www.cropwatch.org

THE FIRST TRULY INDEPENDENT WATCHDOG FOR THOSE WORKING WITH NATURAL AROMATIC MATERIALS

E: info@cropwatch.org T: ++44 (0)7771 872 521

Towards a Cropwatch 'Fragrant Lichen' Bibliography: [Oakmoss/Treemoss/Cedarmoss etc.]

Compiled by Cropwatch May 2009 v1.10
[to be progressively extended].

Contents

Atranol & Chloroatranol
Atranorin
Oakmoss General
Oakmoss – Chemistry
Oakmoss Chinese
Oakmoss – Contact Allergy
Oakmoss – Inhalation Toxicity
Oakmoss – Phototoxicity
Treemoss
Cedarmoss
Other Fragrant Lichens - ***Everniastrum cirrhatum***
Evernia mesomorpha
Parmelia nepalensis.

Atranol & Chloroatranol.

Atranol & chloroatranol are sensitsers, generated from the depsides atranorin & chloroatranorin by hydrolysis to haematommic acid & chlorohaematommic acid, respectively, followed by decarboxylation (the decarboxylation is an easy process). Ethyl haematommate and chlorohaematommate are formed from the atranorins by ethanolysis during the processing of the concrete oil with ethanol. They do not degrade as easily to the atranols. Whatsoever, in the latter case, the process is called a decarbethoxylation. - *Thanks to Daniel Joulain for correcting the previous information presented here.*

Bourgeois G., Suire C., Vivas N. & Vitry C. (1999) "Atraric acid, a marker for epiphytic lichens in the wood used in cooperage: Identification and quantification by GC/MS/(MS)." *Analusis* **27**, 281-283. Abstract. A phenolic derivative, atraric

acid, was identified by GC/MS and quantified by GC/MS/MS in the outer parts of wood from oaks specifically colonised by lichens. This compound was correlated to the presence of a bitter depside, atranorin, which is a natural metabolite of the lichen species belonging to the genus *Parmelia.*

Bossi R., Rastogi S.C., Guillaume B., Gimenez-Arnau E., Johansen J.D., Lepoittevin J.-P.& Menne T. (2004) "A liquid chromatography-mass spectrometric method for the determination of oak moss allergens atranol and chloroatranol in perfumes." *Journal of Separation Science* **27**(7-8), 537-540. Abstract. This paper describes a validated liquid chromatographic-tandem mass spectrometric method for quantitative analysis of the potential oak moss allergens atranol and chloroatranol in perfumes and similar products. The method employs LC-MS-MS with electrospray ionization (ESI) in negative mode. The compounds are analysed by selective reaction monitoring (SRM) of 2 or 3 ions for each compound in order to obtain high selectivity and sensitivity. The method has been validated for the following parameters: linearity; repeatability; recovery; limit of detection; and limit of quantification. The limits of detection, 5.0 ng/mL and 2.4 ng/mL, respectively, for atranol and chloroatranol, achieved by this method allowed identification of these compounds at concentrations below those causing allergic skin reactions in oak-moss-sensitive patients. The recovery of chloratranol from spiked perfumes was 96±4%. Low recoveries (49±5%) were observed for atranol in spiked perfumes, indicating ion suppression caused by matrix components. The method has been applied to the analysis of 10 randomly selected perfumes and similar products.

Johansen J.D., Andersen K.E., Svedman C., Bruze M., Bernard G., Giménez-Arnau E., Rastogi S.C., Lepoittevin J.P., Menné T. (2003) "Chloroatranol, an extremely potent allergen hidden in perfumes: a dose-response elicitation study." *Contact Dermatitis* **49**(4):180-4. Abstract. Oak moss absolute is a long-known, popular natural extract widely used in perfumes. It is reported as the cause of allergic reactions in a significant number of those with perfume allergy. Oak moss absolute has been the target of recent research to identify its allergenic components. Recently, chloroatranol, a hitherto unknown fragrance allergen, was identified in oak moss absolute. The objective was to assess the clinical importance of chloroatranol as a fragrance allergen by characterizing its elicitation profile. 13 patients previously showing a positive patch test to oak moss absolute and chloroatranol were included, together with a control group of 10 patients without sensitization to either of the 2 materials. A serial dilution patch test was performed on the upper back with concentrations ranging from 200 to 0.0063 p.p.m. of chloroatranol in ethanol. Simultaneously, the participant performed an open test simulating the use of perfumes on the volar aspect of the forearms in a randomized and double-blinded design. A solution with 5 p.p.m. chloroatranol was used for 14 days, and, in case of no reaction, the applications were continued for another 14 days with a solution containing 25 p.p.m. All test subjects (13/13) developed an allergic reaction at the site of application of the solution containing chloroatranol. Among them, 12/13 (92%) gave a positive reaction to the 5 p.p.m. solution and 1 to 25 p.p.m. None of the controls reacted

(P < 0.001). The use test was terminated at median day 4. The dose eliciting a reaction in 50% of the test subjects at patch testing was 0.2 p.p.m. In conclusion, the hidden exposure to a potent allergen widely used in perfumes has caused a highly sensitized cohort of individuals. Judged from the elicitation profile, chloroatranol is the most potent allergen present in consumer products today.

Johnson I.R. (2004) *Chloroatranol: Local lymph node assay. Report n° GM7817.* Central Toxicology Laboratory, UK-Cheshire, 11 March 2004.

Johnson, I.R (2004) *Atranol: Local Lymph Node Assay. Report n° GM7816*. Central Toxicology Laboratory, UK-Cheshire, 11 March 2004.

Johansen J.D., Bernard G., Giménez-Arnau E., Lepoittevin J.P., Bruze M., Andersen K.E..(2006) "Comparison of elicitation potential of chloroatranol and atrano 2 allergens in oak moss absolute." *Contact Dermatitis*. **54**(4):192-5 Abstract. Chloroatranol and atranol are degradation products of chloroatranorin and atranorin, respectively, and have recently been identified as important contact allergens in the natural fragrance extract, oak moss absolute. Oak moss absolute is widely used in perfumery and is the cause of many cases of fragrance allergic contact dermatitis. Chloroatranol elicits reactions at very low levels of exposure. In oak moss absolute, chloroatranol and atranol are present together and both may contribute to the allergenicity and eliciting capacity of the natural extract. In this study, 10 eczema patients with known sensitization to chloroatranol and oak moss absolute were tested simultaneously to a serial dilution of chloroatranol and atranol in ethanol, in equimolar concentrations (0.0034-1072 microM). Dose-response curves were estimated and analysed by logistic regression. The estimated difference in elicitation potency of chloroatranol relative to atranol based on testing with equimolar concentrations was 217% (95% confidence interval 116-409%). Both substances elicited reactions at very low levels of exposure. It is concluded that the differences in elicitation capacity between the 2 substances are counterbalanced by exposure being greater to atranol than to chloroatranol and that both substances contribute to the clinical problems seen in oak moss absolute-sensitized individuals.

Rastogi S.C., Bossi R., Johansen J.D., Menné T., Bernard G., Giménez-Arnau E., Lepoittevin J.P.. (2004) "Content of oak moss allergens atranol and chloroatranol in perfumes and similar products." *Contact Dermatitis* **50**(6),367-70. Abstract. Chloroatranol and atranol have been identified as the main allergens in the fragrance material of botanical origin, oak moss absolute. A previous study has shown that nearly all individuals sensitized to chloroatranol will elicit to 5 microg/ml. in a repeated open application test and that 50% will get a reaction to 0.15 micro g/ml under patch test conditions. Thus, chloroatranol is known as a potent allergen. The aim of the current investigation was to quantify exposure to chloroatranol and the chemically related substance atranol in some popular perfumes, eaux de parfum and eaux de toilette available on the European market. In total, 31 products were analysed by liquid chromatography-electrospray ionization-tandemmass spectrometry (LC-ESI-MS-MS) for their contents of atranol and chloroatranol. The 2 substances were found in 87% (n =

27) of the products. The median concentration of atranol in perfumes was 0.502 micro g/ml and 0.012 micro g/ml in eaux de toilette, and 0.235 micro g/ml and 0.006 micro g/ml for chloroatranol, respectively, in perfumes and eaux de toilette. Chloroatranol was found at a maximum concentration of 53 micro g/ml and atranol at one of 190 micro g/ml. The wide exposure to oak moss allergens, together with significant amounts of these potent allergens in at least half of perfumes and some eaux de toilettes explains the high frequencies of oak moss absolute allergy. It is suggested that regulations should be introduced aimed directly at these substances, and not just at oak moss absolute. ***Cropwatch comments:*** This pre-supposes the hypothesis that atranol & chloratranol are, indeed, the potent allergens that the authors suggest is correct, and that the importance of determining their individual concentration in product over-rides the importance of determining the overall oakmoss concentration. During their review of oakmoss in perfumery, Joulain & Tabbachi (2009) present a critical review of the oakmoss toxicology which brings in a wider selection of evidence, questioning the assumptions made in the paper above.

