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Synthesis of Allylic Hydroperoxides and EPR Spin-Trapping Studies on the Formation of Radicals in Iron Systems as Potential Initiators of the Sensitizing Pathway

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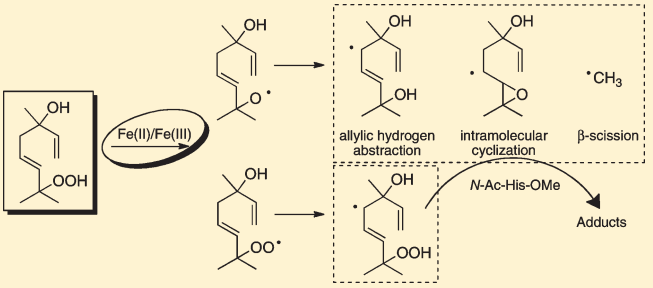
Supporting Information

ABSTRACT: Many terpenes used as fragrance compounds autoxidize when exposed to air, forming allylic hydroperoxides that have the potential to be skin contact allergens. To trigger the immunotoxicity process that characterizes contact allergy, these hydroperoxides are supposed to bind covalently to proteins in the skin via radical pathways. We investigated the formation of reactive radical intermediates from 7-hydroperoxy-3,7-dimethylocta-1,5-dien-3-ol and 2-hydroperoxylimonene, responsible for the sensitizing potential acquired by autoxidized linalool and limonene. Both compounds were synthesized through new short and reproducible synthetic pathways. The hydroperoxide decomposition catalyzed by Fe(II)/Fe(III) redox systems, playing a key role in degradating peroxides in vivo, was examined by spin-trapping-EPR spectroscopy. Alkoxyl and carbon-centered free radicals derived from the hydroperoxides were successfully trapped by the spin-trap 5,5-dimethyl-1-pyrroline *N*-oxide, whereas peroxyl radicals were characterized by spin-trapping studies with 5-diethoxyphosphoryl-5-methyl-1-pyrroline *N*-oxide. Using liquid chromatography combined with mass spectrometry, we demonstrated the formation of adducts, via radical mechanisms induced by Fe(II)/Fe(III), between the hydroperoxides and *N*-acetylhistidine methyl ester, a model amino acid that is prone to radical reactions. Free radicals derived from these hydroperoxides can thus induce amino acid chemical modifications via radical mechanisms. The study of these mechanisms will help to understand the sensitizing potential of hydroperoxides.



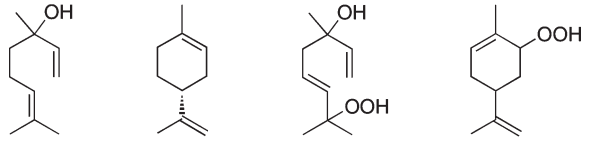
INTRODUCTION

Terpenes, principally mono- and sesquiterpenes, are common fragrance compounds of natural origin used in the production of fine perfumes, but also in a large variety of fragranced cosmetic, household, and industrial products. Some terpenes are known to be converted by autoxidation into allylic hydroperoxides which have the potential to cause skin contact allergy.[1] This is the case for the acyclic terpene alcohol linalool **1** and for the cyclic terpene hydrocarbon *R*-(+)-limonene **2** (Chart 1). Both occur in many essential oils, such as those of lavender and citruses, respectively. Linalool and *R*-(+)-limonene are among the most used fragrance compounds in scented products. A study on the quantitative chemical composition of a variety of deodorants (vapo- and aerosol spray, roll-on), purchased at retail outlets in five European countries, showed that 97% contained linalool.[2] *R*-(+)-Limonene was the most detected fragrance substance when analyzing the chemical composition of 59 domestic and occupational products intended for hand exposure and was present in 71% of 300 cosmetic products examined.[3,4]

Linalool and *R*-(+)-limonene are not themselves sensitizing compounds, but they do autoxidize to allylic hydroperoxides that have been shown to be potent sensitizers in the oxidation mixtures formed upon air exposure. In particular, 7-hydroperoxy-3,7-dimethylocta-1,5-dien-3-ol **3** and 2-hydroperoxylimonene **4** have been identified as the primary forms responsible for the sensitizing potential of autoxidized linalool **1** and limonene **2**, respectively (Chart 1).[5−7]

Allergic contact dermatitis (ACD) is an immunologically based disease resulting from skin sensitization to a chemical.

Chart 1. Structures of Linalool 1, *R*-(+)-Limonene 2, 7-Hydroperoxy-3,7-dimethylocta-1,5-dien-3-ol 3, and 2-Hydroperoxylimonene 4



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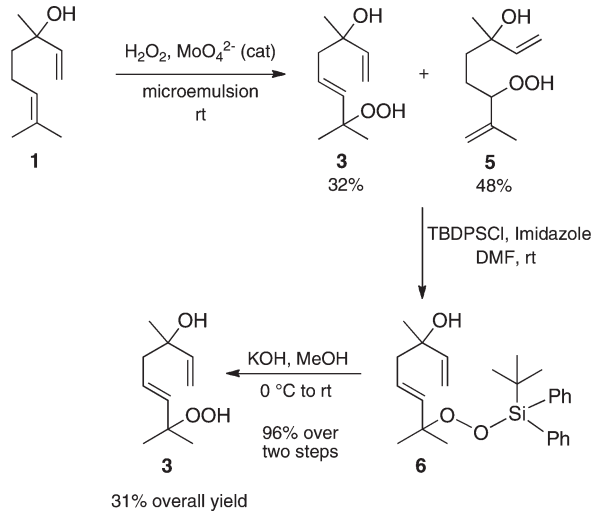
The sensitization process starts by the chemical modification of epidermal proteins by the allergen or hapten. Processing of the hapten−protein complex by immunocompetent skin antigen-presenting cells ensures the presentation of the altered peptides to naïve T-lymphocytes in the lymph nodes. T-lymphocyte subpopulations with T-cell receptors specific for the chemical modification are then selected and will be activated following a second contact with the allergen. Individuals sensitized to the chemical will thus be predisposed to develop ACD, characterized by the appearance of erythema and edema, after a new skin exposure to the same chemical.[8,9] The established mechanism for the initial hapten−protein complex formation is the reaction of an electrophilic function present on the allergen with nucleophilic residues on proteins. Instead, allergenic allylic hydroperoxides are believed to react through mechanisms involving radical intermediates.[10−12]

Many studies have been reported recently on the identification and possible involvement in ACD of radicals derived from allylic hydroperoxides using chemical traps.[10−12] Indeed, homolytic cleavage of the O−O and O−H bonds of hydroperoxides, having bond dissociation energies that are relatively weak (around 42 and 88 kcal mol^{-1}, respectively[13]), is an easy process that affords alkoxyl and peroxyl radicals, which rapidly can lead via intramolecular cyclization, fragmentation, or hydrogen abstraction to the formation of carbon-centered radicals. Today, there is a real belief that this kind of oxygen- and carbon-centered radicals could be important for the binding of those haptens containing hydroperoxide groups with skin proteins. Previous studies have already examined radical formation from allylic hydroperoxides found in oxidation mixtures of limonene, including **4**.[14,15] Chemical trapping experiments were performed using tetraphenylporphyrin-Fe^{3+} (TPP-Fe^{3+}) as initiator, and 1,1,3,3-tetramethylisoindolin-2-yloxyl (TMIO) known to trap exclusively carbon-centered radicals by forming alkoxyamine products. Studies were complemented by preliminary EPR experiments using photolysis for radical initiation. Herein we report a complete identification of radicals issued from the allergenic acyclic tertiary allylic hydroperoxide **3**, derived from linalool, compared to the cyclic and secondary allylic hydroperoxide **4**, derived from limonene, by using the spin-trapping technique combined with EPR spectroscopy, specifically employed for the identification of transient radicals in chemical and biological systems. 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) and 5-diethoxyphosphoryl-5-methyl-1-pyrroline *N*-oxide (DEPMPO) were used as spin traps in organic/aqueous media, and Fe(II)/Fe(III) redox systems were used as initiators of the radical reactions, as they are described to play a key role in the degradation of peroxides in vivo.[16] Further reactivity studies of **3** and **4** toward *N*-acetylhistidine methyl ester in iron systems, by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS), showed the formation of adducts via radical pathways, suggesting that these radicals could play an important role for the binding of the hydroperoxides to skin proteins to form antigenic structures.

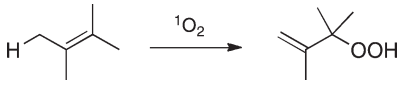
RESULTS AND DISCUSSION

Synthesis of Allylic Hydroperoxides. In order to carry out the EPR spin-trapping and reactivity studies, hydroperoxides **3** and **4** were synthesized, as the formation of hydroperoxides by autoxidation of terpenes, not always completely reproducible, gives low yields and the products are not easy to isolate from the oxidation mixtures. Even though compound **3** has already been isolated from linalool autoxidation mixtures, it has never been previously prepared by synthesis to our knowledge.[17] On the other hand, the synthesis of compound **4** has been reported in the literature but with moderate overall yields (20−30%) that we sought to improve.[14]

Scheme 1. Synthesis of 3



Scheme 2. The Schenck Reaction



a. Allylic Hydroperoxide ***3***. The synthetic route developed for the generation of **3** is illustrated in Scheme 1. We targeted the synthesis of **3** by subjecting linalool **1** to the ene or 'Schenck reaction', allowing the preparation of allylic hydroperoxides from alkenes containing allylic hydrogens via singlet oxygen ($^1O_2$) reaction (Scheme 2).

Concentrating on the factors that control the stereoselectivity and regioselectivity of the $^1O_2$−alkene ene reaction, Alberti and Orfanopoulos demonstrated that in the case of trisubstituted alkenes, including a geminal dimethyl substituent, the allylic hydrogens next to the large alkyl substituent are in general more reactive than those next to the small group.[18] Therefore, the 'Schenck reaction' of linalool **1** was expected to lead mostly to compound **3**, having the hydroperoxide on the more substituted carbon.

