



**HAL**  
open science

## From biosynthesis in plants to post-biosynthetic enzymatic conversion. Generation of odor-impact rose oxides from citronellol-rich essential oils

Mathilde Lecourt, Giordiana Chietera, Frédéric Jullien, Bernard Blerot,  
Sylvain Antoniotti

### ► To cite this version:

Mathilde Lecourt, Giordiana Chietera, Frédéric Jullien, Bernard Blerot, Sylvain Antoniotti. From biosynthesis in plants to post-biosynthetic enzymatic conversion. Generation of odor-impact rose oxides from citronellol-rich essential oils. *Botany Letters*, In press, 170 (1), pp.15-27. 10.1080/23818107.2022.2100476 . hal-03838969

**HAL Id: hal-03838969**

**<https://hal.science/hal-03838969v1>**

Submitted on 4 Nov 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# From biosynthesis in plants to post-biosynthetic enzymatic conversion. Generation of odor-impact rose oxides from citronellol-rich essential oils.

---

Mathilde Lecourt<sup>1</sup>, Giorgiana Chietera<sup>2</sup>, Frédéric Jullien<sup>3</sup>, Bernard Blerot<sup>2</sup> and Sylvain Antoniotti<sup>1,\*</sup>

1 Institut de Chimie de Nice, Université Côte d'Azur, CNRS, Parc Valrose, CEDEX 2, 06108 Nice, France.

2 LMR Naturals by IFF, Parc d'Activité les Bois de Grasse, 18 Avenue Joseph Honoré Isnard, 06130 Grasse, France.

3 Université de Lyon, CNRS – UMR 5079, Laboratoire BVpam – 42000 Saint-Etienne, France.

\* Correspondence: [sylvain.antoniotti@univ-cotedazur.fr](mailto:sylvain.antoniotti@univ-cotedazur.fr)

## Abstract

Citronellol is a monoterpene alcohol biosynthesized by various plant species belonging to different families of Angiosperm. Bioinspired by the metabolism of *Rosa sp.* able to produce (–)-*cis*-rose oxide from citronellol, we have studied and optimized a laccase-catalyzed oxidation of (±)-, (*R*), and (*S*)-citronellol into rose oxides diastereomers in the presence of mediators. The reaction was found to be diastereomerically *cis*-selective but completely non-enantioselective. The laccase-mediator system was then applied on citronellol-containing essential oils such as lemongrass (*Cymbopogon citratus*) and geranium (*Pelargonium graveolens*) essential oils in order to modify their composition beyond the plant metabolism and increase their rose oxides content, thereby tuning their olfactory properties.

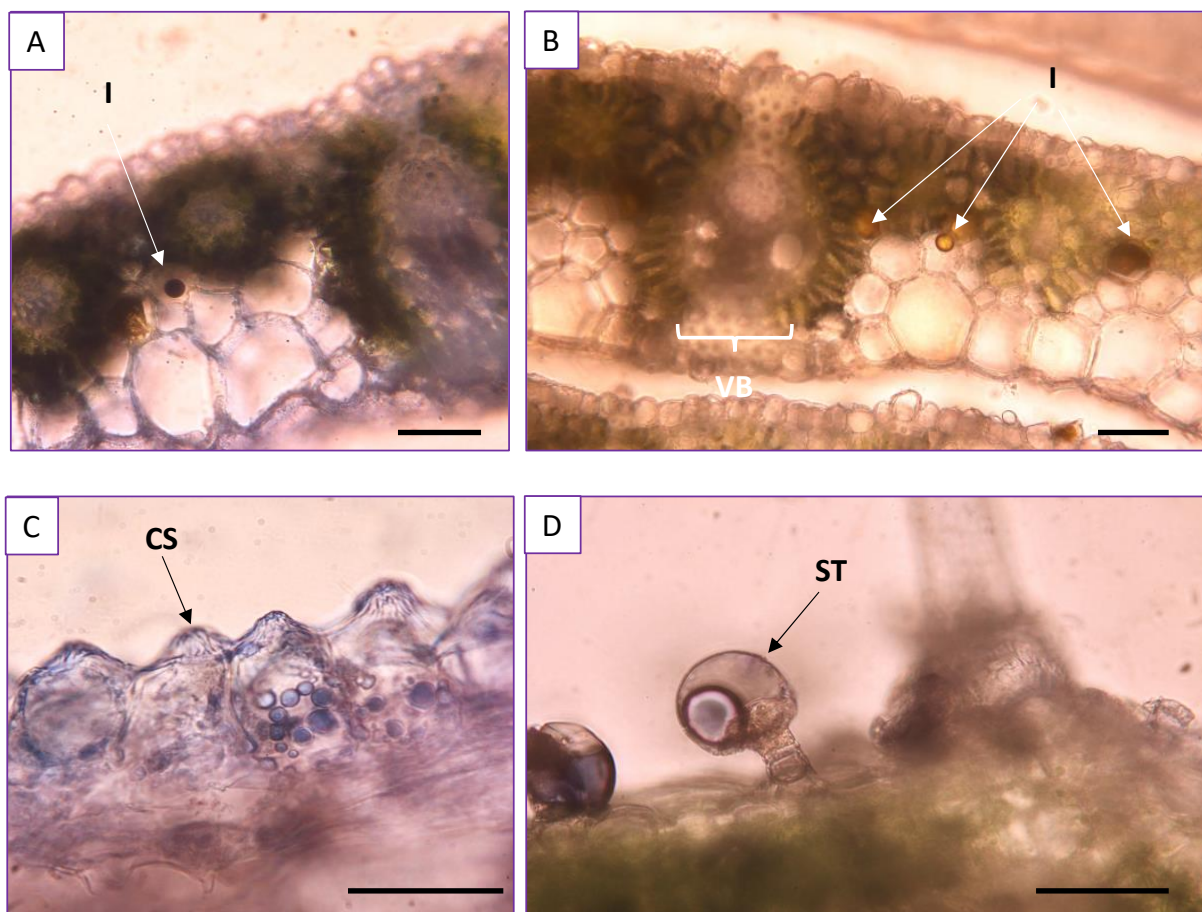
**Key-words:** biocatalysis, essential oils, fragrance, oxidation, rose, citronellol, rose oxide.

## I. Introduction

Plants are able to set up diverse biosynthetic mechanisms to fill various secondary functions such as communication, pollination or defense. (Cheng et al. 2007; Singh B and Sharma 2015) One of these mechanisms is to biosynthesize volatile organic molecules that can be emitted in order to interact with other living organisms. Emitted molecules belong to volatile organic compounds (VOCs): they typically display low molecular weight, high vapor pressure at ambient temperature and lipophilic properties. These features allow VOCs to be easily released in the environment through cell membranes (Dudareva et al. 2013).

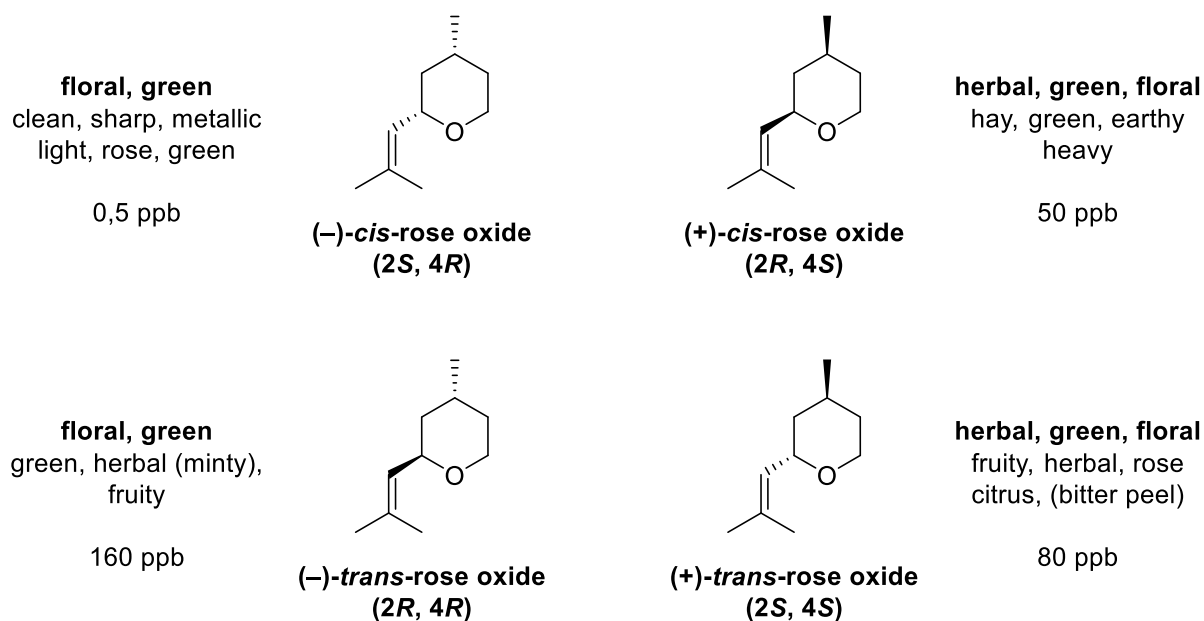
VOCs' family gather compounds from different secondary metabolic pathways such as terpenoids, phenylpropanoids, fatty acid derivatives and other small molecules. Terpenoids are composed by the assembly of two precursors: isopentenyl-phosphate (IPP) and dimethylallyl-diphosphate (DMAPP). In plants these units are typically synthesized through two different pathways: the methylerythritol phosphate (MEP) and the mevalonic acid (MVA) (Vranová et al. 2013). Depending on the number of units assembled, a range of terpenes can be created. Monoterpenes are built upon a 10-carbon skeleton which can be further complexified by oxygenation, oxidation, cyclization, isomerization, hydrogenation and other modifications (Banthorpe et al. 1972). Biosynthesis, storage and emission of monoterpenoids are tightly regulated by gene expression, induced by plant developmental stage or response toward environmental conditions (Dudareva et al. 2013; Abbas et al. 2017).

