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SkinResQU
Centre for Skin Research
Gothenburg

Abiotic Transformation of Fragrance Ingredients

Clinical studies, dermatological observations and toxicological considerations

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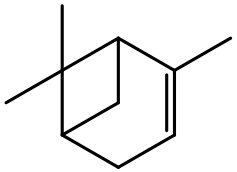
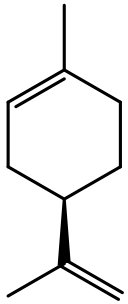
Turpentine

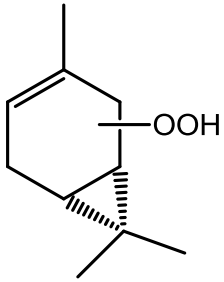
- Investigations of Scandinavian turpentine causing allergic contact dermatitis
- Autoxidation of monoterpenes
- Studies of contact allergenic effects from pure and oxidized turpentine



α-Pinene



***S*-Limonene**

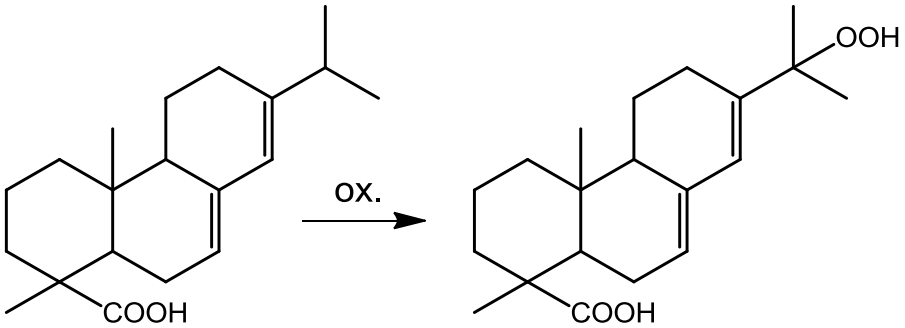


Δ^3-Carene hydroperoxide

Hellerström, S. *Acta Dermato-Venereol.* 1939: *20*: 657
Hellerström S, et al. *J Invest Dermatol* 1955: *24*: 217-224
Pirilä V, Siltanen E. *Dermatologica* 1958: *117*:1-8
Widmark, G. *Svensk Kem. Tidskr.* 1975: *69*: 175-184

Colophony

- Chemical identification of allergens
- Sensitization experiments
- Clinical studies



Abietic acid **15-Hydroperoxy abietic acid**

Karlberg A-T. Colophony: Rosin in unmodified and modified form. In T. Rustemeyer, P. Elsner, S.M. John, H.I. Maibach (eds) *Kanerva's Occupational Dermatology*, DOI 10 1007/978-3-642-02034-3_41, Springer Verlag Berlin Heidelberg 2012

Common Fragrance Terpenes as Prehaptens and/ or Prohaptens

Investigated

- Linalool
- Limonene
- Linalyl acetate
- Caryophyllene
- Geraniol
- Terpinene
- Cinnamic alcohol

Theses

- Maria Sköld 2005
- Carina Bäcktorp 2007
- Moa Andresen Bergström 2007
- Johanna Bråred Christensson 2009
- Lina Hagvall 2009
- Staffan Johansson 2009

- -

- Johanna Rudbäck 2013
- Ida Belogorcev Niklasson 2013

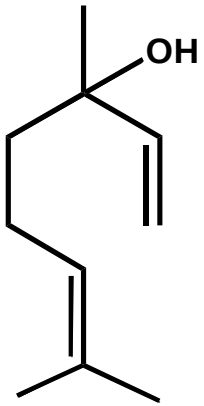
Common Fragrance Terpenes as Prehaptens and or Prohaptens

