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IDEA Meeting on **Pre- and Pro-haptens**

Discussion on relevance of cross reactivity between fragrance hydroperoxides

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Cross reactivity

Definition:

The receptor of a memory cell for antigen **1** cannot distinguish between antigen **1** and an antigen **2** created from another hapten and will thus react also to antigen **2**.

Cross reactivity

- Haptens A and B are chemically and structurally similar.
- A is metabolised to a compound similar to B.
- B is metabolised to a compound similar to A.
- A and B are metabolised to the same compound.

However,

- Small changes in structure and configuration could prevent from cross reactivity.

Cross reactivity

- True cross reactivity studies must be performed experimentally under controlled exposure conditions.
- So far the most reliable are guinea pig studies
  - Live animals
  - Both induction and elicitation
  - No concomitant exposure

➢ Clinical studies can only give indications since no control of the exposure

Cross reactivity studies of terpene hydroperoxides

Haptens formed outside skin by abiotic oxidation

- The primary oxidation products

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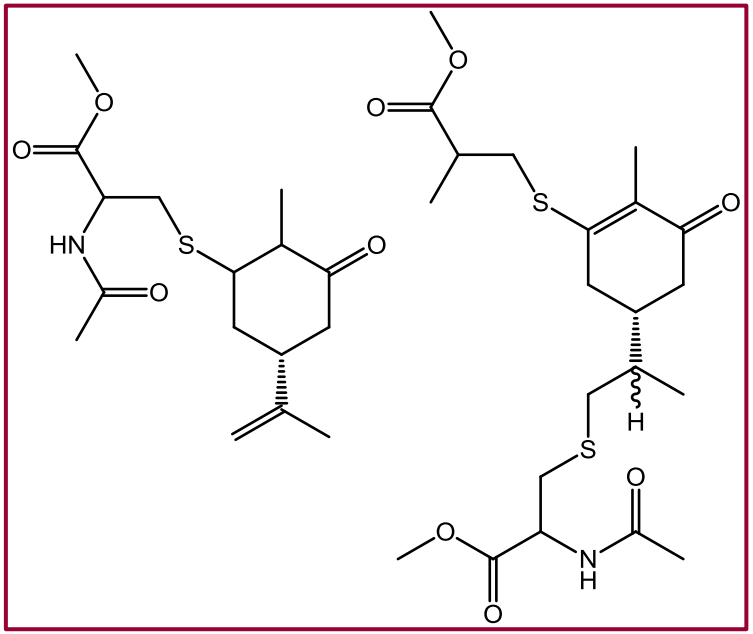
Specific immunogens are formed between terpene–OOH and amino acids

- ✓Involvement of carbon centered, alkoxy, and peroxyl radicals has been demonstrated with radical trappers
- ✓Investigations with peptides and nucleophiles show specific complex formation with limonene-2-OOH
- ✓Presence of iron complexes necessary (Fe(II)/Fe(III))



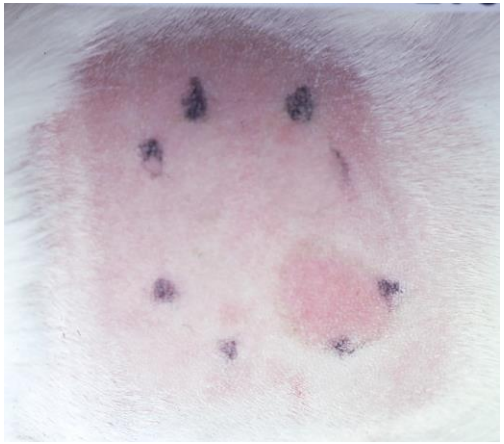
Identified adducts from reaction mixture with Lim-2-OOH, FE(III)TPPCl and NAc-Cys-OMe *

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
Kao D, et al. Toxicol. Res., 2014: 3: 278-289

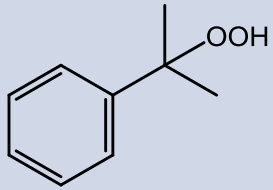
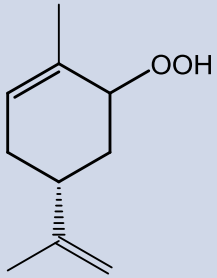
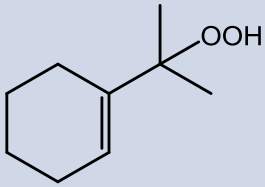
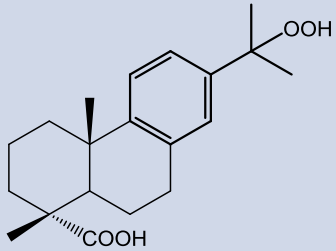
Cross reactivity studies of terpene hydroperoxides in guinea pigs

