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Identification of radical species derived from allergenic 15-hydroperoxyabietic acid and insights into the behaviour of cyclic tertiary allylic hydroperoxides in Fe(II)/Fe(III) systems

Elena Giménez-Arnau [a,*], Laure Haberkorn [a], Loris Grossi [b], Jean-Pierre Lepoittevin [a]

[a] Institut de Chimie de Strasbourg (CNRS-ULP), Laboratoire de Dermatochimie, Clinique Dermatologique, CHU, 1 Place de l'Hôpital, 67091 Strasbourg, France
[b] Dipartimento di Chimica Organica 'A. Mangini', Università di Bologna, Viale Risorgimento 4, 40136 Bologna, Italy

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ABSTRACT

To understand the skin sensitization mechanism of 15-hydroperoxyabietic acid, the major allergen in colophony, we first examined the formation of potential reactive radicals derived from its reaction with light, heat and TPP-Fe^{3+}. Trapping with 1,1,3,3-tetramethylisoindolin-2-yloxyl nitroxide confirmed the formation of carbon-centred radicals derived from allyloxyl/allylperoxyl radicals as a consequence of the hydroperoxide scission. Particular interest was further given to the reactivity with Fe(II)/Fe(III) due to the biological importance of haem containing enzymes. Using a monocyclic 15-hydroperoxyabietic acid-like compound as a model of allergenic allylic hydroperoxides, we evidenced, by the ESR spin-trapping technique, the competition between carbon and oxygen-centred radicals formed in the presence of Fe(II)/Fe(III) in organic/aqueous media. We complemented the study by showing the possibility of formation, via a radical mechanism induced by ferric chloride, of an adduct between the allylic hydroperoxide and *N*-acetyl-cysteine ethyl ester. The results gave new knowledge on the possible generation of highly reactive radicals that could lead to the formation of antigenic structures.

1. Introduction

The accepted mechanism for antigen formation in the allergic contact dermatitis pathology is the generation of covalent bonds between electrophilic chemical functions present on allergens, low-molecular weight compounds penetrating the skin barrier, and nucleophilic residues on skin proteins.[1] The processing of such hapten–protein complex by immunocompetent skin antigen-presenting cells and the transmission of this information to T-cells in the lymph nodes lead to erythema and oedema, the major clinical signs of the pathology.[2] Initially harmless molecules can be converted into electrophilic derivatives and, therefore, possibly allergenic compounds, via metabolic processes mainly based on oxido-reduction reactions,[3] or non-enzymatically as for the reaction with atmospheric oxygen.[4] Terpenes, common compounds of natural origin containing double bonds and, consequently, prone to oxidation on air exposure, belong to this category of allergens. In fact, many allylic hydroperoxides derived from autoxidation of terpenes are known to be strong skin sensitizers.[5–7] However, allylic hydroperoxides are not electrophiles and represent, therefore, an alternative mode of reaction with proteins to form antigenic structures, probably via the involvement of reactive radical intermediates that have gained increased importance in the discussion of hapten–protein binding in the latest years.[1]

In the course of our studies on allergenic allylic hydroperoxides, we have been interested in 15-hydroperoxyabietic acid **1** (Fig. 1), identified as the major allergen in colophony, a naturally occurring material obtained from coniferous trees and used in multiple products due to its stickiness, emulsifying and isolating properties.[8] 15-Hydroperoxyabietic acid **1** is the most important oxidation

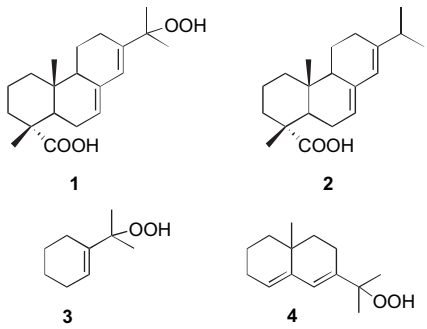

Figure 1. Chemical structures of 15-hydroperoxyabietic acid **1**, abietic acid **2** and model compounds **3** and **4**.

* Corresponding author. Tel.: +33 3 88 35 06 64; fax: +33 3 88 14 04 47.
E-mail address: egimenez@chimie.u-strasbg.fr (E. Giménez-Arnau).

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derivative in air of abietic acid **2**, which is the principal diterpene resinic acid constituting colophony.[9]

In the context of contact allergy and to understand the interaction of **1**-like compounds with proteins, we started our studies by reporting the identification of potential reactive radicals derived from two model compounds, **3** and **4**, that were found to be also strong sensitizers.[10,11] Allylic hydroperoxides are known to form allyloxyl and allylperoxyl radicals, which rapidly decompose via intramolecular cyclization, fragmentation or hydrogen abstraction to form carbon-centred radicals.[12] Carbon-centred radicals derived from the monocyclic model **3** were completely identified using a combination of two spin-trapping techniques, the former leading to neutral compounds and the second allowing the detection of radical intermediates by ESR. For converting radical species induced by light, heat or TPP-Fe^{3+} into neutral molecules, the stable 1,1,3,3-tetramethylisoindolin-2-yloxyl nitroxide **5** was used as trap. For the ESR spin-trapping studies, the allylic alcohol precursor of the hydroperoxide was used. In particular, the allyloxyl radical was generated, in situ, by photolysis of the corresponding nitrite, formed by reacting the parent allylic alcohol with *t*-BuONO, which was also acting as the spin trap. The formation of epoxy alkyl radicals via intramolecular cyclization of the parent allyloxyl radical was proven, as well as the abstraction of hydrogens from different allylic positions as evidenced by the trapping of the corresponding allylic radicals.[11] Similar studies carried out with the bicyclic model **4** and the epoxy alkyl radical derived from it showed the existence of resonance hybrids of an allyl type radical due to the presence of the conjugated double bond (Fig. 2).[10]

To complement our previous studies, we now report the potentially reactive carbon-centred radicals that can be derived from allergenic 15-hydroperoxyabietic acid **1**, identified by radical trapping by employing **5** and using light, heat or TPP-Fe^{3+} as radical inducer. The structure of the isolated adducts was determined using one- and two-dimensional NMR. In parallel, being iron the most abundant metal in humans, and for understanding the reactivity of **1** with TPP-Fe^{3+}, we studied more in detail the behaviour of such cyclic tertiary allylic hydroperoxides in Fe(II)/Fe(III) systems by ESR spectroscopy. But, since **1** was found to be unstable, and, therefore, pure samples for ESR experiments could not be obtained, the model compound **3** was used together with DMPO and DEPMPO as spin traps. We confirmed the formation of carbon-centred radicals, as well as the formation of allyloxyl/peroxyl radicals, the latter was not evidenced by **5** since it was able to trap just carbon-centred radicals. Finally, being cysteine the most labile amino acid in biological radical reactions, we studied the reaction of **3** with *N*-acetyl-cysteine ethyl ester catalyzed by ferric chloride. An adduct was formed by the reaction of the lateral chain of *N*-acetyl-cysteine ethyl ester, through the thiyl radical, with a carbon-centred free radical in an allylic position of **3**. The evidence of carbon and oxygen-centred radicals' formation, which can be derived from allergenic allylic hydroperoxides, together with the possible degradation of these compounds induced by the cysteine–ferric chloride system, gave new insights into the in situ generation of highly reactive radicals in the epidermis. These species could lead to the formation of antigenic structures, the first step of the allergic contact dermatitis mechanism.

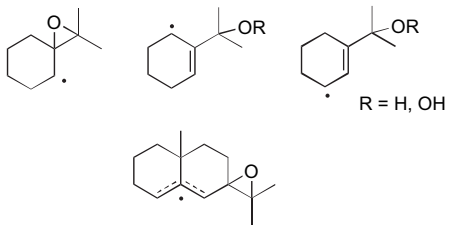


Figure 2. Carbon-centred radicals derived from previously studied model compounds **3** and **4**.

2. Results and discussion

2.1. Radical trapping experiments with 15-hydroperoxyabietic acid

It is well known that 1,1,3,3-tetramethylisoindolin-2-yloxyl nitroxide **5** couples with carbon-centred radicals to give stable non-radical alkoxyamine products, which can be characterized by using a full range of spectroscopic techniques.[13] In this study, we evaluated the formation of carbon-centred radical species derived from 15-hydroperoxyabietic acid **1** by the aminoxyl radical trapping technique using nitroxide **5** as a scavenger. The advantages of this nitroxide are several: it exhibits a chemical inertness, uncommon for most radicals, it is thermally stable, symmetrical (leading to a simplified NMR spectrum of the products) and has a suitable UV absorbing chromophore.

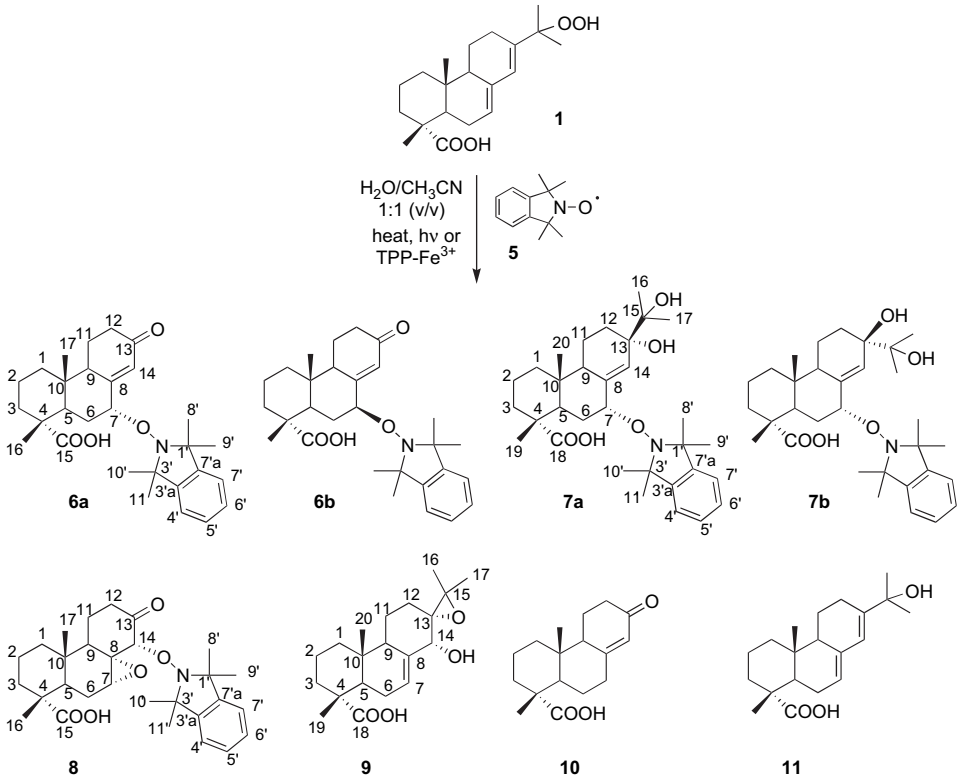
We carried out the experiments in a 1:1 (v/v) H_2O/CH_3CN mixture. Non-polar organic solvents can mimic the reaction conditions within, for example, a membrane, but the reactivity of allylic hydroperoxides in aqueous solutions is also of interest, since it is assumed that many biological processes proceed at aqueous/organic medium interface. Moreover, the skin could also be seen as a semi-organic medium mixing hydrophilic and hydrophobic properties. Radical formation was directly induced by thermal or photochemical cleavage of **1** and by using the iron(III) porphyrin complex TPP-Fe^{3+}. Reactions' proceed was followed by TLC until complete disappearance of **1**. The reaction products (Scheme 1) were isolated by column chromatography on silica gel and their chemical structures identified by NMR (^1H, ^{13}C, DEPT, COSY, NOESY, ^1H–^{13}C HSQC and HMBC).