Citronellol is a monoterpene alcohol biosynthesized by a wide diversity of plant species belonging to different families of Angiosperm from both mono and dicotyledonous taxons (See S.I.). No clear monophyletic clade of plants producing citronellol could be seen in an evolution tree of angiosperm. Citronellol is often produced in low amount in most of plants except in rose (Rosaceae), lemongrass (Poaceae), pelargonium (Geraniaceae) and eucalyptus (Myrtaceae). Citronellol and derivatives like rose oxide are biosynthesized in different structures as single idioblast cell in lemongrass, sub-dermal cavities in eucalyptus, (Goodger et al. 2010) conic epidermal cell in rose petals (Rasouli et al. 2018) and secretory trichomes in pelargonium (Figure 1) (Botha and McCrindle 2000). Because of its chiral center, citronellol is found either as (*R*)-citronellol or (*S*)-citronellol enantiomers. In geranium and rose oils, (*S*)-citronellol is the major enantiomer, whereas (*R*)-citronellol dominates in *Boronia citriodora* (Surburg and Panten 2006). Citronellol biosynthesis has been demonstrated to derive from geraniol in *Caenorhabditis plicata*, (Haiyang Xu et al. 2017) and a similar pathway in pelargonium can be suggested in accordance with the characterization of a geraniol synthase in the species (Blerot et al. 2018). Nevertheless, this pathway is still under debate (Bergman M et al. 2021). Citronellol is not an end-product but undergoes further modifications through esterification or glycosylation to produce several citronellyl esters and citronellol glycosides. If monoterpenoids are stored in cells before their emission, they can indeed be glycosylated (Watanabe et al. 1998). Sugar moiety would be hydrolyzed when plant stage of development would favor monoterpenoids emission (Francis and Allcock 1969; Noriaki et al. 1999). Moreover, citronellol can undergo oxidative cyclization to produce rose oxides.



**Figure 1.** Localization of essential oil containing citronellol in different secretory structures stained by Nadi reagent. (David and Carde 1964) A and B Lemongrass leaves with iodoblast (I); C, conic cells (CS) of rose petals; D, secretory trichome (ST) of pelargonium roseum. In lemongrass, oil could be stained in A as deep purple droplet or observed as gold spot in unstained transverse cuttings (bar: 50  $\mu\text{m}$ ). Idioblasts were mostly closed to vascular bundles (VB). Essential oil droplets in rose conic cells were of light purple (bar: 15  $\mu\text{m}$ ). In pelargonium, essential oil accumulated in subcuticular space of glandular trichomes as huge droplet (bar: 50  $\mu\text{m}$ ).

Rose oxide is a cyclic monoterpene ether occurring only in some species as Rosaceae in rose (*Rosa damascena*), (Verma et al. 2011) and blackcurrant (*Ribes nigrum* L.), (Mikkelsen and Poll 2002). Geraniaceae with the example of Pelargonium roseum (Wüst et al. 1998) and Poaceae in *Cymbopogon* genus (Kreis and Mosandl 1994). This molecule is most often in low abundance in plant extract and can exist as four stereoisomers in relative different amounts. These isomers were separately synthesized and their properties described in the literature (Figure 2) (Yamamoto et al. 2002).



**Figure 2.** Rose oxide stereoisomers and their associated olfactory properties (Yamamoto et al. 2002).

Rose oxide stereoisomers have close olfactory descriptors but large gaps in terms of odor threshold (massic or volumic fractional amount of material necessary in average to be detected by the Human olfactory system, typically expressed in ppb). A factor 320 between (-)-*cis*-rose oxide and (-)-*trans*-rose oxide odor threshold was thus determined. If one focuses on the selectivity, *cis*-rose oxide is more abundant than *trans*-rose oxide in plant. *Trans*-rose oxide is found as sole isomer only in eucalyptus *citriodora*. In pelargonium and rose essential oil, passion fruit flavor and litchi flavor, (Werkhoff et al. 1993) (-)-*cis*-rose oxide dominates. In pelargonium rosat, citronellol metabolism preferentially produces *cis*-rose oxide forms (Wüst et al. 1998). Pelargonium should also be able to convert citronellyl diphosphate and citronellyl  $\beta$ -D-glucoside into *cis*-rose oxides. (Wüst et al. 1999) In grape berries (*Vitis vinifera* L.) feeding experiments with labeled molecules underline that (-)-*cis*-rose oxide was the major stereoisomer with a (-)-*cis*/(+)-*cis* enantiomeric ratio of 9/1 (Luan et al. 2005).

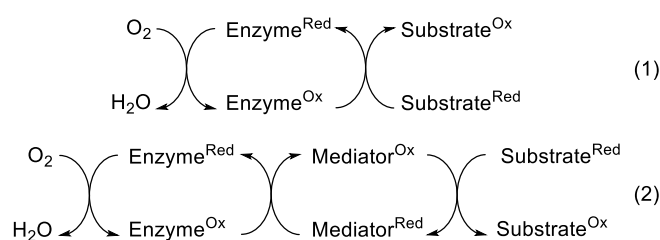
Olfactory importance of each compound in a mixture is linked to its odor threshold, its relative proportion and modulated by the combinatorial treatment of the mixture by olfactory receptors (Malnic et al. 1999). That is why each molecule has an olfactory nonlinear effect on the odor mix, leading to a complex essential oil bouquet. (-)-*cis*-rose oxide is considered an odor-impact molecule of rose, together with 2-phenylethanol, citronellol, geraniol,  $\beta$ -damascone,  $\beta$ -damascenone,  $\beta$ -ionone and nerol. Although not encompassing the whole complexity of rose odor, it is a mandatory contributor to the global perfume of rose with camphoreous and spicy facets. This points out the importance of the contribution of each compound from an odorant natural complex substance to the global odor. This contribution can be developed by multiple aspects: volatility affected by and affecting other components, direct odor, synergy and antagonism with other odorants, and so on.

Terpenoids biosynthesis and emission has various purposes: pollinators attraction, direct or indirect defense response or communication. (Cheng et al. 2007; Singh B and Sharma 2015) Concerning citronellol, it was demonstrated that honeybees (*Apis mellifera carnica*) were able

to discriminate two optical isomers of  $\beta$ -citronellol (Laska and Galizia 2001). Moreover, in orchids (*C. plicata*) wasp pollinator were significantly more attracted by (S)-citronellol (Haiyang Xu et al. 2017). No clear data are reported on the ecological role of rose oxide even if a role in pollinator attraction is suggested because their emission occurs mainly during flower opening in *Rosa damascena* (Noriaki et al. 1999).

(-)-*cis*-rose oxide is of interest to the perfume industry, as an important olfactory contributor to rose odor (Mannschreck and von Angerer 2011). The diversity of stereoisomers synthesis was reviewed (Singh U et al. 2017; Ziarani et al. 2020). Some reactions developed to access rose oxides include chemoenzymatic reactions like the use of a lipase (from *Pseudomonas cepecia*) to produce (+) and / or (-)-rose oxide precursors through a resolution step (Taneja et al. 2005). A non-stereospecific conversion of (*R*)-citronellol by a chloroperoxidase (from *Caldariomyces fumago*) to rose oxide diastereoisomers allowed to replace industrial photo-oxidation, the new process requiring less energy (Piantini et al. 2011). Not only being transformed by enzymes, citronellol can be also biotransformed by whole cells. Citronellol biotransformation by *Aspergillus* sp. and *Penicillium* sp. was performed to produce rose oxides and other compounds (Demyttenaere et al. 2004). (*R*)-citronellol (resp. (*S*)) was converted into the two (+)-rose oxides (resp. (-)). The *cis* form was favored in all cases, but modification by an entire metabolic system induced by-products formation such as linalool, limonene and nerol oxide. *Pseudomonas* strains were also used to convert citronellol (Recco Pimentel et al. 2012).

Enzymes are proteins playing a significant role in cell metabolism, endowed with biocatalytic properties. Their structure includes an active site that can bind with a substrate and induce a reaction. Enzyme can lower activation energy of reactions and be specific to one substrate in a pool and selective in the product formed. They are classified according to the type of reaction they catalyze. Laccase is an enzyme which oxidizes a substrate, or promotes the oxidation of a substrate through the intervention of a chemical mediator, in the presence of molecular oxygen as electron acceptor and thus terminal oxidant (Figure 3) (Monti et al. 2011).



