -   | -  | -  |
| 0.010% ac            | -           | -   | -  | +   | -  | -   | +   | -   | -   | -   | (+) | -   | -   | -  | -  |
| 0.0032% ac           | NT          | NT  | NT | ++  | NT | NT  | -   | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |
| 0.0010% ac           | NT          | NT  | NT | ++  | NT | NT  | -   | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |
| 0.00032% ac          | NT          | NT  | NT | +   | NT | NT  | -   | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |
| 0.00010% ac          | NT          | NT  | NT | -   | NT | NT  | -   | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |
| <b>Chloroatranol</b> |             |     |    |     |    |     |     |     |     |     |     |     |     |    |    |
| 0.050% ac            | ++          | -   | -  | NT  | -  | ++  | NT  | +   | -   | NT  | (+) | +   | (+) | -  | -  |
| 0.010% ac            | -           | -   | -  | ++  | -  | +   | NT  | (+) | -   | ++  | (+) | -   | -   | -  | -  |
| 0.0032% ac           | NT          | NT  | NT | ++  | NT | NT  | +   | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |
| 0.0010% ac           | NT          | NT  | NT | (+) | NT | NT  | +   | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |
| 0.00032% ac          | NT          | NT  | NT | -   | NT | NT  | -   | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |
| 0.00010% ac          | NT          | NT  | NT | -   | NT | NT  | -   | NT  | NT  | NT  | NT  | NT  | NT  | NT | NT |

NT, not tested.

The concentrations are given as % (wt/wt) for pet. preparations and as % (wt/vol) for acetone (ac) solutions.

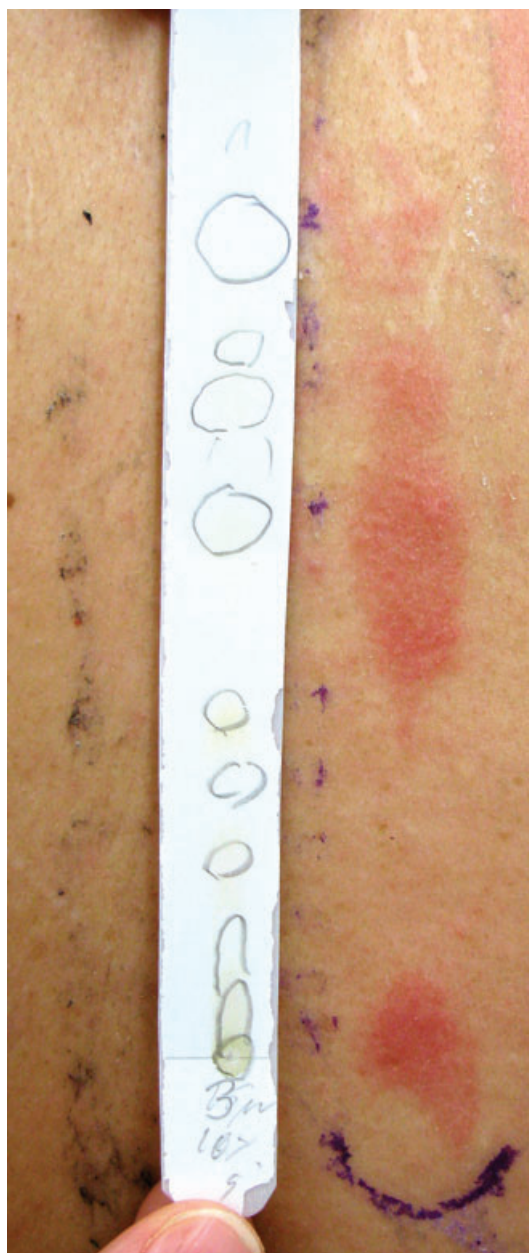


**Fig. 1.** Positive reactions in subject 6 to components of sample A (untreated oak moss absolute) on a thin-layer chromatography strip.