Atranorin

Bourgeois G., Suire C., Vivas N. & Vitry C. (1999) "Atraric acid, a marker for epiphytic lichens in the wood used in cooperage: Identification and quantification by GC/MS/(MS)." *Analusis* **27**, 281-283.

Dahlquist I. & Fregert S. (1980) "Contact allergy to atranorin in lichens and perfumes." *Contact Dermatitis*. **6**(2), 111-9. Abstract. Atranorin, one of the most common lichen substances, gave positive patch test reactions in eight subjects (1%) in a routine series. These subjects also reacted to fumarprotocetraric acid and some of them to evernic acid. Stictic acid and usnic acid gave negative reactions. The lichen oak moss *Evernia prunastri* and an oak moss perfume gave positive reactions. Thin-layer chromatography and a spot test indicated that atranorin is present in oak moss perfumes which are made from oak moss and tree moss. Contact with oak moss perfumes and lichens in nature may cause atranorin allergy. None of the eight subjects had a history of light sensitivity or atopy and none had chronic facial eczema.

Dalquist I. & Fregert S. (1981) “Atranorin and oak moss contact allergy.” *Contact Dermatitis* **7**(3), 168 – 169. Abstract. Atranorin, one of the most common lichen substances, gave positive patch test reactions in eight subjects (1 %) in a routine series. These subjects also reacted to fumarprotocetraric acid and some of them to evernic acid. Stictic acid and usnic acid gave negative reactions. The lichen oak moss *Evernia prunastri* and an oak moss perfume gave positive reactions. Thin-layer chromatography and a spot test indicated that atranorin is present in oak moss perfumes which are made from oak moss and tree moss. Contact with oak moss perfumes and lichens in nature may cause atranorin allergy. None of the eight subjects had a history of light sensitivity or atopy and none had chronic facial eczema.

Dahlquist I. & Fregert S. (1980) "Contact allergy to atranorin in lichens and perfumes" *Contact Dermatitis* **6**(2), 111 – 119. Abstract. Atranorin, one of the

most common lichen substances, gave positive patch test reactions in eight subjects (1 %) in a routine series. These subjects also reacted to fumarprotocetraric acid and some of them to evernic acid. Stictic acid and usnic acid gave negative reactions. The lichen oak moss Evernia prunastri and an oak moss perfume gave positive reactions. Thin-layer chromatography and a spot test indicated that atranorin is present in oak moss perfumes which are made from oak moss and tree moss. Contact with oak moss perfumes and lichens in nature may cause atranorin allergy. None of the eight subjects had a history of light sensitivity or atopy and none had chronic facial eczema.

Hiserodt R.D., Swijter D.F.H & Mussinan C.J. (2000) "Identification of atranorin and related potential allergens in oakmoss absolute by high-performance liquid chromatography–tandem mass spectrometry using negative ion atmospheric pressure chemical ionization." *Journal of Chromatography A* **888**(1-2),103-111 Abstract. This paper describes the first high-performance liquid chromatographic–tandem mass spectrometric method for the identification of atranorin and related potential allergens in oakmoss absolute. Oakmoss absolute is ubiquitous in the fragrance industry and is a key component in many fine perfumes. However, oakmoss absolute causes an allergic response in some individuals. Research is focused toward establishing the identity of the compounds causing the allergic response so a quality controlled oakmoss with reduced allergenic potential can be prepared. Consequently a highly selective and specific analytical method is necessary to support this effort. This is not available with the existing HPLC methods using UV detection.

Kumar S.K.. & Muller K. (1999) “Lichen metabolites. 1. Inhibitory action against leukotriene B_4 biosynthesis by a non-redox mechanism.” *Journal of Natural Products* **62**(6), 817-820. ***Cropwatch comments***: Atranorin, diffractaic acid, and (c)-protolichesterinic acid inhibit leukotriene B4 biosynthesis in poymorphonuclear leukocytes

Lorenzi S., Guerra L., Vezzani C. & Vincenzi C. (2006) "Airborne contact dermatitis from atranorin" *Contact Dermatitis* **32**(5), 315 - 316.

Sandenberg M. & Thune P. (2008) "The sensitizing capacity of atranorin" *Contact Dermatitis* **11**(3), 168-173. Abstract. The allergenic potential of the aromatic lichen substance atranorin has been investigated by means of the guinea pig maximization test of Magnusson & Kligman. Sensitivity was induced in 30% of the animals, which corresponds to a moderate allergenic capacity (grade III). This is in agreement with the clinically-observed frequency of 1.5% among our patients. A modified photoallergy test on the same animals was performed, but irradiation did not increase the number of positive reactions, 4 patients with proven contact sensitivity to atranorin, evernic, usnic or physodic acid, were examined with different dilutions from 0.001 to 0.1%. Irradiation of the test series did not provoke any clear-cut photoallergic reaction.

Vicente C., Fontaniella B., Millanes A.M., Sebastián B. & Legaz M.E. (2003) "Enzymatic production of atranorin: a component of the oak moss absolute by

immobilized lichen cells." *Int J Cosmet Sci.* **25**(1-2), 25-9. Abstract. Cells of the lichen, *Evernia prunastri*, immobilized in calcium alginate were able to produce the depside atranorin from acetate. The synthesis of the depside was enhanced by molecular oxygen and NADH. This enhancement suggested the participation of an oxidase and an alcohol dehydrogenase to produce an aldehyde-substituted phenolic acid, hematommic acid, as the most probable precursor of atranorin. The participation of both enzymes was confirmed by loading immobilized cells with sodium azide, an inhibitor of several metallo-oxidases, and pyrazole, an inhibitor of alcohol dehydrogenase, which impeded atranorin production and accumulated beta-methyl orsellinate (after azide loading) or its alcohol derivative (after pirazole treatment).

Oakmoss General.

Oakmoss absolute (Mousse de chêne), concrete, resinoid etc. are derived from the lichen *Evernia prunastri* (L.) Arch. (Fam. Usneaceae) which grows mainly on the bark of oak trees, but also to some extent on spruces & pine trees. Nine thousand tons of oakmoss lichen is gathered annually in S. Europe, in France (formerly in the forests around Fontainebleau), as well from Calabria, Bohemia, Morocco, Algeria, and the area of former Yugoslavia & Bulgaria (Burfield 2000); however this figure may be overstated – Joulain (2002) mentions a figure of 3,000 tons, and Huneck (2001) reported that for the year 1997, 1900 tons of *Pseudevernia furfuracea* and 700 tons of *Evernia prunastri* were processed at Grasse – *Cropwatch Newsletter* Aug 2008. .

Actander S (1960). “Oak Moss” in *Perfume and Flavor Materials of Natural Origin.* Elisabeth NJ (USA) 1960: 446-46.

Actander S. (1960) “Treemoss absolute.” *Perfume and Flavor Materials of Natural Origin*. Elisabeth NJ (USA), 627-630

Ahad A.M., Goto Y., Kiuchi F., Tsuda Y., Kondo K. & Sato T. (1991) "Nematocidal Principles in "Oakmoss Absolute" and Nematocidal Activity of 2,4-Dihydroxybenzoates." *Chemical & Pharmaceutical Bulletin* **39**(4) 1043-1046 Abstract. Nematocidal principles obtained from oakmoss absolute were identified as methyl 2,4-dihydroxy-3,6-dimethylbenzoate (2), ethyl 3-formyl-2,4-dihydroxy-6-methylbenzoate (4), and ethyl 5-chloro-3-formyl-2,4-dihydroxy-6-methylbenzoate (7). In relation to their structures, the nematocidal activity of 2,4-dihydroxybenzoates of methyl to tetradecyl was tested and the strongest activity was found in the octyl ester (minimal lethal concentration=13 μM).

Bats J-P., Mouliness J-J., Lamidey A.-M. Countiere D. & Arnaudo J-F. (1990) "Continous process for oakmoss extraction." *Perf & Flav*. **15**(6), 15e-16.

Guenther, E. (1952) “Concrete and absolute of aakmoss.” in *The Essential Oils* Vol VI, 179 - 191. Van Norstrand N.Y.