**Figure 3.** Enzyme activity by direct catalysis (1) or indirect catalysis (2) involving a mediator.(Monti et al. 2011)

Laccases mostly come from white rot fungi after their release of the cells to achieve ligninolytic activity and thus their isolation is easy (Yoshida 1883; Schlosser et al. 1997; Baldrian 2006). Structural and mechanistic studies have shown that slight modifications of the structure could imply variations in the laccase redox potential and its subsequent catalytic activity (Piontek et al. 2002; Morozova, Shumakovich, Gorbacheva, et al. 2007).

Laccases are active on a large range of substrates – even more when a mediator is used, which makes it of high interest for industrial processes (Morozova, Shumakovich, Gorbacheva, et al. 2007; Witayakran and Ragauskas 2009). More generally, enzymes are selective and specific biocatalysts, working under soft conditions, and capable of widening their substrate

scope by various forms of catalytic promiscuity (Hult and Berglund 2007). Their use in industrial processes in purified form or in whole cell processes can lower production's cost and hazard. Various enzymes have already been used to modify natural complex substances such as essential oils (Antoniotti et al. 2008; Bouhlef et al. 2012; Notar Francesco et al. 2017), absolutes, (Bouges et al. 2018) essential oils distillation coproducts, or biobased raw materials (Schalk et al. 2012; Diaz-Chavez et al. 2013).

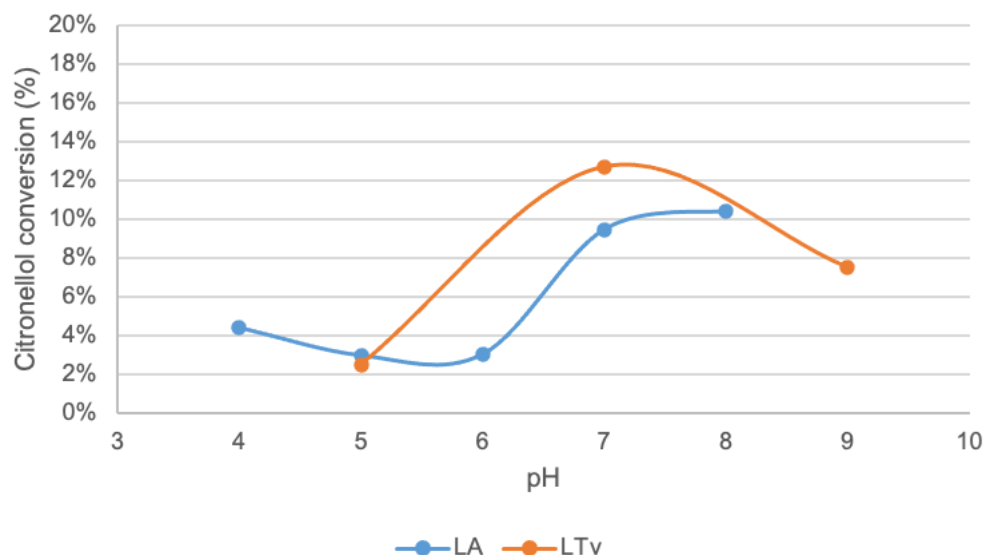
Lemongrass (*Cymbopogon citratus*) essential oil is known for its repellent, (Peach et al. 2019) antioxidant, (Sinha et al. 2011) and genotoxic (Sinha et al. 2014) properties. This plant has been used around the world for its bioactive properties, like in Brazil, Ghana or Singapore (Karami et al. 2021). Essential oil composition was therefore studied, pointing out some monoterpenoids – geraniol, citral, limonene, sesquiterpenoids – longifolene, selina-6-en-4-ol, and phenylpropanoids. Geranium (*Pelargonium graveolens*) essential oil has antioxidant and anticancer effects *in vitro* (Fayed 2009). Its essential oil contains mainly *iso*-menthone and *epi*- $\alpha$ -cardinol, but also citronellol and *cis* and *trans*-rose oxides (Fekri et al. 2021). Various geranium chemotypes were identified depending on monoterpenoids they preferentially accumulate ((-)-isomenthone, geraniol, or (-)-citronellol), (Bergman ME et al. 2019) pointing out metabolic pathways variation between *P. graveolens* hybrids. These two essential oils contain a large proportion of citronellol. However, citronellol conversion in derivatives such as rose oxides seems low.

In this paper, we describe the conversion of citronellol to rose oxides catalyzed by a laccase-mediator system (LMS). In a tentative to go beyond the metabolism of lemongrass and geranium, their citronellol-containing essential oils were treated by the LMS to promote citronellol conversion into rose oxides within the mixture. Modification of the olfactory properties was controlled and characterized by trained perfumers.

## II. Results

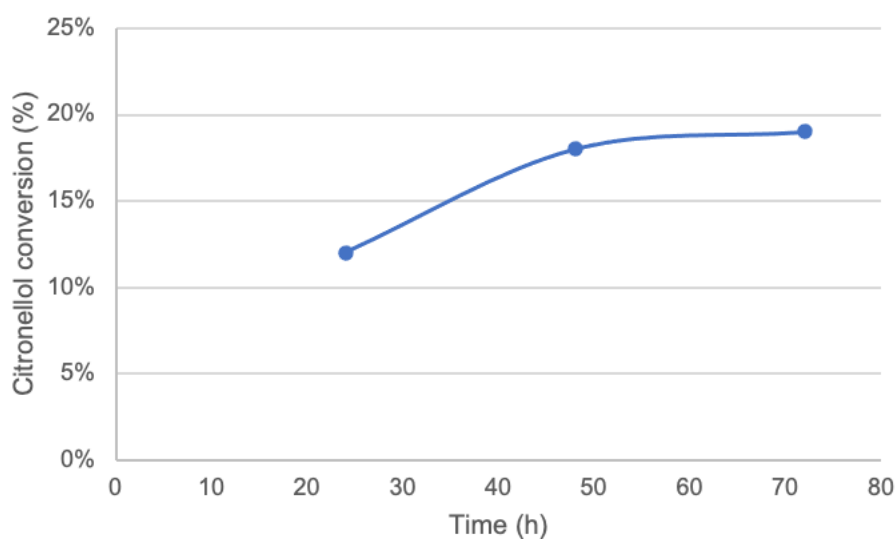
### II.A. Controls and screening of operating conditions

Conversion of citronellol **1** was initially considered on pure substance using a laccase – mediator system (LMS) with gas chromatography coupled with mass spectrometry (GC-MS). Experimental conditions such as pH, reaction time, enzyme ratio, mediator type and ratio were screened in order to maximize citronellol conversion. It is known that pH modulation has an effect on the enzyme charge, which can affect its activity (Leonowicz et al. 1984). In the case of the laccase obtained from *Trametes* sp. (Amano Enzyme, LA) or from *Trametes versicolor* (Sigma-Aldrich, LTV) combined with 1-hydroxybenzotriazole (HOBt) as mediator, screening of various pH from 4 to 9 did not induced a significant variation of citronellol conversion (Figure 4). Although slightly better at pH7, the rest of the study will be performed at pH5 which is considered optimal for the enzyme.



**Figure 4.** pH effect on citronellol conversion. (LA or LTv 5%, HOBt 10%, acetate buffer pH 4 – 8, citronellol 0,25 M, O<sub>2</sub> atmosphere, 40 °C, 24 h incubation, 200 rpm (rotations per minute)).

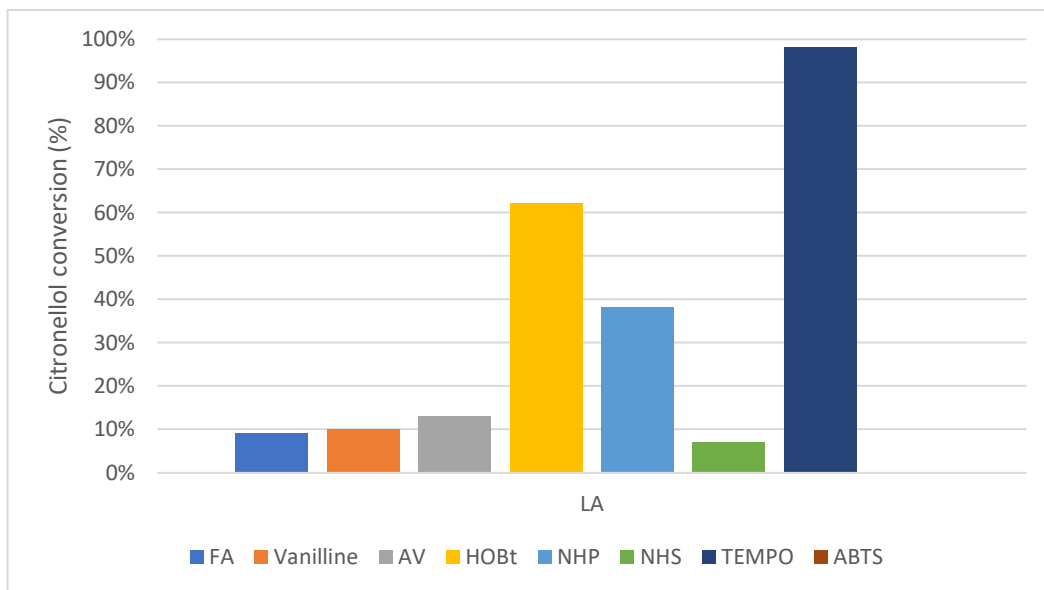
The effect of reaction time was investigated (Figure 5). Indeed, substrate conversion rate is linked to substrate concentration in the medium but also to LMS turnover (Aracri et al. 2009). In our case, a range of 24 – 72 h of reaction did not significantly impact citronellol conversion.