- No general cross reactivity found
- Specific cross-reactivity pattern demonstrated
- Cross reactivity when overall structural similarity



Positive reaction at challenge testing in guinea pigs

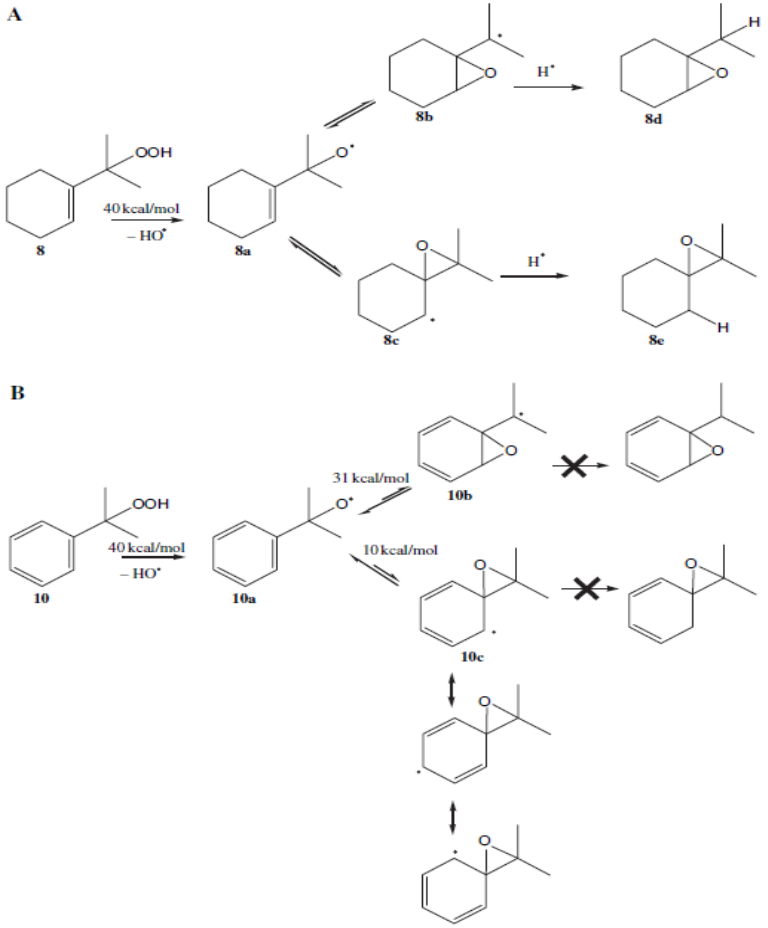
Cross reactivity pattern in guinea pigs

Induction compounds:	Challenge compound 1	Challenge compound 2	Challenge compound 3	Challenge compound 4
	Cumene-OOH 	Limonene-2-OOH 	Cyclohexene-OOH 	15-Hydroperoxy dehydroabietic acid 
<u>Group A</u> Cumene-OOH	**pos**	**neg**	**pos**	**neg**
<u>Group B</u> Limonene-2-OOH	**neg**	**pos**	**neg**	**NT**

*Bråred Christensson et al. Contact Dermatitis 2006: 55: 230–237

Cross reactivity pattern

Supported by determination of the formation energies of the intermediary radicals.

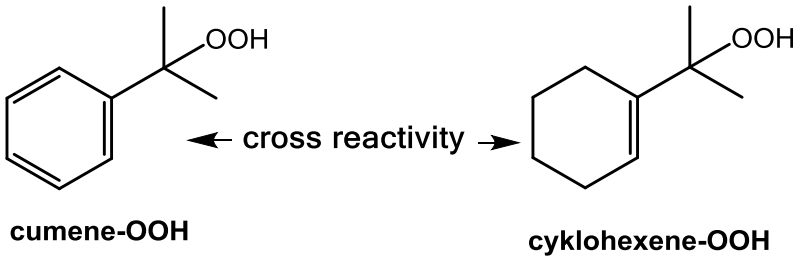


Bråred-Christensson J. et al. Contact Dermatitis 2006: 55: 230-237

Cross reactivity pattern

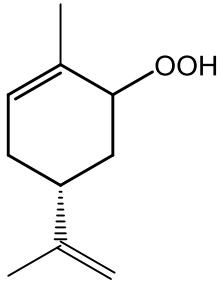
Thus!