According to Cosmetic Products Regulation of the EU, oak moss absolute has to be declared with its INCI name when present in leave-on products at levels >10 ppm and when present in rinse-off products at levels >100 ppm. The Cosmetic Products Regulation does not regulate the levels of oak moss absolute in cosmetic products or the levels of chloroatranol and atranol in the absolutes (6). According to the International Fragrance Association

(IFRA) standard on oak moss extracts, the maximum concentration allowed in cosmetic products under this self-regulation is 0.1%. Since 2008, there has also been an IFRA restriction on the concentrations of atranol and chloroatranol in oak moss extracts, which must not exceed 100 ppm each (7).

The aim of the present study was to compare the elicitation capacity of a traditional oak moss absolute with that of an oak moss absolute with reduced levels of atranol and chloroatranol in compliance with the IFRA standard. Furthermore, we wished to investigate the pattern of reactivity towards the components of the two samples separated on thin-layer chromatography (TLC) strips used for patch testing.

## Material and Methods

### Study population

Fifteen subjects (11 females, 4 males, mean age 50 years, range 25–67 years) diagnosed with contact allergy to oak moss absolute at our department between 2006 and 2008 were enrolled in the study. The strength of the original patch test reaction to oak moss absolute was scored as + in 5 subjects, as ++ in 7 subjects, and as +++ in 3 subjects.

### Patch test preparations

Two samples of oak moss absolute were used in the study. Both samples were provided by Robertet (Grasse, France). Sample A was a traditional oak moss absolute containing, in total, 2.7% atranol and chloroatranol; sample B had been treated by the manufacturer in order to reduce the content of atranol and chloroatranol to a total concentration of 0.0066% (66 ppm). Samples A and B were patch tested in serial dilutions prepared at our laboratory. 2.0% (wt/vol) stock solutions of samples A and B in acetone (VWR International S.A.S, Fontenay-sous-Bois, France) were further diluted by a factor of  $\sqrt{10}$  to the following concentrations: 0.63%, 0.20%, 0.063%, 0.020%, 0.0063%, 0.0020%, 0.00063%, 0.00020%, and 0.000063% (wt/vol). Eleven of the subjects with negative or doubtful reactions to sample B at 2.0% were also tested with sample B at 6.3%.

Atranol and chloroatranol (Laboratoire de Dermatologie, University Louis Pasteur, Strasbourg, France) were each dissolved and diluted in acetone to a concentration of 0.010% (wt/vol). Subjects reacting positively to the 0.010% preparations of atranol and/or chloroatranol on D3 or D4 were additionally tested with dilutions of atranol and/or chloroatranol at 0.0032%, 0.0010%, 0.00032%, and 0.00010% (wt/vol). Subjects

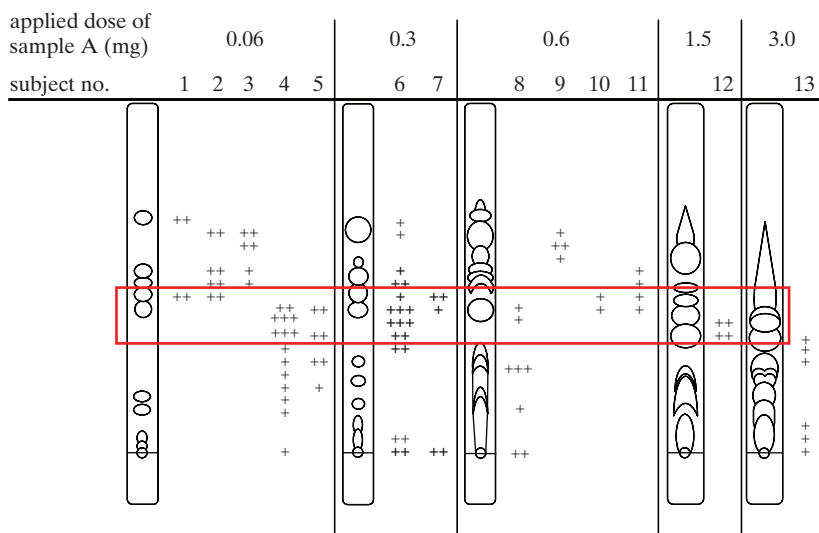


**Fig. 2.** Distribution of positive reactions to thin-layer chromatography strips of sample A (untreated oak moss absolute). The approximate area where atranol and chloroatranol appear on the chromatograms is marked in red.