**Figure 5.** Reaction time effect on citronellol conversion. (LA 40%, HOBt 70%, acetate buffer pH 5, citronellol 0,25 M, O<sub>2</sub> atmosphere, 40 °C, 24 – 72 h incubation, 200 rpm).

LMS allows the oxidation of a wide range of substrates, but implies various oxidation routes, regarding the type of mediator (Morozova, Shumakovich, Shleev, et al. 2007). An array of mediators including both natural and synthetic molecules was thus tested in this reaction (Figure 6).

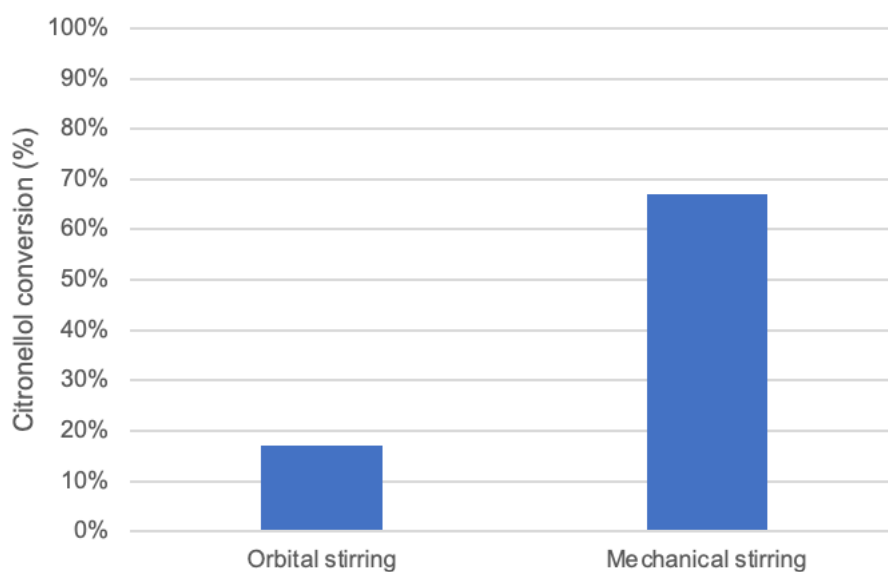




**Figure 6.** Mediator effect on citronellol conversion. (LA 10%, mediator 20%, acetate buffer pH 5, citronellol 0,25 M, O<sub>2</sub> atmosphere, 25 °C, 24 h incubation, 200 rpm, FA: ferulic acid, VA: violuric acid, HOBt: hydroxybenzotriazole, NHP: N-hydroxyphthalimide, NHS: N-hydroxysuccinimide, TEMPO: 2,2,6,6-tetramethylpiperidine-1-oxyl, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)).

Low conversion was observed with ABTS, NHS, FA, vanillin and VA, whereas good conversion was noted for TEMPO, HOBt and NHP. HOBt was selected for the rest of the study over TEMPO which besides higher conversion led to the formation of undesired oxidation products such as citronellal and citronellic acid (see below).

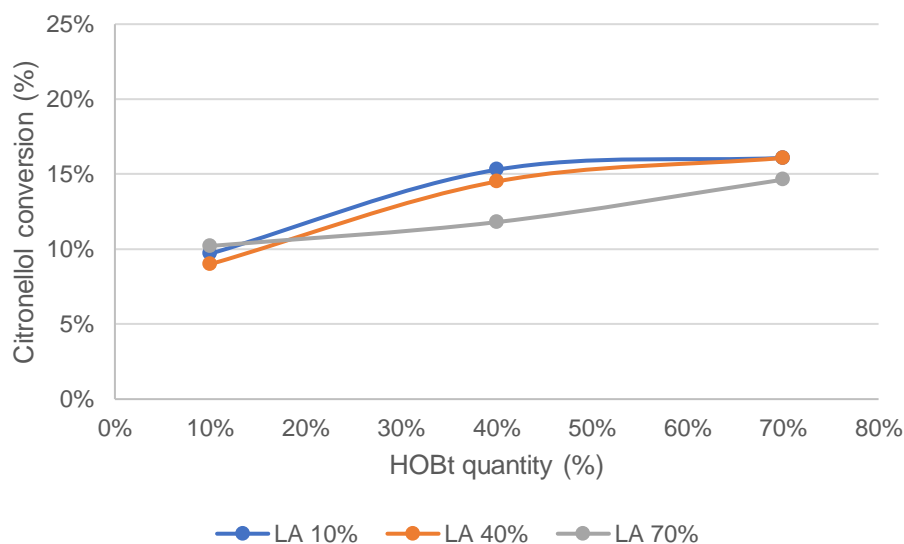
Due to the poor solubility of citronellol in the aqueous buffer, the mode of agitation was investigated: stirring with a magnetic bar (mechanical stirring) or with a platform shaker (orbital stirring). The results are summarized in Figure 7.



**Figure 7.** Agitation effect on citronellol conversion. (LA 10%, HOBt 20%, acetate buffer pH 5, citronellol 0,25 M, O<sub>2</sub> atmosphere, 40 °C, 24 h incubation, orbital agitation (200 rpm) or mechanical agitation).

Mechanical stirring demonstrated to be better adapted to the system with conversion improved from 17 to 67%.

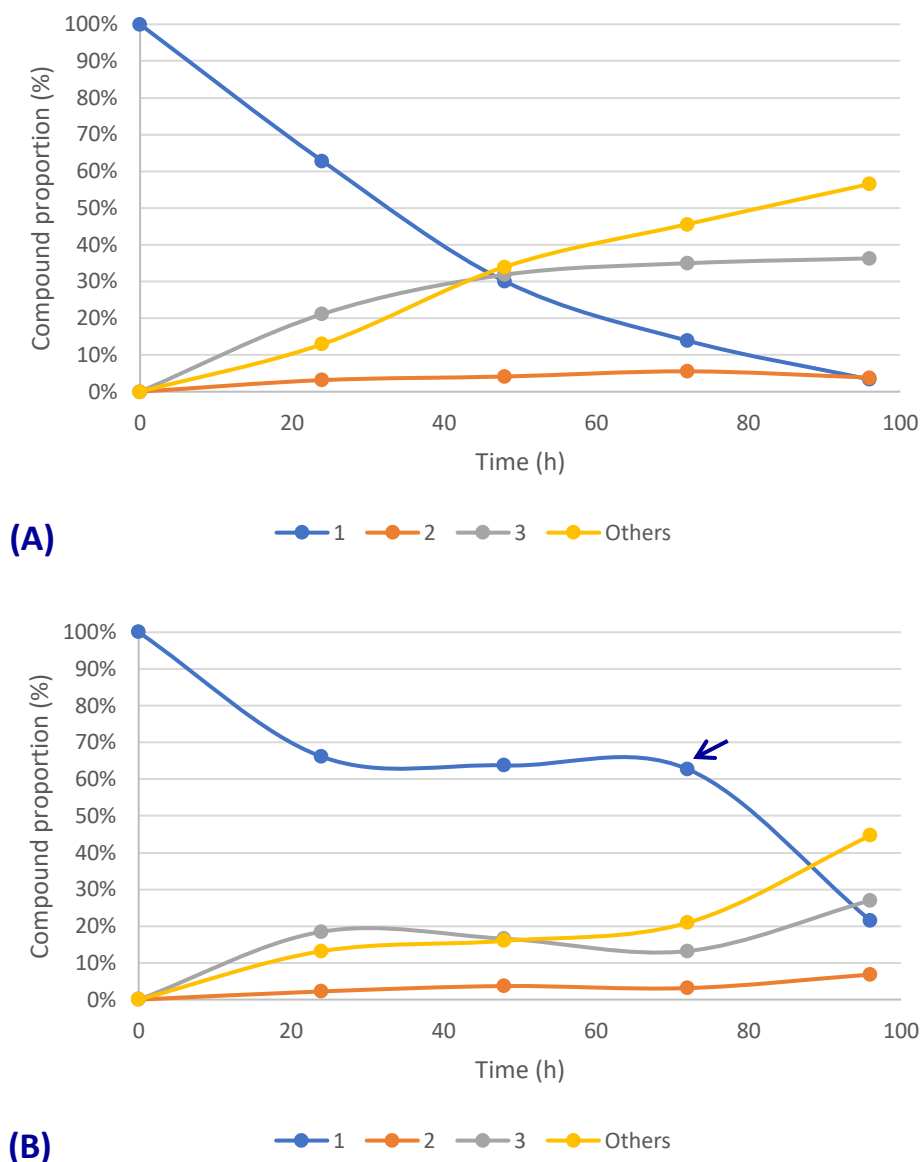
Laccase and mediator quantities were modulated in a series of experiments to observe the effect on citronellol conversion (Figure 8). Even if enzyme and mediator were multiplied by seven, conversion increased by less than 10%.



**Figure 8.** Effect of enzyme and mediator quantities on citronellol conversion. (LA 10 – 70%, HOBt 10 – 70%, acetate buffer pH 5, citronellol 0,25 M, O<sub>2</sub> atmosphere, 40 °C, 24 h incubation, 200 rpm).