Joulain D. & Tabacchi R. (2009) “Lichen extracts as raw materials in perfumery. Part 1: oakmoss.” *Flavour & Fragrance Journal* (in advance of publication).

Abstract. A comprehensive review is presented on extracts of a lichen, oakmoss (*Evernia prunastri*), that are used in the fragrance industry. Analytical aspects are discussed in detail, from both qualitative and quantitative standpoints, mainly in relation to the industrial processing of the lichen. It is shown that more than 170 constituents have been identified so far in oakmoss extracts, including 47 depsides or depside-derived compounds and 25 triterpenes or steroids. A survey of industrially relevant synthetic products with an oakmoss odour is included. Toxicology issues related to the use of oakmoss extracts in cosmetics and fragrance formulations are critically reviewed. ***Cropwatch comments:*** Thoroughly recommended: as probably representing the most comprehensive review of oakmoss chemistry to date. More than this, the paper raises serious questions about the robustness of published research surrounding toxicological (esp. sensitization) issues with oakmoss extracts, and atranol & chloroatranol.

Huneck S. & Yoshimura I., (1996) "*Identification of Lichen Substances*" Springer.

Lutnaes B.F., Bruun T. & Kjøsen H (2004) "(22S)-6-O-acetyl-21betaH-hopane-3beta,6beta,22,29-tetrol from oakmoss (*Evernia prunastri*)." *Nat Prod Res*. **18**(4), 379-85. Abstract A novel hopanoid triterpene, (22S)-6-O-acetyl-21betaH-hopane-3beta,6beta,22,29-tetraol, was isolated from oakmoss (*Evernia prunastri* (L.) Ach.), as identified from 1H, 13C, DEPT, COSY, NOESY, HSQC and HMBC NMR, MS and IR spectroscopy. During recrystallisation a new compound, 30-nor-6-O-acetyl-3beta,6beta-dihydroxy-21alphaH-hopan-22-one, was formed by a formal loss of methanol from the dihydroxypropyl moiety. No biological activity was found for the naturally occurring compound upon testing against a series of fish and human pathogenic bacteria.

Moxham T.H. (1986) "The commercial exploitation of lichens for the perfume industry." *Progress in Essential Oil Research –Proceedings of the ISEO Holzminden Sept 1985* ed. E.-J. Brunke. pp491-504.

Moxham T.H. (19BO) "Lichens and perfume manufacture." *British Lichen Society Bulletin* **47**, 1-2.

Moxham T.H. (1981) "Lichens in the perfume Industry ". *Dragoco Report* **2**,, 3l-39

Moxham T. H. (1981) "To Europe for a study in lichens." *Soap, Perfumery & Cosmetics* **54**(6), 323.

Müller K. (2001) "Pharmaceutically relevant metabolites from lichens." *Appl Microbiol Biotechnol* **56**, 9-16. Abstract. Lichen metabolites exert a wide variety of biological actions including antibiotic, antimycobacterial, antiviral, antiinflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects. Even though these manifold activities of lichen metabolites have now been recognized, their therapeutic potential has not yet been fully explored and thus remains pharmaceutically unexploited. In this mini-review, particular attention is paid to the most common classes of small-molecule constituents of lichens, from both the chemical viewpoint and with regard to possible therapeutic implications.

In particular,aliphatic acids, pulvinic acid derivatives, depsides and depsidones, dibenzofuans, anthraquinones, naphthoquinones as well as epidithiopiperazinediones are described. An improved access to these lichen substances in drug discovery high-throughput screening programs will provide impetus for identifying novel lead-compounds with therapeutic potential and poses new challenges for medicinal chemistry

Racine P.H., Hartmann V.E., Tollard d'Audiffret Y. (2007) "Antioxidant properties of wax from Yugoslavian oakmoss (*Evernia prunastri*)." *Int J. Cosm. Sci* **2**(6), 305-313. Abstract. Wax from Yugoslavian oakmoss resulting from the industrial benzene extraction of the vegetable matter was extracted by solvents of different polarities. The wax and the extracts were tested for antioxidant activities using (+) limonene as peroxidizable test substrate and were found to have such activity. The extracts are more active than the wax itself. Although not directly usable because of still too low activity, the wax and the extracts contain a small amount of substance with antioxidant activity comparable to that of the usual synthetic antioxidants.

Sarin Y.K. (1977) "Techno-economic evaluation of Indigenous lichens as raw material for aromatic resinoids." *Annual Report I976, Regional Research Laboratory, Jamnu-Kashmir.*

Turin L. (2007) "Due Credit" *NZZ Folio* 04/07. ***Cropwatch comments:***: Luca Turin relates the saga of customer anger over Guerlain's reformulation of Mitsouko to conform to the EU's oakmoss restrictions. Turin goes on to describe how Guerlain brought in Edouard Fléchier to rework the perfume and how the new Mitsouko conforms to all the rules and smells sensational. Yeah right.

Oakmoss - Chemistry

Avalos A. & Vicente C. (1987) "The occurrence of lichen phenolics in the photobiont cells of *Evernia prunastri*." *Plant Cell Reports* **6,** 74-76 Abstract. Photobiont cells of *Evernia prunastri* were isolated by filtration through a bed of Sepharose 2B. Algal preparations did not contain fungal contamination, as revealed by the absence of mannitol. Isolated photobionts contain the four *Evernia* phenolics, although repeated superficial washes with polyvinylpyrrolidone remove from these cells 81% atranorin, 51% ehloroatranorin, 9L% evernic acid and 82% usnic acid.

Boelens M. (1997): "Production, chemistry and sensory properties of natural isolates" in *Flavours and Fragrances* ed Kjarl A.D. Swift publ. Royal Society of Chemistry Publications 1997..

Boelens M. (1993) "Formation of volatile compounds from oakmoss" *Perf & Flav* **18**(1) 27-30.

Elix J.A. & S Norfolk S. (1975) "Synthesis of para-β-orcinol depsides." *Australian Journal of Chemistry* **28**(5) 1113 - 112. Abstract. The unambiguous syntheses of the lichen depsides norobtusatic acid, obtusatic acid, 4-O-demethyl-barbatic acid,

barbatic acid, diffractaic acid, chloroatranorin, baeomycesic acid and squamatic acid are reported.

Filho L.X., Pereira E.C., Vicente C., & Legaz M.-E. (2004) "Synthesis of methyl-3-orsellinate by organic synthesis or by altered biosynthetic pathways using lichen immobilisates." *ARKIVOC* 2004 (vi) 5-11. Abstract Methyl-3-orsellinate can be generated from methyl-3-orcinol through the nitration of aryl function to be then reduced to amine group. This last derivative could be the substrate of a diazotonization for the corresponding diazonium salt. After this, a cyanide group could be introduced by nucleophilic substitution using potassium cyanide and then, the carboxyl function could be produced after hydrolysis. Alternatively, methyl-3-orsellinate could be produced by alginate-immobilized, atranorin-producing lichen cells loaded with acetate and treated with sodium azide. Acetate is used by acetyl-CoA carboxylase and aromatic synthase (methyl transfer) to produce methyl-3-orsellinate and its oxidation to haematommoyl alcohol is inhibited by sodium azide..

Gavin J, Nicollier G. & Tabacchi R (1978) “Volatile constituents of oakmoss *Evernia-Prunastri*”. *Helvetica Chimica Acta* **61**, 352-7.

Gavin J. & Tabacchi R. (1975) "Isolation & identification of phenolic & monoterpene compounds of oakmoss (*Evernia prunastri* (L.) Ach)." *Helvetica Chimica Acta* **58**(1), 190-194. Abstract. The composition of different oakmoss extracts was investigated by gas chromatography /mass spectroscopy. A series of 38 compounds – especially phenol derivatives and monoterpenes – was identified, 22 of which have not yet been reported as constituents of oakmoss.

Hassuni I. & Razzouk H. (2005) "Olivetol : Constituent of lichen *Evernia prunastri* Ach. or “oakmoss”. *Physical and Chemical News* **26**, 98-103

Hassuni I. & Razzouk H. (2006) “Isolation and identification of two new compounds in *Evernia prunastri* Ach. or oakmoss.” *Physical and Chemical News* **29**, 131-134. Abstract. Ce travail a trait à l'isolement et l'identification du BHT (hydroxytoluène butylé ou 2, 6-ditert-butyl-p-crésol) et du veratrate de méthyle (3,4 dimethoxy benzoate de méthyle), extraits pour la première fois de la mousse de chêne.