who were negative to the 0.010% preparations of atranol and/or chloroatranol on D3 or D4 were tested with 0.050% (wt/vol) preparations of atranol and/or chloroatranol. Furthermore, all subjects were tested with 0.1% (wt/vol) petrolatum preparations of the lichen allergens atranorin, usnic acid, and evernic acid (Chemotechnique Diagnostics, Vellinge, Sweden).

The levels of atranol and chloroatranol in samples A and B were determined by gas chromatography–mass spectrometry (GCMS) (8). Both samples A and B and atranol and chloroatranol standards were dissolved in pyridine (Acros Organics, Geel, Belgium) and derivatized with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (Acros Organics) before they were injected into the GCMS apparatus.

### Thin-layer chromatography

Patch testing with TLC strips was performed according to the procedure described by Bruze et al. (9). Samples A and B dissolved in acetone were applied on TLC strips cut from a Merck TLC silica gel 60 F254 plastic roll (Merck, Darmstadt, Germany). On the basis of the strength of previous patch test reactions, different doses, ranging from 0.06 to 3.0 mg of oak moss absolute diluted in acetone, were applied on the TLC strips. When the TLC patch tests were negative or doubtful at the first reading, the subjects were additionally tested with TLC strips containing a higher dose of sample A or B. The samples were eluted on the TLC strips with a mobile phase consisting of 86% (vol/vol) chloroform (Merck) and 14% (vol/vol) acetonitrile (VWR International, Leuven, Belgium). For each subject, the TLC strips were prepared in duplicate; one strip was used for patch testing, and the other was

used as a template when the tests were read. The TLC strips were inspected under ultraviolet light at 254 and 366 nm. The visualized spots were marked with a pencil. The TLC strips were prepared as close in time to the patch test as possible, normally on the same day or the day before. The TLC strips were wrapped in aluminium foil, and stored in a refrigerator until they were used for patch testing. The TLC retardation factor values of atranol and chloroatranol were examined when they were applied as individual substances and in spiked oak moss absolute samples.

### Patch testing

Patch testing was performed with 8-mm Finn Chambers<sup>®</sup> (Epitest, Helsinki, Finland) on Scanpor<sup>®</sup> tape (Norgeplaster A/S, Oslo, Norway). The test chambers were loaded immediately before they were applied to the backs of the participants (10). 20 mg of the pet. preparations was applied in the test chambers (11) and 15 µl of the acetone solutions was micropipetted onto the filter paper discs in the test chambers. All 15 subjects were tested with TLC strips of sample A, and 11 of the subjects were also patch tested with TLC strips of sample B. The TLC strips were applied on the upper back of the subjects with Scanpor<sup>®</sup> tape. The test chambers and TLC strips were removed after 48 hr, and the readings were performed on D3 or D4 and on D7. The tests were scored according to the International Contact Dermatitis Research Group criteria (12).

### Ethics

The study was approved by the Regional Ethical Review Board in Lund, Sweden. Written informed consent was obtained from each participant.