Most commonly, $^1O_2$ is generated by photosensitization of molecular oxygen in its ground state, but it can also be generated in high yields using a chemical source. A mild chemical method to quantitatively generate $^1O_2$, developed by Aubry and Cazin in the 1980s, consists of the disproportionation of hydrogen peroxide induced catalytically by sodium molybdate in alkaline aqueous solutions.[19] With this method, a high flux of $^1O_2$ may be generated in situ at room temperature without the need for light and photochemical equipment. However, it is known that the H_2O_2/MoO_4^{2-} system also acts as an efficient double bond epoxidizing agent under acidic and neutral conditions.[20] In order to avoid this pH-dependent competitive reaction, we decided to use a microemulsion consisting of water, dichloromethane (organic phase), sodium dodecyl sulfate (surfactant), and

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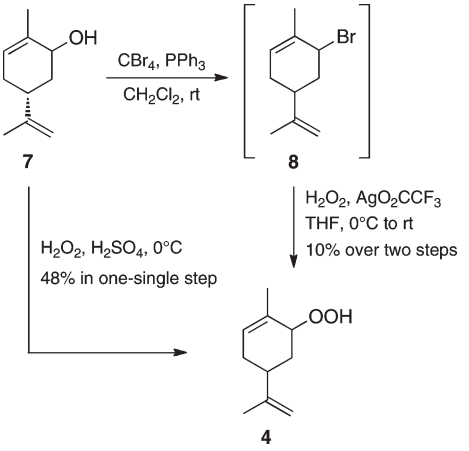
butanol (cosurfactant) as reaction medium, previously described as particularly suitable for the peroxidation of hydrophobic substrates. Also, this water-in-oil microemulsion was reported to limit the direct interaction between the substrate and the peroxomolybdate intermediates in a way in which the olefin epoxidation is minimized.[21,22] The treatment of linalool **1** with H_2O_2/MoO_4^{2-} reactants, generating $^1O_2$ in the described microemulsion, afforded not only the expected allylic hydroperoxide **3** but also the structural isomer **5**. In a 80% yield reaction, a ratio 40:60 of **3** and **5**, respectively, was measured by ^{1}H NMR. This result was in opposition to the previous Alberti and Orfanopoulos observations, in which the presence of a large alkyl substituent on the double bond should have favored the formation of **3**.[18] Yet, the hypothetical steric hindrance of this group seemed to be minimized because of the possibility for free rotation. Much work has been dedicated to the elucidation of the mechanism of the ene reaction for substrates with several allylic hydrogen atoms, and even if significant regioselectivity can be achieved by choosing appropriate structural features within the allylic substrate,[18] there are still examples where allylic hydrogen abstraction may take place at all possible sites, resulting in mixtures of isomeric allylic hydroperoxides.

All attempts to separate **3** from isomer **5** by column chromatography (silica gel, silica gel permeated with silver nitrate, alumina) were unsuccessful. On the basis of a study of Clark et al. in which regioisomer allylic hydroperoxides were selectively protected by using *tert*-butyldimethylsilyl chloride and imidazole in DMF, we suggested the chemical derivatization of **3** and **5** by protection of the hydroperoxide functionality as silyl ethers.[23] We employed *tert*-butyldiphenylsilyl chloride (TBDPSCl) as a protective agent because we thought the steric hindrance of the bulky TBDPS group could favor the selective protection of one of the hydroperoxides. The first trials ended with the protection of both hydroperoxides, and again it was not possible to isolate the peroxysilanes formed. However, it was possible to resolve kinetically the selective protection of the hydroperoxides when treating much more diluted DMF solutions of the **3** and **5** mixture (0.13 M) with TBDPSCl (1.2 equiv) and imidazole (2 equiv), as **3** was protected much faster than **5**, affording the peroxysilane **6**, which was easy to separate from nonprotected **5**. However, it was very difficult to completely remove the excess TBDPSCl. Thus, no absolute yield is given for the formation of **6**, which was purified as much as possible and then deprotected with a methanolic solution of potassium hydroxide to afford **3**. The optimized yield for this two-step method for protection and deprotection of the hydroperoxide was 96%. In summary, a simple three-step route was thus developed for the synthesis of **3** with an overall yield of 31%.

b. Allylic Hydroperoxide ***4***. The use of the Schenck reaction for the synthesis of hydroperoxide **4** from *R*-(+)-limonene **2** resulted in the generation of multiple isomeric allylic hydroperoxides of similar polarity, from which **4** was a minor product that was difficult to isolate. As previously reported, secondary allylic hydroperoxides can be synthesized by S_N2 nucleophilic substitution from the corresponding alcohol, such as the formation of **4** from (−)-carveol **7**.[14] The alcohol functionality is converted into a good leaving group and then substituted by hydrogen peroxide. We tried this methodology via the known silver ion-assisted reaction of the alcohol-corresponding halide and hydrogen peroxide.[24] (−)-Carveol **7** was treated with CBr_4 and PPh_3 in dichloromethane. Unstable crude product **8** was immediately treated with silver trifluoroacetate (1.2 equiv) and at least a 10-fold excess of hydrogen peroxide in a THF solution (Scheme 3). But we only recovered 10% of expected hydroperoxide **4** together with starting product **7**. Finally, **4** was surprisingly obtained in a higher yield and in one step by using the most classical method for preparation of tertiary allylic hydroperoxides, that is the quenching with hydrogen peroxide of a carbocation generated from a hydroxyl function under acidic conditions.[25] Even if secondary carbocations are less favorable than tertiary carbocations for such reactions, the treatment of (−)-carveol **7** with hydrogen peroxide in the presence of H_2SO_4 afforded **4** in a 48% yield (Scheme 3). To our knowledge, this hydroperoxide had never been isolated in more than 30% yield.

Scheme 3. Synthesis of 4



Radical Species Issued from 3 and 4: EPR Spin-Trapping Studies. Our initial investigations on the identification of radical species issued from allergenic allylic hydroperoxides started several years ago by combining two radical trapping techniques, the first leading to neutral compounds and the second allowing the detection of radical intermediates by EPR.[11,12,26] For conversion into neutral molecule radical species obtained from the hydroperoxides by the action of several radical initiators (light, heat, TPP-Fe^{3+}), the stable TMIO was used as a trap. On the other hand, combined with EPR spectroscopy, the spin-trapping technique is considered one of the best methods for the detection of short-lived radicals and is commonly employed for the detection of free radicals in biological media. The EPR spin-trapping technique we initially employed was based on the use of the allylic alcohol precursor of the hydroperoxide. The same allyloxyl radical that could derive from the hydroperoxide was generated in situ by photolysis of the nitrite formed by reacting the alcohol with *tert*-BuONO, which also played the role of the spin trap. Major drawbacks of these methods are that they are specific for the identification of carbon-centered radicals and do not allow direct identification of derived oxygen-centered radicals (e.g., alkoxyl, peroxyl, and hydroxyl). Also, the parent alcohol, and not directly the hydroperoxide of interest, was used for the EPR studies. A solution was initially found by making use of the EPR spin-trapping technique and of DEPMPO as a spin trap, as one of its key features is the high persistence of its oxygen-centered radical adducts, especially the superoxide and the alkylperoxyl radicals.[27] The formation of alkylperoxyl radicals from direct photolysis of hydroperoxide **4** was in this way demonstrated.[14] However, although photochemical cleavage of hydroperoxides is quite facile, it is unlikely to happen in vivo. Reductive cleavage of peroxides and hydroperoxides has been reported to occur not only with reduced metals, in particular Fe(II), but also with cytochrome

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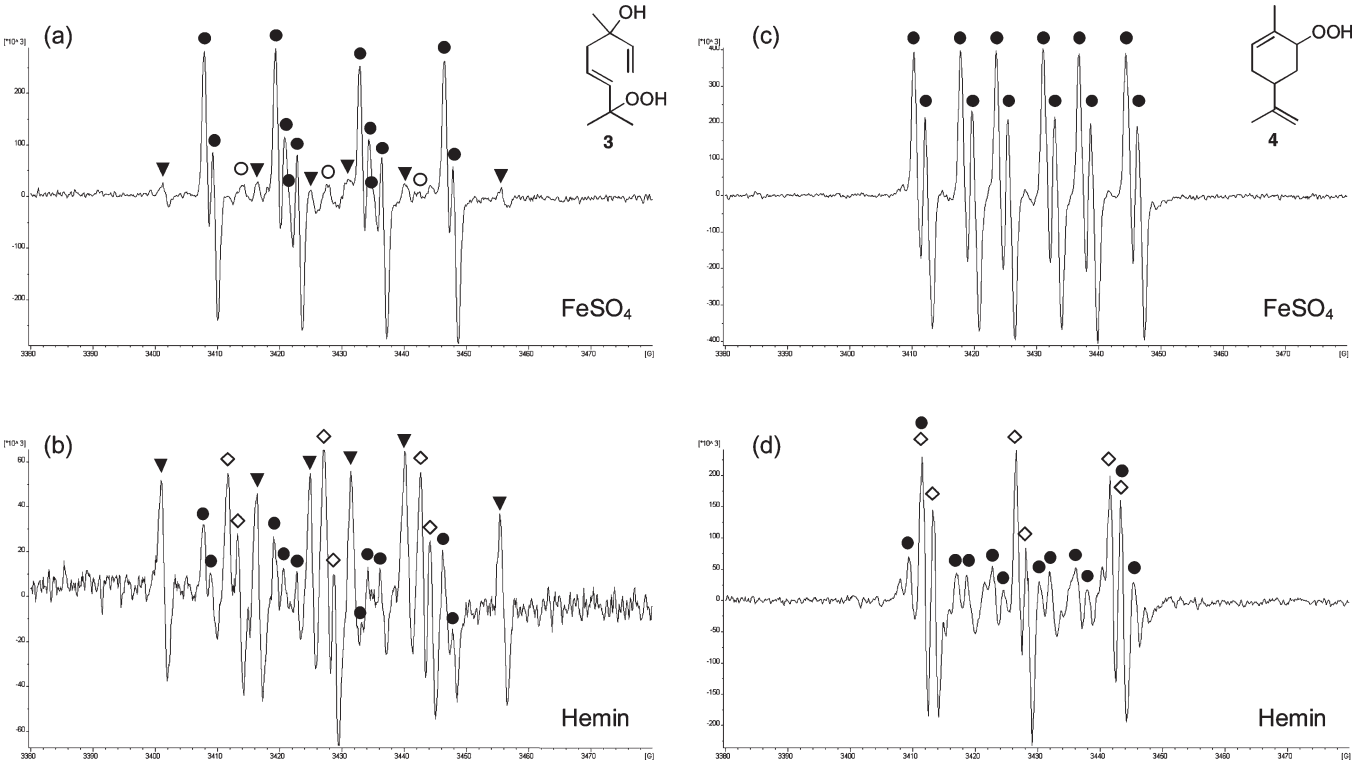