## II.B. Total conversion of citronellol and products identification

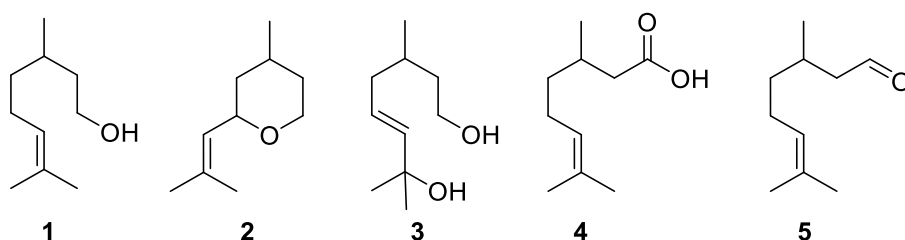
Previous experiments showed that citronellol conversion did not reach 100%. Enzyme or mediator deactivation, or both, had to be considered (Lecourt et al. 2021). Following this hypothesis and in order to improve conversion of citronellol, two experiments were performed in parallel, with either enzyme or mediator being added every 24 h (Figure 9).



**Figure 9.** Kinetic plot of citronellol conversion and products formation, with daily enzyme addition (A) or mediator addition (B) (initially LA 5%, HOBt 10%, acetate buffer pH 5, citronellol 0,25 M, O<sub>2</sub> atmosphere, 25 °C, magnetically stirred). At each point, aliquots were withdrawn and analyzed and addition of enzyme (A, 5%) or mediator (B, 10%) was performed. The arrow indicates an addition of both enzyme and mediator. Proportion of citronellol and products determined by GC-MS.

With enzyme daily addition (A), citronellol conversion was regular with a pseudo linear consumption until total conversion. With mediator daily addition (B), citronellol conversion reached a plateau at ca. 65% that could be broken by the simultaneous addition of enzyme and mediator.

Products isolation by column chromatography allowed to identify rose oxides **2** and 3,7-dimethyl-oct-5-en-1,7-diol **3** as main products (Figure 10). Two other products formed upon the oxidation of the alcohol function of citronellol were identified: citronellic acid **4** and citronellal **5**.



**Figure 10.** Structure of citronellol and oxidation products.

### II.C. Enantioselectivity of citronellol conversion

Citronellol is partially converted into rose oxides by the LMS. In GC-MS analysis, two peaks could be attributed to rose oxides, obtained as 2 stereoisomers, as confirmed by injection of commercial authentic standards. Enantioselective gas chromatography and method optimization allowed to unambiguously identify the four stereoisomers (see SI for details). In order to better understand the reaction stereospecificity, (*R*) and (*S*)-citronellol were independently submitted to oxidation by the LMS (Table 1).

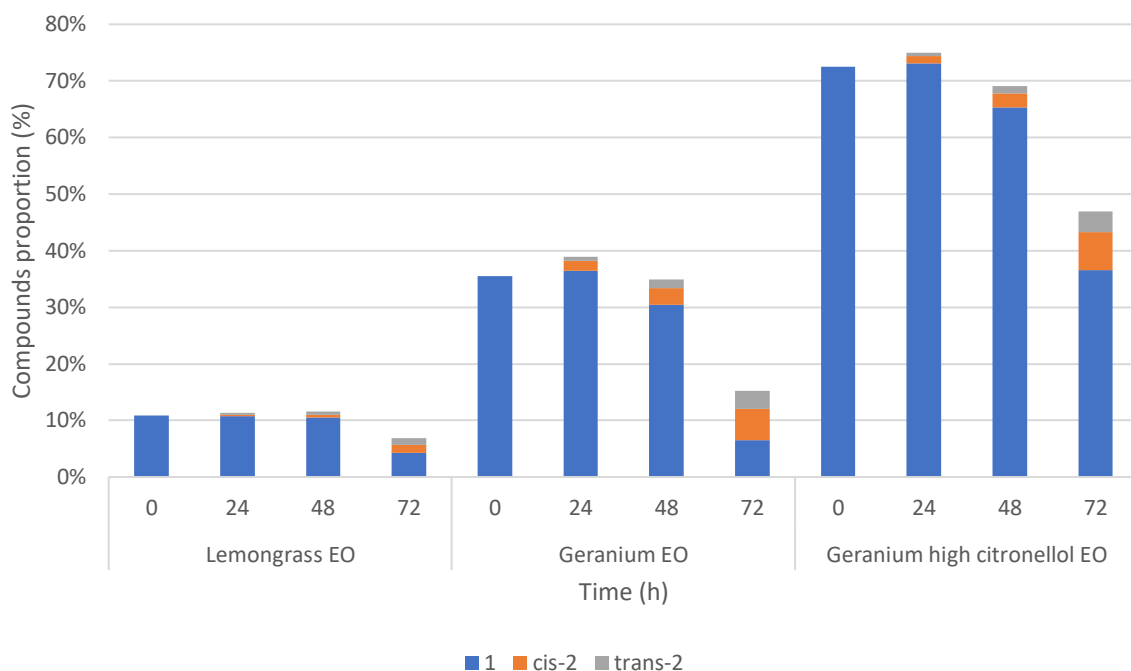
**Table 1.** Rose oxides 2 proportions observed upon citronellol oxidation by the LMS (LA 10%, HOBt 20%, acetate buffer pH 5, citronellol 0,25 M, O<sub>2</sub> atmosphere, 25 °C, 24 h incubation, 200 rpm). Percentages were determined by enantioselective GC-MS analysis.

Entry	Substrate	(+)- <i>cis</i> -2	(-)- <i>cis</i> -2	(-)- <i>trans</i> -2	(+)- <i>trans</i> -2
1	<i>R</i> -(+)- $\beta$ -citronellol	65,0%	-	-	35,0%
2	<i>S</i> -(-)- $\beta$ -citronellol	-	65,0%	35,0%	-
3	( $\pm$ )-citronellol	35,4%	35,3%	13,9%	15,3%

For each isomer, the *cis* diastereomer of rose oxide was preferentially formed from citronellol oxidation. With racemic citronellol, *cis* form remained predominant, but no preference was visible between the (-) and (+) forms indicating that the reaction proceeded without enantioselectivity.

### II.D. Application of LMS to essential oils enzymatic conversion

Two essential oils (EOs) and a distillate rich in citronellol were submitted to the reaction with the LMS according to the previously described procedure. A fraction of citronellol was converted into rose oxides together with other products formed upon the non-specific oxidation of other constituents (Figure 11). The initial quantity of citronellol in each material was variable. Again, rose oxides were mostly formed as the *cis* diastereomers.



**Figure 11.** Oxidation of citronellol within essential oils by the LMS, for three different reaction times. (LA 20 – 60%, HOBT 20 – 60%, acetate buffer pH 5, 457  $\mu$ L of essential oil, O<sub>2</sub> atmosphere, 25 °C, 24 h incubation, 200 rpm).

In a second time, mediator removal from the modified essential oils was performed. Modified essential oils were filtered over a pad of silica gel with a specific elution sequence to allow both mediator removal and chemical diversity conservation, as indicated by GC-MS analysis (see S.I.).

Finally, olfactory properties of the modified essential oils were evaluated by a panel of trained perfumers. Only the geranium oils were of interest for the panel (Table 2). Filtration over silica did not show any effect on olfactory properties. Olfactory descriptors were variable depending on the incubation time. The extract with the longest time of incubation exhibited a strong rose oxide odor, which made it interesting.

**Table 2.** Olfactive evaluation of geranium essential oil and its derivatives. EO: essential oil, Silica Tr: silica treatment, ER: enzymatic reaction, “–”: no particular interest, “\*”: moderate interest, “\*\*\*”: high interest.

	Interest	Comments
<i>Geranium EO</i>	Control	–
<i>Geranium EO, Silica Tr</i>	Control	More fruity, geranyl acetate, liquorous
<i>Geranium EO, ER 24 h, Silica Tr</i>	*	Pennyroyal (minty), more citronella
<i>Geranium EO, ER 48 h, Silica Tr</i>	–	Plastic, carbitol, minty, dirty
<i>Geranium EO, ER 72 h, Silica Tr</i>	**	Mostly rose oxide

### III. Discussion

Citronellol is a monoterpenyl alcohol which can be converted into other derivatives, for example by oxidation. In the context of green and sustainable chemistry, the use of laccases is appropriate to convert citronellol into oxidized products with limited footprints, conventional

oxidation processes being typically waste-generating (Lecourt et al. 2021). However, the presence of laccase alone did not allow to convert citronellol. A redox mediator was required along with the laccase to allow for the oxidation to proceed. Some work was further needed to optimize the formation of rose oxides with the LMS.

Variation of pH from 4 to 9 did not show significant effect on citronellol conversion and therefore further experiments were all conducted at pH5. Laccase as well as mediator activities were not affected by possible isoelectric modifications of the enzyme under different pH. Similarly, increasing the reaction time from 24 to 72 h only poorly affected citronellol conversion. After 48 h, conversion reached a plateau, meaning that LMS activity stopped after such reaction time.