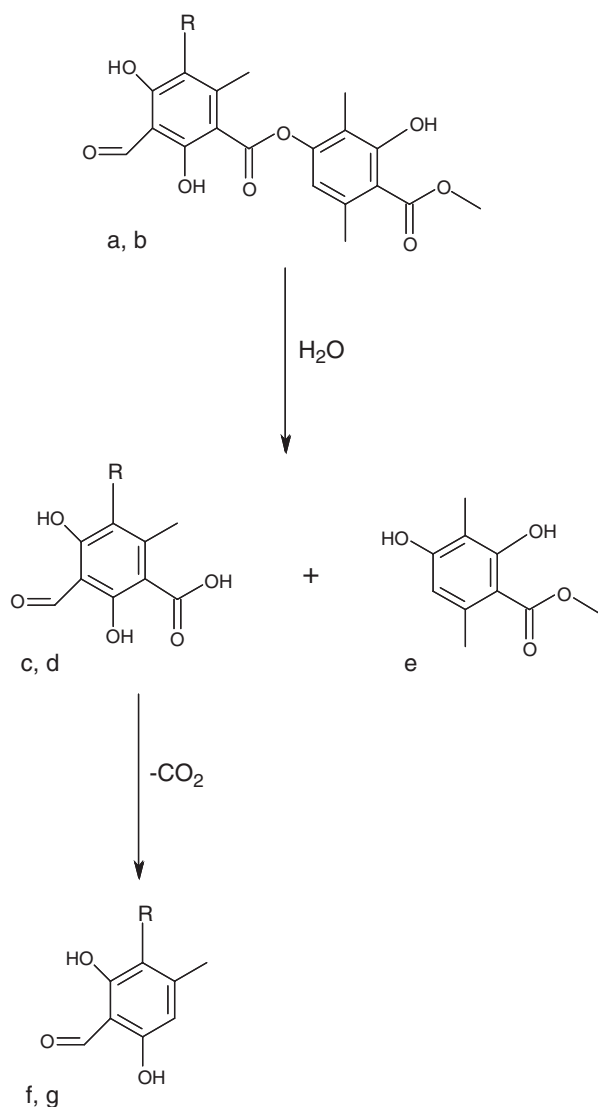


**Fig. 3.** Decomposition of atranorin (a, R = H) and chloroatranorin (b, R = Cl) by hydrolysis, giving haematommic acid (c, R = H) and chlorohaematommic acid (d, R = Cl), together with methyl  $\beta$ -orceinolate (e). Haematommic acid and chlorohaematommic acid are further decarboxylated to atranol (f, R = H) and chloroatranol (g, R = Cl).

## Statistics

McNemar's test (exact version, two-sided) was used to compare the numbers of oak moss-allergic subjects reacting to the patch tests of samples A and B. Differences were considered to be significant at  $p < 0.05$ .

The minimum eliciting concentrations (MECs) were compared between samples A and B. The positive patch test reactions were not always continuous. When the negative and/or doubtful reactions were followed by at

least the same number of positive reactions, the lowest concentration giving a positive reaction was considered to be the MEC. Otherwise, the last positive concentration above the negative or doubtful reactions was considered to be the MEC (13). When the ratio between the MEC of sample B and the MEC of sample A was calculated for the subjects totally negative to sample B, it was assumed that they would test positively if they were tested with a preparation of sample B at a concentration corresponding to a step further up in the dilution series, i.e. the highest tested concentration multiplied by a factor of  $\sqrt{10}$ .

## Results

The results of the patch tests with the dilution series of samples A and B are summarized in Table 1. All 15 subjects reacted to sample A at concentrations of  $\leq 2.0\%$ , and 2 of 15 subjects reacted to sample B at 2.0% ( $p < 0.001$ ). Among subjects with negative or doubtful reactions to sample B at 2.0%, 4 of 11 showed a positive reaction when the test concentration of sample B was raised to 6.3%. The MECs were 0.002% for sample A and 2.0% for sample B. The ratio between the MEC of sample B and that of sample A varied between 10 and 3200 in the individual subjects. The numbers of subjects with positive reactions to the additionally tested individual substances were 7 for chloroatranol, 6 for atranol, 4 for atranorin, 2 for evernic acid, and 1 for usnic acid.

Positive reactions to the TLC strips of sample A were observed in 13 of 15 subjects. Only 1 of 11 subjects tested with TLC strips of sample B showed a positive reaction. The reactions to the TLC strips of sample A were distributed all over the area where the components of the oak moss absolute had migrated (Figs. 1 and 2).