Figure 1. Experimental EPR spectra obtained from the reaction of **3** or **4** with $FeSO_4$, (a and c) or hemin (b and d), in the presence of DMPO and in CH_3CN. Computer simulation of spectra led to the following parameters. (a) Signal (●) a_N = 13.57 G, $a_{H\beta}$ = 11.50 G, $a_{H\gamma}$ = 1.37 G (g = 2.0065) for DMPO/RO• or DMPO/ROO•; signal (▼) a_N = 15.23 G, $a_{H\beta}$ = 23.93 G (g = 2.0061) for DMPO/R• carbon-centered adduct; signal (○) a_N = 14.01 G (g = 2.0065) for **10**. (b) Signal (●) a_N = 13.57 G, $a_{H\beta}$ = 11.50 G, $a_{H\gamma}$ = 1.37 G (g = 2.0065) for DMPO/RO• or DMPO/ROO•; signal (▼) a_N = 15.23 G, $a_{H\beta}$ = 23.93 G (g = 2.0061) for DMPO/R• carbon-centered adduct; signal (◇) a_N = 15.53 G, $a_{H\beta}$ = 1.60 G (g = 2.0063) for **12**. (c) Signal (●) a_N = 13.28 G, $a_{H\beta}$ = 7.52 G, $a_{H\gamma}$ = 1.86 G (g = 2.0064) for DMPO/RO• or DMPO/ROO•. (d) Signal (●) a_N = 13.28 G, $a_{H\beta}$ = 7.52 G, $a_{H\gamma}$ = 1.86 G (g = 2.0064) for DMPO/RO• or DMPO/ROO•; signal (◇) a_N = 15.05 G, $a_{H\beta}$ = 1.65 G (g = 2.0064) for **12**.

enzymes and related Fe(III)-heme-containing models and described to proceed via both homolytic and heterolytic pathways.[28,29] As Fe(II)/Fe(III) redox cycles are common in biological media, we have therefore started to study the behavior of allergenic allylic hydroperoxides in the presence of Fe(II)/Fe(III) systems by EPR spectroscopy and the spin-trapping technique. Preliminary studies were conducted with a model monocyclic tertiary allylic hydroperoxide together with DMPO and DEPMPO, both spin traps being known to distinguish oxygen-centered (peroxyl/alkoxyl) and carbon-centered radical adducts.[30−32] In the presence of $FeSO_4$, a typical reagent for the reduction of hydroperoxides in Fenton's reaction, and Fe(III) derivatives such as $FeCl_3$ and because of the ubiquitous nature of heme complexes such as Fe(III)-porphyrins TPP-Fe^{3+} and hemin, described to induce cleavage of the hydroperoxide functionality,[33,34] we confirmed the formation of carbon-centered radicals and of oxygen-centered allyloxyl and peroxyl radicals in organic/aqueous media.[10] We have now applied this methodology to 7-hydroperoxy-3,7-dimethylocta-1,5-dien-3-ol **3** and 2-hydroperoxylimonene **4**. Experiments were carried out in CH_3CN or in a 1:1 (v/v) H_2O/CH_3CN mixture. Nonpolar organic solvents can mimic the reaction conditions in a membrane for example, but because many biological reactions proceed at aqueous/organic medium interfaces the reactivity of allylic hydroperoxides in aqueous solutions is also of interest. The skin could also be seen as a semiorganic medium with hydrophilic and hydrophobic properties.

a. Influence of Fe(II)/Fe(III) Radical Initiation. Reactions were carried out in CH_3CN and in the presence of $FeSO_4$, TPP-Fe^{3+}, or hemin. In some cases, the recorded EPR spectra when using DEPMPO corroborated the results obtained with DMPO. However, in many other cases the superimposition of all EPR signals could not help to elucidate the adducts obtained. Therefore, results shown here are mainly those obtained with DMPO. Figure 1 shows the EPR spectra obtained from the reaction of **3** and **4** with $FeSO_4$ or hemin and in the presence of DMPO.

DMPO-trapped radicals with hfc values of a_N = 13.57 G, $a_{H\beta}$ = 11.50 G, $a_{H\gamma}$ = 1.37 G (g = 2.0065) and a_N = 13.28 G, $a_{H\beta}$ = 7.52 G, $a_{H\gamma}$ = 1.86 G (g = 2.0064) indicate the formation of oxygen-centered radicals, regardless of the Fe species used for radical initiation. While DMPO/•OH and DMPO/•OOH adducts are well-defined with characteristic hfc values, the attribution of other structures of DMPO oxygen-centered radical adducts, such as alkoxyl and peroxyl adducts, has been described to be controversial.[35,36] The observed hfc values were, in principle, in good agreement with those reported for the trapping of tertiary peroxyl radicals such as those derived from cumene hydroperoxide.[37] However, according to the literature, many DMPO peroxyl radical adducts (DMPO/ROO•) have virtually the same hfc as alkoxyl radical adducts (DMPO/RO•), raising the issue of correct assignement to peroxyl radical adducts. The same phenomenon has been described to happen with DEPMPO spin-trapping.[30,38] The accurate discrimination between spin-trapping of ROO• and of RO• seems to be therefore uncertain when using DMPO or DEPMPO. However, it is well-known that when alkylperoxyl radicals are trapped by DEPMPO, the EPR spectra

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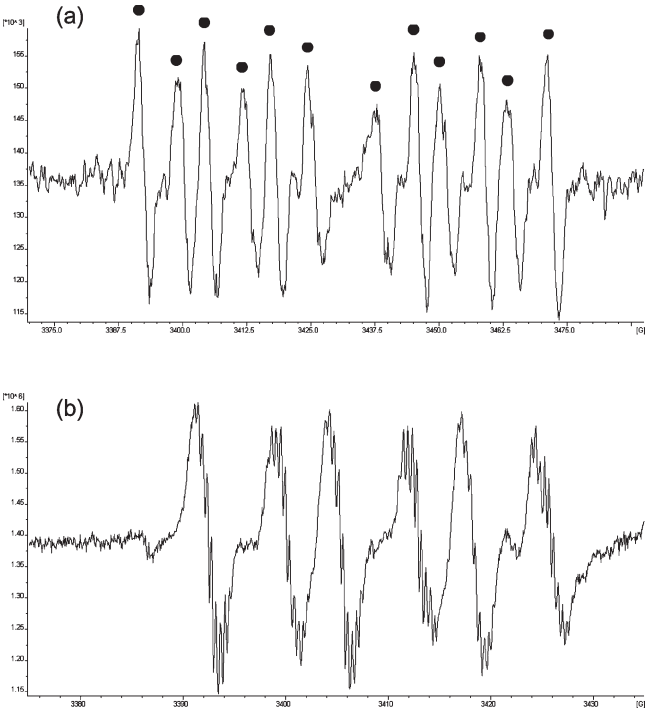
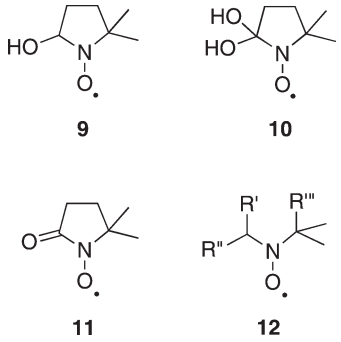


Figure 2. Experimental EPR spectra of DEPMPO spin adducts formed from reaction of **4** with $FeSO_4$. (a) The *trans*-DEPMPO/ROO• isomer is observed with coupling constants a_P = 46.45 G, a_N = 12.80 G, $a_{H\beta}$ = 7.54 G (g = 2.0065). (b) Half EPR spectrum of the *trans*-DEPMPO/ROO• spin adduct recorded at low modulation amplitude (1 G). The lines were split by weak couplings with the pyrrolidine ring hydrogens.

exhibit a dramatic alternate line width when recorded with low modulation amplitude.[38] In order to test if alkylperoxylradicals were present in the reaction mixtures, we also performed the spin-trapping experiments with DEPMPO at low modulation amplitude. As an example, Figure 2 shows the EPR spectra obtained from the reaction of **4** with $FeSO_4$ in CH_3CN and in the presence of DEPMPO (modulation frequence 100 kHz, modulation amplitude 1 G).

The EPR spectrum shown in Figure 2a was attributed to spin adducts arising from the addition of formed alkylperoxyl radicals ROO• on both faces of DEPMPO, as described in the literature.[39,40] This addition can lead to trans and cis diastereoisomers. The main signal observed, with twelve EPR lines (•) and exhibiting an alternating line width effect, corresponds to the trans diastereoisomer resulting from addition of the alkylperoxyl radical on the less hindered face of DEPMPO. Indeed, the hfc values found a_P = 46.45 G, a_N = 12.80 G, and $a_{H\beta}$ = 7.54 G (g = 2.0065) matched well with patterns described for example for the trans spin adducts of *tert*-BuOO• or superoxide with DEPMPO and analogues.[39,40] In the recorded spectrum, the small shoulder seen on the left side of some of the twelve lines probably corresponds to the minor cis diastereoisomer, hidden by the signals of the major *trans*-DEPMPO/ROO• isomer.[14] When the spectra were recorded at low modulation amplitude (Figure 2b), the *trans*-DEPMPO/ROO• adduct clearly displayed the alternating line width mentioned above and showed the characteristic resolution of the long-range γ-hydrogen splittings from the pyrrolidine ring (i.e., $a_{H\gamma}$ = 0.45 and 1.01 G). This phenomenon has been described to be generated by a slow down rotation around the O−O peroxyl bond only possible in the case of the *trans*-DEPMPO/ROO• spin adduct. In this way, the spin adduct acquires different conformational changes resulting in various isomer forms for which the hfc values are different. The same is not observed when trapping alkoxyl RO• radicals.[38,40] These results corroborated the presence of alkylperoxylradicals ROO• issued from the hydroperoxides in the reaction mixtures. However, the simultaneous presence of alkoxyl RO• radicals for which spin-trapping signals could be subjacent to those of the ROO• spin adducts cannot be excluded.

