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Identification of alkyl radicals derived from an allergenic cyclic tertiary allylic hydroperoxide by combined use of radical trapping and ESR studies

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

[a]*Laboratoire de Dermatochimie, Université Louis Pasteur, UMR 7123, Clinique Dermatologique, CHU, 67091 Strasbourg, France*

[b]*Dipartimento di Chimica Organica 'A. Mangini', Università di Bologna, Viale Risorgimento 4, 40136 Bologna, Italy*

Dedicated to Professor C. Benezra (1939–1992) in memorium

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Abstract—Alkyl radicals derived from 1-(1-hydroperoxy-1-methylethyl)cyclohexene, an allergenic cyclic tertiary allylic hydroperoxide, were identified using a combination of radical trapping and ESR studies. Radical trapping experiments were carried out in aqueous acetonitrile solutions containing the stable scavenger agent 1,1,3,3-tetramethylisoindolin-2-yloxyl, and light, heat and TPP–Fe^{3+} were used as radical inducers. ESR spin-trapping studies were performed on the allylic alcohol precursor of the hydroperoxide, which generated the same allyloxyl radical by in situ photolysis of the corresponding nitrite formed in the presence of *t*-BuONO (which also played the role of spin-trap). The formation and trapping of carbon-centered radicals derived from the allyloxyl radical, as well as from the peroxyl radical, are described. The generation of these highly reactive radicals in the epidermis could lead to the formation of antigenic structures, the first step of the allergic contact dermatitis mechanism. © 2002 Published by Elsevier Science Ltd.

1. Introduction

The major mechanism of antigen formation involved in the allergic contact dermatitis (ACD) pathology is the reaction of an electrophilic function, present on the allergen, and nucleophilic residues on proteins to form covalent bonds.[1] However, over the past few years, the radical mechanism, although it has never been firmly established, has gained increasing interest in the discussion of the mechanism of hapten–protein binding.[2]

The high sensitizing potential of allylic hydroperoxides derived from the autoxidation of terpenes is well known. It was already demonstrated, fifty years ago, that several hydroperoxides derived from the autoxidation of Δ^3-carene were responsible for the allergenic activity of turpentine.[3] More recently, Karlberg et al. have extensively reported on the allergenic potential of allylic hydroperoxides derived from the autoxidation of *d*-limonene, used as a perfume and flavoring agent,[4] or the autoxidation of abietic acid, the main resinic acid of colophony.[5,6]

In the course of our investigations on allergenic allylic hydroperoxides we have shown that radical reactions could be important for the binding of haptens containing hydroperoxide groups with skin proteins.[7–9] When treated with a variety of metal complexes such as $FeCl_3$, copper(II) trifluoromethanesulfonate[10] or the *meso*-tetraphenylporphyrin iron(III) chloride complex (TPP–Fe^{3+}),[11] allylic hydroperoxides are known to form allyloxyl radicals that readily rearrange into carbon-centered epoxyalkyl radicals that may react with molecular oxygen and then decay to hydroxy derivatives. In a previous study, a simple model compound to investigate the interaction of the allergenic

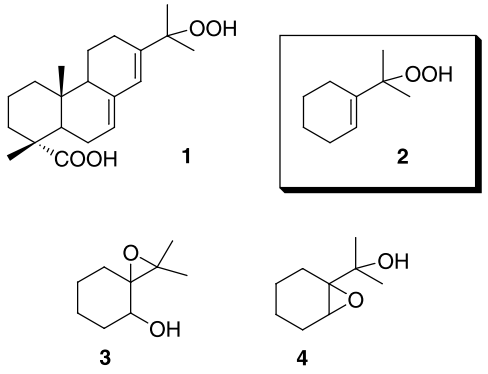

Figure 1. Chemical structures of 15-hydroperoxyabietic acid **1**, 1-(1-hydroperoxy-1-methylethyl)cyclohexene **2** and its rearrangement epoxides **3** and **4**.