The retardation factor values of atranol and chloroatranol observed when oak moss absolute samples spiked with atranol and chloroatranol were applied to the TLC strips differed slightly from those observed when atranol and chloroatranol were applied individually. Furthermore, the retardation factor values were affected by the amount of sample applied to the TLC strips. The approximate region where atranol and chloroatranol appeared on the TLC strips is marked in Fig. 2. Eleven subjects reacted to spots within this region, and, in total, 11 subjects showed positive reactions to other areas of the chromatograms.

On analysis by GCMS, approximately 2.5% (25 000 ppm) of atranol and 0.93% (9300 ppm) of chloroatranol were found in sample A. Approximately 90 ppm of atranol and 20 ppm of chloroatranol were found in sample B.

## Discussion

The raw material used for oak moss absolutes is the lichen *E. prunastri*, which is collected from oak trees in the south-central regions of Europe, as well as in Morocco and Algeria. Each year, ~700 tons of the lichen are processed in France. After being harvested, the lichen is desiccated and then humidified with water prior to the extraction procedure with organic solvents. The solvents used are either hexane or mixtures of hexane and more polar solvents, mainly acetates. The crude solvent extracts, called resinoids, are further treated with ethanol in order to obtain the absolutes, which are then used in fragrance compositions. The absolutes may also be subjected to physical treatments such as discoloration with charcoal or high-vacuum distillation (5). The chemical composition of natural extracts is often complex, and >170 substances have been identified in oak moss extracts. Some of these substances are not present in the crude lichen, but are formed during the processing of the extracts. When the dried lichen is treated with water, phenyl benzoate derivatives such as atranorin and chloroatranorin are hydrolysed, and further decarboxylation results in the formation of atranol and chloroatranol (Fig. 3). The degradation of odourless phenyl benzoates is essential for the olfactory properties of the oak moss absolutes. Orcinol monomethyl ether and methyl  $\beta$ -orcinol carboxylate, which are considered to be the dominant odour constituents of oak moss absolutes, are formed from evernin in a pathway analogous to the one described in Fig. 3. The phenyl benzoates may also be degraded through a transesterification reaction with ethanol (3–5).

Several substances found in lichens have been reported as contact allergens, including atranorin, evernic acid, fumarprotocetraric acid, and usnic acid (14–17). More recently, atranol and chloroatranol have been identified as strong allergens and methyl  $\beta$ -orcinolcarboxylate has been identified as a weak allergen in oak moss absolute (3).

Chloroatranol has been shown to elicit allergic reactions at ppb level in patch tests and at ppm levels in a repeated open application test (18). The elicitation capacity of chloroatranol has been found to be 2.2 times higher than that of atranol (19). Rastogi et al. have found levels of atranol and chloroatranol in perfumes that are likely to be able to elicit allergic contact dermatitis in sensitized individuals (20).

The contents of atranol and chloroatranol in untreated oak moss absolutes have been reported to be in the ranges of 2.4–2.9% and 0.9–1.4%, respectively (3, 5, 21). The contents of atranol and chloroatranol in the IFRA-compliant oak moss absolutes offered on the market today are below 100 ppm (0.01%) each. Consumers using

cosmetic products containing these extracts are exposed to atranol and chloroatranol at concentrations of  $\leq 0.1$  ppm. The IFRA standard on oak moss absolute is part of a self-regulating system for the fragrance industry members of IFRA. Thus, oak moss absolutes with qualities other than those of oak moss absolutes manufactured in accordance with the IFRA standard may be available on the market.

Several methods for reducing the content of sensitizers such as atranol and chloroatranol in oak moss extracts have been reported, involving, for example, chromatographic methods (22), treatment with amino acids (21), or binding to an insoluble polymer support (23). The two latter methods act on the aldehyde function in atranol and chloroatranol, but there are also other aldehydes present in the oak moss extracts, and it is also likely that their contents are reduced with the treatment mentioned above.

Nardelli et al. performed patch testing with a 1% pet. preparation of oak moss absolute treated with a polymer-based method that reduced the contents of atranol and chloroatranol to <75 and <25 ppm, respectively. Nevertheless, 8 of 14 oak moss-allergic individuals reacted to the treated sample, and the authors concluded that the described treatment 'reduces the allergenic elicitation potential in previously sensitized individuals only to a minor extent', and that the residual amounts of atranol and chloroatranol are 'unsafe for the consumer' (23).