Heide ter R., Provatoroff N., Traas P.C., Valois P.J. van der Plasse N., Wobben H.J. & Timmer R. (1975) “Qualitative analysis of the odoriferous fraction of oakmoss (*Evernia prunastri* (L.) Ach.).” *J. Agric. Food Chem* **23**(*5)* p 950 *et seq*. Abstract. The composition of the important odoriferous fractions of a commercial oakmoss extract was investigated. The extract had been prepared by steam distillation of an alcoholic extract from Yugoslavian oakmoss (*Euernia prunastri* (L.) Ach.).After removal of the main component ethyl everninate, the residual material was separated by gradient elution over silica gel. Four fractions were analyzed. These gave by combination a product with olfactive properties very similar to those of oakmoss absolute. Analysis was performed by GC-MS combination using glass SCOT columns. Further characterization was

accomplished by preparative GC followed by spectral analysis. In some cases confirmation was obtained by synthesis. Carbonyls, phenols, and acids were isolated by chemical methods. A total number of 61 components was identified, 49 of which were not reported previously as constituents of oakmoss extract. A number of aromatic compounds formed by alcoholysis of depsides was found.

ter Heide R., Provatoroff N., Traas P.C,. de Valois P. J., Wobben H.J. & Timmer R. (1975) "*Analyse qualitative de la fraction odorante de la mousse de chêne*" *Parfums, Cosmétiques, Arômes* **3**, 61-74. ***Cropwatch comments***: The presented analysis indicates presence of irone isomers corresponding to pollution with orris! Thanks to Daniel Joulain for pointing this out.

Hiromu K. & S Yoshichika S. (1984) "Study on the oakmoss, synthesis of orcinol monomethyl ether of orcinol monomethyl ether." *Kinki Daigaku Rikogakubu Kenkyu Hokoku*, 1984.

Joulain D. (2002) “Stable Isotopes for determining the origin of flavour & fragrance components: recent findings.” In: *Advances in flavours & fragrances from the sensation to the synthesis.*ed. Karl A.D. Swift publ. Royal Soc. Chem 2002. Section 5 is: “The case of a natural raw material for fragrances: lichen resinoids.”

Joulain D., Guillamon N., Casazza A. & Tabacchi R. (2005) “New insight in the knowledge of the qualitative and quantitative composition of oakmoss resinoids.” *36th International Symposium on Essential Oils, 4-7 September, Budapest, Hungary.* Abstract. Oakmoss absolute oil is prepared from *Evernia prunastri* resinoids.. Although it has previously been subject to thorough analytical studies, these are mostly qualitative in nature and very little has been published, so far, on the quantitative composition of any oakmoss extract (1)(2). Using GC-MS with chemical derivatization and LC-MS, we report herein some quantitative data obtained from a commercial absolute oil. We also revisited some aspects of the qualitative analysis of oakmoss and now report that the thujone (3) and irone (4) isomers previously described in the literature, cannot be detected in a laboratory-made extract from pure *Evernia prunastri* lichen. This suggests that earlier results were either inaccurate or were derived from contaminated products. Further to our previous measurements of natural abundance of 2H and 18O in lichen substances, we report new data on site-specific natural abundance 13C in oakmoss components, measured by quantitative NMR. Lastly, using the recent described technique of ALEX/GC-MS, we show that the quantitative determination of atranol and chloroatranol in oakmoss extracts containing reduced levels of these two allergens, can be achieved without significant contamination of the instrumentation.

Neelakantan S., Padmasani R. & Seshadri T.R. (1965) "New reagents for the synthesis of depsides methyl evernate, methyl lecanorate, evernic acid and atranorin." *Terahedron* **21**(12), 3531-3536. Abstract. Lichen depsides have now been synthesized by making use of two reagents, dicyclohexyl-carbodiimide (DCC) and trifluoroacetic anhydride. Methyl evernate, methyl lecanorate and

evernic acid have been prepared in satisfactory yields. Altranorin has been synthesized for the first time.

Nicollier G. & Tabacchi R. (1976) "Isolation & identification of evernine in oakmoss (*Evernia prunastri* (l.). Ach.)" *Helvetica Chimica Acta* **59**(8), 2979-2983.

Nicollier GMR, Tabacchi R, Gerlach H, Thalmann A (1978) "Synthesis of Evernin.". *Helvetica Chimica Acta* **61**, 2899-904

Nicollier G., Tabacchil R., Gavin J., Breton J.L. & Gonzalec A.G. (1979) "Triterpenoids from Oakmoss (*Evernia prunastri* (L.) Ach) . Part 5." *Helvetica Chimica Acta* **62** (3), 807-810.

Nicollier G., Rebetz M., Tabacchi R. (1979) "Identification & synthesis of new depsides isolated from oakmoss (*Evernia prunastri* (L.) Ach). Part 4." *Helvetica Chimica Acta* **62**(3), 711-717.

Renaud J.M., Nicollier G. & Tabacchi R. (1980) *"*Mousse de Chêne (*Evernia prunastri* L. Ach.) et mousse d'arbre (*Pseudevernia furfuracea* L. Zopf): comparaison de quelkque extraits.*" Proceedings of the 8th International Congress of Essential Oils, Cannes Grasse, October 1980*, Paper Nr. 72, pages 201-205.

Schulz H. & Albroscheit G. (1989) "Characterization of oakmoss products used in perfumery by high-performance liquid chromatography" *Journal of Chromatography A* 466, 1989, Pages 301-306 Abstract An high-performance liquid chromatographic (HPLC) method was developed to identify and to quantify characteristic substances in commercially available oakmoss products. The procedure offers a rapid and reliable method for routine process and/or quality control. The identity of the registered peaks was confirmed using HPLC coupled on-line with ultraviolet—visible spectroscopy as well as by spectral analysis (1H and 13C nuclear magnetic resonance and mass spectrometry) of the isolated substances. Whereas a freshly prepared laboratory extract of *Evernia prunastri* contains mainly evernic acid, heating at 118°C results in decomposition products such as evernyl, orsellic acid, everninic acid and other phenol derivatives. The results indicate that the often used gas chromatographic method is not readily applicable to the study of lichen compounds, because these are not sufficiently volatile or too unstable at elevated temperatures.

Shiseido Co. Ltd. European Patent EP19860106811 (publ. 1991) "A process of obtaining a hypo-allergenic moss oil." Claims: A process of obtaining a hypo-allergenic moss oil by removing ethylhematommate, ethylchlorohematommate and chloroatranorin from a starting moss oil, obtained by the extraction from epiphytic moss on the bark of trees, with at least one treatment selected from the group consisting of chromatography, solvent extraction, countercurrent partition, and membrane separation and a further treatment consisting of a catalytic hydrogenation treatment or both a catalytic hydrogenation treatment and an alkaline treatment.

Tabacchi R. & Tsoupras G (1986) ""*Sur la présence de triterpènes dans quelques lichens*" in *Progress in Terpene Chemistry* ed D. Joulain, Editions Frontières (1986), 293-305

Terajima Y., Ichikawa H., Tokuda K. & Nakamura, S. (1988) "Quantitative analysis of oakmoss oil." In: *Flavors and fragrances: a world perspective* eds B.M. Lawrence, B.D. Mookherjee and B.J. Willis. *Proceedings of the 10th International Congress of Essential Oils, Fragrances and Flavors, Nov 16-20, 1986, Washington, D.C.*

Tesevic, V.D. (2000) "Supercritical carbon dioxide extraction of oak moss *Evernia prunastri* L." Thesis Univerzitet u Novom Sadu, Novi Sad (Yugoslavia). Tehnoloski fakultetnovi Sad (Yugoslavia). Abstract. Oak moss extract is widely applied in perfume industry because of its fixative characteristics. In this work it has been examined the use of supercritical carbon dioxide for extracting the oak moss for obtaining extracts which can be supstitution to benzoln extract. The use of this extragens belongs to the field of new technology and has been proven to be highly selective, ecological and whose traces in the product- extract does not have any harmful effects on the user.

Yonetani K. (1981) "Oakmoss & Treemoss". *Koryo* **131**, 60-64.

Oakmoss Chinese

Ding D. (1988) "Oakmoss and treemoss in China" *Perf & Flav.* **13**(5), 13-16.

Gao Y, Liu BZ, Zhu XL, Shi L, Chen JL, Gong M, Zhang LG. (2000) "[A study on the chemical components of essential oil of oak moss concrete by gas chromatography/mass spectrometry]" *Se Pu.* **18**(3), 251-3. Abstract. The essential oil of oak moss concrete was extracted by volatile oil content equipment. The chemical compositions and their relative contents were analyzed by GC and GC/MS. A Supelco-5 fused silica capillary column (30 m x 0.32 mm i.d.; 0.25 micron thickness) and a flame ionization detector (FID) were employed in GC analysis. The temperature program included temperature increase of 4 degrees C/min from 50 degrees C to 250 degrees C, and a 10 min isothermal period at 250 degrees C. Mass spectra were obtained by electron impact at 70 eV and a source temperature of 170 degrees C. Twenty-four volatile compounds of oak mass concrete were identified, which comprised more than 83% of volatile fraction. The major components were diethyl phthalate, alpha-terpineol, cedrane and linalool.