Chart 2. Structures of DMPO Degradation Products Detected in the EPR Spectra



Most interestingly, when the experiments were conducted with compound **3**, a signal corresponding to the trapping of carbon-centered radicals was also observed, especially when using Fe(III)-porphyrins such as hemin (Figure 1a and 1b). The experimental signal was in good agreement with simulations that used the calculated parameters a_N = 15.23 G, $a_{H\beta}$ = 23.93 G (g = 2.0061), $a_H > a_N$, being characteristic of trapped carbon-centered radicals derived from the decomposition of organic hydroperoxides.[41] Finally, degradation of the spin-trap itself was also detected in the spectra with the identification of known oxidation products **10** (a_N = 14.01 G, g = 2.0065) and **11** (a_N = 6.90, $a_{H\gamma}$ = 3.45 G, g = 2.0073; spectra not shown), and probably *sec*-alkyl-*tert*-alkyl nitroxide derivative **12** (a_N = 15.53 G, $a_{H\beta}$ = 1.60 G, g = 2.0063) (Chart 2).[42,43]

b. Influence of the Solvent. The same experiments described above were conducted in 1:1 (v/v) H_2O/CH_3CN solutions. Compared to the results obtained in CH_3CN, the outlines of the spectra were modified considerably (Figure 3). In all cases, the EPR spectra showed the presence of DMPO/R• carbon-centered radical adducts characterized by hfc of $a_H > a_N$. As carbon-centered radicals were in general not trapped when CH_3CN was used as solvent, it might be expected that in CH_3CN oxygen-centered radicals were trapped before subsequent rearrangement processes. However, in H_2O/CH_3CN solutions the spin-trapping process seemed to be slower, allowing enough time for rearrangements to take place and thus accounting for the formation of carbon-centered radicals mainly. Only the reaction of hydroperoxide **4** with $FeSO_4$ permitted the identification of DMPO/RO•/ROO• adducts, formed before potential rearrangement of the oxygen-centered radicals (Figure 3c).

In H_2O/CH_3CN, while it was not observed at all in CH_3CN, the monohydroxyl DMPO adduct **9** was clearly identified (Figure 3a, 3c, and 3d), together with the dihydroxyl adduct **10** in the case of hydroperoxide **4** reactions (Figure 3c and 3d) (Chart 2).

c. Mechanistic Interpretations. The formation of oxygen-centered radicals ROO•/RO• derived from the reactions of hydroperoxides **3** and **4** with Fe(II)/Fe(III) species was demonstrated by EPR spin-trapping using DMPO and DEPMPO as spin-traps. However, it was not possible to determine the preferential formation of alkylperoxyl ROO• or alkoxyl RO• radicals but rather to

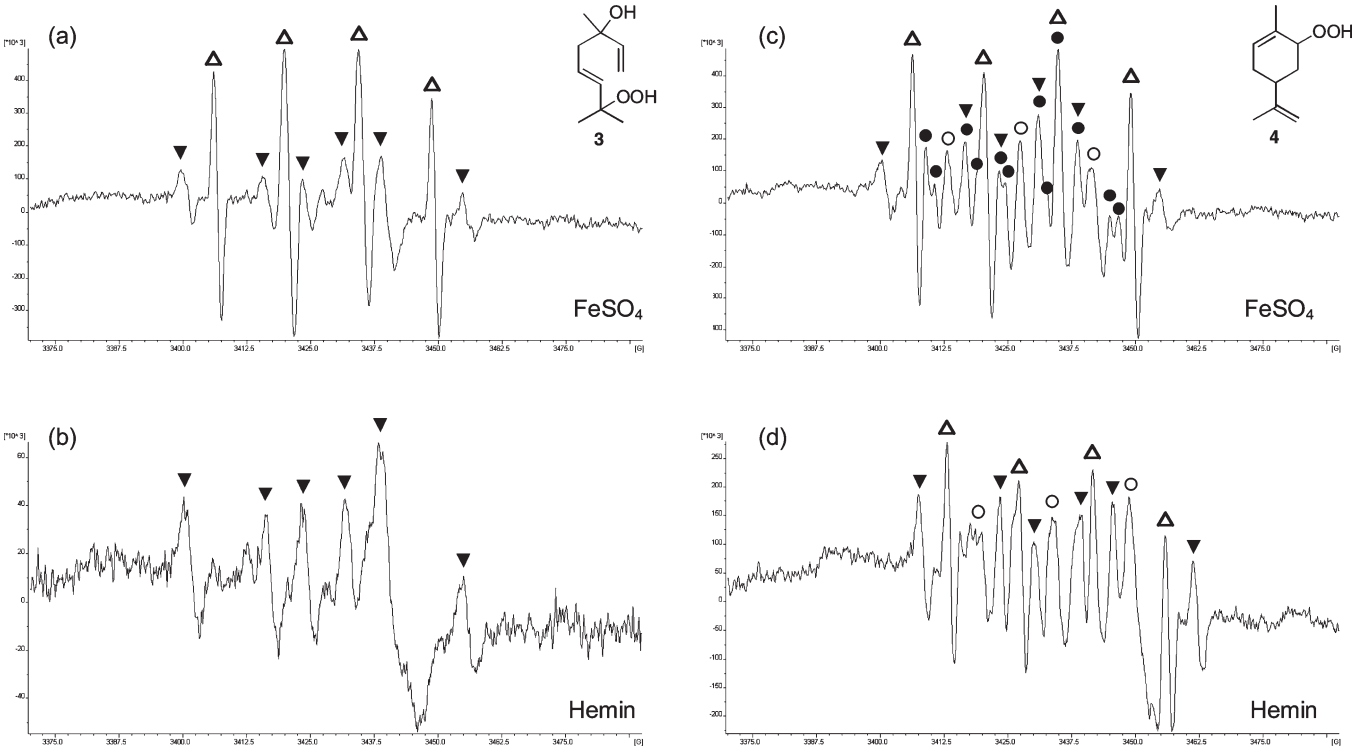
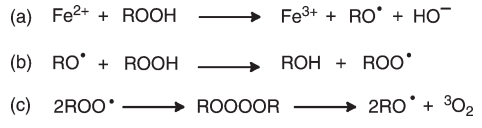
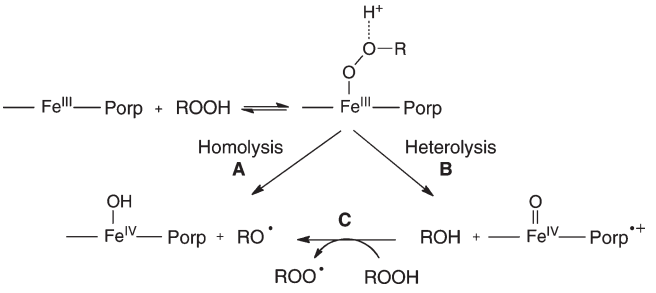


Figure 3. Experimental EPR spectra obtained from the reaction of **3** or **4** with $FeSO_4$, (a and c) or hemin (b and d), in the presence of DMPO and in 1:1 (v/v) H_2O/CH_3CN. Computer simulation of spectra led to the following parameters: (a) Signal (▼) a_N = 15.92 G, $a_{H\beta}$ = 24.35 G (g = 2.0059) for DMPO/R• carbon-centered adduct; signal (△) a_N = 14.20 G, $a_{H\beta}$ = 14.20 G (g = 2.0062) for **9**. (b) Signal (▼) a_N = 15.92 G, $a_{H\beta}$ = 24.35 G (g = 2.0059) for DMPO/R• carbon-centered adduct. (c) Signal (▼) a_N = 15.47 G, $a_{H\beta}$ = 23.36 G (g = 2.0062) for DMPO/R• carbon-centered adduct; signal (●) a_N = 13.28 G, $a_{H\beta}$ = 7.52 G, $a_{H\gamma}$ = 1.86 G (g = 2.0064) for DMPO/RO• or DMPO/ROO•; signal (△) a_N = 14.20 G, $a_{H\beta}$ = 14.20 G (g = 2.0062) for **9**; signal (○) a_N = 13.74 G (g = 2.0064) for **10**; (d) signal (▼) a_N = 15.47 G, $a_{H\beta}$ = 23.36 G (g = 2.0062) for DMPO/R• carbon-centered adduct; signal (△) a_N = 14.20 G, $a_{H\beta}$ = 14.20 G (g = 2.0062) for **9**; signal (○) a_N = 13.74 G (g = 2.0064) for **10**.

Scheme 4. Competitive Formation of Oxygen-Centered Radicals in ROOH/Fe(II) Reactions



Scheme 5. Homolytic/Heterolytic O−O Bond Cleavage of Hydroperoxides by Fe(III) Porphyrin Complexes



accept the competitive existence of both in the reaction mixtures. Indeed, it is commonly accepted that hydroperoxides decompose in the presence of ferrous salts, simultaneously affording alkoxyl RO• radicals and hydroxide ions in a process similar to the Fenton reaction (Scheme 4, entry a). However, these alkoxyl radicals can easily further abstract the hydrogen of the hydroperoxide ROOH functionality to lead to alkylperoxyl ROO• radicals (Scheme 4, entry b). Furthermore, ROO• radicals are known to undergo bimolecular self-reactions to form tetroxide intermediates (R-OOOO-R) which decompose irreversibly, leading again to the formation of alkoxyl RO• radicals and dioxygen (Scheme 4, entry c).[10,41] ROO• and RO• can thus coexist together in the reaction mixtures.

In the case of Fe(III) porphyrin complexes, there is no clear mechanistic consensus on both the possible homolytic and heterolytic O−O bond cleavage of the hydroperoxide ROOH functionality. Almarsson and Bruice proposed a straight homolytical cleavage of the O−O bond of an initially formed Fe(III) porphyrin-ROOH complex, leading directly to RO• radicals and to a ferryl-oxo complex (Scheme 5, pathway A).[44] However, Traylor et al. proposed that the O−O bond would be cleaved in an heterolytic way, leading to the generation of ROH and the formation of a high-valent Fe(IV) oxo porphyrin cation radical intermediate (Scheme 5, pathway B).[45] They also suggested a different mechanism for the product distribution of O−O bond homolysis.[46] The heterolytic O−O bond cleavage being the starting process, they proposed it can be followed by a fast reaction between the Fe(IV) oxo porphyrin cation radical intermediate and the hydroperoxide, leading to the product distribution of O−O bond homolysis and to ROO• radicals (Scheme 5, pathway B followed by C). Also, as shown in Scheme 4 (entry c), ROO• radicals can evolve and result in the formation of RO• radicals. ROO• and RO• could thus coexist together in the reaction mixtures.