We present data on patch testing with serial dilutions of two samples of oak absolute extracts containing atranol and chloroatranol at different levels. Sample B was of current commercial quality, containing, in total, 66 ppm of atranol and chloroatranol, whereas sample A was a traditional oak moss absolute, with a total concentration of atranol and chloroatranol of 2.7% (27 000 ppm).

The elicitation capacity of sample B was considerably lower than that of sample A. Nevertheless, some oak moss-allergic individuals reacted positively to this sample. For these individuals, the positive patch tests could be explained by reactions either to the low levels of atranol and/or chloroatranol or to other substances present in oak moss absolute. The ratio between the MEC of sample B and that of sample A varied between 10 and 3200 in the individual subjects. This variation might indicate the importance of allergens other than atranol and chloroatranol. If the difference in reactivity towards samples A and B resulted only from the different levels of atranol and chloroatranol in the samples, a ratio of the MECs in the same order of magnitude as the ratio between the concentrations of atranol and chloroatranol in the samples would be expected.

Although the total level of atranol and chloroatranol (<1000 ppb) in the 1% pet. preparation used in the

study by Nardelli et al. was similar to or lower than that in the 2.0% solution of sample B, containing, in total, 1300 ppb of atranol and chloroatranol, more reactions were observed in the previous study. This might indicate that the method used by the manufacturer to reduce the content of atranol and chloroatranol in sample B to a larger extent also reduces the contents of other allergens. However, there are several differences between the studies with regard to the origin of the absolutes, the concentrations and vehicles used, the time of patch test reading, and the reactivities of the study populations towards the untreated oak moss samples.

Our GCMS investigations of samples A and B confirmed that the total concentrations of atranol and chloroatranol were in the same order of magnitude as the concentrations stated by the manufacturer. We observed slightly higher concentrations, which might be explained by the breakdown of atranorin/chloroatranorin into atranol/chloroatranol during storage of the samples or by the heat in the GCMS analysis.

The patch tests with TLC strips of sample A indicated the presence of oak moss allergens other than atranol and chloroatranol. We observed reactions in several regions of the chromatograms other than those where atranol and chloroatranol appeared. Chemical investigations of the spots giving positive reactions are needed in order to identify the substances responsible for the reactions.

Only 1 patient reacted to the TLC strip of sample B. The spots giving positive reactions were located in the region where atranol and chloroatranol are expected on the TLC strip. Theoretically, it is possible that more reactions would appear if a higher dose of sample B were applied to the TLC strip. This was not done because the separation is impaired when too much of the sample is applied on the TLC strip. Besides, the fact that we observed only one positive reaction to the TLC strips of sample B could possibly be explained by an unspecific treatment method also reducing the contents of allergens other than atranol and chloroatranol.

This study shows a statistically significant lowering of the patch test reactivity to the oak moss absolute with a reduced content of atranol and chloroatranol as compared with the untreated sample. Whether this difference in patch test reactivity also implies a lower reactivity after daily exposure to treated oak moss absolutes can be investigated in usage tests. It is possible that some oak moss-allergic individuals react to products containing oak moss absolute with a reduced content of atranol and chloroatranol, depending on a high degree of reactivity towards these substances or contact allergy directed towards other sensitizers in oak moss absolute. The TLC patch tests indicate the presence of several other sensitizers in oak moss absolute.