Cross reactivity due to structural similarity in accordance with what is seen for other haptens.

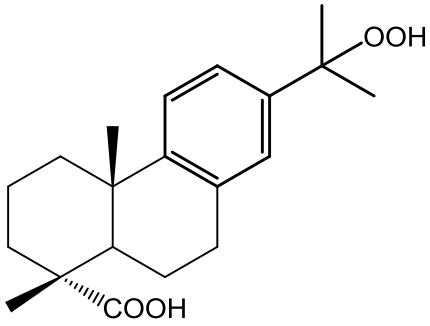


cumene-OOH **cyklohexene-OOH**

No unspecific 'cross reactivity' due to hydroperoxide was seen



limonene-OOH



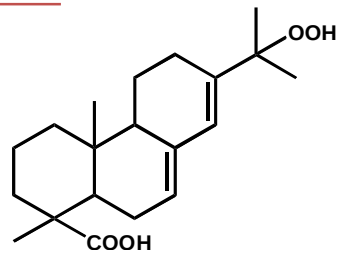
15-hydroperoxydehydroabietic acid

Bråred-Christensson J. et al. Contact Dermatitis 2006: 55: 230-237

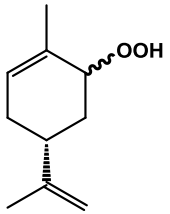
Cross reactivity studies of terpene hydroperoxides in allergic individuals

29 individuals allergic to colophony tested with:

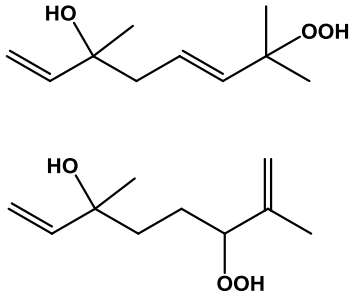
- ✓15-Hydroperoxyabietic acid
  (important hapten in colophony)



- ✓Limonene-2-hydroperoxide



- ✓Linaloolhydroperoxides



Bråred-Christensson J. et al. Contact Dermatitis 2006: 55: 230-237

Cross reactivity studies of terpene hydroperoxides in allergic individuals

No unspecific 'cross reactivity' due to hydroperoxide was seen

- ✓28/ 29 individuals reacted to colophony at retesting
- ✓13/29 (36%): colophony + 15-hydroperoxyabietic acid (15HPA)
- ✓1/29: colophony, 15HPA + limonene-2-OOH
- ✓1/29: colophony + limonene-2-OOH
- ✓1/29: colophony + linalool-OOH
- ✓Nobody reacted to both limonene-2-OOH and linalool-OOH
- ✓Nobody reacted to all three tested hydroperoxides

Bråred-Christensson J. et al. Contact Dermatitis 2006: 55: 230-237

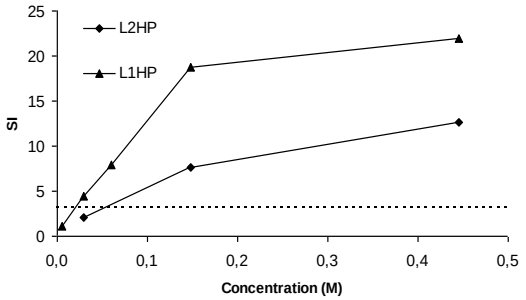
Observe

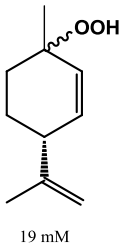
1/29 reacted to linalool-OOH = 3.4%
2/29 reacted to limonene-2-OOH = 6.9%

Screening in consecutive patients:
Pos to oxidized linalool (6.9%)
Pos to oxidized limonene (5.2%).