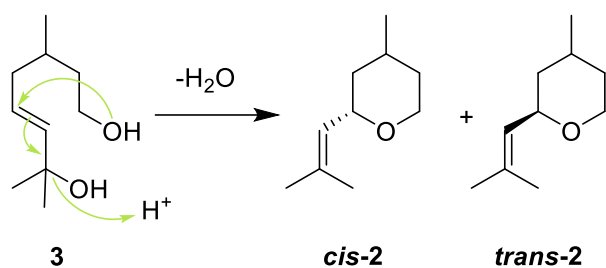
Mediators screening resulted in a diversity of conversion rates. Natural mediators such as ferulic acid, vanillin and violuric acid were not able to promote citronellol oxidation. Among synthetic mediators, N-hydroxysuccinimide and ABTS did not exhibit significant activity neither. N-hydroxyphthalimide and HOBt converted citronellol respectively with 38 and 62%. The case of TEMPO is interesting because it allows almost total citronellol conversion, although with a different selectivity in terms of products formation. Indeed, LMS with TEMPO as the mediator converted citronellol mostly into citronellic acid, without producing rose oxides, thus following a distinct reaction mechanism. Eventually, HOBt gave the best conversion rate toward rose oxides.

Because of the moderate solubility of citronellol in aqueous systems (ca. 0.2 M), the method of stirring was slightly investigated. It was found that orbital stirring with a platform shaker did not allow optimal mixing of citronellol in the aqueous acetate buffer. Mechanical stirring with a magnetic stirrer in the reactor was tested and allowed citronellol conversion increase from 7 to 67%.

Laccase and mediator quantities were screened simultaneously. Nevertheless, increasing enzyme and/or laccase quantity did not improve citronellol conversion, even though a 7-fold increase.

Enzyme and/or mediator deactivation was thus investigated because no total citronellol conversion was achieved after one cycle of reaction. Addition of enzyme or mediator was performed daily in parallel with citronellol conversion monitoring. With the daily addition of enzyme to the reaction medium, citronellol conversion followed a linear pace as for a pseudo 1<sup>st</sup> order reaction. In the case of mediator addition, conversion reached 34% after 24 h but did not evolve for the next 48 h despite multiple mediator additions. After 72 h, fresh mediator and laccase were added to the medium, allowing the conversion to restart. This suggests laccase early deactivation, to what mediator addition could not change anything, and influence of the solubilization of organic partners in aqueous medium. Proportion of side-products also increased during the experiment. Indeed, oxidation state with the products was variable and a longer reaction time induced new products formation, each of them formed in small proportions.

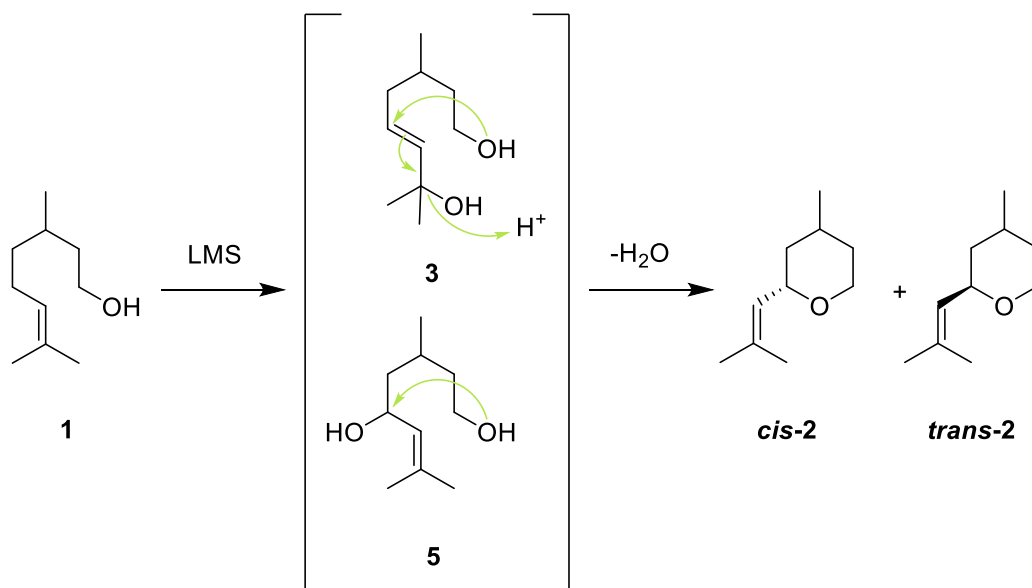
The main products formed by the LMS was dihydroxylated product **3**, along with rose oxides **2**. Citronellal **4** was produced in small amounts. Dihydroxylated product **3** has already been described in the literature as a metabolic precursor of rose oxide in *Rosa damascena* Mill. (Knapp et al. 1998; Winterhalter et al. 1998). This compound **3** can be cyclodehydrated under acidic conditions following either a S<sub>N</sub>2' or S<sub>N</sub>1' mechanism (Figure 12) (Knapp et al. 1998).



**Figure 12.** Conversion of 3 into 2 under acidic conditions.

In plants, *cis* form of rose oxide is most frequently observed. Enantioselectivity study was initiated in order to understand such phenomenon. (*R*) and (*S*)-citronellol were submitted to the LMS and both gave around 65% of *cis*-rose oxide and 35% of *trans*-rose oxide. From one citronellol enantiomer (e.g. (*R*)-(+)-citronellol), only two rose oxide stereoisomers were formed ((+)-*cis* and (+)-*trans*-rose oxide): the absolute configuration at the methyl-substituted  $sp^3$  carbon atom was conserved during the oxidation. Conversely, when a racemate was used, proportions of *cis* and *trans*-rose oxide in the product remained the same: the LMS was not concerned by stereoselectivity. Moreover, the result underline that the ratio of (*R*) and (*S*) citronellol is determinant to obtain the suitable (2*S*,4*R*)-(-)-*cis*-rose-oxide. This ratio is dependent on the cultivar.

The *cis*-rose oxide diastereoisomer remained predominant, whichever citronellol enantiomer was used. This result was already noticed when each citronellol enantiomer was biotransformed by a strain of *Aspergillus niger* (Demyttenaere et al. 2004). Reaction pathway suggests that cyclisation of **3** by  $SN_2'$  mechanism implies *trans* pre-positioning of the hydroxyl group relatively to the methyl group to minimize steric hindrance at the transition state upon ring-closure process. This results in a *cis* relationship between the methyl group and the newly formed 2-methylpropenyl group after the cyclisation (Figure 13). The dihydroxylated intermediate **5** was hypothesized, obtained by direct allylic oxidation of citronellol. This compound that was not isolated and would mostly lead to *trans*-rose oxide by cyclization; oxidation would preferentially form a *trans*-product **5**, further leading to *trans*-rose oxide by  $SN_2$  and Walden inversion of the stereocenter (Figure 13).



**Figure 13.** Hypothetical oxidation and cyclisation route towards rose oxide stereoisomers.

Finally, the LMS was used directly on citronellol-containing essential oils in order to promote the oxidation of their citronellol content in a post-biosynthetic conversion. The aim of this application was to create a modified essential oil, blended with newly formed rose oxides, only relying on pre-existing contents within the essential oil.

In our case, essential oil modification by LMS allowed the formation of rose oxides to the extent of citronellol conversion. Other compounds were impacted by the LMS such as geraniol and citronellal. Essential oil complexity did not impact *cis*-rose oxide formation, which was still predominant. Before the olfactory evaluation, silica treatment was performed and optimized to specifically remove the mediator from the modified essential oil. Mediator removal was essential to limit odor pollution and preserve safety for the evaluators. This treatment was optimized on non-modified essential oils and the chemical analysis of the composition followed by an olfactory evaluation confirmed the successful removal of HOBt without impacting olfactory properties.

Olfactory evaluation by a panel of trained experts focused on geranium essential oil. Odor descriptors were found to be reaction-time dependent in relation with the evolution of the chemical composition. The most interesting modified essential oil was the one obtained after the longest reaction time. It contained more rose oxides, with typical olfactory facets indeed perceived by the panel.

#### IV. Conclusion

Citronellol oxidation by the LMS allowed for the formation of rose oxides. Experimental conditions significantly influencing the reaction outcome were found to be the mediator type and the mixing technique. Kinetic studies showed that laccase activity was limited after 24 h of reaction and that addition of fresh enzyme was required to reach total conversion. Reaction stereoselectivity analysis highlighted that *cis*-rose oxide was preferentially formed with a 65:35 ratio to *trans*-rose oxide. No enantioselectivity was observed, certainly ruling out a purely-



enzymatic process. Finally, application of the system to essential oils with a high citronellol content such as geranium essential oil allowed the formation of rose oxides in the modified oil. Olfactory description of these modified oils was performed and showed some interesting facets of rose oxide overlaying the typical geranium odor in an original manner. This augmented essential oil, doped in rose oxides by an *in vitro* transformation, draws attention to a new way to improve natural products by a semi-natural process in route to the development of novel processes and ingredients for perfumery in the context of sustainability.

## V. Acknowledgements

This work was supported by Université Côte d'Azur, Université de Lyon, and CNRS. ML's PhD fellowship is co-funded by IFF-LMR and French Région SUD. We are grateful to Atsushi Tanaka (Amano Enzyme) for a loan of Amano Enzyme M120 laccase. No potential competing interest was reported by the authors.