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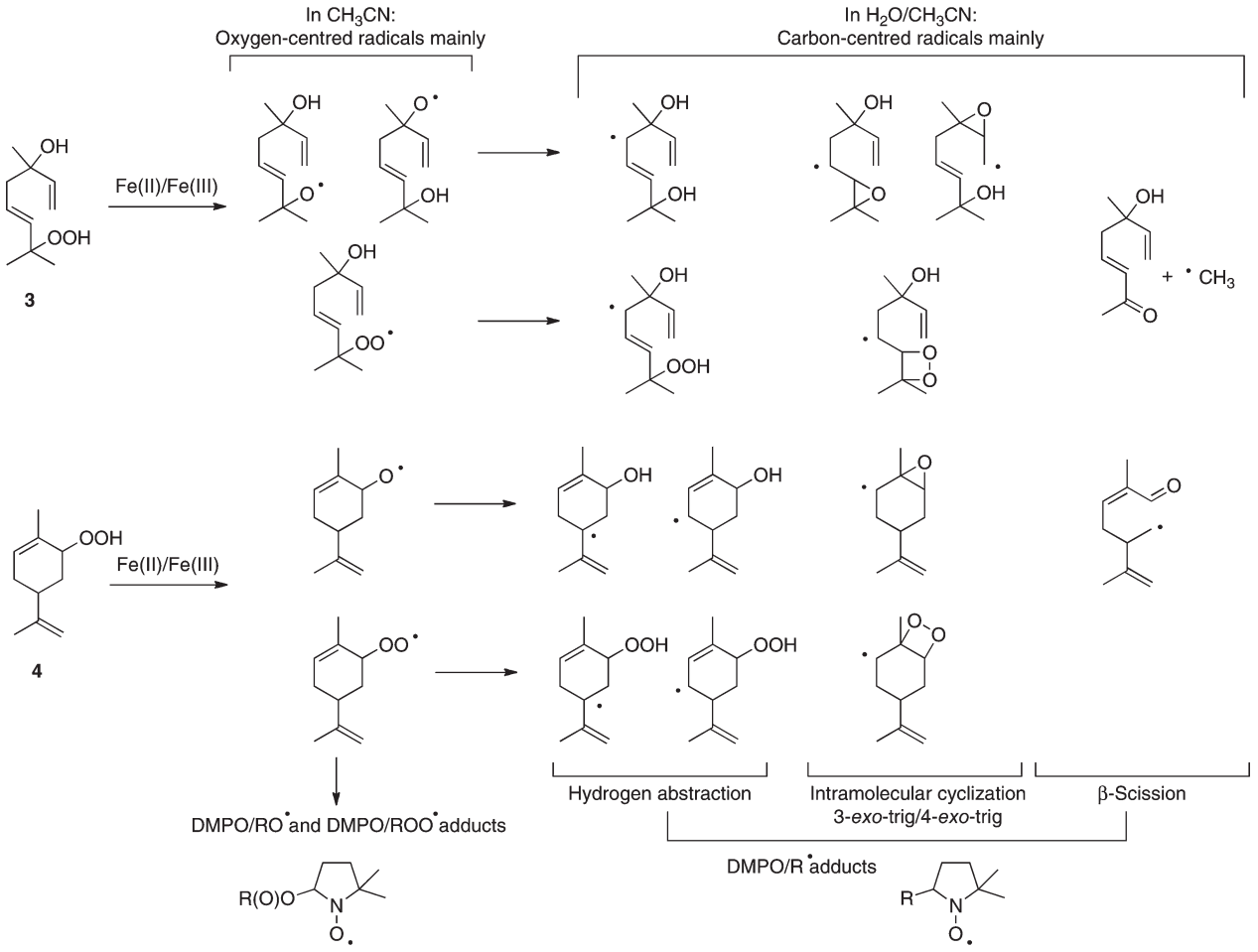
Scheme 6. Reactivity of Hydroperoxides 3 and 4 with Fe(II)/Fe(III): Potential Oxygen/Carbon-Centered Radicals Revealed by Spin-Trapping EPR Studies in H_2O and H_2O/CH_3CN Media



For many other authors, one pathway would not necessarily rule out the other pathway. Partitioning between heterolysis and homolysis would basically be affected by the electronic nature of the iron porphyrin complexes (i.e., electronic nature of axial ligands) and also by the electron-withdrawing or -donating character of the ROOH substituent.[47,48] Electron-deficient iron porphyrin complexes show a propensity to cleave the O—O bond heterolytically, while electron-rich iron porphyrin complexes such as TPP-Fe^{3+} do it homolytically. It has also been shown that hydroperoxides containing electron-donating *tert*-alkyl groups such as *t*-BuOOH tend to be cleaved homolytically, whereas electron-withdrawing substituents such the acyl group in *m*-CPBA seem to facilitate O—O bond heterolysis. This approach leads us initially to favor the hypothesis of a homolytic cleavage of the O—O bond of compounds **3** and **4** by TPP-Fe^{3+} for example, nevertheless without excluding the heterolysis possibility. Indeed, unfortunately the present data do not allow us to make a definite statement concerning a homolytic or heterolytic mechanism for O—O bond cleavage when using hemin, for instance, and therefore on the precise nature of oxygen-centered radicals formed. The coexistence of both the peroxyl ROO• and the alkoxyl RO• radicals could thus also be possible in the presence of Fe(III) species.

The rearrangement of oxygen-centered radicals to form R• carbon-centered radicals is thoroughly discussed in the literature. Concerning allyloxyl radicals, there is a competition between the 3-exo-trig cyclization leading to the formation of oxiranylcarbinyl radicals and the β-scission leading to the formation of carbonyl compounds and alkyl radicals.[49] These two processes are extremely fast, and the relative rate of obtention of each one has been described to depend on the number and nature of alkyl substituents present on the carbon atom in a position α to the oxygen atom. An allyloxyl radical having a dimethylated α-C, such as the one derived from **3**, would have a strong preference for the 3-exo-trig cyclization, while a secondary allyloxyl radical, such as the one derived from **4**, would have a preference for the β-scission favored by the alkyl chain length in α. Concerning allylperoxyl radicals, they can follow a 4-exo-trig cyclization by addition to the double bond and lead to the formation of carbon-centered radicals with an unstable dioxetane structure known to cleave to yield carbonyl compounds.[10,12] Finally, the abstraction of hydrogens from different allylic positions is also an important pathway for the formation of carbon-centered radicals from RO• and ROO• radicals issued from hydroperoxides.[12] In this work, the spin-trapping experiments carried out with **3** and **4** seemed to show, except if other signals were overlaid, the formation of a single species of carbon-centered radicals. Unfortunately, we could not determine precisely the chemical structure corresponding to that species. As described above, different potential carbon-centered radicals could thus be formed and are shown in Scheme 6. At this stage, it was not possible to distinguish between oxiranylcarbinyl radicals, dioxetane radicals, and radicals derived from allylic hydrogen abstraction.

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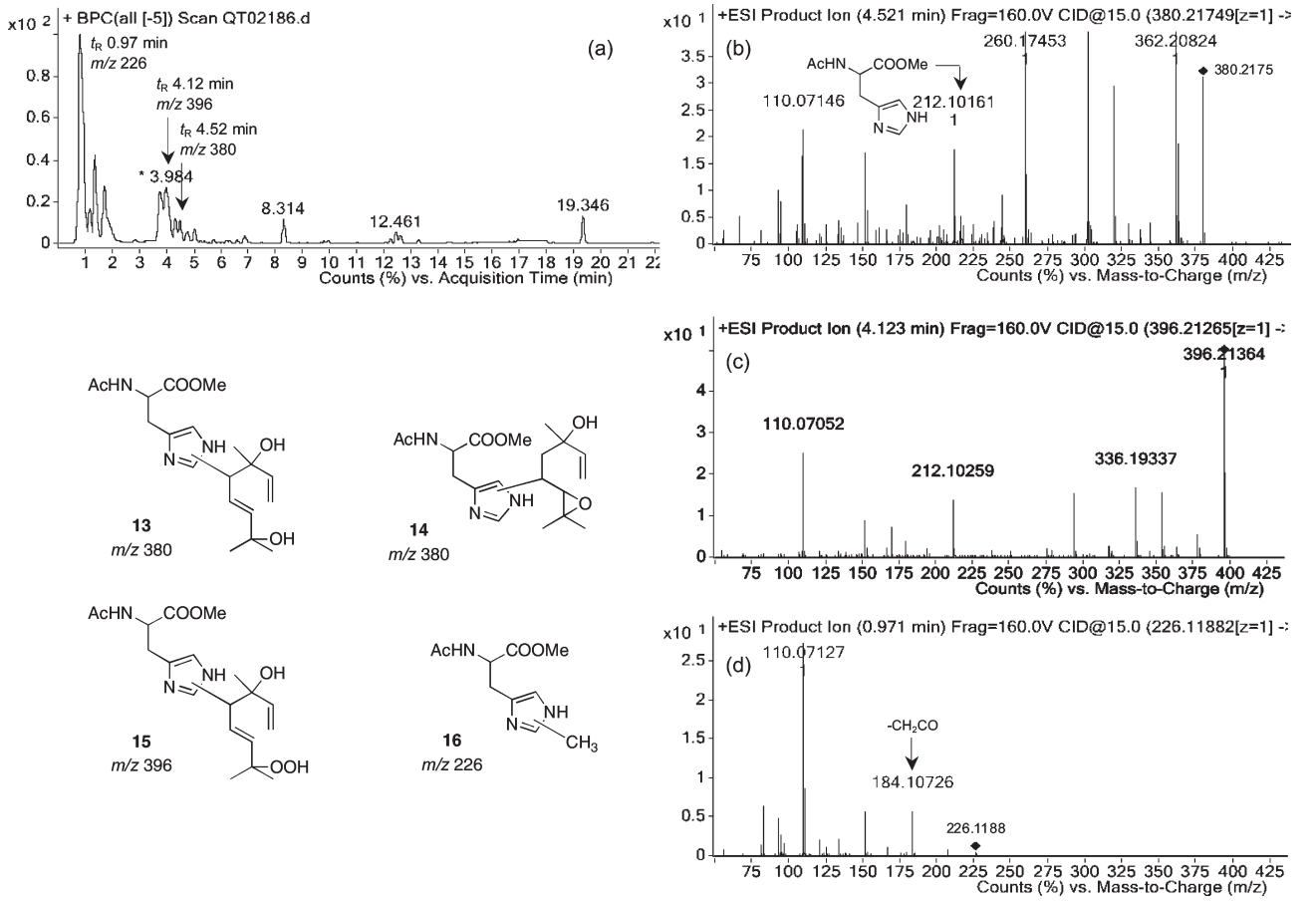

Figure 4. Reaction of **3** with *N*-Ac-His-OMe studied by LC-ESI-MS/MS, and potential chemical structures proposed for adducts formed. (a) LC chromatogram for the reaction catalyzed by $FeSO_4$. Peaks at retention times (t_R) of 0.97, 4.12, and 4.52 min had associated m/z values of 226, 396, and 380, respectively. (b) ESI-MS/MS spectrum obtained from the analysis of m/z 380. (c) ESI-MS/MS spectrum obtained from the analysis of m/z 396. (d) ESI-MS/MS spectrum obtained from the analysis of m/z 226.