Mao D., Liu Q., Hou C. & Wang D. (2005) "[Analysis of volatile constituents in oakmoss concrete by GC-MS with solid phase microextraction]." *Se Pu*. **23**(3), 323

Oakmoss - Contact Allergy

Aalto-Korte K., Lauerma A.& Alanko K (2005). "Occupational allergic contact dermatitis from lichens in present-day Finland." *Contact Dermatitis* **52**(1), 36-8. Abstract. Lichens are abundant in forests, living on trees, soil, stones and rocks.

They contain usnic acid and other lichen acids that are contact allergens. Lichens and liverworts cause woodcutter's dermatitis, eczema that appears in the forest on the bare skin areas, especially in cold and wet weather. Occupational allergic contact dermatitis from lichens occurs in forestry and horticultural workers and in lichen pickers. Lichens can cause immediate allergy, contact urticaria, rhinitis and asthma and probably also photoallergic contact dermatitis. Lichens are used for the manufacture of oak moss absolute, a fragrance constituent. Oak moss absolute contains lichen acids and is one of the commonest contact allergens. Lichen acid allergy develops either from contact with lichens or from fragrances. We describe 4 cases of occupational allergic contact dermatitis from lichens during the past decade: 2 were farmers and 2 gardeners. 3 of them had allergic reactions to fragrance mix and oak moss absolute. Lichen contact allergy is an old, partly forgotten, syndrome that should be remembered for symptoms in contact with barked wood or wood dust.

Basketter D.A., Wright Z.M., Warbrick E/V. *et al*. (2001). *Contact Dermatitis* **45**, 89–94.

Bernard G., Giménez-Arnau E., Rastogi S.C., Heydorn S., Johansen J.D., Menné T., Goossens A., Andersen K. & Lepoittevin J.P. (2003) "Contact allergy to oak moss: search for sensitizing molecules using combined bioassay-guided chemical fractionation, GC-MS, and structure-activity relationship analysis." *Archives of dermatological research* **295**(6), 229-235 Abstract. In addition to pure synthetic fragrance materials several natural extracts are still in use in the perfume industry. Among them oak moss absolute, prepared from the lichen *Evernia prunastri* (L.) Arch., is considered a major contact sensitizer and is therefore included in the fragrance mix used for diagnosing perfume allergy. The process of preparing oak moss absolute has changed during recent years and, even though several potential sensitizers have been identified from former benzene extracts, its present constituents and their allergenic status are not clear. In the study reported here, we applied a method developed for the identification of contact allergens present in natural complex mixtures to oak moss absolute. The method is based on the combination of bioassay-guided chemical fractionation, gas chromatography-mass spectrometry analysis and structure-activity relationship studies. Our first results showed that atranol and chloroatranol, formed by transesterification and decarboxylation of the lichen depsides, atranorin and chloroatranorin, during the preparation of oak moss absolute, are strong elicitants in most patients sensitized to oak moss. Methyl-β-orcinol carboxylate, a depside degradation product and the most important monoaryl derivative of oak moss from an olfactory standpoint, was also found to elicit a reaction in most patients.

Buckley D.A., Rycroft R.J.G., White I.R. & McFadden J.P. (2002) "Contaminating resin acids have not caused the high rate of sensitivity to oak moss." *Contact Dermatitis* 47(1),19-20. Abstract. Commercially available oak moss absolute patch test material has recently been shown to contain resin acids of the type found in colophony (colophonium). We wished to assess whether the high

frequency of positive patch tests to oak moss absolute at this institute was likely to reflect significant contamination by resin acids. The rate of positive reactions to colophony among our oak moss-allergic patients patch tested during 1984–2000 was retrospectively investigated. 25,395 patients were tested to the European standard series during this period and 1,963 (7.7%) were allergic to the fragrance mix. 342 of these patients were allergic to oak moss absolute, of whom 73 (21.3%) were allergic to colophony. In comparison, 115 (13.4%) of 861 fragrance mix-positive but oak moss-negative patients were allergic to colophony ($p = 0.0002$, Fisher's exact test). This strongly statistically significant association between oak moss absolute and colophony shows only a small increase in rates of allergy to colophony in oak moss-positive patients. Thus we conclude that contaminating resin acids have not alone caused the high rate of sensitivity to oak moss.

Calnan C.D. (1979) “Perfume dermatitis from the cosmetic ingredients oakmoss and hydroxycitronellal.” *Contact Dermatitis* **5**(3), 194.

Ehret C., Maupetit P., Petrzilka M *et al*. (1992) *Int. J. Cosmet. Sci*. **14**, 121–130.

Ehret C.P. Maupetit M. Petrzilka G. Klecak (2007) "Preparation of an oakmoss absolute with reduced allergenic potential" *International Journal of Cosmetic Science* **14**(3), 121 - 130. Abstract Oakmoss absolute, an extract of the lichen *Evernia prunastri*, is known to cause allergenic skin reactions due to the presence of certain aromatic aldehydes such as atranorin, chloratranorin, ethyl hematommate and ethyl chlorohematommate. In this paper it is shown that treatment of Oakmoss absolute with amino acids such as lysine and/or leucine, lowers considerably the content of these allergenic constituents including atranol and chloratranol. The resulting Oakmoss absolute, which exhibits an excellent olfactive quality, was tested extensively in comparative studies on guinea pigs and on man. The results of the Guinea Pig Maximization Test (GPMT) and Human Repeated Insult Patch Test (HRIPT) indicate that, in comparison with the commercial test sample, the allergenicity of this new quality of Oakmoss absolute was considerably reduced, and consequently better skin tolerance of this fragrance for man was achieved.

EP0202647: "A process of obtaining a hypo-allergenic moss oil." Abstract. Hypo-allergenic moss oil from which ethyl hematommate and/or ethyl chlorohematommate or atranorin and/or chloroatranorin are substantially removed. This hypo-allergenic moss oil can be produced by chromatography separation, solvent extraction, countercurrent partition, and/or membrane separation or catalytic hydrogenation treatment and/or alkaline treatment.

Ford R.A. & Api A.M. (1990) “An Investigation of the potential for allergic contact sensitisation of several oakmoss preparations.” *Contact Dermatitis* **23**, 249.

Fregert S. & Dahlquist I. (1983) “Patch testing with oakmoss extract.” *Contact Dermatitis* **9**, 227.

Goossens A, Nardelli A, Bernard G et al. Paper No. PB24, presented at the *9th Congress of the European Society of Contact Dermatitis*, Estoril, Lisbon, 28–30 May 2008.

Goncalo S. (1987) “Contact sensitivity to lichens and compositae in frullania dermatitis.” *Contact Dermatitis* **16**, 84-86.

Gonçalo S, Cabral F, Gonçalo M. (1988) "Contact sensitivity to oak moss." *Contact Dermatitis.* **19**(5):355-7. Abstract. Oak moss allergy was the principle allergen in contact sensitivity to perfumes (45%); 31 patients reacting to oak moss were studied. The sensitivity was attributed to contact with perfumes in 20, lichens in 7 and unknown in 4. Atranorin was the most frequent allergen, followed by usnic, evernic and fumarprotocetraric acids. Concomitant allergy occurred to several lichen acids and also to balsam of Peru, colophony and other fragrance components

Held J.L., Ruszkowski A.M., DeLeo V.A.. (1988) “Consort contact dermatitis due to oak moss.” *Arch Dermatol* **124,** 261-2.

Hostynek J.J. & Maibach H.I. (2004) "Thresholds of elicitation depend on induction conditions. Could low level exposure induce sub-clinical allergic states that are only elicited under the severe conditions of clinical diagnosis*?" Food and Chemical Toxicology* **42**(11),1859-1865 Abstract While numerous studies have examined dose/response relationships occurring in the experimental induction of contact allergic dermatitis, fewer have examined the effects of varying the doses of both induction and challenge. Recently published studies have however done this and they all show the same remarkable observation: the threshold of elicitation decreases as the doses used to induce the allergy increase. This has important implications. One is that it may be more complicated to determine clear threshold doses below which allergic responses are not seen. It is also proposed that normal exposure to weak allergens such as some fragrance materials may induce “sub-clinical” allergic states which will not be elicited under these same exposure conditions but which may become apparent under the more severe conditions of clinical diagnosis. This may explain why the prevalence of Patch test reactions to some fragrance materials is apparently increasing in the absence of any clearly documented “epidemic” of consumer complaints.