*2.1.1. Structural analysis of compounds **6a**, **6b**, **7a**, **7b** and **8***

The chemical structure of adducts resulting from radical trapping by **5** was determined by two-dimensional (2D) NMR. ^1H–^{13}C HSQC experiments allowed the correlation of each proton to a carbon atom through ^1J(C, H). Then, the skeleton of the molecules was build thanks to the ^2J(C, H) and ^3J(C, H) long-range couplings observed in the HMBC spectra. Finally, the relative stereochemistry of the diverse substituents on the adducts was resolved using NOESY data on ^1H–^1H correlations through space.

Analysis of the one-dimensional (1D) ^1H and ^{13}C NMR spectra of isolated diastereomers **6a** and **6b** showed the presence of a new carbonyl chemical function at C-13 instead of the **1** isopropyl fragment. Also, the disappearance of the **1** vinyl proton at 5.42 ppm, the new set of signals in the 6.8–7.2 ppm region corresponding to the scavenger aromatic protons and the signals for a proton in position α to an alkoxyamine for each diastereomer at 4.10 and 4.39 ppm, respectively, were in agreement with the formation of an adduct between **5** and a carbon-centred radical derived from **1** at C-7. The relative stereochemistry of **6a**, having H-7 (4.10 ppm) on an equatorial position, was established by NOESY ^1H–^1H correlations between H-7 and H-14 (6.20 ppm), and H-7 and the two H-6 (1.46 and 1.79 ppm). The absence of a NOESY cross-peak between H-7 (4.39 ppm) and H-14 (6.89 ppm) in adduct **6b**, together with H-7 being correlated with H-5 (1.86 ppm), H-6_{eq} (2.54 ppm) and H-9 (1.41 ppm), were in favour of H-7 being on an axial position (Fig. 3). Mass spectrometric analysis of **6a** and **6b** showed an *m*/*z* value of 466 that supported the structures elucidated by the NMR analysis.

Diastereomers **7a** and **7b** were also isolated easily by column chromatography on silica gel. As for **6a** and **6b**, the disappearance of the **1** vinyl proton at 5.42 ppm was observed in the 1D ^1H NMR spectra, and new signals appeared instead for a proton in position α to an alkoxyamine for each diastereomer at 4.27 and 4.20 ppm,



Scheme 1. Trapping of carbon-centred radicals derived from **1**.

respectively. This was in agreement with the formation of an adduct with **5** at C-7. On another hand, the chemical shifts of carbon atoms C-13 (141.6 ppm) and C-15 (81.9 ppm) of **1** did move to 73.8 and 74.2 ppm in the case of one diastereomer, and to 74.1 and 73.7 ppm in the case of the other one. These values were characteristic of carbons bearing a tertiary alcohol. NOESY experiments concluded the axial orientation of scavenger **5** in both **7a** and **7b**. In the two structures, H-7 showed a cross-peak with the vinyl H-14 and the two H-6 protons. The relative stereochemistry distinguishing **7a** from **7b** was defined on C-13. Adduct **7a** had the isopropyl group on an axial position as protons of the methyl groups C-16 (1.16 ppm) and C-17 (1.24 ppm) were correlated with protons of the C-20 methyl group (0.69 ppm). Adduct **7b** had the isopropyl group on an equatorial position as protons of the methyl groups C-16 (1.19 ppm) and C-17 (1.21 ppm) were correlated with protons of a methyl group belonging to **5** and with the vinyl proton H-14 (6.12 ppm) (Fig. 3). Mass spectrometric analysis of **7a** and **7b** showed an *m*/*z* value of 526 that supported the structures elucidated by the NMR analysis.

Complete NMR analysis of adduct **8** indicated the loss of the isopropyl group of **1**, its replacement by a carbonyl chemical function and the disappearance of all vinyl protons. Moreover, the quaternary carbon C-8 of **1** at 134.7 ppm had also disappeared. Again, new signals in the aromatic region corresponding to the scavenger (6.89–7.09 ppm) and also a signal corresponding to a proton in position α to an alkoxyamine (3.96 ppm) were observed. A new tertiary carbon at 56.9 ppm and a new quaternary carbon at 58.6 ppm indicated the epoxidation of the C-7/C-8 double bond of **1**. As a consequence, it was concluded that **5** could only be bound at C-14. The relative stereochemistry of the substituents on positions

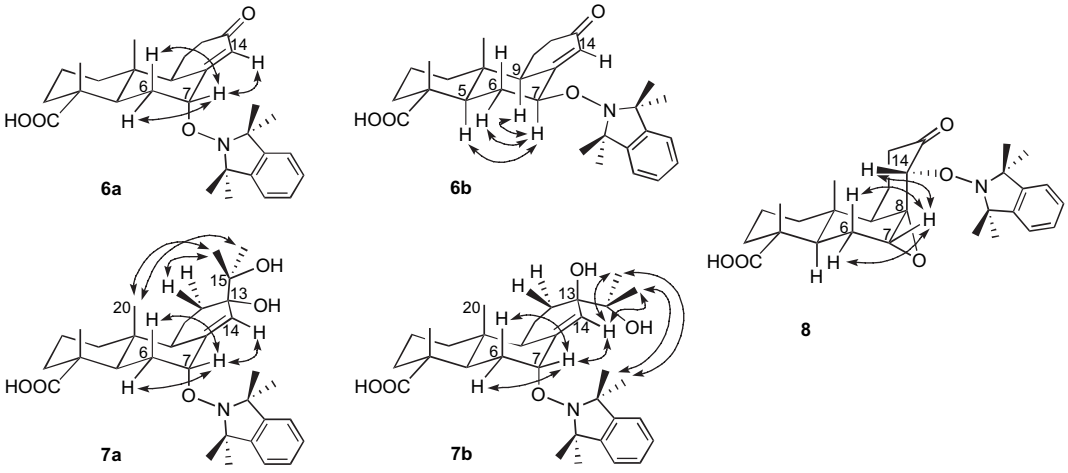


Figure 3. Main NOESY effects observed in compounds **6a**, **6b**, **7a**, **7b** and **8**.

7, 8 and 14 was established thanks to the data furnished by the NOESY experiments, being proton H-7 (3.31 ppm) correlated with H-14 (3.96 ppm) and the two H-6 protons (1.61 and 1.88 ppm) (Fig. 3). Mass spectrometric analysis of **8** showed an *m*/*z* value of 482 that supported the structure elucidated by the NMR analysis.

2.1.2. Structural analysis of compounds *9*–*11*

Each compound was identified by comparison with the reference materials obtained from a control experiment carried out without **5**. Compound **9** was found to be an α hydroxy epoxide. The NMR spectra showed the loss of the C-13/C-14 double bond of the starting material and there were no signals that could be assigned to scavenger **5**. However, a new tertiary carbon at 70.8 ppm, with a related proton at 3.97 ppm, was pointed out. These chemical shifts were typical of a carbon atom bearing a hydroxyl chemical group and of a proton in geminal position to this hydroxyl. Also, the chemical shifts of C-13 (141.6 ppm) and C-15 (81.9 ppm) of **1** did move to 66.5 and 63.1 ppm, respectively, which were typical values of quaternary carbon atoms belonging to an epoxide. The relative stereochemistry of the substituents on positions 13 and 14 was established thanks to the data furnished by the NOESY experiments. The C-14 hydroxyl chemical group was on an axial position. The epoxide was orientated to the α side of the molecule as protons of the methyl group at C-16 (1.09 ppm) were correlated with H-12_{eq} (1.82 ppm) and those of the methyl group at C-17 (1.26 ppm) with H-14 (3.97 ppm). Finally, the structures of unsaturated ketone **10** and tertiary alcohol **11** were well known as these compounds were characterized in previous work on the hemisynthesis of **1**.[14]

2.1.3. Mechanistic interpretations

Reaction products were formed in different yields depending on the method used to induce radical generation. As shown in Table 1, in the presence of TPP-Fe^{3+} (1:1 (v/v) H_2O/CH_3CN solvent, room temperature, 1 h) the decomposition of allylic hydroperoxide **1** was very fast but only compounds **6a**, **7a** and **7b** were isolated. In contrast, both the thermal (1:1 (v/v) H_2O/CH_3CN solvent, reflux, 22 h) and the photochemical (1:1 (v/v) H_2O/CH_3CN solvent, UV–vis, 20 h) decomposition of **1** resulted slower, leading to several reaction products and in the same percentage. The only difference was the formation of compound **8** when the reaction was induced photochemically. In general, the overall yield of trapped radicals was very similar for both the thermal and the photochemical reactions. A complementary experiment in the absence of **5** was carried out. Obviously, in this case, only compounds resulting from the decay of carbon and oxygen-centred radicals were isolated. Among them, **9** and **11** in a 3% and 5% yields, respectively. However, the formation of other compounds in a very low yield, even if not isolated, cannot be excluded.

As models for the haem-containing enzymes, reactions of iron(III) porphyrin complexes with oxidants, such as peroxyacids and hydroperoxides, have been extensively studied with the intention of elucidating the mechanisms of O–O bond activation and oxygen atom transfer reactions. Despite intensive investigation on haem-hydroperoxide reactions for the last three decades, a clear mechanistic consensus has not evolved on the homolytic or heterolytic O–O bond cleavage by iron porphyrin complexes. One of the frequently used mechanistic tools to distinguish the homolytic/heterolytic O–O bond cleavage of hydroperoxides is to analyze the products formed in the epoxidation of olefins by iron porphyrin complexes and hydroperoxides.[15–17] It is commonly accepted that a high yield of epoxidation indicates the formation of a high-valent iron(IV) oxo porphyrin cation radical intermediate **12** via heterolytic O–O bond cleavage (Scheme 2, pathway A), while the generation of a ferryl-oxo complex **13** via O–O homolysis of the hydroperoxide bond affords low yield or none olefin epoxidation (Scheme 2, pathway B). Traylor and co-workers suggested a different mechanism for the product distribution of O–O bond homolysis.[15,18] It was proposed that the heterolytic O–O bond cleavage was the starting process, followed by a fast side reaction between **12** and the hydroperoxide evidenced in the product distribution of O–O bond homolysis (Scheme 2, pathway A followed by C). Furthermore, experiments conducted with oxidant mixtures let to conclude that even if **12** is reacting fast with ROOH, the low epoxidation due to the homolytic O–O bond cleavage cannot be excluded.