Aside from detected known DMPO oxidation products **10** and **11**, the hydroxylated DMPO derivative **9** was also observed but only in H_2O/CH_3CN mixtures. This nitroxide radical would result from the nucleophilic addition of water to the double bond of DMPO complexed to ferric ions present in solution, as previously described.[50]

Reactivity with *N*-Acetylhistidine Methyl Ester Catalyzed by Fe(II)/Fe(III). Analyses of amino acid residues from enzymes and of their related antioxidant activity have demonstrated that those having an aromatic moiety in the side chain are prone to radical reactions. The aromatic imidazole ring of histidine, a common coordinating ligand in metalloproteins and radical enzymes, has two unsaturated double bonds and therefore could react with metabolic free radicals (R•) by a free radical addition reaction. It has been proposed that in electron-transfer processes and biological redox reactions, including oxidative stress, histidine radicals are formed as transient intermediates. EPR spectroscopic evidence of the oxidation of histidine in aqueous solution by addition of OH• radical to position C5 of the imidazole ring has been reported in an H_2O_2—Fenton system over a large range of pH values, attesting to its antioxidant potential.[51] The formation of OH• radicals and other reactive oxygen species in the reaction of nickel(II)-histidine complexes with hydroperoxides has also been reported.[52] Very recently, the reactivity of a large excess of hydroperoxide **4** with protected histidine in the presence of a catalytic amount of TPP-Fe^{3+} has been reported in the literature.[53] The authors showed for the first time the formation of an adduct with this amino acid and, up to previous studies, proposed the probable addition of a carvone moiety (major decomposition product of **4**) to histidine. Studies with angiotensin I, having two histidine residues in the peptide sequence, corroborated this result through the detection of an adduct bound at one of the two residues. Thus, we also tried to trap radical intermediates issued from allylic hydroperoxides **3** and **4** using a histidine derivative as trapping agent. The reactions were conducted in the presence of a catalytic amount of either Fe(II) or Fe(III). An excess of the amino acid was used, as these are conditions supposed to happen in vivo. Both the amine and carboxyl groups of histidine were protected to mimic its structure as part of a protein sequence but also to favor the reactivity of the side chain exclusively and to avoid the eventual reactivity of the amine terminus with decomposition products of the test compounds that contained carbonyl chemical groups (i.e., carvone for hydroperoxide **4** via Schiff base formation).

Compounds **3** or **4** and *N*-acetylhistidine methyl ester (*N*-Ac-His-OMe, 2 equiv) in a deaerated 1:1 (v/v) H_2O/CH_3CN mixture were treated with a catalytic amount of $FeSO_4$ or $FeCl_3$ (0.1 equiv), at room temperature. TLC indicated that the hydroperoxides were not completely consumed even after 7 days. The reactions were then stopped, and the solvent was removed under reduced pressure. The crude products were analyzed by LC-ESI-MS/MS.

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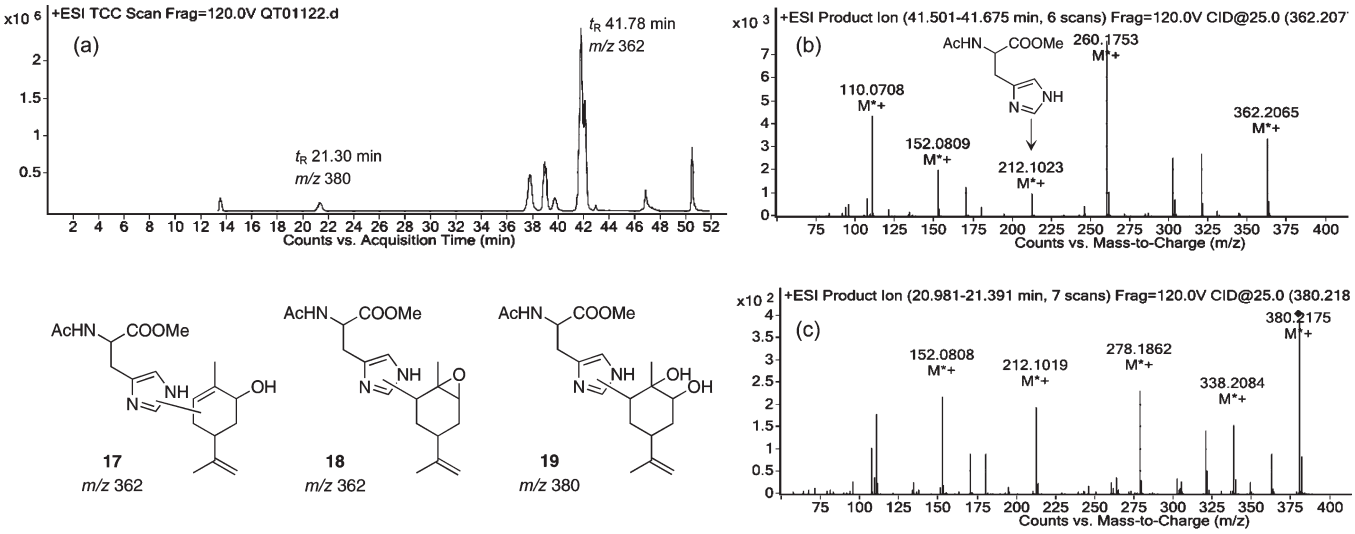

Figure 5. Reaction of **4** with *N*-Ac-His-OMe as studied by LC-ESI-MS/MS, and potential chemical structures proposed for adducts formed. (a) LC chromatrogram for the reaction catalyzed by $FeCl_3$. Peaks at retention times (t_R) of 21.30 and 41.78 min had associated *m/z* values of 380 and 362, respectively. (b) ESI-MS/MS spectrum obtained from the analysis of *m/z* 362. (c) ESI-MS/MS spectrum obtained from the analysis of *m/z* 380.

a. Allylic Hydroperoxide ***3***. The reaction of **3** with *N*-Ac-His-OMe in the presence of Fe(II)/Fe(III) was rather slow (reaction stopped after 7 days without complete disappearance of **3**). The LC-MS spectra profiles of the crude products were practically identical under both experimental conditions and showed the formation of a large number of compounds. Among all recorded LC-peaks, three of them had a related MS spectrum that could fit with the generation of *N*-Ac-His-OMe adducts, with $[M + H]^+$ molecular ions at *m/z* 226, *m/z* 396, and *m/z* 380 values, respectively (Figure 4a). The peak corresponding to *m/z* 226 was quite important, whereas peaks at *m/z* 396 and 380 were of lower magnitude. To get further information, complementary ESI-MS/MS analyses of these signals were carried out. First, all ESI-MS/MS spectra showed a fragment ion at *m/z* 110 due to the immonium ion related to histidine (Figure 4b−d).[54] Immonium ions, of general structure $[H_2N{=}CH\text{-}R]^+$ where R is the amino acid side chain, are characteristic fingerprints of amino acid fragmentations in tandem mass spectrometry. This result was a first indication of the formation of adducts involving *N*-Ac-His-OMe and hydroperoxide **3**. Also, $[M + H]^+$ molecular ions at *m/z* 380 and at *m/z* 396 produced a fragment ion with an associated *m/z* value of 212, characteristic of a loss of *N*-Ac-His-OMe (Figure 4b and 4c). From a mechanistic point of view, and on the basis of the EPR results described above, the O−O bond cleavage of the hydroperoxide functionality by Fe(II) and Fe(III) can form allyloxyl radicals RO•. These can further evolve to carbon-centered radicals either by allylic hydrogen abstraction or by 3-exo-trig cyclization leading to the formation of oxiranylcarbinyl radicals. In both cases, a radical-based reaction with a side chain of *N*-Ac-His-OMe would give adducts with an associated value of *m/z* 380. Therefore, **13** and **14** could be principally suggested for *m/z* 380 (Figure 4). However, further studies allowed neither the elucidation of the exact chemical structure of the adduct nor the precise position of addition on the side chain of *N*-Ac-His-OMe. The molecular ion at *m/z* 396 could correspond to compound **15**, the mass increment of 16 with regard to *m/z* 380 indicating the presence of an oxygen atom in the addition. Indeed, the hydroperoxide O−H bond cleavage can form allylperoxyl radicals ROO• that can also evolve through hydrogen abstraction to form carbon radicals in allylic positions. The *m/z* value of 226 fitted well with the addition of a methyl group (Δmass 15) into the side chain of *N*-Ac-His-OMe to form adduct **16**. Mechanistically, the RO• radicals formed could in parallel follow a β-scission process, releasing, in this way, reactive methyl radicals that could be trapped by the amino acid. But this result remains just a hypothesis, as we were not able to exactly elucidate the precise position of methyl addition on the side chain of *N*-Ac-His-OMe, and ESI-MS/MS analysis of the molecular ion at *m/z* 226 only corroborated the loss of the acetyl group of methylated *N*-Ac-His-OMe that formed the fragment ion at *m/z* 184 (Figure 4d). Even so, reaction of **3** with *N*-Ac-His-OMe in Fe(II)/Fe(III) systems could be representative of the three main possible mechanisms explaining the evolution of the initially formed RO• radicals to carbon-centered radicals, i.e., allylic hydrogen abstraction, 3-exo-trig cyclization, and β-scission processes. Finally, no oxidation products of *N*-Ac-His-OMe were detected in any of the experimental conditions tested.