Johansen J.D., Heydorn S., & Menné T. (2002) "Oak moss extracts in the diagnosis of fragrance contact allergy" *Contact Dermatitis*. **46**(3):157-61.Abstract. Oak moss absolute is one of the eight ingredients of the fragrance mix (FM) used for diagnosing perfume allergy.Oak moss absolute is an extract prepared from the lichen *Evernia prunastri* growing on oak trees. It has been shown that the oak moss patch test material from one producer contained resin acids which are ingredients of another lichen, tree moss. Resin acids, e.g. abietic acid and dehydroabietic acid, are also the main allergens in colophonium. The aim of the study was to assess whether the contamination of oak moss absolute and thus the FM with resin acids had affected their diagnostic value so that they, instead

of indicating fragrance allergy, had become indicators of allergy to resin acids and thus colophonium. Two studies were undertaken. First the relationship between patch test reactions to FM, oak moss absolute, both with contents of resin acids, and colophonium were assessed in 885 consecutive patients. A significant relationship between reactions to colophonium and FM was seen ($p < 0.001$) as well as a significant relationship between oak moss absolute and colophonium ($p < 0.001$). The relationship between colophonium and FM was still significant when all reactions to oak moss absolute were disregarded ($p < 0.001$), showing a relationship also between colophonium and fragrance ingredients other than oak moss absolute. Second, 119 consecutive patients were tested with an old and a new version of oak moss absolute containing resin acid (0.05%) and no measurable resin acid, respectively, and with the corresponding FM. No overall difference in reactivity to the old and new version of oak moss absolute/FM was seen. It is concluded the diagnostic value of oak moss absolute as indicator fragrance contact allergy has been and is unaffected by the resin acid contamination.

Johnson, I.R., Oakmoss (absolute): Local Lymph Node Assay. Report n° GM7788. Central Toxicology Laboratory, UK-Cheshire, 14 January 2004

Johnson, I.R., Treemoss (absolute): Local Lymph Node Assay. Technical toxicology report n° GM7789. Central Toxicology Laboratory, UK-Cheshire, 25 March 2004

Kanerva L, Jolanki R. & Estlander T (1999).” Hairdresser's dermatitis caused by oak moss in permanent waving solutions.” *Contact Dermatitis* **41**, 55-56.

Kieć-Swierczyńska M., Krecisz B., & Swierczyńska-Machura D (2006) “Contact allergy to fragrances.” *Medycyna pracy.* **57**(5),:431-7. Abstract. BACKGROUND: The incidence of allergy induced by fragrances, leading mostly to the development of contact dermatitis and urticaria chronica, has been growing in a large number of countries. In general, allergy is of non-occupational nature, however, it can also have traits of occupational exposure. The fragrance mix is used in screening for allergy to aromas, but it frequently produces false positive or false negative results of the test. The aim of the study was to assess whether the fragrance mix is suitable for detecting allergy to aromas in persons with suspected occupational allergy and also to analyze types of allergy to separate fragrances. MATERIAL AND METHODS: In a group of 1937 patients diagnosed in the Nofer Institute of Occupational Medicine in Lódź, the incidence of allergy to fragrance mix was assessed. They underwent dermatological examinations and tests with use of the European standard kit (Chemotechnique Diagnostics, Malmö, Sweden) in the years 2000-2005. In addition, in using fragrance series (Chemotechnique Diagnostics, Malmö, Sweden). RESULTS: The positive reaction to fragrance mix was found in 99 (5.1%) patients (women--5.2% and men--4.9%). In 57.6% of patients, allergy to balsam of Peru was also observed. Allergy to at least one aroma was diagnosed in 82 (72%) persons tested with an expanded fragrance series. In the remaining 23 (28%) patients, patch tests proved to be false positive. In the latter group, 17 patients showed negative

results of the test to balsam of Peru. In the study group, 65% of patients reacted to 1, 2 or 3 and 36% to more aromas. The following allergens sensitized most frequently: cynnamic alcohol, hydroxycitronellal, cinnamic aldehyde, isoeugenol, eugenol, Ylang-Ylang oil, oakmoss absolute, and jasmine. Allergic contact dermatitis was diagnosed in 69.7%, urticaria chronica in 5.1% of patients, atopic dermatitis in 4%, whereas in 21.2% no skin lesions were observed. CONCLUSIONS: The results of the study confirmed observations of other authors that the usefulness of fragrance mix in the diagnosis of allergy to aromas is limited (a high number of false positive results of skin tests). We noticed that a combined reaction to fragrance mix and balsam of Peru is helpful in diagnosis in persons whom a complete allergic diagnosis cannot be performed. We also found less frequent hypersensitivity to oakmoss absolute and more frequent to cinnamic aldehyde and alcohol, hydroxycitronellal, eugenol, isoeugenol in Lódź than in studies reported by other authors. the group of patients with positive tests, the type of allergy to individual aromas was analyzed

Laquieze S., (2004) “Cutaneous tolerance and sensitising potential of the solution "Absolue mousse de chene RAM Lot 1" performed in 100 subjects during 6 weeks according to the Marzulli-Maibach method.” PERITESCO, Paris. Reference 2644-41-DPL. 2.08.2004, RIFM 2004i #48063

Lepoittevin J.P., Meschkat E., Huygens S. & Goosens A. (2000) "Presence of resin acids in "Oakmoss" patch test material: a source of misdiagnosis?" *J Invest Dermatol*. **115**(1), 129-30.

van Loveren H., Cockshott A,, Gebel T., Gundert-Remy U., de Jong, Wm JH., Matheson J., McGarry H., Musset L., Selgradeg M-J. K. & Vicker C. (2008) "Skin sensitization in chemical risk assessment: Report of a WHO/IPCS international workshop focusing on dose–response assessment." *Regulatory Toxicology and Pharmacology* 50(2), 155-199. Abstract. An international workshop was held in 2006 to evaluate experimental techniques for hazard identification and hazard characterization of sensitizing agents in terms of their ability to produce data, including dose–response information, to inform risk assessment. Human testing to identify skin sensitizers is discouraged for ethical reasons. Animal-free alternatives, such as quantitative structure–activity relationships and in vitro testing approaches, have not been sufficiently developed for such application. Guinea pig tests do not generally include dose–response assessment and are therefore not designed for the assessment of potency, defined as the relative ability of a chemical to induce sensitization in a previously naive individual. In contrast, the mouse local lymph node assay does include dose–response assessment and is appropriate for this purpose. Epidemiological evidence can be used only under certain circumstances for the evaluation of the sensitizing potency of chemicals, as it reflects degree of exposure as well as intrinsic potency. Nevertheless, human diagnostic patch test data and quantitative elicitation data have provided very important information in reducing allergic contact dermatitis risk and sensitization in the general population. It is therefore recommended that clinical data, particularly dose–response data derived from

sensitized patients, be included in risk assessment.***Cropwatch comments***: Claim made that new treemoss qualities containing lower amounts of atranol & chloroatranol are under test.

Rademaker M. (20000) "Allergy to lichen acids in a fragrance." *Australas J Dermatol* **41**(1), 50-1. Abstract. A 48-year-old clerical officer with a recurrent facial eruption had positive patch test reactions to nickel, fragrance mix and lichen acid mix. On testing to individual ingredients of fragrance mix and lichen acid mix, she had 2+ reactions to oak moss, which is thought to be the main allergen in fragrance mix, and to usnic acid, which is one of a number of lichen acids comprising oak moss. Avoidance of fragrance use resulted in clearing of the eruption but, subsequently, an acute vesicular flare on her face and hands occurred after exposure to lichen on garden shrubs.