## VI. Experimental section

**Chemicals:** Chemicals, organic solvents, and laccase from *Trametes versicolor* were purchased from Sigma-Aldrich and used as received. Distilled water was obtained by filtration on an Elix® Reference 3 system with a Progard®S2 cartridge. Laccase M120 (LA) was obtained as a kind loan of Amano Enzyme Inc. (United Kingdom). Enzymes were stored at –18°C. The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. Essential oils were a kind gift of LMR Naturals (Grasse, France).

**Analysis:** <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker Avance 400 spectrometer. Chemical shifts, reported in ppm, were referenced relative to TMS based on the residual signals of the solvents (<sup>1</sup>H and <sup>13</sup>C). GC/MS analyses were carried out with an Agilent 7820A chromatograph coupled to an Agilent 5977B MS detector (Agilent, California). Samples were analysed on a capillary column SAPIENS-5MS (10 m × 0.10 mm × 0.10 μm, 5% diphenyl-95% dimethylpolysiloxane, Teknokroma, Spain). Operating conditions: carrier gas: He, constant flow, 20 mL/min; injection temp., 250°C, injection volume, 0.2 μL, split ratio, 1:50; temp. program: 50 °C held 1 min, 50 °C to 280 °C at 10 °C/min, then held isothermal (10 min), ion source temp., 230 °C; transfer line temp., 300°C; ionization energy, 70 eV; electron ionization mass spectra were acquired over the mass range of 35–550 amu. Enantioselective-GC analyses were performed with a Hydrodex® β-TBDM column (50 m x 0,25 mm, Macherey-Nagel, Germany). Program: 50 °C held 2 min, 50 °C to 150 °C at 1 °C/min, 150 °C to 200 °C at 25 °C/min, then held isothermal (6 min). Identification of the components was based on computer matching against commercial libraries (NIST15, Wiley), laboratory MS libraries built up from pure substances, MS literature data, or comparison with authentic samples.

Incubation was performed with an orbital shaker incubator (C24 serie, M1247-0004 model, New Brunswick Scientific, New Jersey). Magnetic stirring was performed with a magnetic stirring hot plate IKA, C-MAG HS 7 model.

**Standard procedure for the enzymatic oxidation of citronellol:** In a 20 mL vial were introduced citronellol (1 mmol, 0.25 M), mediator (20 mol%) and laccase (10 wt%) in a pH5 20 mM aqueous acetate buffer (4 mL). Reaction medium was incubated under oxygen atmosphere using incubator shaker with orbital agitation (24 h, 40 °C, 200 rpm). Work up consisted in

EtOAc extraction of the aqueous phase (5×2 mL). Organic phases were pooled, dried over MgSO<sub>4</sub> and concentrated in vacuo. GC/MS and <sup>1</sup>H-NMR analysis were performed to determine the reaction outcome on crude products.

*Monitoring of citronellol oxidation:* Enzymatic oxidation was carried out on a larger scale. Citronellol (12.75 mmol, 0.25 M), HOBt (1.28 mmol, 173.1 mg) and laccase (98.5 mg) were introduced in a pH5 20 mM acetate buffer (50 mL). The mixture was stirred under oxygen atmosphere in a 25 °C oil bath. After 24 h, 1 mL of the mixture was submitted to the above-described work-up and reaction progress was estimated by GC/MS analysis. In case of partial conversion, laccase (case A) or mediator (case B) addition was performed (up to 4 additions for a total of: case A: 173.1 mg of HOBt and 1349.0 mg of laccase, case B: 843.4 mg of HOBt and 202.2 mg of laccase). Upon completion of the reaction with full conversion, workup was performed as described before.

For essential oil modification, 457 µL of essential oil were engaged with laccase (78.1 mg) and HOBt (70.2 mg, 0,519 mmol) for 24 h. Reaction mixture was extracted or re-engaged for additional 24 or 48 h (with addition of laccase (20%) and mediator (20%) every 24 h).

*Silica-based rapid filtration of modified essential oil:* 0.54 g of modified essential oil was filtrated on a pad of 6.0 g of silica gel. Elution was performed with 50 mL of petroleum ether/diethyl ether (50:50). Eluted fraction was dried over MgSO<sub>4</sub> and concentrated in vacuo.

## VII. References

- Abbas F, Ke Y, Yu R, Yue Y, Amanullah S, Jahangir MM, Fan Y. 2017. Volatile terpenoids: multiple functions, biosynthesis, modulation and manipulation by genetic engineering. *Planta*. 246(5):803-816.
- Antoniotti S, Fernandez X, Duñach E. 2008. Reaction design for evaluation of the substrate range of hydrolases. *Biocatalysis and Biotransformation*. 26(3):228-234.
- Aracri E, Colom JF, Vidal T. 2009. Application of laccase-natural mediator systems to sisal pulp: An effective approach to biobleaching or functionalizing pulp fibres? *Bioresource Technology*. 100(23):5911-5916.
- Baldrian P. 2006. Fungal laccases – occurrence and properties. *FEMS Microbiology Reviews*. 30(2):215-242.
- Banthorpe DV, Charlwood BV, Francis MJO. 1972. Biosynthesis of monoterpenes. *Chemical Reviews*. 72(2):115-155.
- Bergman M, Bhardwaj M, Phillips MA. 2021. Cytosolic geraniol and citronellol biosynthesis require a Nudix hydrolase in rose-scented geranium (*Pelargonium graveolens*). *The Plant Journal*. 107(2):493-510.
- Bergman ME, Chávez Á, Ferrer A, Phillips MA. 2019. Distinct metabolic pathways drive monoterpene biosynthesis in a natural population of *Pelargonium graveolens*. *Journal of Experimental Botany*. 71(1):258-271.
- Blerot B, Martinelli L, Prunier C, Saint-Marcoux D, Legrand S, Bony A, Sarrabère L, Gros F, Boyer N, Caissard JC et al. 2018. Functional Analysis of Four Terpene Synthases in Rose-Scented *Pelargonium* Cultivars (*Pelargonium × hybridum*) and Evolution of Scent in the *Pelargonium* Genus. *Front Plant Sci*. 9:1435.
- Botha BM, McCrindle CME. 2000. An appropriate method for extracting the insect repellent citronellol from an indigenous plant (*Pelargonium graveolens* L'Her) for potential use by resource-limited animal owners. *Journal of the South African Veterinary Association*. 71(2):103-105.
- Bouges H, Monchot A, Antoniotti S. 2018. Enzyme-Catalysed Conversion of Atranol and Derivatives into Dimeric Hydrosoluble Materials: Application to the Preparation of a Low-Atranol Oakmoss Absolute. *Cosmetics*. 5(4):69-80.

Bouhleb C, Dolhem GA, Fernandez X, Antoniotti S. 2012. Model Study of the Enzymatic Modification of Natural Extracts: Peroxidase-Based Removal of Eugenol from Rose Essential Oil. *Journal of Agricultural and Food Chemistry*. 60(4):1052-1058.

Cheng A-X, Lou Y-G, Mao Y-B, Lu S, Wang L-J, Chen X-Y. 2007. Plant Terpenoids: Biosynthesis and Ecological Functions. *Journal of Integrative Plant Biology*. 49(2):179-186.

Demyttenaere JCR, Vanoverschelde J, De Kimpe N. 2004. Biotransformation of (R)-(+)- and (S)-(-)-citronellol by *Aspergillus* sp. and *Penicillium* sp., and the use of solid-phase microextraction for screening. *Journal of Chromatography A*. 1027(1):137-146.

Diaz-Chavez ML, Moniodis J, Madilao LL, Jancsik S, Keeling CI, Barbour EL, Ghisalberti EL, Plummer JA, Jones CG, Bohlmann J. 2013. Biosynthesis of Sandalwood Oil: *Santalum album* CYP76F Cytochromes P450 Produce Santalols and Bergamotol. *PLoS One*. 8(9):e75053.

Dudareva N, Klempien A, Muhlemann JK, Kaplan I. 2013. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytologist*. 198(1):16-32.

Fayed S. 2009. Antioxidant and Anticancer Activities of Citrus reticulata (Petitgrain mandarin) and Pelargonium graveolens (Geranium) Essential Oils. *Research Journal of Agriculture and Biological Sciences*. 5:740-747.

Fekri N, El Amir D, Owis A, AbouZid S. 2021. Studies on essential oil from rose-scented geranium, Pelargonium graveolens L'Hérit. (Geraniaceae). *Natural Product Research*. 35(15):2593-2597.

Francis MJO, Allcock C. 1969. Geraniol  $\beta$ -D-glucoside; occurrence and synthesis in rose flowers. *Phytochemistry*. 8(8):1339-1347.

Goodger JQD, Heskes AM, Mitchell MC, King DJ, Neilson EH, Woodrow IE. 2010. Isolation of intact subdermal secretory cavities from *Eucalyptus*. 6(1):20.

Haiyang Xu, Björn Bohman, Darren C. J. Wong, Claudia Rodriguez-Delgado, Adrian Scaffidi, Gavin R. Flematti, Ryan D. Phillips, Eran Pichersky, Rod Peakall. 2017. Complex Sexual Deception in an Orchid Is Achieved by Co-opting Two Independent Biosynthetic Pathways for Pollinator Attraction. *Current Biology*. 27(13):1867-1877.e1865.