Keywords: allergenic hydroperoxide; allylic hydroperoxide; radicals and radical reactions; radical trapping; electron spin resonance.

* Corresponding author. Tel.: +33-3-88-35-06-64; fax: +33-3-88-14-04-47; e-mail: jplepoit@chimie.u-strasbg.fr

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15-hydroperoxyabietic acid **1**-like terpenes with proteins, the 1-(1-hydroperoxy-1-methylethyl)cyclohexene **2**, was synthesized (Fig. 1).[7] Its allergenic potential, with that of the epoxides **3** and **4** that can derive from its rearrangement (Fig. 1), was evaluated.

Compound **2** was found to be a strong sensitizer while both epoxides, that could have been suspected to react as electrophiles, failed to induce a significant sensitization. These results ruled out a possible nucleophilic–electrophilic mechanism involving epoxides as the actual sensitizers derived from such tertiary allylic hydroperoxide. However, the rearrangement of the allylic hydroperoxide is highly probable and, since the rearranged compounds are not active, it may be supposed that a radical intermediate able to react with skin proteins is formed during the process.

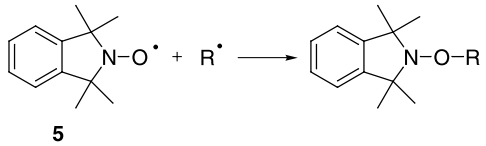
In this paper, we report the complete identification of the alkyl radicals deriving from the allylic hydroperoxide **2**. A radical trapping technique employing the stable nitroxide 1,1,3,3-tetramethylisoindolin-2-yloxyl, in an aqueous acetonitrile medium, has been used. The structure of the adducts obtained was determined using one- and two-dimensional NMR techniques (^{1}H, ^{13}C, DEPT, COSY, NOESY, and ^{1}H–^{13}C correlations HSQC and HMBC). A complementary ESR spin-trapping technique for the detection of alkyl radical intermediates formed from the **2** derived allyloxyl radical is also described. It confirmed the results obtained with the radical trapping technique. The in situ generation of these highly reactive radicals in the epidermis could lead to the formation of antigenic structures, the first step of the ACD mechanism.

2. Results and discussion

2.1. Radical trapping experiments

The 1,1,3,3-tetramethylisoindolin-2-yloxyl[12] **5** and similar species[13] couple with carbon-centered radicals to give stable non-radical alkoxyamine products, that can be characterized by use of a full range of spectroscopic techniques (Scheme 1). Solomon et al. have used this selective coupling reaction to trap and identify reactive intermediates in the initial stages of free radical polymerizations of commercial and theoretical importance.[14,15]

Allyloxyl radicals, derived from homolytic cleavage of allylic hydroperoxides, are known to be reactive species which can lead to the formation of carbon-centered radicals through hydrogen abstraction, intramolecular cyclization, or fragmentation. In this study, we have evaluated the formation of alkyl radical species derived from **2** by the aminoxyl radical trapping technique using the nitroxide **5** as a scavenger. The advantages of this nitroxide over more readily accessible nitroxides are several: it exhibits a chemical inertness quite uncharacteristic of most other free radicals, it is thermally stable, symmetrical (so as to generate a minimum number of NMR signals in the products) and has a suitable UV absorbing chromophore.