Methods and investigations performed

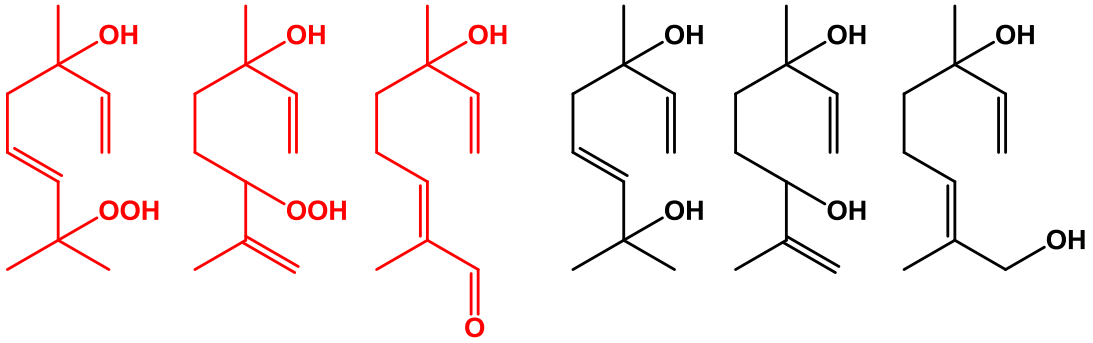
- Air exposure
- Chemical analysis
- Synthesis of reference compounds and compounds for allergy testing
- Studies of sensitisation potential in vivo
- Theoretical calculations of mechnisms
- Clinical testing to investigate relevance
- Studies on cross reactivity and metabolic activation

Oxidation Products of Linalool

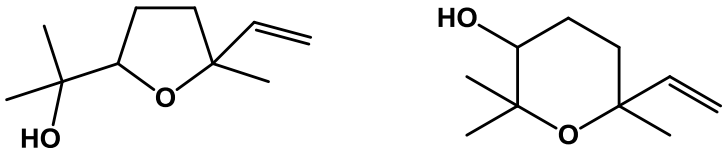
Allergenic



Linalool



hydroperoxides aldehyde alcohols

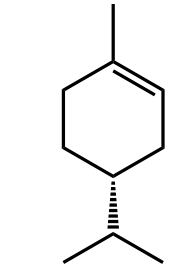


oxides

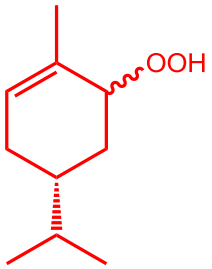
Sköld et al. Chem Res Toxicol 2004, *17*, 1697-1705

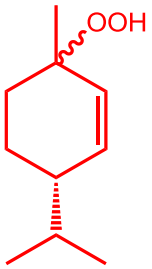
Oxidation Products of *R*- Limonene

Allergenic

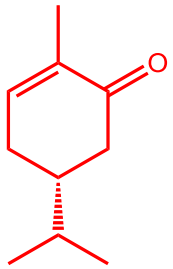


***R*- Limonene**

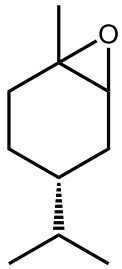




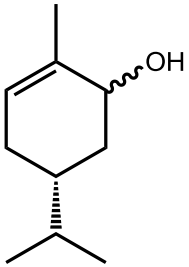
hydroperoxides

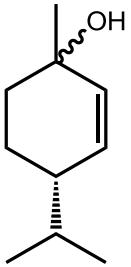


carvone

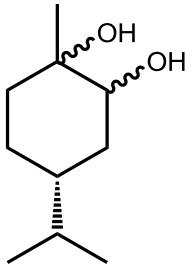


epoxide





alcohols



diol

Nilsson et al. *Chromatographia* 1996, *42*, 199-205

LLNA Results from Pure and Oxidized Compounds

Compound	EC3 values* (% w/v) Oxidized	Non oxidized
Cinnamyl alcohol	4.9 (2 w)	20.1
Geraniol	4.4 (10 w)	22.4
	5.8 (45 w)	
R- Limonene	3.0 (10 w)	30
Linalool	9.4 (10 w)	46
	4.8 (45 w)	
Linalyl acetate	3.6 (10 w)	25
α-Terpinene	0.94 (3 w)	8.9

***A lower EC3 value corresponds to a higher sensitizing potency**

Contact Allergic Reactions in Consecutive Dermatitis Patients

Autoxidation status not reported but it has been intended to be low.