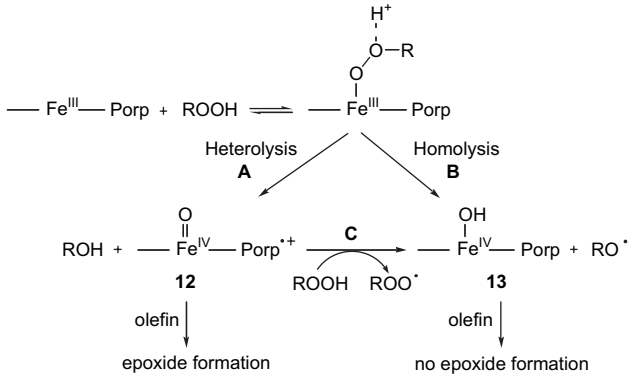
In the reaction between TPP-Fe^{3+} and **1**, the epoxidation of the double bonds was not observed. On the contrary, the presence of both the peroxyl (ROO•) and the alkoxyl (RO•) radicals was necessary to account for adducts **6a**, **7a** and **7b**. Therefore, the O–O bond cleavage had to take place and possibly through pathways A and C (Scheme 2). The peroxyl radical ROO• formed could rearrange via intramolecular addition to the C-13/C-14 double bond to a carbon-centred radical with a dioxetane structure, which is known to cleave to get acetone and a carbonylic compound.[19] The radical formed at C-14, due to the presence of the allylic double bond, coexisted with the resonance hybrid form containing the carbon-centred radical at C-7, which when trapped by **5** led solely to the diastereomer **6a** (Scheme 3). On another hand, also the alkoxyl radicals RO• could undergo a cyclization and lead to an α-oxiranylcarbinyl radical in resonance with other allyl radicals due to the presence of the allylic double bond. In an aqueous medium, the trapping of this radical by **5** and the following epoxide ring opening can account for the formation of diastereomers **7a**/**7b** (Scheme 3).

Under thermal or photochemical action, hydroperoxides can cleave homolytically to produce RO• and hydroxyl (HO•) radicals. The latter, via hydrogen abstraction from unreacted hydroperoxide molecules, accounts for ROO• formation,[20] even if direct homolysis of the O–H bond of the hydroperoxide group under heating or irradiation conditions has also been reported.[21,22] Alkoxyl RO• and peroxyl ROO• radicals so-formed can account for the formation of all the derivatives **6a**/**6b** and **7a**/**7b** (Scheme 3).

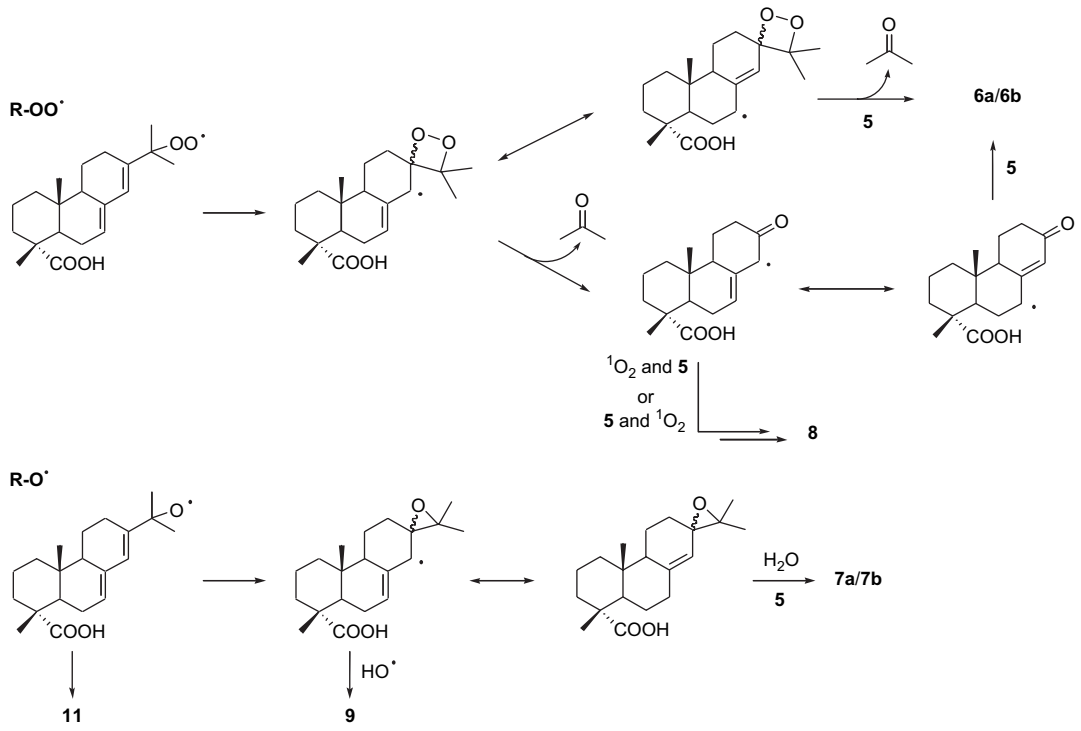
Table 1
Yields of the products formed in the radical trapping experiments with **1** according to the radical inducer used

Radical inducer	Time[a] (h)	Products yield (%)							
		6a	**6b**	**7a**	**7b**	**8**	**9**	**10**	**11**
TPP-Fe^{3+}	1	7	—	2	2	—	—	—	—
Heat	22	4	3	1	6	—	5	10	3
Heat[b]	5	—	—	—	—	—	3	17	5
hν	20	4	3	1	6	3	5	10	3

[a] The reactions were followed by TLC and stopped after complete disappearance of **1**.
[b] Control experiment was carried out in the absence of **5**.



Scheme 2. Heterolytic or homolytic mechanism for the O–O bond cleavage of hydroperoxides by iron(III) porphyrin complexes.



Scheme 3. Mechanistic interpretation for the generation of **6a**/**6b**, **7a**/**7b**, **8**, **9** and **11**.

As mentioned above, only **6a** was isolated after 1-h reaction between **1** and TPP-Fe^{3+} (complete disappearance of **1**). However, additional NMR follow-up of the reaction, in deuterated chloroform, showed the appearance of signals belonging to **6b** concomitantly to the decrease in the intensity of signals attributed to **6a** and reaching the same concentration in the reaction mixture after 2 days. We hypothesized that both keto diastereomers were in equilibrium through an enol non-isolated intermediate as proved by NMR experiments conducted in deuterated benzene, in which the equilibrium between **6a** and **6b** was reached after 7 days. Finally, **1** resulted much less reactive when the process was induced by heat or light and its decomposition took place in circa 20 h. This allowed enough time for the **6a**/**6b** equilibrium to be reached and then the possibility to isolate both diastereomers. Concerning adducts **7a**/**7b**, only the carbon-centred radical at C-7 was trapped by **5** and that is probably because of the steric hindrance of the scavenger and of the isopropyl group. The relative stereochemistry of substituents on C-13 was defined by the epoxide ring opening and not by the cyclization of the alkoxyl radical. Indeed, only the α-oxiranylcarbinyl radical resulting from the cyclization of the alkoxyl radical on the α-side of the molecule was trapped by HO•, a less bulky group able to trap rapidly the carbon-centred radical at C-14 accounting for the formation of **9** (Scheme 3).

Compound **8** was detectable only when the parent peroxyl radical was formed via photolysis. In particular, to account for the carbonyl group at C-13 the decomposition of an intermediate dioxetane as described for **6a**/**6b** has to be invoked and the resulting radical at C-14 trapped by **5**. The formation of the C-7α/C-8α epoxide could be explained by photo-oxidation of the double bond in the presence of singlet dioxygen (Scheme 3). The mechanism of this reaction, even if not completely well understood, has been described on the basis of the formation and evolution of intermediates of the perepoxide kind.[23,24]

A possible mechanism explaining the formation of α,β-unsaturated ketone **10** would involve the heterolytic fragmentation of the hydroperoxide combined with a migration of the vinyl group, which is known as the Hoch rearrangement, a facile reaction of allylic hydroperoxides to afford carbonylic compounds.[25] Even if this reaction, in general, occurs under acidic catalysis, several studies describe the possible degradation of allylic hydroperoxides in accordance with this mechanism by heating.[26,27] Thus, the formation of **10** in experiments carried out by heating or by photolysis cannot be disregarded as it is obtained with significant yields. Finally, the tertiary alcohol **11** can be accounted for by hydrogen abstraction of the corresponding alkoxyl radical RO•.

2.2. ESR spin-trapping experiments with model compound 3 in the presence of Fe(II)/Fe(III)

Combined with ESR spectroscopy, the spin-trapping technique, nowadays considered as the best method for the detection of short-lived radicals, has been extensively used for the detection of free radicals generated in biological milieu.[28] This method is based on the reaction of a spin trap with a radical to form a more stable radical adduct that can be detected by ESR. Cyclic nitrones, especially pyrroline *N*-oxides, exhibit very interesting properties as spin traps. Among them, 5-diethoxy-phosphoryl-5-methyl-1-pyrroline *N*-oxide (DEPMPO) has gained wider acceptance because of the very characteristic ESR spectra that are observed upon reaction with free radicals. The hyperfine coupling constants (hfc) afford valuable information on the radical trapped and today DEPMPO is recognized as the most efficient spin trap for superoxide in vivo. Indeed, the HOO–DEPMPO• adduct results much more persistent compared to the analogous HOO–DMPO• adduct obtained with 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO).[29] Moreover, the DEPMPO allows an easy distinction between oxygen-centred adducts (peroxyl/alkoxyl) and carbon-centred adducts.[30] Thus, to get more information on the reactivity of allergenic cyclic tertiary allylic hydroperoxides with iron systems, experiments were conducted using the stable model compound **3** and DEPMPO, and the results compared with those obtained using DMPO.

Figure 4 shows the ESR spectra obtained from the reaction of **3** with Fe(II) in the presence of DEPMPO or DMPO, in CH_3CN or in a 1:1 (v/v) H_2O/CH_3CN mixture. Using DEPMPO in a CH_3CN solution

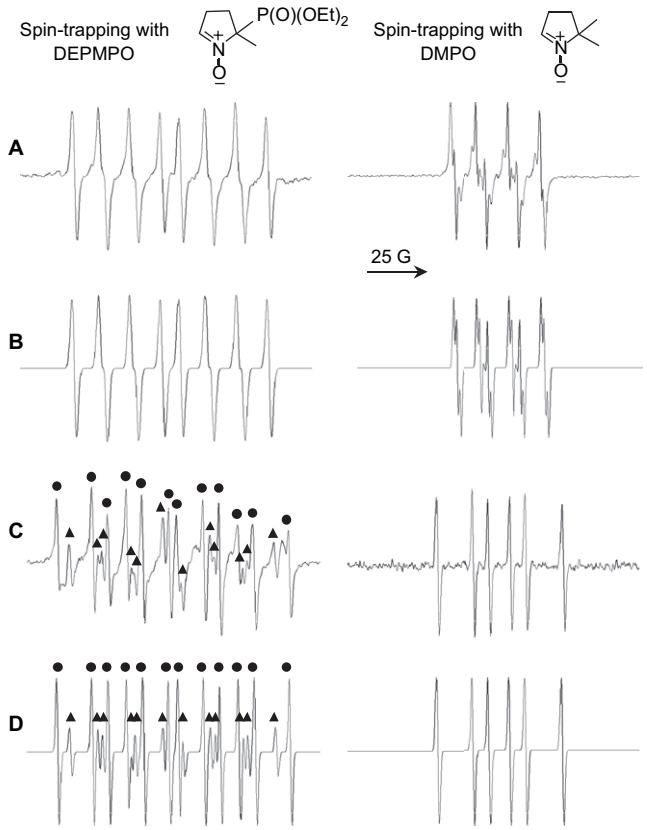


Figure 4. ESR spectra obtained from the reaction of **3** with Fe(II) in the presence of DEPMPO or DMPO, in CH_3CN or in a 1:1 (v/v) H_2O/CH_3CN mixture. ESR settings: microwave power 1 mW, microwave frequency 9 GHz, modulation frequency 100 KHz, modulation amplitude 1 G, scan width 100 G, conversion time 25 ms, receiver gain 1×10^5. (A) A solution in CH_3CN of compound **3** (60 mM) and the spin trap (90 mM) was reacted with $FeSO_4$ heptahydrate (2 mg). (B) Computer simulation of spectra of panel A. The best fit was obtained with coupling constants a_N=13.17 G, a_H=11.14 G and a_P=45.24 G (g=2.0064) for RO–DEPMPO• or ROO–DEPMPO•, and a_N=14.15 G, a_H=10.60 G and a_H=1.49 G (g=2.0063) for RO–DMPO• or ROO–DMPO•. (C) A 1:1 (v/v) H_2O/CH_3CN solution of compound **3** (60 mM) and the spin trap (90 mM) was reacted with $FeSO_4$ heptahydrate (2 mg). (D) Computer simulation of spectra of panel C. A composite spectrum was obtained when using DEPMPO with coupling constants a_N=13.81 G, a_H=11.92 G and a_P=47.79 G (g=2.0064) for RO–DEPMPO• or ROO–DEPMPO• (△), and a_N=14.81 G, a_H=21.72 G and a_P=47.33 G (g=2.0059) for a DEPMPO-carbon-centred adduct (○). For DMPO the best fit was obtained with coupling constants a_N=15.51 G, a_H=22.26 G (g=2.0059) for a DMPO-carbon-centred adduct.