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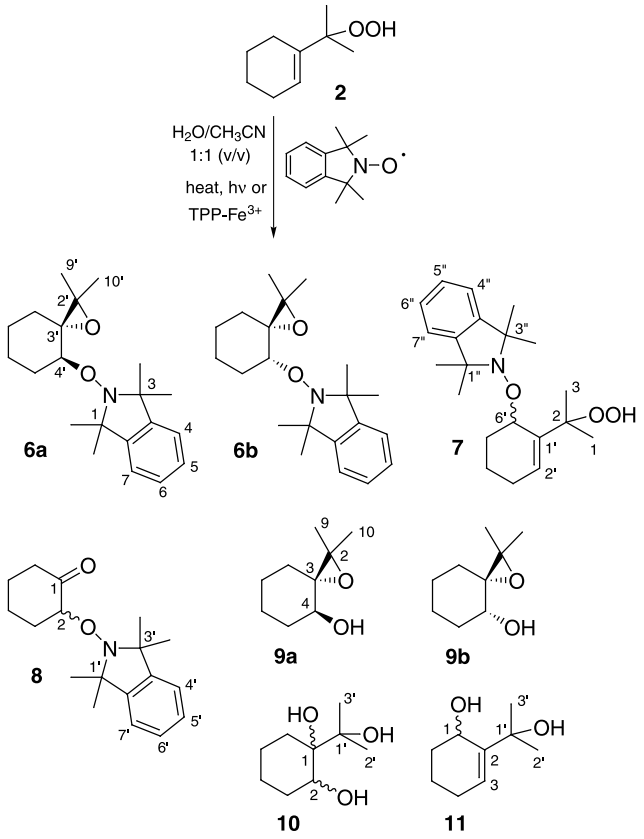
Scheme 1. Formation of stable alkoxyamine products by radical trapping with **5**.



Scheme 2. Trapping of radicals derived from compound **2**.

Most of the reported radical trapping experiments concerning mainly polyunsaturated fatty acid hydroperoxides have been carried out in organic solvents.[16] Very first experiments on the decomposition of **2** were performed in dichloromethane unsuccessfully: no products resulting from radical trapping by **5** were detected. While non-polar organic solvents may in a way mimic the reaction conditions within, for example, a membrane, the reactivity of allyloxyl radicals in aqueous solution is nevertheless also of interest, since it is assumed that many biological processes proceed at an aqueous and organic media interface. We carried out the experiments in a 1:1 (v/v) H_2O/CH_3CN mixture that allowed easy solubilization of both **2** and **5**. These conditions are, of course, still far away from those existing in in vivo systems, but the skin, could be seen as a semi-organic medium mixing hydrophilic and hydrophobic properties.

Radical formation was induced by direct thermal or photochemical cleavage of **2**, and by use of a metal complex such as TPP–Fe^{3+}. The reactions were followed by thin layer chromatography until complete disappearance of the hydroperoxide. Solvents were then removed under reduced pressure and the reaction compounds were separated, by column chromatography on silica gel, into two fractions (polar and non-polar). Each fraction was further purified by column