Rastogi S.C., Johansen J.D. & Bossi R. (20070 "Selected important fragrance sensitizers in perfumes--current exposures." *Contact Dermatitis* **56**(4), 201-4. Abstract. Contact allergy to fragrance ingredients is frequent. Recommendations and regulations of some of the most frequent and potent fragrance allergens have recently been introduced. To investigate current exposures to 4 important fragrance allergens in hydroalcoholic cosmetic products. 25 popular perfume products of Danish as well as international brands were purchased from the Danish retail market. Contents of 4 important fragrance allergens, isoeugenol, hydroxy-iso-hexyl 3-cyclohexene carboxaldehyde (HICC, Lyral), were determined by gas chromatography-mass spectrometry, and atranol and chloro-atranol were determined by liquid chromatography-tandem mass spectrometry. Isoeugenol was found in 56%, HICC in 72%, atranol in 59%, and chloro-atranol in 36% of the 22 eau de toilette/eau de parfum products. The concentrations of isoeugenol were, in all products, below the recommended maximum concentration of 0.02%. HICC reached a maximum of 0.2%, which is 10-fold higher than maximum tolerable concentration considered safe by the EU Scientific Committee. The median concentrations of atranol and chloro-atranol in the investigated products were similar to those found in similar products in 2003. A significant decrease in the frequency of presence of chloro-atranol in the products was observed. There is still a wide-spread exposure to potent fragrance allergens in perfumes

Romaguera C, Vilaplana J, Grimalt F. (1991) “Contact dermatitis from oak moss.” *Contact Dermatitis* **24**, 224-25

Salo, H., Hannuksela, M., Hausen, B. (1981) “Lichen picker's dermatitis (*Cladonia alpestris* (L.) Rab.)”, *Contact Dermatitis* 7, 9, 1981.

SCCP (2008) *Opinion on oak moss/tree moss (sensitization only).* See http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_131.pdf

SCCP (2004) *Opinion on Atranol and Chloroatranol present in natural extracts (e.g. oak moss and tree moss extract).* See http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_006.pdf

Schmidt R.J. (1996) “Allergic contact dermatitis to liverworts, lichens, and mosses”. *Seminars in Cutaneous Medicine & Surgery* **15**(2), 95-102.

Schnuch A, Uter W, Geier J, Lessmann H. & Frosch PJ. (2007) "Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVDK and review of the literature." *Contact Dermatitis* **57**(1), 1-10. Abstract. To study the frequency of sensitization to 26 fragrances to be labelled according to current European regulation. During 4 periods of 6 months, from 1 January 2003 to 31 December 2004, 26 fragrances were patch tested additionally to the standard series in a total of 21 325 patients; the number of patients tested with each of the fragrances ranged from 1658 to 4238. Hydroxymethylpentylcyclohexene carboxaldehyde (HMPCC) was tested throughout all periods. The following frequencies of sensitization (rates in %, standardized for sex and age) were observed: **tree moss** (2.4%), HMPCC (2.3), **oak moss** (2.0), hydroxycitronellal (1.3), isoeugenol (1.1), cinnamic aldehyde (1.0), farnesol (0.9), cinnamic alcohol (0.6), citral (0.6), citronellol (0.5), geraniol (0.4), eugenol (0.4), coumarin (0.4), lilial (0.3), amyl-cinnamic alcohol (0.3), benzyl cinnamate (0.3), benzyl alcohol (0.3), linalool (0.2), methylheptin carbonate (0.2), amyl-cinnamic aldehyde (0.1), hexyl-cinnamic aldehyde (0.1), limonene (0.1), benzyl salicylate (0.1), gamma-methylionon (0.1), benzyl benzoate (0.0), anisyl alcohol (0.0). 1) Substances with higher sensitization frequencies were characterized by a considerable number of '++/+++' reactions. 2) Substances with low sensitization frequencies were characterized by a high number of doubtful/irritant and a low number of stronger (++/+++) reactions. 3) There are obviously fragrances among the 26 which are, with regard to contact allergy, of great, others of minor, and some of no importance at all.

Stinchi C., Gulrhini V., Ghetter E., Tosti A. (2006) "Contact dermatitis from lichens" *Contact Dermatitis* **36**(6), 309 - 310 .

Thune P., Solberg Y., McFadden N., Stærfelt F. & Sandberg M. (1982) “Perfume allergy due to oak moss and other lichens. *Contact Dermatitis* **8**, 396-400. Abstract. During a period of 2 1/2 years, 7 of 2000 patients routinely tested at our laboratory revealed contact allergy to oak moss in perfumes. All reacted to a mixture of different lichens and to some specific lichen compounds. The sensitivity was probably induced by cosmetics containing lichen substances. The following 3 compounds caused reactions in all patients tested: atranorin, evernic and usnic acids. 3 patients were photosensitive, but stronger reactions were elicited by prolonged contact during occlusion of the patches and complete protection against light, rather than by irradiation alone. The data suggest that the sensitizing capacity of the lichen compounds is primarily of a contact rather than of a photocontact nature.

Thune P. & Sandberg M. (1987) “Allergy to lichen and compositae compounds in perfumes. Investigations on the sensitizing, toxic and mutagenic potential,” *Acta Derm Venereol Suppl* (Stockh), **134**, 87.

Uter W., Gefeller O., Geier J. & Schnuch A., IVDK (2001) "Limited concordance between "oakmoss" and colophony in clinical patch testing." *Invest Dermatol.* **116**(3), 478-492.

Wood, B., Rademaker, M. (1996) "Allergic contact dermatitis from lichen acids." *Contact Dermatitis*, **34**, 370.

Oakmoss – Inhalation Toxicity.

Fujiwara R., Komori T., Noda Y., Kuraoka T., Shibata H., Shizuya K., Miyahara S., Ohmori M., Nomura J. & Yokoyama M.M. (1998)"Effects of a long-term inhalation of fragrances on the stress-induced immunosuppression in mice." *Neuroimmunomodulation* **5**(6), 318-22. Abstract. The aim of this study was to determine the effects of the long-term application of various fragrances on the suppression of immune response induced by high-pressure stress in mice. The immune response was analyzed based on plaque-forming cell (PFC) count, using mice sensitized with sheep red blood cells. The decreased PFC involving thymic involution induced by high-pressure stress in mice was restored by exposing the stressed mice to tuberose, lemon, oakmoss and labdanum for 24 h following exposure to stress. The decreased PFC and thymic involution from stress were restored by exposure to lemon and oakmoss, but not to tuberose and labdanum when the mice were exposed to those fragrances continuously for 3 weeks before the stress was given, followed by exposure to the same fragrances for 24 h after the stress. The decreased PFC and thymic involution from stress were restored by exposure to lemon and labdanum for 24 h after the stress, but not to tuberose over 3 weeks before the stress was given. These data suggest that the neuroimmunomodulatory effects of fragrances may be affected by tolerance depending on the kinds of fragrances in the case of a long-term application.

Oakmoss - Phototoxicity

Fernández de Corres L. (1986) "Photosensitivity to oak moss." *Contact Dermatitis* **15**, 118.

Fernández de Corres L, Munoz D, Leaniz-Barrutia I, Corrales J.L. (1983) "Photocontact dermatitis from oak moss." *Contact Dermatitis* **9**(6), 528-29.

Placzek M., Frömel W., Eberlein B., Gilbertz K-P. & Przybilla B. (2007) "Evaluation of phototoxic properties of fragrances" *Acto-dermato-Venereologica* **87**, 312-316. Abstract. Fragrances are widely used in topical formulations and can cause photoallergic or phototoxic reactions. To identify phototoxic effects, 43 fragrances were evaluated in vitro with a photohaemolysis test using suspensions of human erythrocytes exposed to radiation sources rich in ultraviolet (UV) A or B in the presence of the test compounds. Haemolysis was measured by reading the absorbance values, and photohaemolysis was calculated as a percentage of total haemolysis. Oakmoss caused photo-haemolysis of up to 100% with radiation rich in UVA and up to 26% with radiation rich in UVB. Moderate UVA-induced haemolysis (5–11%) was found

with benzyl alcohol, bergamot oil, costus root oil, lime oil, orange oil, alpha-amyl cinnamic aldehyde and laurel leaf oil. Moderate UVB-induced haemolysis was induced by hydroxy citronellal, cinnamic alcohol, cinnamic aldehyde, alpha-amyl cinnamic aldehyde and laurel leaf oil. The phototoxic effects depended on the concentration of the compounds and the UV doses administered. We conclude that some, but not all, fragrances exert photo-toxic effects in vitro. Assessment of the correlation of the clinical effects of these findings could lead to improved protection of the skin from noxious compounds ***Cropwatch comments***: Joulain & Tabbachi (2009) note that the oakmoss used in this study is of uncertified origin.

Thune P. (1977) "Allergy to lichens with photosensitivity." *Contact Dermatitis* **3**, 213-214.

Thune, P. & Solberg Y. J. (1980) "Photosensitivity and allergy to aromatic lichen acids, Compositae oleoresins and other plant substances." *Contact Dermatitis* **6**, 64, 1980.

Treemoss.