(Fig. 4A), the main trapped radical was characterized by hfc values (a_N=13.17 G, a_H=11.14 G and a_P=45.24 G; g=2.0064) that let to hypothesize the presence of an oxygen-centred radical, in particular, the adduct of the superoxide to the DEPMPO.[29,30] As ROO–DEPMPO• adducts are in general characterized due to their close similarity to that of the HOO–DEPMPO• adduct, the trapping of an ROO• radical could be suggested. However, similar values of hfc are reported for adducts to DEPMPO of alkoxyl radicals, such as *tert*-butyloxyl and radicals derived from peroxidized lipids, and then the possibility of trapping of an RO• radical cannot be excluded.[30] This was confirmed in experiments conducted in the presence of DMPO, which let to detect a radical species characterized by hfc (a_N=14.15 G, a_H=10.60 G and a_H=1.49 G; g=2.0063) that were in good agreement with those reported for tertiary peroxyl radicals, such as those derived from *tert*-butyl or cumene hydroperoxide, trapped by DMPO.[31] Later, the spin-trapping literature reported that many DMPO peroxyl radical adducts have virtually the same hfc as alkoxyl radical adducts, raising the issue of correct assignment to peroxyl radical adducts.[32] Based on ^{17}O-labelling, but also on liquid chromatography/ESR, electrospray-ionization mass spectrometry and tandem mass spectrometry, further studies characterized the previously assigned *tert*-butylperoxyl and cumylperoxyl DMPO adducts, formed from the reaction of Fe(II) with *tert*-butyl or cumene hydroperoxide, as the result of the trapping of the *tert*-butyloxyl and cumyloxyl radicals, respectively.[33,34] In accordance with the described controversial assignment of peroxyl and alkoxyl radical adducts, with both spin traps, it was necessary to consider the possibility of having the concurrent presence of both types of oxygen-centred radicals. Actually, from the mechanistic point of view, a Fenton-like reaction with Fe(II) will at first produce RO• radicals, which easily can abstract the hydrogen of the hydroperoxide group (ROO–H) to lead to ROO• radicals. These radicals are known to undergo bimolecular self-reactions to form a tetroxide intermediate, ROOOOR, which decomposes irreversibly to give alkoxyl RO• radicals and dioxygen (Scheme 4).[31]

When experiments with DEPMPO were conducted in H_2O/CH_3CN (1:1 (v/v)) as solvent, the ESR spectrum (Fig. 4C) showed the presence of an oxygen-centred adduct, besides a species characterized by hfc (a_N=14.81 G, a_H=21.72 G and a_P=47.33 G; g=2.0059) typical of carbon-centred radical adducts.[35] The formation of a carbon-centred radical was also confirmed when DMPO was used, resulting in the formation of a carbon-centred DMPO adduct (a_N=15.51 G and a_H=22.26 G).[36] As carbon-centred radicals were not trapped when CH_3CN was used as solvent, it might be expected that oxygen-centred radicals are trapped before rearranging. On the other hand, in H_2O/CH_3CN as solvent, the spin-trapping process should result slower, thus allowing enough time for the rearrangement to take place, and then account for the formation of carbon-centred radicals.

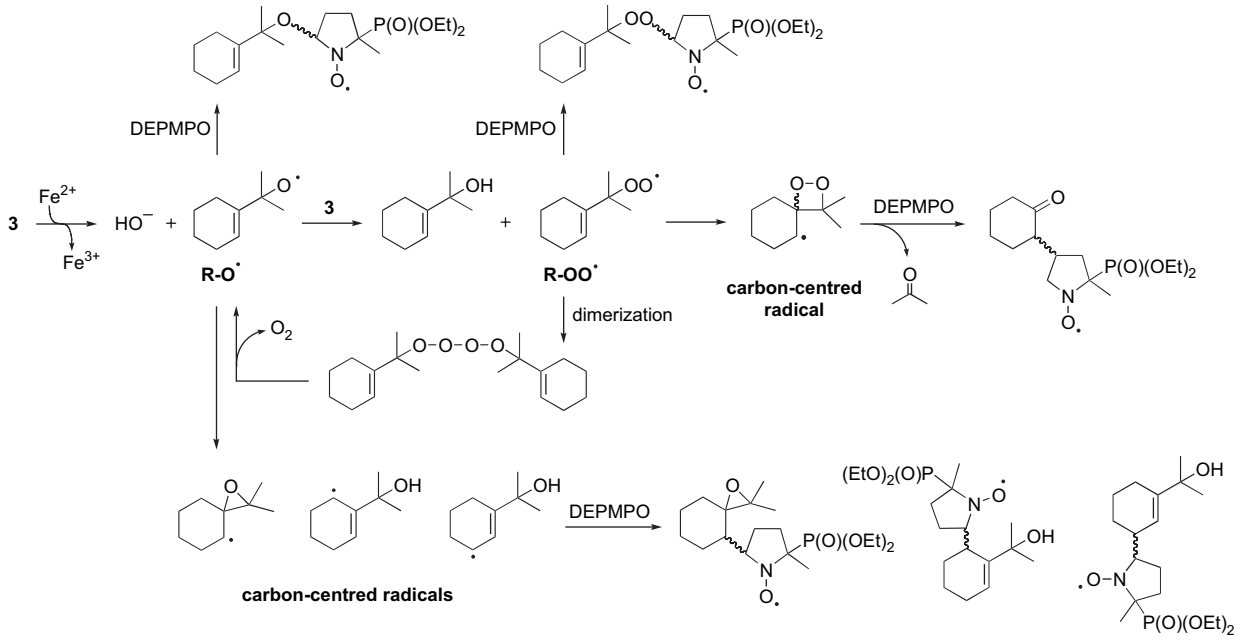
Allyloxyl radicals have been shown to undergo a rapid cyclization to give α-oxiranylcarbinyl radicals, as well as a β-scission to yield alkyl radicals and conjugated ketones or aldehydes. In the case of tertiary allyloxyl radicals, as the one derived from **3**, it has been shown that the ratio between the amount of oxiranylcarbinyl and alkyl radicals formed is 6 to 1, confirming that the formation of the oxiranylcarbinyl radical is the fastest process.[37] On another hand, we evidenced in previous studies that other hydrogens, mainly in allylic positions, can also undergo abstraction.[11] Finally, we have proved for **1** (Scheme 3), as previously for **3**,[11] that peroxyl radicals can rearrange, via intramolecular addition to a double bond, leading to a carbon-centred radical with a dioxetane structure that can cleave to get a carbonylic compound. Therefore, the possible formation of several carbon-centred radicals in the reaction medium must be considered (Scheme 4).

ESR spin-trapping experiments were also carried out with Fe(III) derivatives, such as $FeCl_3$, and due to the ubiquitous nature of haem complexes also with Fe(III)-porphyrins TPP-Fe^{3+} and haemin. When **3** was reacted with $FeCl_3$, in the presence of DEPMPO in CH_3CN or in a 1:1 (v/v) H_2O/CH_3CN solvent, the ESR spectra showed that an oxygen-centred radical was the main trapped species in organic solvents, while in an organic/aqueous mixture also carbon-centred radicals could be trapped (Fig. 5). As shown in Scheme 2, peroxyl ROO• and alkoxyl RO• radicals could be obtained from the controversial homolytic/heterolytic O–O bond cleavage by iron porphyrin complexes. These oxygen-centred radicals can further evolve to form carbon-centred radicals as indicated in Scheme 4.

Thus, ESR spin-trapping experiments confirmed the formation of carbon-centred radicals and evidenced the formation of allyloxyl and peroxyl radicals even if cannot be trapped by **5**.

2.3. Addition of *N*-acetyl-cysteine ethyl ester to model compound 3 catalyzed by ferric chloride

Analyses of amino acid residues from enzymes, and from other proteins treated with lipid hydroperoxides, have demonstrated that



Scheme 4. Potential nitroxide radicals that could be obtained by DEPMPO spin-trapping of oxygenated and/or carbon-centred radicals derived from compound **3**.

cysteine is one of the most labile residues. Thus, we tried to trap radical intermediates issued from allylic hydroperoxide **3** using a cysteine derivative as trapping agent. Similar experiments had been already conducted with fatty acids containing a hydroperoxide group and were suggested as possible models for the interactions of allylic hydroperoxides with proteins.[38] More precisely, several products resulted from the reaction of linoleic acid hydroperoxide with cysteine catalyzed by iron ion ($FeCl_3$), some of which were the result of a thio-bond formation between the thiyl radical derived from the lateral chain of cysteine and a carbon-centred radical derived from the hydroperoxide.[39]

N-Acetyl-cysteine ethyl ester was selected as the reactant because of its resemblance with cysteine when located in a protein

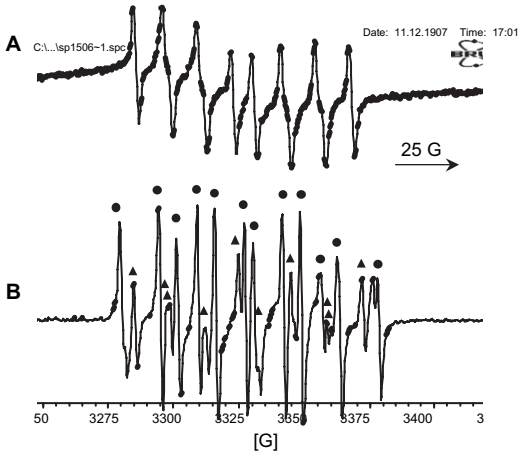
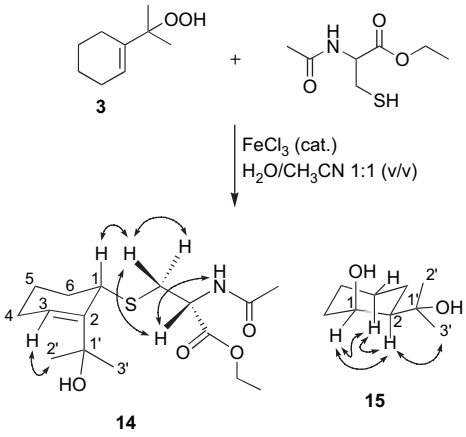


Figure 5. ESR spectra of DEPMPO radical adducts from the reaction of **3** with Fe(III). ESR settings: microwave power 1 mW, microwave frequency 9 GHz, modulation frequency 100 KHz, modulation amplitude 1 G, scan width 100 G, conversion time 25 ms, receiver gain 1×10^5. (A) A solution in CH_3CN of compound **3** (60 mM) and the spin trap (90 mM) was reacted with $FeCl_3$ (2 mg). In the computer simulation the best fit was obtained with coupling constants a_N=13.17 G, a_H=11.14 G and a_P=45.24 G (g=2.0064) for RO–DEPMPO• or ROO–DEPMPO•. (B) A 1:1 (v/v) H_2O/CH_3CN solution of compound **3** (60 mM) and the spin trap (90 mM) was reacted with $FeCl_3$ (2 mg). A composite spectrum was obtained with coupling constants a_N=13.81 G, a_H=11.92 G and a_P=47.79 G (g=2.006) for RO–DEPMPO• or ROO–DEPMPO• (Δ), and a_N=14.81 G, a_H=21.72 G and a_P=47.33 G (g=2.0059) for a DEPMPO-carbon-centred adduct (O).