## References

- 1 Larsen W G. Perfume dermatitis. A study of 20 patients. *Arch Dermatol* 1977; **113**: 623–626.
- 2 Uter W, Geier J, Frosch P, Schnuch A. Contact allergy to fragrances: current patch test results (2005–2008) from the Information Network of Departments of Dermatology. *Contact Dermatitis* 2010; **63**: 254–261.
- 3 Bernard G, Gimenez-Arnau E, Rastogi S C et al. Contact allergy to oak moss: search for sensitizing molecules using combined bioassay-guided chemical fractionation, GC-MS, and structure–activity relationship analysis. *Arch Dermatol Res* 2003; **295**: 229–235.
- 4 Boelens M H. Formation of volatile compounds from Oakmoss. *Perf Flav* 1993; **18**: 27–30.
- 5 Joulain D, Tabacchi R. Lichen extracts as raw materials in perfumery. Part 1: oakmoss. *Flavour Frag J* 2009; **24**: 49–61.
- 6 Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. *Off J Eur Union* 2009; **L342**: 59–209.
- 7 International Fragrance Association. Code of practice 43rd Amendment. Standard on Oakmoss extracts, 2008. Available at: [www.ifraorg.org](http://www.ifraorg.org) (last accessed 30 October 2012).
- 8 Lammintausta K, Zimerson E, Hasan T et al. An epidemic of furniture-related dermatitis: searching for a cause. *Br J Dermatol* 2010; **162**: 108–116.
- 9 Bruze M, Frick M, Persson L. Patch testing with thin-layer chromatograms. *Contact Dermatitis* 2003; **48**: 278–279.
- 10 Mowitz M, Zimerson E, Svedman C, Bruze M. Stability of fragrance patch test preparations applied in test chambers. *Br J Dermatol* 2012; **167**: 822–827.
- 11 Bruze M, Isaksson M, Gruvberger B, Frick-Engfeldt M. Recommendation of appropriate amounts of petrolatum preparation to be applied at patch testing. *Contact Dermatitis* 2007; **56**: 281–285.
- 12 Wilkinson D S, Fregert S, Magnusson B et al. Terminology of contact dermatitis. *Acta Derm Venereol* 1970; **50**: 287–292.
- 13 Hindsén M, Bruze M, Christensen O B. The significance of previous allergic contact dermatitis for elicitation of delayed hypersensitivity to nickel. *Contact Dermatitis* 1997; **37**: 101–106.
- 14 Dahlquist I, Fregert S. Contact allergy to atranorin in lichens and perfumes. *Contact Dermatitis* 1980; **6**: 111–119.
- 15 Thune P, Solberg Y, McFadden N, Stærfelt F, Sandberg M. Perfume allergy due to oak moss and other lichens. *Contact Dermatitis* 1982; **8**: 396–400.
- 16 Sandberg M, Thune P. The sensitizing capacity of atranorin. *Contact Dermatitis* 1984; **11**: 168–173.
- 17 Gonçalves S, Cabral F, Gonçalves M. Contact sensitivity to oak moss. *Contact Dermatitis* 1988; **19**: 355–357.
- 18 Johansen J D, Andersen K E, Svedman C et al. Chloroatranol, an extremely potent allergen hidden in perfumes: a dose–response elicitation study. *Contact Dermatitis* 2003; **49**: 180–184.
- 19 Johansen J D, Bernard G, Gimenez-Arnau E, Lepoittevin J P, Bruze M, Andersen K E. Comparison of elicitation potential of chloroatranol and atranol–2 allergens in oak moss absolute. *Contact Dermatitis* 2006; **54**: 192–195.

- 20 Rastogi S C, Bossi R, Johansen J D, Menné T, Bernard G, Gimenez-Arnau E, Lepoittevin J P. Content of oak moss allergens atranol and chloroatranol in perfumes and similar products. *Contact Dermatitis* 2004; **50**: 367–370.
- 21 Ehret C, Maupetit P, Petrzilka M, Klecak G. Preparation of an oakmoss absolute with reduced allergenic potential. *Int J Cosmet Sci* 1992; **14**: 121–130.
- 22 Terajima Y, Tokuda K, Nakamura S, Uehara K, Ichikawa H, Iwakami S. *A Process of Obtaining a Hypo-Allergenic Moss Oil*. US patent No 4663080; 1987.
- 23 Nardelli A, Gimenez-Arnau E, Bernard G, Lepoittevin J P, Goossens A. Is a low content in atranol/chloroatranol safe in oak moss-sensitized individuals? *Contact Dermatitis* 2009; **60**: 91–95.