Compound	Test conc. (%)	n Positive/n tested (%)	References
Limonene	2	0/1200	Santucci B, et al. *Contact Dermatitis* 1987: 16: 93-95
Limonene		3/2396 (0.1%)	Schnuch A, et al. *Contact Dermatitis* 2007: 57: 1-10
R-S-Limonene		11/1241 (0.88%)	Uter W, et al. *Contact Dermatitis* 2010: 63: 254-261
Limonene		0/320	van Oosten E J, et al. *Contact Dermatitis* 2009: 61: 217-23
R-S-Limonene		3/2396 (0.1%)	Schnuch A, et al. *Contact Dermatitis* 2007: 57: 1-10
Linalool	30	0/179	de Groot A C, et al. *Contact Dermatitis* 1985: 12: 87-92
	20	3/1825 (0.2%)	Uter W, et al. *Contact Dermatitis* 2010: 63: 254-261
	10	2/320 (0.6%)	van Oosten E J, et al. *Contact Dermatitis* 2009: 61: 217-23
	10	4/792 (0.5%)	Fregert S, Hjorth N. *Contact Dermatitis Newsletter* 1969: 5: 85-86
	5 and 1	0/100	Frosch P J, et al. *Contact Dermatitis* 1995: 33: 333-342
Linalool, stabilised	10	7/2401 (0.3%)	Schnuch A, et al. *Contact Dermatitis* 2007: 57: 1-10
	10	2/985 (0.2%)	Uter W, et al. *Contact Dermatitis* 2010: 63: 254-261
Linalyl acetate	1, 5	0/100	Frosch P J, et al. *Contact Dermatitis* 1995: 33: 333-342
	10	4/1855 (0.2%)	Frosch P J, et al. *Contact Dermatitis* 2002: 47: 78-85

FOUND: < 1% positive reactions

Contact Allergic Reactions in Dermatitis Patients

Compound Autoxidised (ox.)	Bi-or multicentre study	Test conc. (% w/w in pet.)	n Tested	n Positive (%/n tested)
Geraniol (ox.)		2	2179	12(0.55)
		4	655	6 (0.92)
Geraniol (ox.)		6	655	15 (2.3)
		11	653	30 (4.6)
R-Limonene (ox.)	x	3	2273	63 (2.8)
R -Limonene (ox.)	x	3	1812	49 (2.3)
S-Limonene (ox.)	x	3	1812	36 (2.0)
R – and/or S - Limonene (ox.)	x	3	2411	63 (2.6)
R -Limonene (ox.)	x	3 (0.3% lim-OOH)	2900	152 (5.2)
Linalool (ox.)	x	2	1511	20 (1.3)
Caryophyllene (ox.)	x	3.9	1511	2 (0.1)
Myrcene (ox.)	x	3	1511	1 (0.1)
Linalool (ox.)	x	2	1693	14 (0.83)
		4	2075	67 (3.2)
		6	1725	91 (5.3)
		11	1004	72 (7.2)
Linalool (ox.)	x	3	483	11 (2.3)
Linalool (ox.)	x	6 (1% lin-OOH)	2900	200 (6.9)
Linalyl acetate (ox.)		6	1217	13 (1.1)

Karlberg A T, Dooms-Gossens A. *Contact Dermatitis* 1997: 36: 201-6.

Matura M, et al. *J Am Acad Dermatol* 2002: 47: 709-14.

Matura M, et al. *Contact Dermatitis* 2003: 49: 15-21.

Matura M, et al. *Contact Dermatitis* 2005: 52: 320-28.