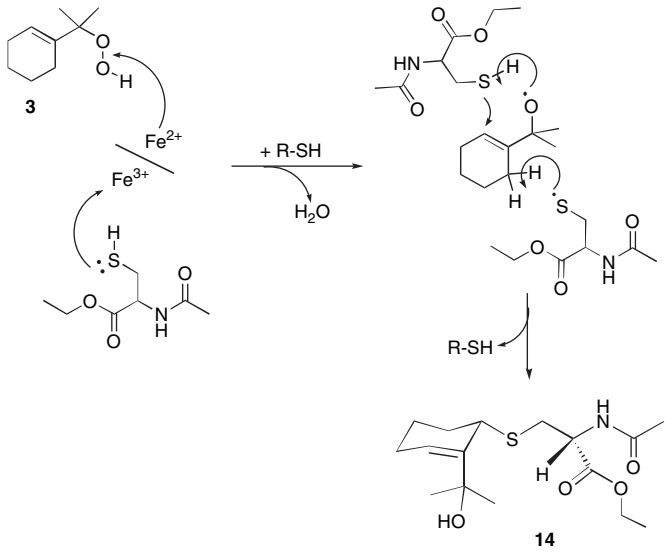
context and also to avoid the Schiff base that could be formed between the free amino group and carbonylic compounds produced in the hydroperoxide decomposition. A catalytic amount of $FeCl_3$ was employed to induce the radical formation. In fact, it has been reported that certain reductants, such as cysteine, induce an increase of the rate of hyroperoxide decomposition in the presence of Fe(III), via reduction of Fe(III) to Fe(II).[40] A redox reaction between the reductant and the hydroperoxide can be thus catalyzed by Fe(II)/Fe(III) ions. Reactions were carried out in argon atmosphere to minimize the competing reaction with oxygen and assuring higher yields of adducts. It is well known that the reaction of fatty acids carrying a hydroperoxide group in their structure with cysteine/$FeCl_3$ in the presence of oxygen results in the formation of numerous oxygenated fatty acids, as well as cystine and oxides of cysteine.[38,40] This has also been described for the Fe(II)/Fe(III)-mediated chemistry of artemisinin and synthetic 1,2,4-trioxanes with glutathione.[41]

Hydroperoxide **3** and *N*-acetyl-cysteine ethyl ester (2 equiv) in a deaerated 1:1 (v/v) H_2O/CH_3CN mixture were treated with a catalytic amount of $FeCl_3$ (0.1 equiv), at room temperature and under argon atmosphere. The reaction, followed by TLC, let to detect the formation of new products after 90 min. However, the starting hydroperoxide was still present in the reaction mixture after 4 days. As the reaction did not evolve after that time, it was stopped and the solvent removed under reduced pressure. The reaction mixture was separated by column chromatography, but only one fraction containing a non-separable mixture of two new products could be isolated, whose structures were determined using one- and two-dimensional NMR techniques, especially ^1H, ^{13}C, DEPT, COSY, NOESY and ^1H–^{13}C HSQC and HMBC experiments (Scheme 5).

An adduct originated from **3** and *N*-acetyl-cysteine ethyl ester was identified from the NMR data as 1-*S*-(*N*-acetyl-cysteine ethyl ester)-2-(1′-hydroxy-1′-methyl-ethyl) cyclohex-2-ene **14**. The HSQC spectrum showed a proton at 6.31 ppm directly correlated to a carbon atom at 123.4 ppm, indicating the existence of the vinylic group (=CH), and also a proton at 4.04 ppm correlated to a carbon atom at 41.2 ppm, chemical shifts characteristic of the –CH in position α to the cysteine thiyl group. The ^{13}C NMR spectrum confirmed the two quaternary carbons C-2 at 139.6 ppm and C-1′ at 75.1 ppm (C–OH). All these positions were corroborated by ^1H–^{13}C



Scheme 5. Ferric chloride catalyzed reaction of **3** with *N*-acetyl-cysteine ethyl ester. The products identified are indicated together with the main NOE effects observed allowing their structural elucidation.



Scheme 6. A possible mechanistic pathway for the formation of adduct **14**.

long-range correlations existing between H-3 at 6.31 ppm, C-1 at 41.2 ppm and C-5 at 20.8 ppm, and between the protons of the methyl groups at 1.32 ppm, C-1′ at 75.1 ppm and C-2 at 139.6 ppm. But more decisive for the structural elucidation of **14** was that the C-1 at 41.2 ppm was correlated via long-range coupling with protons at 2.92 and 3.07 ppm, both belonging to the methylene group (33.7 ppm) of the lateral chain of cysteine. Also, protons at 2.92 and 3.07 ppm were long-range correlated with the α-carbon position of the amino acid at 51.7 ppm. These results were supported by NOE experiments as proton at 4.04 ppm (H-1) exhibited important NOE cross-peaks with protons of the methylene group at 2.92 and 3.07 ppm (Scheme 5). Mechanistically, a redox reaction between *N*-acetyl-cysteine ethyl ester and allylic hydroperoxide **3**, catalyzed by $FeCl_3$, was conducted. Cysteine is known to reduce Fe(III) and hydroperoxides are known to oxidize Fe(II). Under reduction of Fe(III) to Fe(II), thiyl radicals were generated from *N*-acetyl-cysteine ethyl ester. The produced Fe(II) can then generate RO• radicals through a Fenton-like reaction. Easily, RO• radicals could perform an allylic hydrogen abstraction from position 1 as the abstraction of hydrogens from different allylic positions was evidenced previously by the ESR spin trapping of the corresponding allylic radicals.[11] A radical termination reaction between the carbon-centred radical so-formed and the cysteine thiyl radical to afford adduct **14** was unlikely because the concentrations of the transient species were low and the chances of one radical encountering another were small. A suggestion for a mechanistic pathway explaining the formation of **14** could be based on a more probable concerted mechanism (Scheme 6). That would imply that a thiyl radical could abstract an allylic hydrogen[42] and concomitantly a second cysteinyl thiyl radical, formed via an hydrogen abstraction by the alkoxyl radical from a neutral thiol molecule (2 equiv used in the reaction), was adding to the intermediate.

The NMR data of the fraction containing adduct **14** showed also the presence of the non-separable 2-*R*-(1′-hydroxy-1′-methyl-ethyl)-1-*R*-cyclohexanol **15**. The quaternary carbon of the vinyl group of **3** had been replaced by a –CH at 1.38/53.7 ppm bearing the 1′-hydroxy-1′-methyl-ethyl substituent. In parallel, the vinyl =CH carbon had been replaced by a –CH at 3.65/73.3 ppm, chemical shifts that are characteristic of this carbon atom bearing an hydroxy group as substituent. The stereochemistry of the substituents was established on the basis of NOE cross-peaks existing between H-2 at 1.38 ppm, H-1 at 3.65 ppm, axial H-4 at 0.85 ppm and protons of the methyl group at 1.19 ppm. Proton H-1 also displayed an NOE cross-peak with axial H-4. From the mechanistic point of view, the easily formed alkoxyl radical underwent a rapid cyclization to an α-oxiranylcarbinyl radical. This C-1 carbon-centred radical species, trapped by dioxygen, could give a peroxyl radical that after dimerization and loss of dioxygen led to a C-1 alkoxyl radical. Opening of the epoxide and hydrogen abstraction afforded compound **15**.

3. Conclusion

To complement our previous studies on 15-hydroperoxyabietic acid **1**-like compounds **3** and **4**, we now confirm, using radical trapping experiments, the formation of carbon-centred radicals derived from the allergenic bis-allylic tertiary hydroperoxide **1**. Even if the trapping of these radicals by 1,1,3,3-tetramethylisoindolin-2-yloxyl nitroxide **5** does not prove that they are able to form adducts with skin proteins in vivo, their possible generation in the epidermis could be responsible of the formation of antigenic structures that are able to trigger the immunological mechanism of allergic contact dermatitis. As Fe(II)/Fe(III) redox cycles are common in biological media, we initially wanted to test the reactivity of 15-hydroperoxyabietic acid **1** in the presence of Fe(II)/Fe(III), but its instability let it difficult to prepare a pure sample of **1** for ESR experiments. Thus, alternatively, we used the monocyclic allylic hydroperoxide **3** as a model. We confirmed the formation of carbon-centred radicals in the presence of Fe(II)/Fe(III) redox systems and also the formation of oxygen-centred allyloxyl and peroxyl radicals even if not trapped by **5**. Therefore, carbon and oxygen-centred radicals must both be considered as possible haptens of allylic hydroperoxides in allergic contact dermatitis. Finally, and to the best of our knowledge, we report here for the first time the formation of an adduct between an allergenic allylic hydroperoxide and cysteine catalyzed by iron. Identification of this adduct provided evidence on the participation of oxygen-centred allyloxyl radicals, which could abstract hydrogen from an allylic position and coupled with *N*-acetyl-cysteine ethyl ester through a carbon–sulfur bond. These results provided interesting data at the molecular level for this class of allergenic compounds as well as useful insights into the formation of antigenic structures via radical mechanisms.

4. Experimental

Caution. Skin contact with allylic hydroperoxides must be avoided. Since these compounds are skin sensitizing substances, they must be handled with care.

4.1. Chemicals and reagents

15-Hydroperoxyabietic acid **1** and 1-(1-hydroperoxy-1-methyl-ethyl) cyclohexene **3** were synthesized using procedures we had reported previously.[14,43] The free-radical trapping agent 1,1,3,3-tetramethylisoindolin-2-yloxyl nitroxide **5** was prepared using a method described in the literature from *N*-benzylphthalimide.[13] *meso*-Tetraphenylporphyrin iron(III) chloride (TPP-Fe^{3+}) was purchased from Fluka (St. Quentin Fallavier, France). Iron(III) chloride ($FeCl_3$), iron(II) sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$) and polyvinyl alcohol were purchased from Aldrich (St. Quentin Fallavier, France). Haemin and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) were acquired from Sigma (St. Quentin Fallavier, France). 5-Diethoxy-phosphoryl-5-methyl-1-pyrroline *N*-oxide (DEPMPO) was synthesized in two steps from 2-methyl-1-pyrroline according to the method described by Stolze et al.[30] *N*-Acetyl-cysteine ethyl ester was synthesized from *N*-acetyl-cysteine, purchased from Sigma (St. Quentin Fallavier, France), by a classical esterification method using absolute ethanol and thionyl chloride.[44] Spectrophotometric grade acetonitrile (99.8%) was purchased from Carlo Erba (Val de Reuil, France) and used as-delivered. Aqueous solutions were prepared with deionized water. All other chemicals were purchased from Aldrich (St. Quentin Fallavier, France) and used without further purification. All reactions were conducted in flame-dried glassware, under inert atmosphere of argon, and with previously deaerated solvents. Thin layer chromatography (TLC) was carried out on 0.25 mm Merck silica gel plates (60 F_{254}). After elution, the TLC plates were visualized with UV light at 254 nm or sprayed with a solution of phosphomolybdic acid in ethanol (5% w/v) or with a solution of *m*-anisaldehyde (0.5 mL) and *p*-anisaldehyde (0.5 mL) in glacial acetic acid (100 mL), ethanol (85 mL) and concentrated sulfuric acid (5 mL) followed by heating. Compounds were purified by column chromatography on silica gel (Merck 60, 230–400 mesh).