Limonene-1-OOH and Limonene -2-OOH In the LLNA

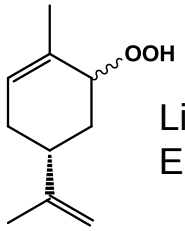
The limonene hydroperoxides differ in sensitizing potency in LLNA





19 mM

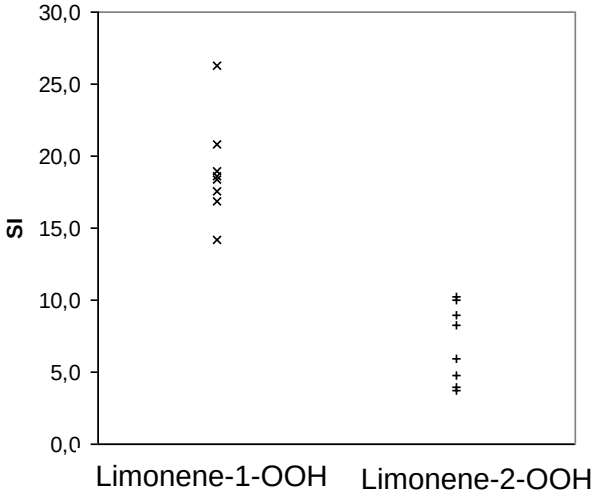
Limonene-1-OOH
EC3= 19 mM



49 mM

Limonene-2-OOH
EC3= 49 mM

Statistically significant



Bråred Christensson et al. Contact Dermatitis: 2008:59:344-52.

Limonene-1-OOH and Limonene -2-OOH

Small clinical study:

7 patients allergic to ox. limonene were patch tested with Lim-1-OOH and Lim-2-OOH

Results:
- 7/7 reacted to Lim-1-OOH
- 3/7 reacted to Lim-2-OOH

- Lim-1-OOH stronger allergen
  - in LLNA
  - more positive patch test reactions in limonene-allergic patients

Bråred Christensson et al. Contact Dermatitis: 2008:59:344-52.

Limonene-1-OOH and Limonene -2-OOH

Enlarged clinical study:

Testing in 763 consecutive patients

Reactions to	Also reactions to Ox. R-lim.	Also reactions to Lim-1-OOH	Also reactions to Lim-2-OOH	No pos reactions to other limonene markers
Ox. R-lim. (3%)*	9 (total)	6	4	2
Lim-1-OOH (0.5%)	6	18 (total)	8	7
Lim-2-OOH (0.5%)	4	8	13 (total)	4

*Content of Lim-1-OOH 0.0003% and of Lim-2-OOH 0.002%

Bråred Christensson J et al Contact Dermatitis 2014:70:291-99

Recent big multicenter studies – what do they say?

Unspecific ’cross reactivity’
due to hydroperoxide?

Please observe!

The clinical studies - on oxidized limonene or oxidized linalool

- ✓ **Mixtures of**:
- ✓ Non-oxidized parent compounds
- ✓ Primary oxidation products
- ✓ Secondary oxidation products
- ✓ Dimers?

International multicentre study

- 2900 consecutive patients tested with ox. limonene and ox. linalool
- 2619/2900 did not react to the ox. terpenes

- **25%** (71/281) of pos. patients reacted to both ox. limonene and ox. linalool

- **75%** (71/281) reacted only to one of them

Bråred Christensson J et al. Submitted

Multicentre study in the U.K.

4731 consecutive patients tested with ox. limonene* and ox. linalool

26% (107/411) of positive patients reacted to both ox. linalool and ox. limonene

74% reacted only to one of the preparations

*The Chemotechnique preparations named ”Hydroperoxides of limonene” and ”Hydroperoxides of linalool” are the same as used in the studies by Bråred Christensson J et al.

Audrian et al. British Journal of Dermatology 2014: 171: 292–297

In contrast:

High frequency of concomitant reactions to citral and geraniol

German multicentre study

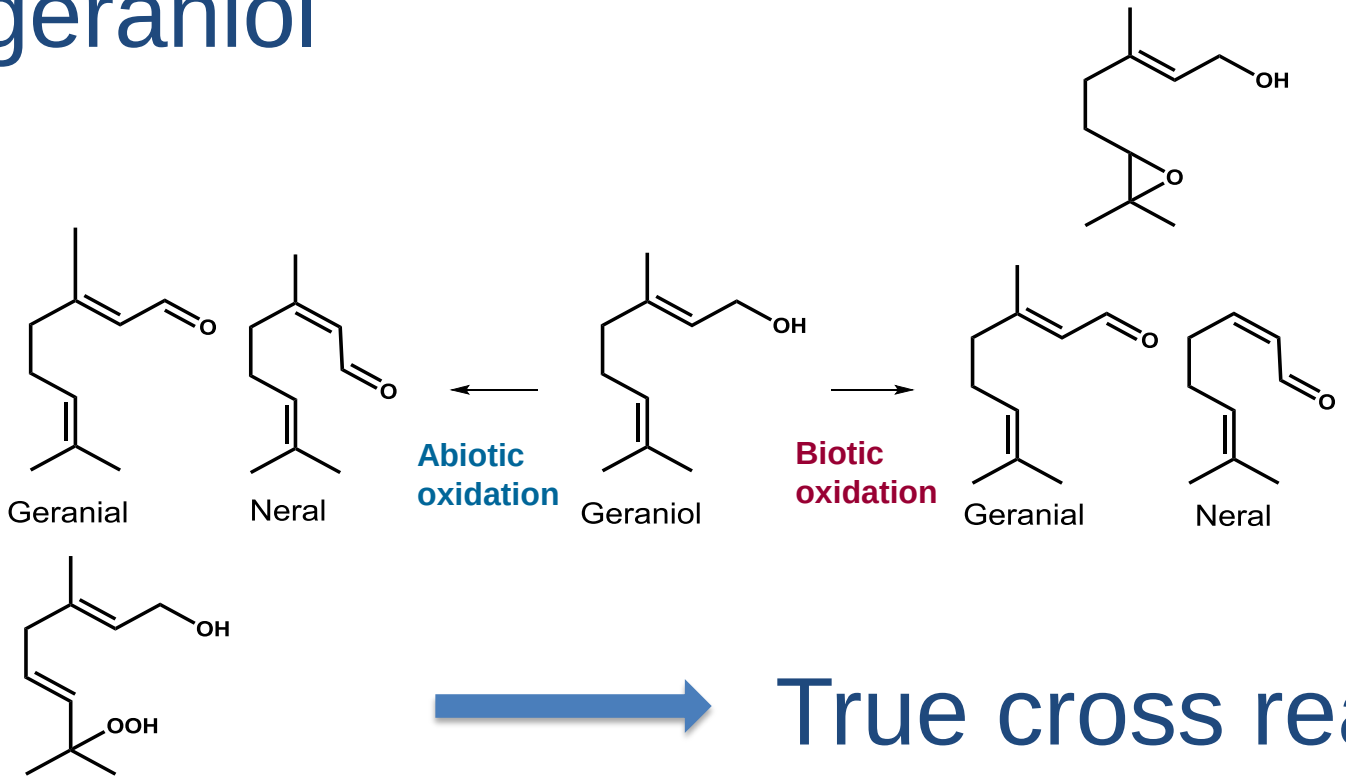
2021 patients patch tested with both geraniol and citral

83.3% of citral-allergic patients reacted also to geraniol

Citral = Geranial + Neral (2: 1)