Matura M, et al. *Contact Dermatitis* 2006: 55: 274-79.

Christensson J B, et al. *Contact Dermatitis* 2010: 62:32-41

Sköld M, et al. *Food Chem Toxicol* 2006: 44: 538-45.

Buckley D A. *Contact Dermatitis* 2011: 64: 240-41

Analysis of Essential Oils

Fragrance compounds in essential oils autoxidize

Turek C, Stintzing FC. Stability of Essential oils: A Review. Comprehensive Reviews. In Food Science and Food Safety 2013: 12 40-53

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A sensitive method for determination of allergenic fragrance terpene hydroperoxides using liquid chromatography coupled with tandem mass spectrometry

J. Sep. Sci. 2013, 36, 1370–1378

Johanna Rudbäck, Nurul Islam, Ulrika Nilsson, Ann-Therese Karlberg

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[2]Department of Analytical Chemistry, Stockholm University, Stockholm, Sweden

Analysis of Essential Oils

Petitgrain oil content: mainly linalyl acetate and linalool

Sweet Orange oil content: mainly limonene

Sample	Compound	LOQ* (ppm)	LOD** (ppm)
Petitgrain oil	Linalool-OOH	1.0	0.3
Petitgrain oil	Linalylacetate-OOH	0.3	0.09
Sweet Orange oil	Limonene-2-OOH	0.6	0.18

* LOQ=Limit of Quantification
**LOD= Limit of Detection

J. Rudbäck *et al*. J. Sep. Sci. 2013, *36*, 1370-1378

Associated Positive Patch Test Reactions

	Positive reactions in the standard series		
	Fragrance mix I	**Myroxylon pereirae**	**Colophony**
91-95. Pos. to oxidized **limonene** (49/2800 pat.)	**41%**	**24%**	**24%**
97-99. Pos. to oxidized **limonene** (63/2273 pat.)	**37%**	**21%**	**22%**
2001. Pos. to oxidized **limonene** (63/2411 pat.)	**33%**	**22%**	**29%**
2002. Pos. to oxidized **linalool** (25/1511 pat.)	**40%**	**20%**	**32%**

Theoretical Explanations

- ✓Concomitant sensitizers?
- ✓True cross reactivity?
- ✓Hydroperoxides form non-specific antigens?

Conclusion

Hydroperoxides form specific immunogenic complexes

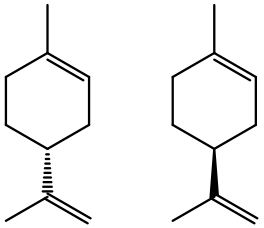
- Concomitant sensitizers? Yes
- True cross reactivity? Yes but not from the specific hydroperoxides
  Many other sensitizers present in the oxidation mixture
- Hydroperoxides form non-specific antigens? Possible in addition to specific antigens

Not only oxidized *R*- but also *S*- limonene is a common cause of contact allergy in dermatitis patients in Europe

Contact Dermatitis 2006: 55: 274–279

Mihaly Matura, Maria Sköld, Anna Börje, Klaus E. Andersen, Magnus Bruze, Peter Frosch, An Goossens, Jeanne D. Johansen, Cecilia Svedman, Ian R. White and Ann-Therese Karlberg

***R*- limonene**



***S*- limonene**

WHY concomitant reactions?

- ✓ **Exposure and sensitization to both enantiomers**
- ✓ **The same allergenic oxidation products can be formed from both enantiomers**

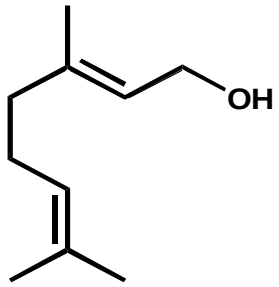
Is Geraniol Really a Hapten?