4.2. Instrumentation

^1H and ^{13}C NMR spectra were recorded on Bruker Avance 300 and Bruker Avance 500 spectrometers. Chemical shifts are reported in parts per million (δ) and are indirectly referenced to Me_4Si via the solvent signals ($CDCl_3$, 7.26 ppm for ^1H and 77.0 ppm for ^{13}C; benzene-d_6, 7.15 ppm for ^1H and 128.6 ppm for ^{13}C). Multiplicities are indicated by s (singlet), d (doublet), t (triplet) and m (multiplet). ^1H coupling constants (J) were obtained by first-order analysis and are reported in hertz. The different types of carbon in the structures have been identified by the DEPT-135 technique. The two-dimensional phase-sensitive ^1H–^1H COSY (correlation spectroscopy) spectra were collected using a standard pulse sequence with 254 ms acquisition time, sweep width 12.25 ppm, 512 complete t_1 data points and 8 scans per increment. The two-dimensional phase-sensitive ^1H–^1H NOESY (nuclear Overhauser effect spectroscopy) spectra were collected using 800 ms mixing time and 254 ms acquisition time, sweep width of 12.25 ppm, 512 complete t_1 data points, and 16 scans per increment. The two-dimensional ^1H–^{13}C HSQC (heteronuclear single-quantum correlation) spectra and ^1H–^{13}C HMBC (heteronuclear multiple-bond correlation) spectra were collected using a standard pulse sequence, with 254 ms acquisition time, 512 complete t_1 data points, 16 (HSQC) or 32 (HMBC) scans per increment and a spectral width in the f_1 dimension of 209.2 ppm. The data were processed using the NMR notebook software (version 2.5; NMRtec S.A.S., Illkirch-Graffenstaden, France). Fast atom bombardment mass spectrometry was carried out on an Autospect spectrometer (Micromass Inc., United Kingdom), in a 3-nitrobenzyl alcohol matrix and using caesium cations for the bombardment in positive and negative ion modes. Electron spin resonance (ESR) spectra were recorded on a Bruker ESP 380 spectrometer equipped with an X-band microwave bridge. Standard spectrometer settings: microwave power 1 mW, microwave frequency 9 GHz, modulation frequency 100 KHz, modulation amplitude 1 G, scan width 100 G, conversion time 25 ms and receiver gain 1×10^5. Hyperfine splitting assignments were obtained by means of computer simulation using the Bruker WINEPR SimFonia software (version 1.25; Rheinstetten, Germany). For the ESR spin-trapping experiments in iron systems, special glassware equipment was designed where the hydroperoxide and the spin trap in solution were initially in a distinct compartment (round-bottom container) to the one of the iron reagent (capillary tube). Both compartments were interlinked in order to allow subsequently mix of the reagents. An ESR spectrum of the reaction mixture was registered directly by placing the capillary tube in the spectrometer cavity.

4.3. Radical trapping experiments with 15-hydroperoxyabietic acid

4.3.1. Generation of radicals by reaction with TPP-Fe^{3+}

To a solution of hydroperoxide **1** (173 mg, 0.52 mmol) in a 1:1 (v/v) mixture of H_2O/CH_3CN (100 mL) were added **5** (197 mg, 1.03 mmol) and TPP-Fe^{3+} (400 mg, 0.57 mmol). The reaction mixture was stirred at room temperature for 1 h (complete disappearance of **1** as shown by TLC, hexane/AcOEt 7:3). The mixture was filtered and the solid obtained was washed with CH_3CN (20 mL). All filtrates together were concentrated under reduced pressure and taken up with the minimum amount of CH_2Cl_2 (40 mL). The solution was then dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was purified at first by column chromatography on silica gel (hexane/AcOEt 7:3) in order to eliminate the excess of TPP-Fe^{3+}. Further column chromatography on silica gel (eluent gradient from hexane/AcOEt 8:2 to AcOEt) allowed the purification, as white solids, of compounds **6a** (16.9 mg, 36.2 μmol, 7% yield), **7a** (4.5 mg, 8.5 μmol, 2% yield) and **7b** (3.6 mg, 6.8 μmol, 2% yield).

4.3.2. Generation of radicals by thermal cleavage

Compound **1** (123 mg, 0.37 mmol) was dissolved in a 1:1 (v/v) mixture of H_2O/CH_3CN (100 mL) together with nitroxide **5** (175 mg, 0.92 mmol). The solution was heated to reflux and the reaction followed by TLC (hexane/AcOEt 7:3) until complete disappearance of the hydroperoxide. After 22 h stirring under reflux, the solution was allowed to cool down to room temperature and concentrated under reduced pressure. The residue obtained was taken up with the minimum amount of CH_2Cl_2 (40 mL). The solution was then dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (eluent gradient from hexane/AcOEt 8:2 to AcOEt) to give, as white solids, compounds **6a** (5.9 mg, 12.6 μmol, 4% yield), **6b** (5.5 mg, 10.4 μmol, 3% yield), **7a** (2 mg, 3.8 μmol, 1% yield), **7b** (9.6 mg, 20.6 μmol, 6% yield), **9** (5.6 mg, 16.7 μmol, 5% yield), **10** (10 mg, 36.1 μmol, 10% yield) and **11** (3.4 mg, 10.6 μmol, 3% yield).

4.3.3. Generation of radicals by photochemical cleavage

Compound **1** (132 mg, 0.39 mmol) was dissolved in a 1:1 (v/v) mixture of H_2O/CH_3CN (100 mL). Nitroxide **5** (188 mg, 0.98 mmol) was added to the solution and the stirred reaction mixture was irradiated for 20 h with a 150 W tungsten filament lamp at a distance of 30 cm. The evolution of the reaction was followed by TLC (hexane/AcOEt 7:3) until complete disappearance of the hydroperoxide. The mixture was concentrated under reduced pressure and the residue obtained was taken up with the minimum amount of CH_2Cl_2 (40 mL). The solution was then dried over magnesium sulfate, filtered and concentrated under reduced pressure. The

crude product was purified by silica gel column chromatography (eluent gradient from hexane/AcOEt 8:2 to AcOEt) to give, as white solids, compounds **6a** (5.9 mg, 12.6 μmol, 4% yield), **6b** (5.5 mg, 10.4 μmol, 3% yield), **7a** (2 mg, 3.8 μmol, 1% yield), **7b** (9.6 mg, 20.6 μmol, 6% yield), **8** (4.4 mg, 9.1 μmol, 3% yield), **9** (5.6 mg, 16.7 μmol, 5% yield), **10** (10 mg, 36.1 μmol, 10% yield) and **11** (3.4 mg, 10.6 μmol, 3% yield).

*4.3.4. Radical trapping experiment in the absence of **5***

Allylic hydroperoxide **1** (96 mg, 0.29 mmol) was dissolved in a 1:1 (v/v) mixture of H_2O/CH_3CN (100 mL). The solution was heated to reflux. The reaction was followed by TLC (hexane/AcOEt 7:3) until complete disappearance of the hydroperoxide. After 5 h stirring under reflux, the solution was allowed to cool down to room temperature and concentrated under reduced pressure. The residue obtained was taken up with the minimum amount of CH_2Cl_2 (30 mL). The solution was then dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (eluent gradient from hexane/AcOEt 6:4 to AcOEt) to give, as white solids, compounds **9** (3.1 mg, 9.2 μmol, 3% yield), **10** (14 mg, 50.6 μmol, 17% yield) and **11** (4.3 mg, 13.5 μmol, 5% yield).

4.4. Characterization of radical trapping adducts derived from 15-hydroperoxyabietic acid

*4.4.1. 7α-(1′1′,3′,3′-Tetramethyl-1′,3′-dihydroisoindol-2′-yloxyl)-13-oxo-deisopropyl-abietan-8(14)-enoic acid (**6a**)*

^{1}H NMR (benzene-d_6, 500 MHz): δ 0.50 (s, 3H, H-17), 0.81 (m, 1H, H-1_{eq}), 1.14 (s, 3H, H-16), 1.23 (m, 2H, H-2), 1.30 (m, 1H, H-1_{ax}), 1.31 (s, 3H, H-8′), 1.34 (s, 3H, H-9′), 1.46 (m, 2H, H-6_{eq} and H-11_{eq}), 1.52 (m, 1H, H-3_{ax}), 1.54 (s, 3H, H-10′), 1.56 (s, 3H, H-11′), 1.58 (m, 1H, H-11_{ax}), 1.79 (m, 1H, H-6_{ax}), 1.83 (m, 1H, H-3_{eq}), 2.16 (m, 1H, H-12_{eq}), 2.33 (m, 1H, H-9), 2.35 (m, 1H, H-12_{ax}), 2.50 (m, 1H, H-5), 4.10 (m, 1H, H-7), 6.20 (d, *J*=1.9 Hz, 1H, H-14), 6.85 (dd, *J*=7.5 and 1.0 Hz, 1H, H-7′), 7.03 (dd, *J*=7.6 and 1.0 Hz, 1H, H-4′), 7.07 (m, 1H, H-6′), 7.12 (m, 1H, H-5′); ^{13}C NMR (benzene-d_6, 125 MHz): δ 15.2 (C-17), 16.8 (C-16), 18.2 (C-2), 20.3 (C-11), 25.6 (C-9′), 25.8 (C-11′), 30.2 (C-8′), 30.6 (C-6), 30.9 (C-10′), 36.5 (C-3), 36.8 (C-12), 38.4 (C-1), 39.1 (C-10), 43.2 (C-5), 47.2 (C-4), 48.1 (C-9), 67.4 (C-1′), 68.3 (C-3′), 83.9 (C-7), 121.6 (C-4′), 122.0 (C-7′), 127.6 (C-6′), 128.2 (C-5′), 130.1 (C-14), 145.0 (C-3′a and C-7′a), 158.4 (C-8), 184.3 (C-15), 197.8 (C-13); FABMS [M+H]: 466.4.