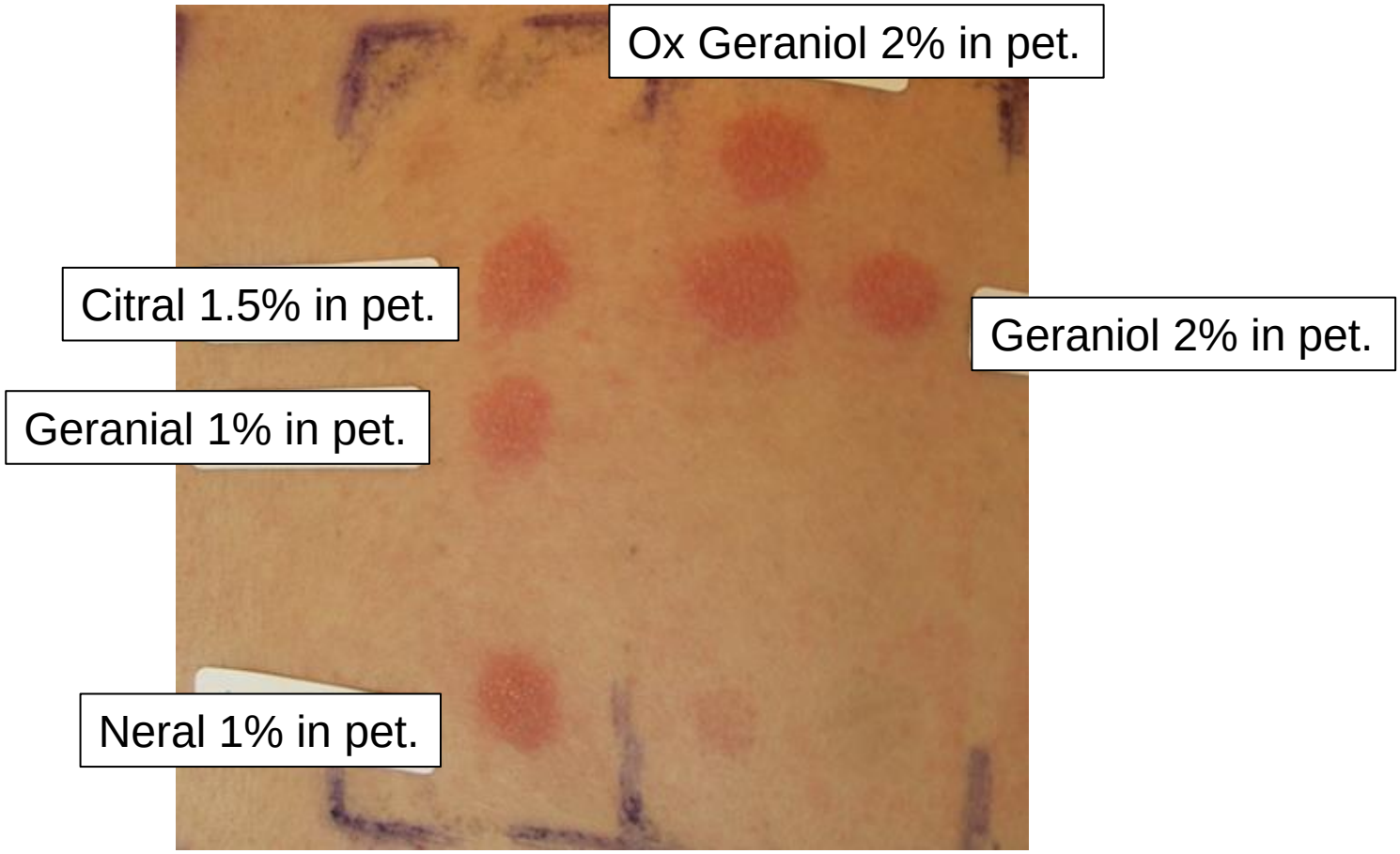
Schnuch et al. Contact Dermatitis 2007:57;1-10

Geranial and neral are formed by oxidation of geraniol



True cross reactivity

1. Hagvall et. al. Chem Res Toxicol. **2007**, *20*, 807-814
2. Hagvall et. al. Toxicol Appl Pharm. **2008**, *233,* 308-313



Hagvall L. et al. Contact Dermatitis 2012:**67**: 20–27
Hagvall L and Bråred Christensson J Contact Dermatitis 2014:**71**:280–288

ox. linalool and ox. limonene

25% (71/281) and **26%** (107/411) in the multicentre studies reacted to both ox. linalool and ox. limonene

- Is this due to mixed exposure?

Limonene and linalool are the most commonly used fragrance compounds - often used together ”tandem exposure”

- Or is this due to unspecific ’cross reactivity’ in clinical practice?

Unspecific ’cross reactivity’ due to hydroperoxide?

Natsch et al.:

”One possibility is that patch test to different terpene hydroperoxides do not only reveal a hapten-specific sensitization but rather a reactive state to other or multiple oxidizing agents.”

Chem Res Toxicol May 2015

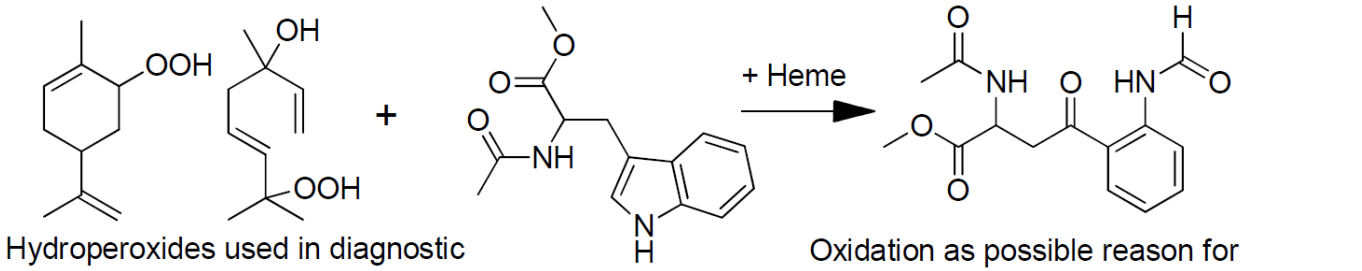
Oxidative Tryptophan modification by terpene- and squalene-hydroperoxides and a possible link to cross-reactions in diagnostic tests