(Mousse d'arbre) Treemoss derivatives (concretes, absolutes) are mainly prepared from the lichen species *Pseudevernia furfuracea* (L.) Zopf. with *Usnea barbata, Parmelia sulcata* and other species often co-gathered in. These tree lichens can both be found living on the barks of firs and pines in Southern and Central Europe including and France and Morocco, & Balkan countries, including former Yugoslavia. Preparation of fragrant treemoss products is carried out in a similar manner to the preparation of oakmoss products, although evidence that isopropanol may be included as a processing solvent is shown by the presence of isopropyl haematommate (which does not exist in lichens) in the analysis of the weakly acidic factions of treemoss absolute (Endo *et al.* 1999). It should be noted that Treemoss products are generally considered inferior to oakmoss products and command a lower purchasing price – *Cropwatch Newsletter* Aug. 2008.

Endo H., Andatsu M. & Ishihara M. (1999) "Chemical components of treemoss absolute", *43rd TEAC Oita Japan*. Abstract. Tree moss is widely used for a perfumery material. To clarify the components of the characteristic m,ossy odor. we analyzed the weakly acidic part of the tree moss absolute Six phenolic compounds, methyl p-orcinol carboxylate (3), 5-pentylresorcinol (4). ethyl hematornmate (5), isopropyl hematommate (6). olivetonide (7), and physodone (8). have been isolated respectively by silica gel chromatography followed by preparative HPLC. Their structures were identified on the bases of their spectroscopic data (MS. ^{t}H- and $^{l'3-}$CNMR). Among them, 6 was found as a new compound. The synthesized compound has possessed a dry, sweet and mossy odor.

Huneck S. (1999) "The significance of lichens and their metabolites." *Naturwissenschaften* **86**, 559-570 (1999). Abstract. Lichens, symbiontic

organisms of fungi and algae, synthesize numerous metabolites, the “lichen substances,” which comprise aliphatic, cycloaliphatic, aromatic, and terpenic compounds. Lichens and their metabolites have a manifold biological activity: antiviral, antibiotic, antitumor, allergenic, plant growth inhibitory, antiherbivore, and enzyme inhibitory. Usnic acid, a very active lichen substance is used in pharmaceutical preparations. Large amounts of *Pseudevernia furfuracea* and *Evernia prunastri* are processed in the perfume industry, and some lichens are sensitive reagents for the evaluation of air pollution.

Huneck S. (2001) *Progr. Chem. Org. Nat. Prod.* **81**, 1.

Johnson, I.R., *Treemoss (absolute): Local Lymph Node Assay. regulatory report n°GM7789*. Central Toxicology Laboratory, UK- Cheshire, 25 March 2004

Joulain D. & Tabacchi R. (2009) “Lichen extracts as raw materials in perfumery. Part 2: treemoss.” *Flavour and Fragrance Journal* **24**, 105-116. Abstract. This is a comprehensive review of extracts from the lichen *Pseudevernia furfuracea* (treemoss) that are used in the fragrance industry. Qualitative and quantitative analytical aspects are critically reviewed and the results are compared to those of the related oakmoss extracts. It is shown that more than 90 constituents have been identified so far in treemoss extracts, including 42 depsides, depsidones or depside-derived compounds, and 42 triterpenes or steroids. Constituents of certain host trees, mainly *Pinus* species, generate specific analytical and toxicological issues which need to be considered in addition to those related to the known degradation products of lichen compounds. A new classification of lichen extracts used as raw materials in fragrance compounding is proposed.

Joulain D. & Guillamon N. (2002) "*Pseudevernia furfuracea* ("treemoss") resinoid in fragrance compounding: Analytical issues." Presented at *46th TEAC Symposium Tokushima, Japan (2002)*

RIFM (Research Institute for Fragrance Materials, Inc.), 2005a. *Treemoss absolute: Local lymph node assay. RIFM report number 50884.* (RIFM, Woodcliff Lake, NJ, USA)

RIFM (Research Institute for Fragrance Materials, Inc.), 2005b. *Treemoss absolute: Local lymph node assay. RIFM report number 50885.* (RIFM, Woodcliff Lake, NJ, USA)

Renaud J-M., Nicollier G. & Tabacchi R., *Proceeedings of the 8th International Congress of Essential Oils*, Cannes-Grasse, paper No. 72, 201-205 (1980)

Tabacchi R. (1983) “Contributions to the knowledge of chemical composition of tree moss *Pseudoervinia furfuranaceae* L. Zopf.” *Proceedings of 9th International Congress of Essential Oils Singapore* 65-68. (1983).

Tabacchi R. & Tsoupras G (1986) “"*Sur la présence de triterpènes dans quelques lichens*" in *Progress in Terpene Chemistry* ed D. Joulain, Editions Frontières (1986)., 293-305

Turk H., Yilmaz M., Tay T., Turk A.O. & Kivanc M. (2006) "Antimicrobial activity of extracts of chemical races of the lichen *Pseudevernia furfuracea* and their physodic acid, chloroatranorin, atranorin, and olivetoric acid constituents." *Z. Naturforsch*. **61c**, 499 - 507. Abstract. The antimicrobial activity and the MIC values of the ethanol, chloroform, diethyl ether, and acetone extracts of the chemical races of *Pseudevernia furfuracea* (var. *furfuracea* and var. *ceratea*) and their physodic acid, chloroatranorin, atranorin, and olivetoric acid constituents have been investigated against some microorganisms. Nearly all extracts of both chemical races showed antimicrobial activity against *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Proteus vulgaris, Staphylococcus aureus, Streptococcus faecalis, Yersinia enterocolitica, Candida albicans, Candida glabrata, Alternaria alternata, Ascochyta rabiei, Aspergillus niger, Fusarium culmorum, Fusarium moniliforme, Fusarium oxysporum, Fusarium solani*, and *Penicillium notatum*. There was no antimicrobial activity of the extracts against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas syringae, Salmonella typhimurium, Alternaria citri, Alternaria tenuissima, and Gaeumannomyces graminis*. Chloroatranorin and olivetoric acid were active against the same microorganisms with few exceptions. Physodic acid was active against about the same bacteria and yeasts and inactive against all of the filamentous fungi tested. Also no activity of atranorinagainst the filamentous fungi was observed.

Cedarmoss.
Cedarmoss qualities are derived from *Pseudevernia furfuracea* Ach. growing on the Atlas cedarwood tree *Cedrus atlantica,* found mainly in the Atlas Mountains of Morocco. Solvent extraction produces the resinoid (cyclohexane is used as solvent by some manufacturers), followed by distillation to produce an 'absolute' although other methods for obtaining the absolute are used. Often sweeter than corresponding oakmoss products, it is used in similar perfumery applications – *Cropwatch Newletter* Aug 2008..

Other Fragrant Lichens - *Everniastrum cirrhatum*
GB Patent No GB2438552 (A). "Ethanolic extracts of *Everniastrum cirrhatum* for use in the treatment of fungal infections. Khanuja S.P.S. *et al.* (Year?) Abstract of GB 2438552 (A): Ethanolic extracts of Everniastrum Cirrhatum comprising methyl-l-orcinol carboxylate of formula (1) & lt ;;may be useful for treating pathogenic fungal infections (in humans) that are resistant to polyene and azole antibiotics such as amphotericin B, nystatin, clotrimazole etc. The methyl-B-orcinol is optionally present in a concentration in a range of 10 to 400 pg/ml. The ethanolic extract may subsequently be extracted using hexane and ethyl acetate with the use of these extract for treating pathogenic fungal infections.

Other Fragrant Lichens - *Evernia mesomorpha*.
Reportedly commercially available from China.

Llano, G.A.. (1951). "Economic uses of lichens." *Ann. Rep. Smiths. Inst.* 385-422. Page 414 – notes use of *E. mesomorpha* "in modern perfumes & cosmetics".

Other Fragrant Lichens – *Parmelia nepalensis.*

Up to 1,000 tons/year of *Parmelia nepalensis* (Taylor) Hale ex Sipman is processed into lichen oil, absolute or extract in Western Nepal, and exported for global perfumery and incense use (although the lichens are also used in traditional systems of medicine) – *Cropwatch Newsletter* Aug 2008.

Kumar S.K., Banskota A.H. & Manandhar M.D. (1996) "Isolation & identification of some chemical constituents of *Parmelia nepalensis*." *Plant Med* **62**(1), 93-94.

Kumar S.K. & Muller K. (1999) “Lichen metabolites. 1. Inhibitory action against eukotriene B4 biosynthesis by a non-redox mechanism.” *Journal of Natural Products* **62**(6), 817-820.

Moxham T.H. (1986) “The commercial exploitation of lichens for the perfume industry.” In *Progress in Essential Oil Research* Walter de Gruyter Berlin – NY (1986).