- Considered to be a weak allergen
- Included in the standard series used for screening of contact allergens,
  the fragrance mix (FM) - but few reactions
- No electrophilic properties
- Susceptible to autoxidation, according to structure

Geraniol

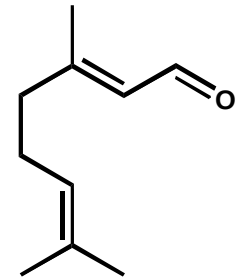
”Interestingly, concomitant reactions between citral and geraniol occurred frequently (83%). This may be due to co-exposure, but probably also to cross-reactions, as both compounds are structurally closely related.”

Schnuch A. et al. Contact Dermatitis 2007: 57: 1-10

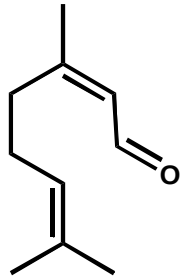


Geraniol

The isomers of citral



Geranial



Neral

Geranial and neral are formed by abiotic and biotic activation of geraniol.

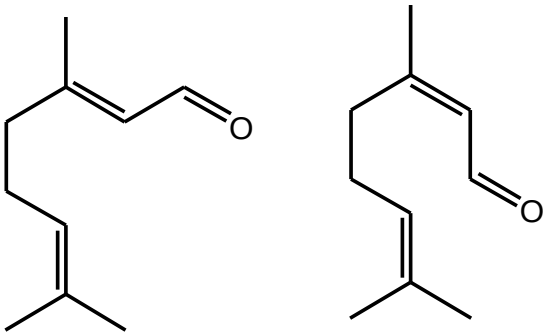
Hagvall L, et al. Chem Res Toxicol 2007: 20: 807-814

Hagvall L. et al. TAAP 2008: 233: 308-13

Citral

Hapten, Prehapten and Prohapten

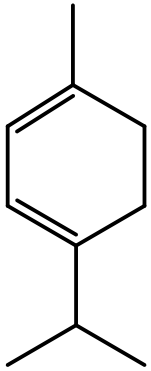
Citral



geranial + neral = citral

Hagvall L, et al. Chem Res Tox 2011: 224: 1507-1515

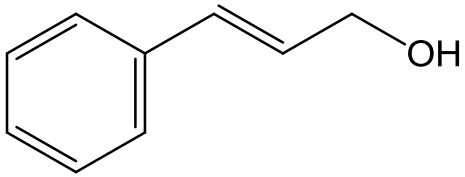
Other fragrance compounds acting as both pre- and prohaptens



α-Terpinene

Andresen Bergström M, et al.
Chem Res Toxicol 2006:19:760-769

Rudbäck J, et al.
Chem. Res. Toxicol. 2012: 25: 713−721



Cinnamic alcohol

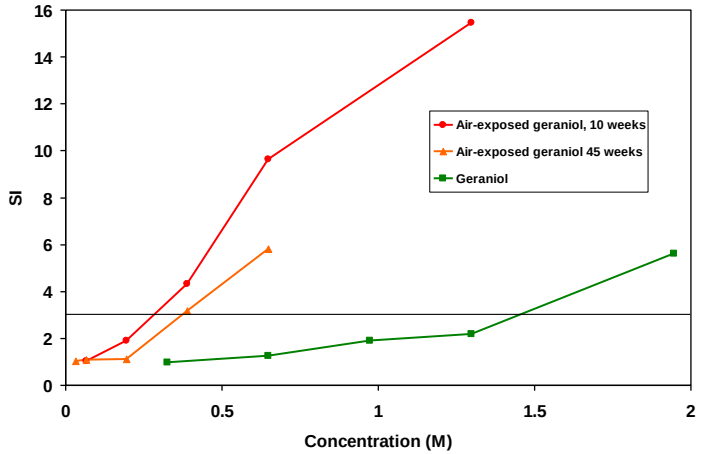
Niklasson B. I. et al.
Contact Dermatitis 2013: 68 (3) : 129-138

Basketter DA, Acta Dermatologica Venereologica 1992: 72: 264-65.