b. Allylic Hydroperoxide ***4***. As indicated above, the reaction of **4** with *N*-Ac-His-OMe, catalyzed by Fe(II)/Fe(III) ions, was also rather slow (reaction stopped after 7 days without complete disappearance of **4**). The LC-MS spectra profiles of the crude products were practically identical in both experimental conditions. Among the recorded peaks, two of them had an associated MS spectrum that could fit with the formation of a *N*-Ac-His-OMe adduct. The observed value of *m/z* 362 suggested the formation of an adduct in the presence of either $FeSO_4$ or $FeCl_3$, detected at relatively high amounts. Another potential adduct at *m/z* 380 was only seen in the presence of $FeCl_3$, detected at smaller amounts (Figure 5a). Complementary ESI-MS/MS analyses of these signals were carried out. As described for *N*-Ac-His-OMe adducts of hydroperoxide **3**, all ESI-MS/MS spectra showed the fragment ion at *m/z* 110 due to the immonium ion related to histidine (Figure 5b and 5c). Morever, both $[M + H]^+$ molecular ions at *m/z* 362 and at *m/z* 380 produced a fragment ion with an associated *m/z* value of 212, characteristic of a loss of *N*-Ac-His-OMe (Figure 5b and 5c). Following the same reasoning as for hydroperoxide **3**, chemical structures **17** and **18** could be suggested for *m/z* 362. The O−O bond cleavage

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of the hydroperoxide functionality by Fe(II) and Fe(III) can form allyloxyl radicals RO•. These can further evolve to carbon-centered radicals either by allylic hydrogen abstraction or by 3-exo-trig cyclization, leading to the formation of oxiranylcarbinyl radicals. In both cases, a radical-based reaction with the side chain of *N*-Ac-His-OMe would give adducts with an associated value of *m*/*z* 362 (Δmass 150). Therefore, **17** and **18** could be principally suggested for *m*/*z* 362 (Figure 5). However, further studies allowed neither the elucidation of the exact chemical structure of the adduct nor the precise position of addition on the side chain of *N*-Ac-His-OMe. In the case of *m*/*z* 380, the mass increment of 18 with regard to *m*/*z* 362 would correspond to the addition of a molecule of H_2O. It may then be suggested that nucleophilic addition of H_2O, present in the reaction solvent, on the electrophilic epoxide functionality of **18** afforded **19** at *m*/*z* 380 (Figure 5). Adduct **19** could thus be indirect evidence of the formation of **18**. Being only observed when $FeCl_3$ catalyzed the reaction, it could be proposed that the formation of oxiranylcarbinyl radicals was favored when using these experimental conditions. However, the simultaneous formation of allyloxyl radicals which would afford **17**-like adducts cannot be excluded. Finally, as for hydroperoxide **3**, no oxidation products of *N*-Ac-His-OMe were detected under any of the experimental conditions tested.

CONCLUSIONS

To complement our previous studies on allergenic allylic hydroperoxides and their supposed ability to form immunogenic structures by reacting with skin proteins through radical mechanisms, we now confirm, by using the spin-trapping technique combined with EPR spectroscopy, the formation of potentially reactive oxygen- and carbon-centered radicals derived from 7-hydroperoxy-3,7-dimethylocta-1,5-dien-3-ol **3** and 2-hydroperoxylimonene **4**. Both hydroperoxides have been identified as being responsible for the sensitizing potential of autoxidized linalool and *R*-(+)-limonene, respectively. Radical initiation by using Fe(II)/Fe(III) redox cycles, common in biological media, confirmed that the generation of these radical intermediates could be possible in vivo, in the epidermis, once the intact hydroperoxide molecules have penetrated into the skin. Even if with the spin-trapping EPR technique the exact chemical structure of carbon-centered radicals formed could not be completely elucidated, when histidine was used as a model amino acid for radical reactions, adducts were formed by reaction of different radical intermediates issued from **3** and **4** with the aromatic imidazole ring. The LC-ESI-MS/MS studies conducted on the radical reactivity of hydroperoxides **3** and **4** with histidine thus completed the spin-trapping EPR data. Reactions with histidine indicated that not just a single species of carbon-centered radicals was formed, as suggested when analyzing the EPR data, but rather many of them. The acyclic tertiary allylic hydroperoxide **3** illustrated ideally the case in which the first formed allyloxyl radical evolves subsequently through the three main mechanisms (hydrogen abstraction, cyclization, and β-scission) to the formation of carbon-centered radicals (allylic, oxiranylcarbinyl, methyl), all reactive toward histidine, whereas for the cyclic and secondary allylic hydroperoxide **4**, the possibilities of rearrangement were more limited. Significantly, this indicates that if a certain number of reactive radical intermediates can issue from these compounds depending on their chemical structure, different potentially immunogenic protein chemical modifications could be induced by these hydroperoxides. Different protein modifications may thus arise that could lead to sensitization.

EXPERIMENTAL SECTION

Caution: Skin contact with allylic hydroperoxides must be avoided because these compounds are skin-sensitizing substances. Hydrogen peroxide and allylic hydroperoxides must be handled with care, as hazardous side reactions are possible.

General Experimental Chemical Procedures. All reagents were obtained from commercial sources and used as received. Commercial linalool **1** and (−)-carveol **7** were available as mixtures of isomers. DMF was dried over phosphorus pentoxide prior to vacuum distillation. Air- or moisture-sensitive reactions were conducted in flame-dried glassware under an atmosphere of dry argon. All reactions leading to the formation of hydroperoxides were carried out protected from daylight. The reactions were followed by TLC performed on 0.25 mm silica gel plates ($60F_{254}$). After migration, the TLC plates were inspected under UV light (254 nm) and then sprayed with a solution containing phosphomolybdic acid (5 g), cerium(IV) sulfate (2 g), and sulfuric acid (12 mL) in water (188 mL), or a solution containing *p*-anisaldehyde (0.5 mL), *o*-anisaldehyde (0.5 mL), sulfuric acid (5 mL), and ethanol (8 mL) in acetic acid (100 mL), followed by heating. Column chromatography purifications were performed using silica 60 (Geduran, 40−63 μm) or previously neutralized silica gel. Neutralized silica was prepared by adding to an homogeneous water solution of silica 60 a saturated solution of $NaHCO_3$ until a pH of about 8. After decantation, the silica precipitate was washed with water to reach a pH of 7, filtered, and then dried in a drying oven for at least 24 h. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. The chemical shifts (δ) are reported in ppm and are indirectly referenced to TMS via the solvent signal ($CDCl_3$: δ ^{1}H = 7.26, δ ^{13}C = 77.16). In the ^{1}H NMR spectra multiplicities are denoted as s (singlet), d (doublet), dd (doublet of doublets), m (multiplet), and br (broad). The different types of carbon in the structures were identified by the DEPT-135 technique. The analytical data for known compounds matched the literature data.

Schenck Reaction: (5*E*)-7-Hydroperoxy-3,7-dimethylocta-1,5-dien-3-ol (3) and 6-Hydroperoxy-3,7-dimethylocta-1,7-dien-3-ol (5). A microemulsion was prepared by adding, drop by drop, an aqueous solution of Na_2MoO_4 (3.38 g in 18 mL of distilled water) into a suspension of sodium dodecyl sulfate (27.9 g) in butanol (34 mL) and dichloromethane (166 mL). The microemulsion became clear after 5 min stirring. A solution of linalool **1** (2.83 g, 17.8 mmol, 1 equiv) in the microemulsion (297 g) was treated at rt with a first portion of hydrogen peroxide (35 wt % in water, 1 mL). The brick red mixture was stirred for about 20 min, until it became light yellow. Another 14 portions of hydrogen peroxide (35 wt % in water, approximately 1 mL each) were successively added every 10 min. In the end, a total of 15.3 mL of aqueous hydrogen peroxide (35%) was used (179 mmol, 10 equiv). The light yellow mixture was stirred overnight at rt and became completely clear. The solvent was removed in vacuo and the crude product obtained dissolved in dichloromethane (900 mL). The suspension was stirred vigorously during 2 days and filtered. The filtrate, partially evaporated in vacuo, was washed with water (3 × 100 mL), and the aqueous layers were extracted with dichloromethane (1 × 100 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated in vacuo. Purification by flash chromatography (petroleum ether/EtOAc 8:2, then 6:4) furnished a mixture 4:6 of compounds **3** and **5** as a yellowish oil (2.64 g, 14.2 mmol, 80%). R_f = 0.24 (petroleum ether/EtOAc 7:3). Compound **3** (mixture of enantiomers): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 3 H), 1.32 (s, 6 H), 2.29 (m, 2 H), 5.06 (dd, J = 10.8, 1.1 Hz, 1 H), 5.19 (dd, J = 17.4, 1.1 Hz, 1 H), 5.58−5.73 (m, 2 H), 5.92 (dd, J = 17.3, 10.7 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 24.5, 27.8, 45.3, 72.9, 82.1, 112.3, 126.6, 138.1, 144.8; CAS registry number [51276-32-5]. Compound **5** (mixture of 4 diastereomers): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28

(s, 6 H), 1.51−1.66 (m, 8 H), 1.72 (bs, 6 H), 4.29 (m, 2 H), 4.99 (m, 4 H), 5.07 (dd, J = 10.8, 1.1 Hz, 2 H), 5.20 (dd, J = 17.4, 1.1 Hz, 1 H), 5.21 (dd, J = 17.4, 1.6 Hz, 1 H), 5.87 (dd, J = 17.4, 10.8 Hz, 1 H), 5.88 (dd, J = 17.4, 10.8 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.6 (2 C), 25.2 (2 C), 28. 1, 28.2, 37.7, 37.9, 73.3 (2 C), 89.5, 89.6, 112.3 (2 C), 114.2, 114.3, 143.7 (2 C), 144.7, 144.8; CAS registry number [51276-31-4]. These spectroscopic data are in agreement with those reported.[7,17]