Andreas Natsch, Roger Emter, Remo Badertscher, Gerhard Brunner, Thierry Granier, Susanne Kern, and Graham Ellis

Chem. Res. Toxicol., **Just Accepted Manuscript** • DOI: 10.1021/acs.chemrestox.5b00039 • Publication Date (Web): 05 May 2015

Hypothetically:

Table of content graphic



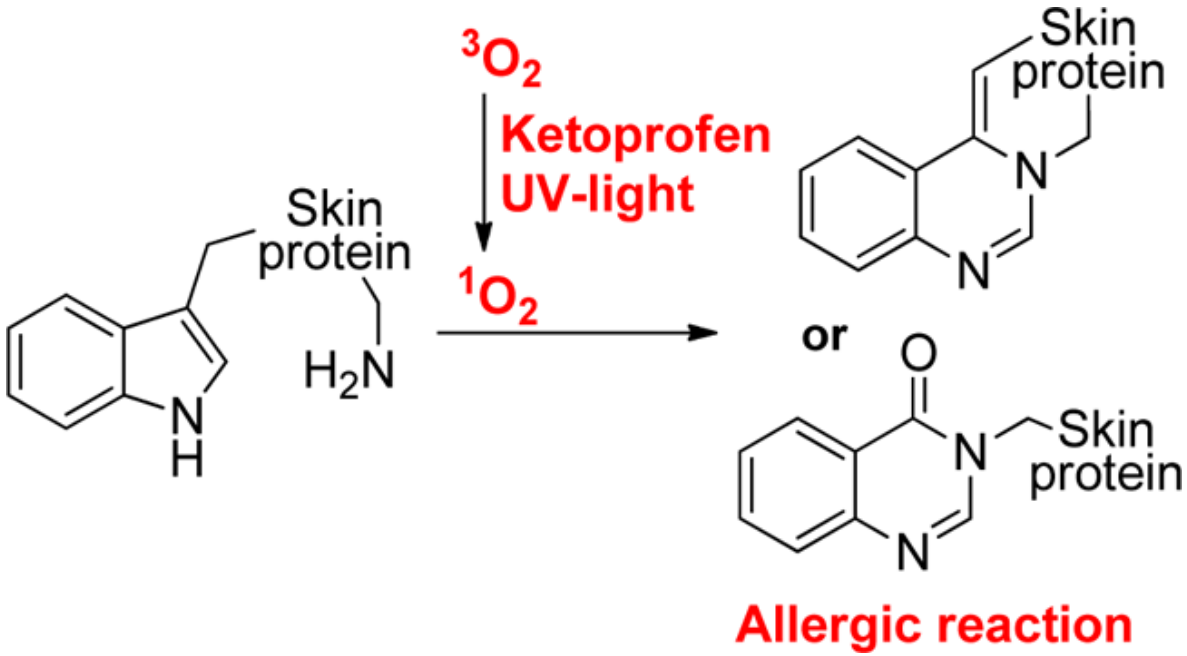
Hydroperoxides used in diagnostic tests for skin allergy

Oxidation as possible reason for crossreactions between hydroperoxides?