*4.4.2. 7β-(1′,1′,3′,3′-Tetramethyl-1′,3′-dihydroisoindol-2′-yloxyl)-13-oxo-deisopropyl-abietan-8(14)-enoic acid (**6b**)*

^{1}H NMR (benzene-d_6, 500 MHz): δ 0.54 (s, 3H, H-17), 0.64 (m, 1H, H-1_{eq}), 1.17 (s, 3H, H-16), 1.22 (m, 2H, H-2), 1.24 (m, 1H, H-1_{ax}), 1.36 (s, 3H, H-9′), 1.41 (m, 1H, H-9), 1.42 (m, 2H, H-11), 1.45 (s, 3H, H-11′), 1.50 (m, 1H, H-3_{eq}), 1.54 (m, 1H, H-6_{ax}), 1.56 (s, 6H, H-8′ and H-10′), 1.64 (m, 1H, H-3_{ax}), 1.86 (m, 1H, H-5), 2.05 (m, 1H, H-12_{eq}), 2.33 (m, 1H, H-12_{ax}), 2.54 (m, 1H, H-6_{eq}), 4.39 (m, 1H, H-7), 6.89 (s, 1H, H-14), 6.90 (m, 1H, H-7′), 7.02 (m, 1H, H-4′), 7.09 (m, 1H, H-6′), 7.18 (m, 1H, H-5′); ^{13}C NMR (benzene-d_6, 125 MHz): δ 15.6 (C-17), 17.1 (C-16), 18.2 (C-2), 20.3 (C-11), 25.6 (C-9′), 25.8 (C-11′), 30.0 (C-8′ and C-10′), 32.4 (C-6), 36.5 (C-12), 36.8 (C-3), 38.0 (C-1), 39.0 (C-10), 45.7 (C-5), 47.4 (C-4), 49.3 (C-9), 67.3 (C-1′), 68.2 (C-3′), 82.5 (C-7), 122.0 (C-4′), 122.2 (C-7′), 124.1 (C-14), 128.0 (C-6′), 128.1 (C-5′), 145.0 (C-3′a and C-7′a), 160.7 (C-8), 184.2 (C-15), 197.2 (C-13); FABMS [M+H]: 466.3, FABMS [M−H]: 464.3.

*4.4.3. 7α-(1′,1′,3′,3′-Tetramethyl-1′,3′-dihydroisoindol-2′-yloxyl)-13α,15-dihydroxy-abietan-8(14)-enoic acid (**7a**)*

^{1}H NMR (benzene-d_6, 500 MHz): δ 0.69 (s, 3H, H-20), 1.00 (m, 1H, H-1_{eq}), 1.16 (s, 3H, H-16), 1.20 (s, 3H, H-19), 1.24 (s, 3H, H-17), 1.35 (m, 2H, H-2), 1.42 (s, 3H, H-9′), 1.45 (s, 3H, H-8′), 1.53 (m, 2H, H-12), 1.57 (m, 1H, H-3_{ax}), 1.60 (m, 1H, H-1_{ax}), 1.61 (s, 3H, H-11′), 1.62 (m, 1H, H-6_{eq}), 1.62 (s, 3H, H-10′), 1.73 (m, 1H, H-11_{eq}), 1.83 (m, 1H, H-11_{ax}), 1.90 (m, 1H, H-6_{ax}), 1.93 (m, 1H, H-3_{eq}), 2.33 (d, *J*=7.8 Hz, 1H, H-9), 2.65 (dd, *J*=13.0 and 2.6 Hz, 1H, H-5), 4.27 (m, 1H, H-7), 6.17 (d, *J*=1.4 Hz, 1H, H-14), 6.91 (m, 1H, H-7′), 7.02 (m, 1H, H-4′), 7.11 (m, 2H, H-5′ and H-6′); ^{13}C NMR (benzene-d_6, 125 MHz): δ 15.7 (C-20), 16.6 (C-19), 17.7 (C-11), 18.4 (C-2), 24.7 (C-17), 24.9 (C-16), 25.4 (C-9′), 25.6 (C-11′), 29.4 (C-12), 30.3 (C-8′), 30.7 (C-10′), 31.7 (C-6), 36.9 (C-3), 38.6 (C-1), 39.9 (C-10), 43.5 (C-5), 45.5 (C-9), 47.4 (C-4), 67.7 (C-1′), 68.2 (C-3′), 73.8 (C-13), 74.2 (C-15), 85.6 (C-7), 121.7 (C-4′), 122.0 (C-7′), 127.5 (C-5′ and C-6′), 128.3 (C-14), 141.8 (C-8), 145.0 (C-3′a and C-7′a), 184.4 (C-18); FABMS [M+H]: 526.4, FABMS [M−H]: 524.3.

*4.4.4. 7α-(1′,1′,3′,3′-Tetramethyl-1′,3′-dihydroisoindol-2′-yloxyl)-13β,15-dihydroxy-abietan-8(14)-enoic acid (**7b**)*

^{1}H NMR (benzene-d_6, 400 MHz): δ 0.69 (s, 3H, H-20), 1.01 (m, 1H, H-1_{eq}), 1.19 (s, 3H, H-16), 1.21 (s, 3H, H-17), 1.23 (s, 3H, H-19), 1.33 (m, 2H, H-2), 1.40 (m, 1H, H-12_{eq}), 1.46 (s, 3H, H-11′), 1.50 (m, 1H, H-1_{ax}), 1.50 (s, 3H, H-8′), 1.55 (m, 1H, H-11_{ax}), 1.57 (m, 1H, H-3_{ax}), 1.59 (m, 1H, H-6_{eq}), 1.60 (s, 3H, H-9′), 1.66 (m, 1H, H-12_{ax}), 1.67 (m, 1H, H-11_{eq}), 1.67 (s, 3H, H-10′), 1.92 (m, 1H, H-6_{ax}), 1.97 (m, 1H, H-3_{eq}), 2.33 (t, *J*=8.1 Hz, 1H, H-9), 2.61 (dd, *J*=13.1 and 2.3 Hz, 1H, H-5), 4.20 (m, 1H, H-7), 6.12 (s, 1H, H-14), 6.92 (m, 1H, H-7′), 7.04 (m, 1H, H-4′), 7.10 (m, 2H, H-5′ and H-6′); ^{13}C NMR (benzene-d_6, 100 MHz): δ 14.2 (C-20), 16.8 (C-19), 17.5 (C-11), 18.2 (C-2), 24.9 (C-17), 25.2 (C-16), 25.8 (C-9′ and C-11′), 29.2 (C-12), 30.6 (C-8′), 31.0 (C-6), 31.5 (C-10′), 36.9 (C-3), 38.0 (C-1), 38.2 (C-10), 43.4 (C-5), 47.1 (C-9), 47.2 (C-4), 67.9 (C-1′), 68.3 (C-3′), 73.7 (C-15), 74.1 (C-13), 84.5 (C-7), 121.8 (C-4′), 121.9 (C-7′), 127.7 (C-5′ and C-6′), 129.9 (C-14), 142.0 (C-8), 145.7 (C-3′a and C-7′a), 184.5 (C-18); FABMS [M+H]: 526.4.

*4.4.5. 14α-(1′,1′,3′,3′-Tetramethyl-1′,3′-dihydroisoindol-2′-yloxyl)-7α,8α-epoxy-13-oxo-deisopropyl-abietic acid (**8**)*

^{1}H NMR (benzene-d_6, 500 MHz): δ 0.63 (m, 1H, H-1_{eq}), 0.73 (s, 3H, H-17), 1.18 (s, 3H, H-16), 1.22 (m, 2H, H-2), 1.34 (m, 1H, H-1_{ax}), 1.35 (m, 1H, H-11_{eq}), 1.37 (s, 3H, H-10′), 1.39 (s, 3H, H-8′), 1.47 (m, 1H, H-9), 1.50 (s, 3H, H-9′), 1.53 (m, 1H, H-3_{ax}), 1.55 (s, 3H, H-11′), 1.61 (m, 1H, H-6_{eq}), 1.74 (m, 1H, H-3_{eq}), 1.88 (m, 1H, H-6_{ax}), 2.00 (m, 1H, H-11_{ax}), 2.10 (m, 1H, H-12_{ax}), 2.13 (m, 1H, H-5), 2.55 (m, 1H, H-12_{eq}), 3.31 (m, 1H, H-7), 3.96 (m, 1H, H-14), 6.89 (m, 2H, H-4′ and H-7′), 7.09 (m, 2H, H-5′ and H-6′); ^{13}C NMR (benzene-d_6, 125 MHz): δ 15.7 (C-17), 17.6 (C-16), 17.8 (C-11), 18.1 (C-2), 24.6 (C-6), 25.9 (C-9′), 25.7 (C-11′), 29.8 (C-8′), 30.6 (C-10′), 34.1 (C-10), 37.1 (C-3), 37.8 (C-12), 38.4 (C-1), 40.4 (C-5), 46.3 (C-4), 51.1 (C-9), 56.9 (C-7), 58.6 (C-8), 67.6 (C-1′), 68.7 (C-3′), 91.5 (C-14), 121.8 (C-4′ and C-7′), 127.5 (C-5′ and C-6′), 144.6 (C-7′a), 144.9 (C-3′a), 183.3 (C-15), 205.0 (C-13); FABMS [M+H]: 482.2, FABMS [M−H]: 480.2.

*4.4.6. 13α,15α-Epoxy-14α-hydroxy-abietic acid (**9**)*

^{1}H NMR (benzene-d_6, 500 MHz): δ 0.73 (s, 3H, H-20), 0.85 (m, 1H, H-1_{eq}), 1.09 (s, 3H, H-16), 1.24 (s, 3H, H-19), 1.25 (m, 1H, H-11_{ax}), 1.26 (s, 3H, H-17), 1.34 (m, 2H, H-2), 1.46 (m, 1H, H-12_{ax}), 1.55 (m, 1H, H-1_{ax}), 1.60 (m, 1H, H-3_{ax}), 1.67 (m, 1H, H-11_{eq}), 1.74 (m, 1H, H-9), 1.82 (m, 1H, H-12_{eq}), 1.83 (m, 1H, H-3_{eq}), 1.96 (m, 2H, H-6), 2.22 (m, 1H, H-5), 3.97 (m, 1H, H-14), 5.79 (m, 1H, H-7); ^{13}C NMR (benzene-d_6, 125 MHz): δ 14.2 (C-20), 16.9 (C-19), 18.3 (C-2), 19.4 (C-11), 20.3 (C-17), 22.2 (C-16), 25.7 (C-6), 26.7 (C-12), 35.1 (C-10), 37.5 (C-3), 38.2 (C-1), 45.2 (C-5), 46.5 (C-4), 50.5 (C-9), 63.1 (C-15), 66.5 (C-13), 70.8 (C-14), 123.8 (C-7), 137.5 (C-8), 183.5 (C-18); FABMS [M−(OH)]: 317.2.

*4.4.7. 13-Oxo-deisopropyl-abietan-8(14)-enoic acid (**10**)*

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.84 (s, 3H, H-17), 1.23 (s, 3H, H-16), 1.41–2.53 (m, 16H, H-1, H-2, H-3, H-5, H-6, H-7, H-9, H-11 and H-

12), 5.88 (br s, 1H, –COO*H*); ^{1}C NMR ($CDCl_3$, 50 MHz): δ 15.6 (C-17), 16.8 (C-16), 17.9 (C-2), 20.4 (C-11), 24.1 (C-6), 34.5 (C-10), 35.2 (C-3), 36.7 (C-1), 36.9 (C-7), 38.3 (C-12), 47.1 (C-4), 47.9 (C-9), 51.7 (C-5), 126.3 (C-14), 165.0 (C-8), 184.3 (C-15), 199.9 (C-13); CAS registry number [5708-85-0].

*4.4.8. 15-Hydroxyabietic acid (**11**)*

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.83 (s, 3H, H-20), 1.18–2.36 (m, 14H, H-1, H-2, H-3, H-5, H-6, H-9, H-11 and H-12), 1.26 (s, 3H, H-19), 1.33 (s, 3H, H-16 or H-17), 1.35 (s, 3H, H-16 or H-17), 5.48 (m, 1H, H-7), 6.07 (s, 1H, H-14); ^{1}C NMR ($CDCl_3$, 50 MHz): δ 14.0 (C-20), 16.7 (C-19), 18.1 (C-2), 22.6 (C-11), 25.7 (C-12), 25.8 (C-6), 28.6 (C-16), 28.7 (C-17), 34.4 (C-10), 37.2 (C-3), 38.3 (C-1), 44.8 (C-9), 46.3 (C-4), 50.7 (C-5), 72.9 (C-15), 122.5 (C-14), 122.9 (C-7), 135.0 (C-8), 144.5 (C-13), 184.3 (C-18); CAS registry number [101821-23-2].