Selective Protection: (5*E*)-7-(*tert*-Butyldiphenylsilylperoxy)-3,7-dimethylocta-1,5-dien-3-ol (6). In a flame-dried, two-necked, round-bottomed flask, under argon, were introduced a mixture of **3** and **5** (200 mg, 1.07 mmol, 1 equiv), dry DMF (5 mL), and imidazole (99.5%, 147 mg, 2.15 mmol, 2 equiv). *tert*-Butyldiphenylsilyl chloride (98%, 0.34 mL, 1.28 mmol, 1.2 equiv) was added, and the reaction mixture was stirred at rt. After 10 days, the yellow reaction mixture was quenched by the addition of water (30 mL), followed by extraction with pentane (3 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated in vacuo. The crude product was purified by flash chromatography (petroleum ether/EtOAc 9:1) to obtain a yellow oil (377 mg) corresponding to compound **6** together with a tiny amount of *tert*-butyldiphenylsilyl chloride. This mixture was directly used in the next step. R_f = 0.26 (petroleum ether/EtOAc 9:1); mixture of enantiomers; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.11 (s, 9 H), 1.24 (s, 3 H), 1.25 (s, 3 H), 1.26 (s, 3 H), 2.20−2.28 (m, 2 H), 5.02 (dd, J = 10.8, 1.3 Hz, 1 H), 5.17 (dd, J = 17.2, 1.3 Hz, 1 H), 5.51−5.68 (m, 2 H), 5.91 (dd, J = 17.4, 10.8 Hz, 1 H), 7.39 (m, 6 H), 7.71 (d, J = 1.5 Hz, 2 H), 7.74 (d, J = 1.3 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.7, 24.6, 24.9, 26.7, 27.5 (3 C), 45.5, 72.5, 82.6, 112.1, 124.8, 127.6 (4 C), 129.8 (2 C), 133.4 (2 C), 136.0 (4 C), 139.7, 144.8; HRMS (ESI) m/z calcd for $C_{26}H_{36}O_3Si$ [M + Na^+] 447.2330, found 447.2300.

(5*E*)-7-Hydroperoxy-3,7-dimethylocta-1,5-dien-3-ol (3) from (5*E*)-7-(*tert*-Butyldiphenylsilylperoxy)-3,7-dimethylocta-1,5-dien-3-ol (6). Compound **6** obtained in the previous step (377 mg), together with a tiny amount of *tert*-butyldiphenylsilyl chloride, was dissolved in methanol (25 mL) and the solution cooled to 0 °C. Potassium hydroxide (241 mg, 4.30 mmol, 4 equiv related to a 1.07 mmol mixture of **3** and **5**) was added all at once. The reaction mixture was allowed to warm to rt and stirred overnight. Water was added (125 mL), and the solution became milky white. The mixture was then neutralized with an aqueous solution of chlorhydric acid (2%, 70 mL) and extracted with diethyl ether (3 × 250 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated in vacuo. The crude product was purified by flash chromatography (petroleum ether/EtOAc 65:35) to obtain **3** as a yellow oil (77 mg, 0.41 mmol, 96% over two steps, calculated from **3** in a 4:6 mixture of **3** and **5**). R_f = 0.24 (petroleum ether/EtOAc 7:3); mixture of enantiomers; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 3 H), 1.32 (s, 6 H), 2.29 (m, 2 H), 5.06 (dd, J = 10.8, 1.1 Hz, 1 H), 5.19 (dd, J = 17.4, 1.1 Hz, 1 H), 5.58−5.73 (m, 2 H), 5.92 (dd, J = 17.3, 10.7 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 24.5, 27.8, 45.3, 72.9, 82.1, 112.3, 126.6, 138.1, 144.8; CAS registry number [51276-32-5]. These spectroscopic data are in agreement with those reported.[17]

2-Hydroperoxylimonene (4). To an aqueous solution of hydrogen peroxide (50%, 85 mL) were added, at 0 °C, some drops of concentrated sulfuric acid and (−)-carveol **7** (97%, 6.0 g, 38.2 mmol). The reaction mixture was vigorously stirred at 0 °C during 2 days, followed by extraction with pentane (5 × 200 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated in vacuo. The crude product was purified by flash chromatography on neutralized silica gel (pentane/diethyl ether 8:2) to obtain **4** as a colorless oil (3.08 g, 18.3 mmol, 48%). R_f = 0.21 (petroleum ether/EtOAc 95:5); mixture of diastereomers; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40−1.51 (m, 2 H), 1.75 (s, 6 H), 1.80 (s, 6 H), 2.13−2.38 (m, 8 H), 4.36 (s, 1 H), 4.53 (m, 1 H), 4.74 (s, 2 H), 4.75 (s, 2 H), 5.64 (m, 1 H), 5.75 (d, J = 5.0 Hz, 1 H), 7.67 (s, 1 H), 8.02 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.3, 21.4, 20.6, 21.0, 31.0, 31.3 (2 C), 32.6, 35.3, 40.7, 82.7, 84.4, 109.2, 109.4, 127.2, 129.4, 129.8, 132.9, 148.8, 149.3; CAS registry number [1045725-54-9]. These spectroscopic data are in agreement with those reported.[14]

EPR Spin-Trapping Studies. DEPMPO was synthesized as reported in the literature.[55] All other reagents were obtained from commercial sources and used as received. Spectrophotometric grade CH_3CN (99.8%) was used, and aqueous solutions were prepared with deionized water. EPR spectra were recorded on a spectrometer equipped with an X-band microwave bridge (10 GHz). The standard spectrometric settings were modulation frequency 100 kHz, microwave power 7 mW, modulation amplitude 1.0 G, 2.2, or 7.1 G, receiver gain 2 × 10^6, scan width 100 or 120 G, and conversion time 25 ms. The g calibration standard was a strong pitch of known g factor (2.0028).[56] For these EPR spin-trapping experiments in iron systems, special glassware was used where the hydroperoxide and the spin-trap in solution were initially in a separate compartment (round-bottom container) from that of the iron reagent (capillary tube). Compound **3** (5 mg, 27 μmol) or compound **4** (5 mg, 30 μmol) and the spin-trap, DMPO (5 mg, 43 μmol) or DEPMPO (5 mg, 21 μmol), were dissolved in 500 μL of deaerated CH_3CN or in 500 μL of a deaerated 1:1 (v/v) H_2O/CH_3CN mixture. The solution was placed in the round-bottom compartment of the EPR glassware. In the capillary tube the Fe(II)/Fe(III) reagent was introduced, $FeSO_4$ (heptahydrate, 3 mg, 11 μmol), TPP-Fe^{3+} (3 mg, 4 μmol), or hemin (3 mg, 4 μmol). The compartments were interlinked to allow the subsequent mix of the reagents. To avoid mixing the hydroperoxides too rapidly when being poured over the iron derivative, a layer of polyvinyl alcohol was used as a filter. In order to carry out the experiments in the absence of oxygen, to avoid the formation of secondary oxidation products, a vacuum line using the standard freeze−thaw technique degassed the whole system. Then, the solution containing **3** or **4** was poured into the capillary tube containing the layer of polyvinyl alcohol and iron underneath. An EPR spectrum of the reaction mixture was recorded directly by placing the capillary tube in the spectrometer cavity. Hyperfine splitting assignments were obtained by means of computer simulation using the Bruker WINEPR SimFonia software (version 1.25; Rheinstetten, Germany) and were in agreement with those reported in the literature.[14,57−60]

Reaction with *N*-Acetylhistidine Methyl Ester Catalyzed by Fe(II)/Fe(III). Allylic hydroperoxide **3** (35 mg, 0.19 mmol) or **4** (300 mg, 1.78 mmol) were dissolved in a deaerated 1:1 (v/v) H_2O/CH_3CN mixture (10 mL for **3**, 100 mL for **4**). *N*-Ac-His-OMe was added to the solutions (2 equiv) together with a catalytic amount of $FeCl_3$ or $FeSO_4$ heptahydrate (0.1 equiv). The reaction mixtures were continuously stirred at room temperature. The reactions were monitored by TLC (0.25 mm silica gel plates, $60F_{254}$). After migration, the TLC plates were inspected under UV light (254 nm) and then sprayed with a solution containing phosphomolybdic acid (5 g), cerium(IV) sulfate (2 g) and sulfuric acid (12 mL) in water (188 mL), or a solution containing *p*-anisaldehyde (0.5 mL), *o*-anisaldehyde (0.5 mL), sulfuric acid (5 mL), and ethanol (8 mL) in acetic acid (100 mL), followed by heating. The reactions were stopped after 7 days, as the starting hydroperoxides were still present in the reaction mixtures and the reactions did not evolve after that time. In order to remove iron salts, the mixtures were filtered on Celite (Celite 545). The solvent was removed under reduced pressure, and the crude products obtained were analyzed by LC-ESI-MS associated with ESI-MS/MS. LC-ESI-MS analyses were performed using a HPLC system equipped with a binary pump, an automatic sample injector, and a diode array absorbance detector scanning 190 to 700 nm. The samples were subjected to reverse phase chromatography on a C18 column (1 × 100 mm; Thermo Hypersil Gold; 1.9 μm particle size) at a flow rate of 0.2 mL/min. Samples were eluted from the column using a mobile phase B (0.01% formic acid in H_2O) and a mobile phase A (CH_3CN) with a gradient. The gradient started at 98% B and was decreased to 10% B after 20 min, was at 10% B during 10 min, and was increased again to 98% B in the last 10 min. The injection volume was 0.1 mL. After passing through the diode array

absorbance detector, the eluent was directed to a connected ion trap mass spectrometer with a standard electrospray source. The ionization method used was ESI in the positive ion mode (heated capillary temperature 325 °C, gas flow 10 L/min, nebulizer gas 40 psi). Full scan mass spectra were acquired in the profile mode scanning m/z 50 to 1000. For the ESI-MS/MS studies, the mass spectrometer was equipped with the ESI source used for the ESI-MS studies described above and with the same source parameter settings. The ionization method used was ESI in the positive ion mode. The isolation width was of 4 m/z. All data were processed using Agilent MassHunter Qualitative Analysis software.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of all compounds. HRMS (ESI) for compound **6**. This material is available free of charge through the Internet at http://pubs.acs.org. This material is available free of charge via the Internet at http://pubs.acs.org.

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