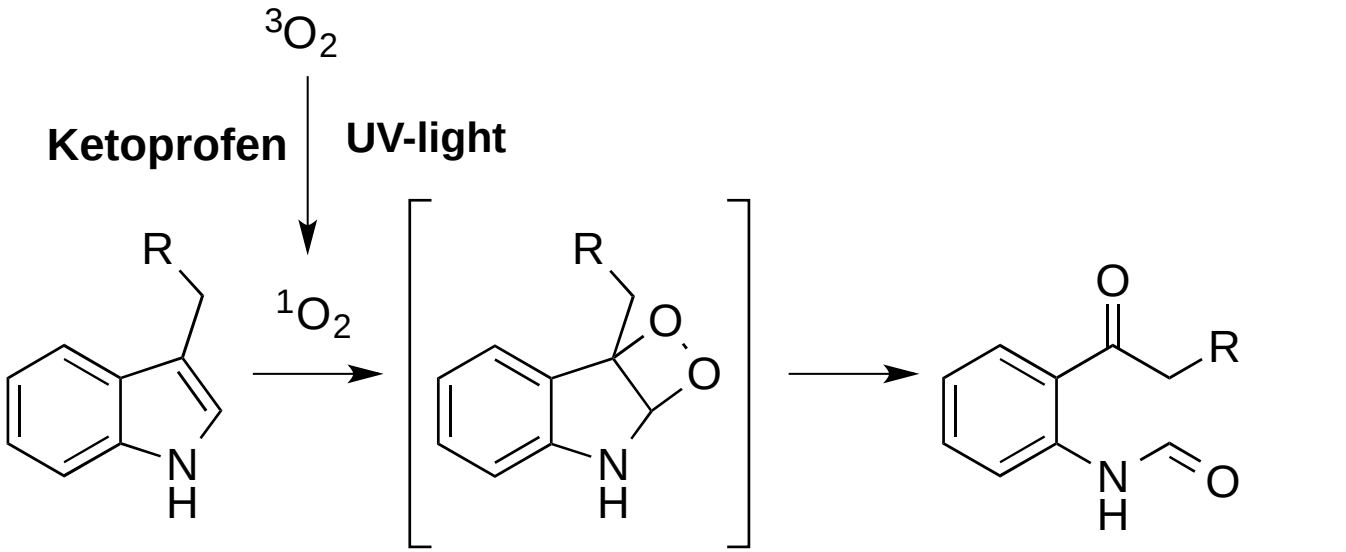
Ketoprofen-Induced Formation of Amino Acid Photoadducts: Possible Explanation for Photocontact Allergy to Ketoprofen.

Isabella Karlsson, Elin Persson, Andreas Ekebergh, Jerker Mårtensson, Anna Börje

Chem Res Toxicol 2014: **27**: 1294−1303



Mechanism for photooxidation of Trp induced by ketoprofen – type II photooxidation (formation of singlet oxygen)



N-formylkynurenine

Isabella Karlsson et al. Ketoprofen-Induced Formation of Amino Acid Photoadducts: Possible Explanation for Photocontact Allergy to Ketoprofen.
Chem Res Toxicol 2014: 27: 1294−1303

Karlsson et al

- **Ketoprofen** acts via the formation of singlet oxygen in a photooxidation process
- Mainly photo allergy, contact allergy rare
- No ketoprofen adducts were found but large amounts of tryptophan- lysine adduct
- 50 % of N-acetyl-O-methyl-Trp was oxidized after **15 min** in presence of ketoprofen (**1 equiv**.), UV-radiation and lysine.
- 60 % of the Trp analog (Me-Indole) was oxidized after **10 min** in presence of ketoprofen (**1 equiv**.) and UV-radiation.

  50% of Trp analog transformed to N-formylkynurenine /kynurenine.
- 25% of the Trp analog (Me-Indole) was oxidized after **30 min** in presence of **UV-radiation only**.

  20% of Trp analog turned into N-formylkynurenine /kynurenine

Natsch et al.

- **Terpene hydroperoxides** act via a radical mechanism, UV-radiation not needed
- Contact allergy commonly seen
- Earlier studies show formation of specific peptide adducts with terpenehydroperoxide
- No experiments including other amino acids in the present study

- 50 % of N-acetyl-O-methyl-Trp was oxidized after **24 h** in presence of hydroperoxides (**10 equiv**.) and heme.

  5% of Trp transformed to

  N- formylkynurenine (major compound formed)
- No experiment with only UV-radiation

Comments

- Different mechanisms for ketoprofen and the terpene hydroperoxides
- Photo allergy not the main issue with fragrance terpenes and oxidized fragrance terpenes
- Tryptophan is easily oxidized by other agens.

Conclusions

True cross reactivity exists due to:

- ✓Close strucural similarity (guinea pig studies)
- ✓Formation of the same haptens by bioactivation or abiotic activation (geraniol, citral)

No unspecific ’cross reactivity’ is seen:

- ✓In cross reactivity studies in guinea pigs
- ✓In directed clinical studies on specific hydroperoxides (abietic acid-OOH, Lim-OOH, Lin-OOH)
- ✓In big screening studies with oxidized terpenes (75% reacted only to one of the tested compounds)

- ✓No theoretical explanation for unspecific ’cross reactivity’ demonstrated.
- ✓Instead massive simultaneous exposure to fragrance terpenes