Hult K, Berglund P. 2007. Enzyme promiscuity: mechanism and applications. *Trends Biotechnol*. 25(5):231-238.

Karami S, Yargholi A, Sadati Lamardi SN, Soleymani S, Shirbeigi L, Rahimi\* R. 2021. A Review of Ethnopharmacology, Phytochemistry and Pharmacology of Cymbopogon Species. *Research Journal of Pharmacognosy*. 8(3):83-112.

Knapp H, Straubinger M, Fornari S, Oka N, Watanabe N, Winterhalter P. 1998. (S)-3,7-Dimethyl-5-octene-1,7-diol and Related Oxygenated Monoterpenoids from Petals of *Rosa damascena* Mill. *J Agric Food Chem*. 46(5):1966-1970.

Kreis P, Mosandl A. 1994. Chiral compounds of essential oils. Part XVII. simultaneous stereoanalysis of cymbopogon oil constituents. *Flavour and Fragrance Journal*. 9(5):257-260.

Laska M, Galizia CG. 2001. Enantioselectivity of odor perception in honeybees (*Apis mellifera carnica*). *Behavioral Neuroscience*. 115(3):632-639.

Lecourt M, Chietera G, Blerot B, Antoniotti S. 2021. Laccase-Catalyzed Oxidation of Allylbenzene Derivatives: Towards a Green Equivalent of Ozonolysis. *Molecules*. 26(19).

Leonowicz A, Edgehill RU, Bollag JM. 1984. The effect of pH on the transformation of syringic and vanillic acids by the laccases of *Rhizoctonia praticola* and *Trametes versicolor*. *Archives of Microbiology*. 137(2):89-96.

Luan F, Mosandl A, Münch A, Wüst M. 2005. Metabolism of geraniol in grape berry mesocarp of *Vitis vinifera* L. cv. Scheurebe: demonstration of stereoselective reduction, E/Z-isomerization, oxidation and glycosylation. *Phytochemistry*. 66(3):295-303.

Malnic B, Hirono J, Sato T, Buck LB. 1999. Combinatorial Receptor Codes for Odors. *Cell*. 96(5):713-723.

Mannschreck A, von Angerer E. 2011. The Scent of Roses and Beyond: Molecular Structures, Analysis, and Practical Applications of Odorants. *Journal of Chemical Education*. 88(11):1501-1506.

Mikkelsen BB, Poll L. 2002. Decomposition and Transformation of Aroma Compounds and Anthocyanins during Black Currant (*Ribes nigrum* L.) Juice Processin. *Journal of Food Science*. 67(9):3447-3455.

Monti D, Ottolina G, Carrea G, Riva S. 2011. Redox Reactions Catalyzed by Isolated Enzymes. *Chem Rev.* 111(7):4111-4140.

Morozova OV, Shumakovich GP, Gorbacheva MA, Shleev SV, Yaropolov AI. 2007. "Blue" laccases. *Biochemistry (Moscow).* 72(10):1136-1150.

Morozova OV, Shumakovich GP, Shleev SV, Yaropolov YI. 2007. Laccase-mediator systems and their applications: A review. *Applied Biochemistry and Microbiology.* 43(5):523-535.

Noriaki O, Hisayoshi O, Tatsuya H, Martin H, Kanzo S, Naoharu W. 1999. Aroma Evolution during Flower Opening in *Rosa damascena* Mill. *Zeitschrift für Naturforschung C.* 54(11):889-895. English.

Notar Francesco I, Filippi J-J, Antoniotti S. 2017. Sustainable Manufacture of a Valuable Fragrance Ingredient: Lipase-Catalyzed Acylation of Vetiver Essential Oil and Chemoselectivity between Sesquiterpene Alcohols. *ChemPlusChem.* 82(3):407-415.

Peach DAH, Almond M, Gries R, Gries G. 2019. Lemongrass and Cinnamon Bark: Plant Essential Oil Blend as a Spatial Repellent for Mosquitoes in a Field Setting. *Journal of Medical Entomology.* 56(5):1346-1352.

Piantini U, Schrader J, Wawrzun A, Wüst M. 2011. A biocatalytic route towards rose oxide using chloroperoxidase. *Food Chemistry.* 129(3):1025-1029.

Piontek K, Antorini M, Choinowski T. 2002. Crystal Structure of a Laccase from the Fungus *Trametes versicolor* at 1.90-Å Resolution Containing a Full Complement of Coppers. *J Biol Chem.* 277(40):37663-37669.

Rasouli O, Ahmadi N, Rashidi Monfared S, Sefidkon F. 2018. Physiological, phytochemicals and molecular analysis of color and scent of different landraces of *Rosa damascena* during flower development stages. *Scientia Horticulturae.* 231:144-150.

Recco Pimentel M, Molina G, Bertucci T, Pastore G. 2012. Biotransformation of Citronellol in Rose Oxide by *Pseudomonas* Spp. *Chemical Engineering Transactions.* 27:295-300.

Schalk M, Pastore L, Mirata MA, Khim S, Schouwey M, Deguerry F, Pineda V, Rocci L, Daviet L. 2012. Toward a Biosynthetic Route to Sclareol and Amber Odorants. *J Am Chem Soc.* 134(46):18900-18903.

Schlosser D, Grey R, Fritsche W. 1997. Patterns of ligninolytic enzymes in *Trametes versicolor*. Distribution of extra- and intracellular enzyme activities during cultivation on glucose, wheat straw and beech wood. *Appl Microbiol Biotechnol.* 47(4):412-418.

Singh B, Sharma RA. 2015. Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. *3 Biotech.* 5(2):129-151.

Singh U, Dwivedi P, Sangwan RS, Mishra BB. 2017. In situ rose oxide enrichment led valorization of citronella (*Cymbopogon winterianus*) essential oil. *Industrial Crops and Products.* 97:567-573.

Sinha S, Biswas D, Mukherjee A. 2011. Antigenotoxic and antioxidant activities of palmarosa and citronella essential oils. *Journal of Ethnopharmacology.* 137(3):1521-1527.

Sinha S, Jothiramajayam M, Ghosh M, Mukherjee A. 2014. Evaluation of toxicity of essential oils palmarosa, citronella, lemongrass and vetiver in human lymphocytes. *Food and Chemical Toxicology.* 68:71-77.

Surburg H, Panten J. 2006. Individual Fragrance and Flavor Materials. *Common Fragrance and Flavor Materials.* p. 7-175.

Taneja SC, Sethi VK, Andotra SS, Koul S, Qazi GN. 2005. Rose Oxides: A Facile Chemo and Chemo - Enzymatic Approach. *Synthetic Communications.* 35(17):2297-2303.

Verma RS, Padalia RC, Chauhan A, Singh A, Yadav AK. 2011. Volatile constituents of essential oil and rose water of damask rose (*Rosa damascena* Mill.) cultivars from North Indian hills. *Natural Product Research.* 25(17):1577-1584.

Vranová E, Coman D, Grisse W. 2013. Network Analysis of the MVA and MEP Pathways for Isoprenoid Synthesis. *Annual Review of Plant Biology.* 64(1):665-700.

Watanabe N, Washio H, Straubinger M, Knapp H, Winterhalter P. 1998. Occurrence of A Glucosidic Progenitor of Rose Oxide In Rose Flowers, *Rosa Damascena* Mill. *Natural Product Letters.* 12(1):5-10.

Werkhoff P, Brennecke S, Bretschneider W, Güntert M, Hopp R, Surburg H. 1993. Chirospecific analysis in essential oil, fragrance and flavor research. *Zeitschrift für Lebensmittel-Untersuchung und Forschung.* 196(4):307-328.

Winterhalter P, Knapp H, Straubinger M, Fornari S, Watanabe N. 1998. Chapter 16, Application of Countercurrent Chromatography to the Analysis of Aroma Precursors in Rose Flowers. *Flavor Analysis*. American Chemical Society; p. 181-192.

Witayakran S, Ragauskas AJ. 2009. Synthetic Applications of Laccase in Green Chemistry. *Adv Synth Catal*. 351(9):1187-1209.

Wüst M, Beck T, Mosandl A. 1999. Conversion of Citronellyl Diphosphate and Citronellyl  $\beta$ -d-Glucoside into Rose Oxide by *Pelargonium graveolens*. *Journal of Agricultural and Food Chemistry*. 47(4):1668-1672.

Wüst M, Rexroth A, Beck T, Mosandl A. 1998. Mechanistic aspects of the biogenesis of rose oxide in *Pelargonium graveolens* L'Héritier. *Chirality*. 10(3):229-237.

Yamamoto T, Matsuda H, Utsumi Y, Hagiwara T, Kanisawa T. 2002. Synthesis and odor of optically active rose oxide. *Tetrahedron Letters*. 43(50):9077-9080.

Yoshida H. 1883. LXIII.—Chemistry of lacquer (Urushi). Part I. Communication from the Chemical Society of Tokio [10.1039/CT8834300472]. *Journal of the Chemical Society, Transactions*. 43(0):472-486.

Ziarani GM, Mohajer F, Jamali SM, Ebrahim NA. 2020. Quantitative and Qualitative Bibliometric Scope Toward the Synthesis of Rose Oxide as a Natural Product in Perfumery. *Current Organic Synthesis*. 17(8):610-624.