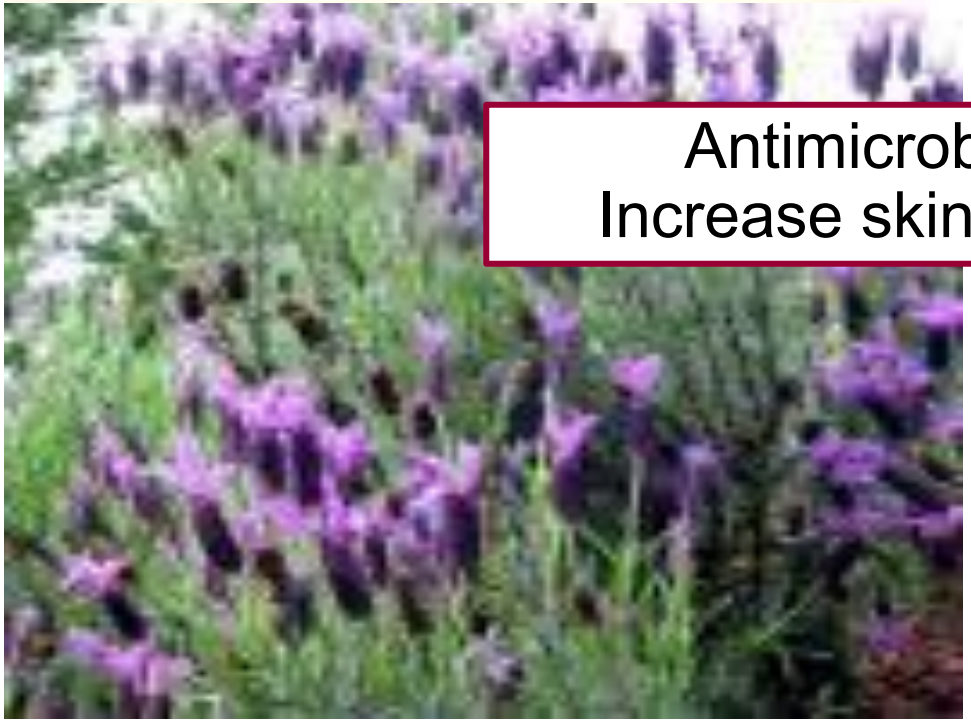
Prehapten and Prohapten

- ✓**Prehaptens:** Possible to prevent activation outside the body to a certain extent by different measures
- ✓**Prohaptens:** The activity is inherent in the molecule
- ✓**Both ways** of activation must be considered for a compound
- ✓Important from the view of **cross reactivity**

LLNA results from oxidized and pure geraniol



Active components to protect/ defend the plant



Antimicrobial effect
Increase skin penetration

What happens when a lot of radicals are formed?

In the Product

Not only in fine fragrances but in
formulated products applied on the skin

On the Skin

When fragrance compounds and scented products are applied

In the Skin

Formation of immunogenic complexes

Lepoittevin J-P, Karlberg A-T. Chem Res Toxicol 1994: 7: 130-133
Johansson S, et al. Chem Res Toxicol 2008: 21: 1536-1547
*Johansson S, et al. Chem Res Toxicol 2009: 22: 1774-1781
Redeby T, et al. Chem Res Toxicol 2010: 23: 203-210
Kao D, et al. J. Org. Chem. 2011: 76: 6188–6200

Not here Today:

Anna Börje organic chemist (radical chemistry, photo activation)

Kristina Luthman medicinal chemist (bioactivation and antigen formation)

Ulrika Nilsson analytical chemist (specialised in terpene analysis)

Lina Hagvall ocupational hygienist (scientific and practical aspects)

Mihaly Matura dermatologist (experienced in clinical testing of fragrance allergens)

Elena Gimenez Arnau chemist (radical chemistry, contact allergy)

Independent scientists with profound knowledge and experience of different aspects of fragrance compounds and their activity in the context of contact allergy