4.5. ESR spin-trapping experiments with model compound 3 in the presence of Fe(II)/Fe(III)

Compound **3** (5 mg, 0.032 mmol) and the spin trap, DMPO or DEPMPO (0.05 mmol), 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 a glassware equipment specially designed for the experiments, whilst in another compartment, a capillary tube, the Fe(II) or Fe(III) derivatives (0.035 mmol). The compartments were interlinked to allow the subsequent mix of the reagents. In order to avoid a fast mix of the hydroperoxide when poured over the iron derivative, a layer of polyvinyl alcohol was used as a filter. The whole system was degassed by a vacuum line using the standard freeze–thaw technique, procedure repeated three times. Then, the solution containing **3** and the spin trap was poured into the capillary tube containing the layer of polyvinyl alcohol and the iron reagent underneath. An ESR spectrum of the reaction mixture was registered directly by placing the capillary tube in the spectrometer cavity.

4.6. Addition of *N*-acetyl-cysteine ethyl ester to the model compound 3 catalyzed by ferric chloride

Allylic hydroperoxide **3** (300 mg, 1.92 mmol) was dissolved in a deaerated 1:1 (v/v) mixture of H_2O/CH_3CN (100 mL). *N*-Acetyl-cysteine ethyl ester (734 mg, 3.84 mmol) and $FeCl_3$ (31 mg, 0.20 mmol) were added to the solution. The reaction mixture continuously stirred for 90 min, at room temperature, let to identify, by TLC (pentane/AcOEt 4:6), new products. As the starting hydroperoxide was still present in the reaction mixture after 4 days, and the reaction did not evolve after that time, the solvent was removed under reduced pressure. The crude product obtained was fractionated by column chromatography on silica gel (pentane/AcOEt 4:6) into three fractions, which were analyzed by NMR (^{1}H, ^{13}C, DEPT, COSY, NOESY, HSQC and HMBC). Only the fraction obtained at an average TLC retention factor of 0.45 (pentane/AcOEt 4:6) was shown to contain the non-separable compounds **14** and **15**.

*4.6.1. 1-S-(N-Acetyl-cysteine ethyl ester)-2-(1′-hydroxy-1′-methyl-ethyl) cyclohex-2-ene (**14**)*

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.12 (m, 1H, H-5_{eq}), 1.13 (m, 1H, H-6_{ax}), 1.26 (m, 3H, –CH_2–CH_3), 1.32 (m, 6H, H-2′ and H-3′), 1.59 (m, 1H, H-6_{eq}), 1.70 (m, 1H, H-4_{ax}), 1.85 (m, 1H, H-5_{ax}), 1.97 (m, 1H, H-4_{eq}), 2.01 (s, 3H, –CO–CH_3), 2.92 (m, 1H, –S–CH_2–), 3.07 (m, 1H, –S–CH_2–), 4.04 (m, 1H, H-1), 4.18 (m, 2H, –CH_2–CH_3), 4.75 (m, 1H, –NH–*CH*–), 6.31 (m, 1H, H-3), 6.40 (s, 1H, –N*H*–); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.11 (–CH_2–CH_3), 20.8 (C-5), 22.9 (–CO–CH_3), 25.7 (C-6), 27.2 (C-4), 29.6 (C-2′ and C-3′), 33.7 (–S–CH_2–), 41.2 (C-1), 51.7 (–NH–*CH*–), 61.6 (–CH_2–CH_3), 75.1 (C-1′), 123.4 (C-3), 139.6 (C-2), 170.0 (–NH–*CO*–), 170.9 (–*CO*–O–).

*4.6.2. 2-R-(1′-Hydroxy-1′-methyl-ethyl)-1-R-cyclohexanol (**15**)*

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.85 (m, 1H, H-4_{ax}), 1.12 (m, 1H, H-5_{eq}), 1.17 (s, 3H, H-2′), 1.19 (s, 3H, H-3′), 1.24 (m, 1H, H-3_{ax}), 1.30 (m, 1H, H-6_{eq}), 1.38 (m, 1H, H-2), 1.63 (m, 1H, H-5_{ax}), 1.67 (m, 1H, H-4_{eq}), 1.70 (m, 1H, H-3_{eq}), 1.95 (m, 1H, H-6_{ax}), 3.65 (m, 1H, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.7 (C-2′), 24.8 (C-3), 25.9 (C-5), 27.6 (C-4), 29.97 (C-3′), 35.8 (C-6), 53.7 (C-2), 73.3 (C-1), 74.2 (C-1′).

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References and notes

1. Lepoittevin, J.-P. *Contact Dermatitis*; Frosch, P. J., Menné, T., Lepoittevin, J.-P., Eds.; Springer: Berlin, Heidelberg, New York, NY, 2006; pp 45–68.
2. Rustemeyer, T.; van Hoogstraten, I. M. W.; von Blomberg, B. M. E.; Scheper, R. J. *Contact Dermatitis*; Frosch, P. J., Menné, T., Lepoittevin, J.-P., Eds.; Springer: Berlin, Heidelberg, New York, NY, 2006; pp 11–43.
3. Smith, C. K.; Hotchkiss, S. A. M. *Allergic Contact Dermatitis: Chemical and Metabolic Mechanisms*; Smith, C. K., Hotchkiss, S. A. M., Eds.; Taylor and Francis: London, 2001; pp 87–117.
4. Karlberg, A.-T.; Basketter, D.; Goossens, A.; Lepoittevin, J.-P. *Contact Derm.* **1999**, *40*, 183.
5. Matura, M.; Sköld, M.; Börje, A.; Andersen, K. E.; Bruze, M.; Frosch, P. J.; Goossens, A.; Johansen, J. D.; Svedman, C.; White, I. R.; Karlberg, A.-T. *Contact Derm.* **2005**, *52*, 320.
6. Sköld, M.; Börje, A.; Harambasic, E.; Karlberg, A.-T. *Chem. Res. Toxicol.* **2004**, *17*, 1697.
7. Matura, M.; Goossens, A.; Bordalo, O.; García-Bravo, B.; Magnusson, K.; Wrangsjo, K.; Karlberg, A.-T. *Contact Derm.* **2003**, *49*, 15.
8. Karlberg, A.-T.; Bohlinder, K.; Boman, A.; Hacksell, U.; Hermansson, J.; Jacobson, S.; Nilsson, J. L. *J. Pharm. Pharmacol.* **1988**, *40*, 42.
9. Joye, N. M.; Lawrence, R. V. *J. Chem. Eng. Data* **1967**, *12*, 279.
10. Mutterer, V.; Giménez-Arnau, E.; Karlberg, A.-T.; Lepoittevin, J.-P. *Chem. Res. Toxicol.* **2000**, *13*, 1028.
11. Giménez-Arnau, E.; Haberkorn, L.; Grossi, L.; Lepoittevin, J.-P. *Tetrahedron* **2002**, *58*, 5535.
12. Dussault, P. *Active Oxygen in Chemistry*; Foote, C. S., Valentine, J. S., Greenberg, A., Liebman, J. F., Eds.; Blackie Academic and Professional: London, Glasgow, New York, NY, 1995; pp 141–203.
13. Griffiths, P. G.; Moad, G.; Rizzardo, E.; Solomon, D. H. *Aust. J. Chem.* **1983**, *36*, 397.
14. Haberkorn, L.; Mutterer, V.; Giménez-Arnau, E.; Lepoittevin, J.-P. *Synlett* **2001**, 1723.
15. Traylor, T. G.; Kim, C.; Fann, W.-P.; Perrin, C. L. *Tetrahedron* **1998**, *54*, 7977.
16. Almarsson, O.; Bruice, T. C. *J. Am. Chem. Soc.* **1995**, *117*, 4533.
17. Labeque, R.; Marnett, L. J. *J. Am. Chem. Soc.* **1989**, *111*, 6621.
18. Traylor, T. G.; Tsuchiya, S.; Byun, Y.-S.; Kim, C. *J. Am. Chem. Soc.* **1993**, *115*, 2775.
19. Frimer, A. A. *Chem. Rev.* **1979**, *79*, 359.
20. Walling, C. *Active Oxygen in Chemistry*; Foote, C. S., Valentine, J. S., Greenberg, A., Liebman, J. F., Eds.; Blackie Academic and Professional: London, Glasgow, New York, NY, 1995; pp 24–65.
21. Roe, A. N.; McPhail, A. T.; Porter, N. A. *J. Am. Chem. Soc.* **1983**, *105*, 1199.
22. Porter, N. A.; Zuraw, P. J. *J. Org. Chem.* **1984**, *49*, 1345.
23. Shimizu, N.; Bartlett, P. D. *J. Am. Chem. Soc.* **1976**, *98*, 4193.
24. Jefford, C. W.; Boschung, A. F. *Helv. Chim. Acta* **1977**, *60*, 2673.
25. Hock, H.; Kropf, H. *Angew. Chem.* **1957**, *69*, 313.
26. Turner, J. A.; Hertz, W. *J. Org. Chem.* **1977**, *42*, 1657.
27. Frimer, A. A.; Rot, D.; Sprecher, M. *Tetrahedron Lett.* **1977**, 1927.
28. Janzen, E. G. *Acc. Chem. Res.* **1971**, *4*, 31.
29. Frejaville, C.; Karoui, H.; Tuccio, B.; Le Moigne, F.; Culcasi, M.; Pietri, S.; Lauricella, R.; Tordo, P. *J. Med. Chem.* **1995**, *38*, 258.
30. Stolze, K.; Udilova, N.; Nohl, H. *Free Radical Biol. Med.* **2000**, *29*, 1005.
31. Chamulitrat, W.; Takahashi, N.; Mason, R. P. *J. Biol. Chem.* **1989**, *264*, 7889.
32. Hanna, P. M.; Chamulitrat, W.; Mason, R. P. *Arch. Biochem. Biophys.* **1992**, *296*, 640.
33. Dikalov, S. I.; Mason, R. P. *Free Radical Biol. Med.* **1999**, *27*, 864.
34. Guo, Q.; Qian, S. Y.; Mason, R. P. *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 862.
35. Khramtsov, V.; Berliner, L. J.; Clanton, T. L. *Magn. Reson. Med.* **1999**, *42*, 228.

36. Sinha, B. K. *J. Biol. Chem.* **1983**, *258*, 796.
37. Grossi, L.; Strazzari, S.; Gilbert, B. C.; Whitwood, A. C. *J. Org. Chem.* **1998**, *63*, 8366.
38. Gardner, H. W.; Plattner, R. D.; Weisleder, D. *Biochim. Biophys. Acta* **1985**, *834*, 65.
39. Gardner, H. W.; Weisleder, D.; Kleiman, R. *Lipids* **1976**, *11*, 127.
40. Gardner, H. W.; Jursinic, P. A. *Biochim. Biophys. Acta* **1981**, *665*, 100.
41. Singh, C.; Gupta, N.; Tiwari, P. *Tetrahedron Lett.* **2005**, *46*, 4551.
42. Lunazzi, L.; Placucci, G.; Grossi, L. *J. Chem. Soc., Chem. Commun.* **1979**, 533.
43. Lepoittevin, J.-P.; Karlberg, A.-T. *Chem. Res. Toxicol.* **1994**, *7*, 130.
44. Atlas, D.; Melamed, E.; Ofen, D. U.S. Patent 5,874,468, 1999.