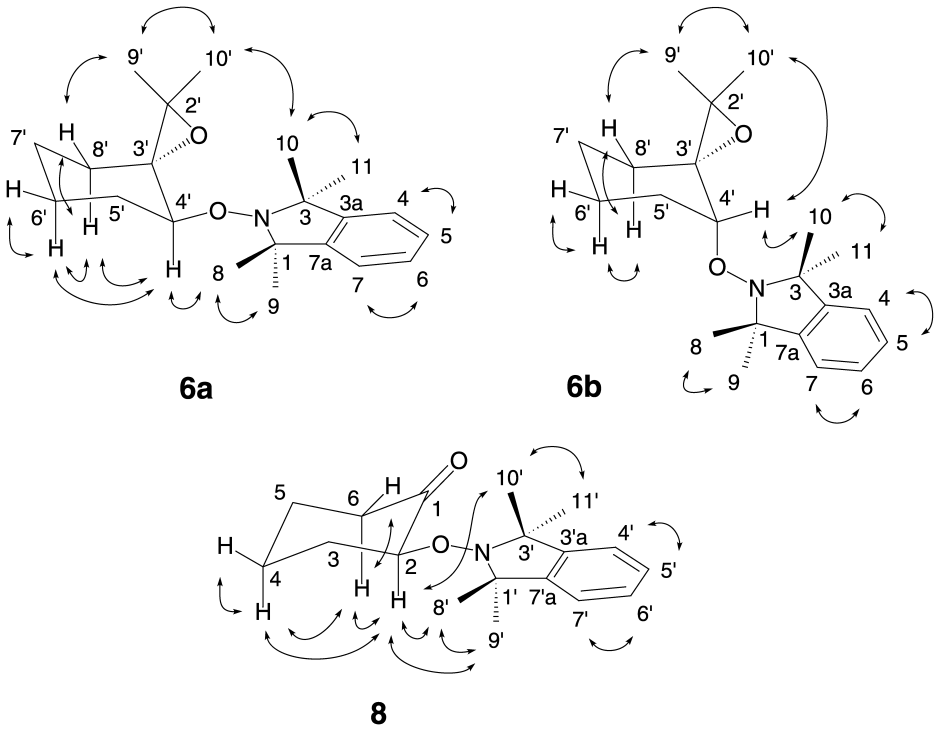


Figure 2. Main NOE effects observed in compounds **6a**, **6b** and **8**.

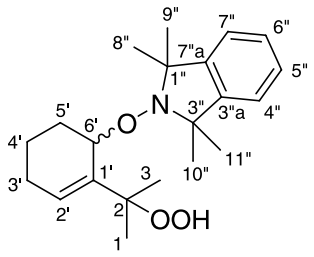
chromatography on silica gel. The non-polar fraction was found to contain adducts resulting from the trapping of formed alkyl radicals by **5**. The polar fraction was found to contain compounds resulting from the decay of alkyl radicals trapped by dioxygen. Each compound of the polar fraction was identified by comparison with reference materials obtained in a control experiment carried out in the absence of **5**. Structures of all isolated and identified radical trapping adducts are shown in Scheme 2.

2.1.1. Structural analysis of compounds 6a, 6b, 7 and 8. The decomposition of **2** in the presence of **5** in H_2O/CH_3CN 1:1 led to a complex mixture of products, no matter the method of generating free radicals employed. Adducts resulting from radical trapping by **5**, such as the non-separable mixture of diastereomers **6a** and **6b**, and compounds **7** and **8**, were isolated from the non-polar fraction.

The 500 MHz ^{1}H NMR spectrum of the non-separable diastereomers **6a** and **6b** showed the disappearance of the vinylic proton of the starting material **2** at 5.77 ppm. A set of signals in the 7.10–7.30 ppm region corresponding to the scavenger aromatic protons was present. The spectrum also exhibited signals for a proton in position α to an alkoxyamine for each diastereomer at 3.87 and 3.92 ppm, respectively. In the 125 MHz ^{13}C NMR spectrum, the disappearance of signals at 139.8 ppm (=*C*) and at 84.1 ppm (*C*–OOH) and the appearance of two new quaternary carbons at 67.6 and 68.1 ppm for one isomer, and at 67.3 and 68.3 ppm for the other isomer, were in agreement with the formation of a 2′,3′-spiro-type epoxide. The HSQC experiments allowed the correlation of proton and carbon nuclei via ^1J(C, H) for each isomer, and the skeleton of each adduct was determined by long-range ^{1}H–^{13}C correlations via ^2J(C, H) and ^3J(C, H) (HMBC experiments). Thus, H-4′ protons at 3.87 ppm (**6a**) and at 3.92 ppm (**6b**) were correlated via long-range coupling with the epoxide quaternary carbons at 67.6 (C-2′) and 68.1 ppm (C-3′), and at 67.3 (C-2′) and 68.3 ppm (C-3′), respectively. These quaternary carbons were also correlated with the methyl protons at 1.33 (H-10′) and 1.43 ppm (H-9′) for isomer **6a**, and with the methyl protons at 1.38 (H-10′) and 1.37 ppm (H-9′) for isomer **6b**. The relative stereochemistry determination was based on observations of nuclear Overhauser effects (NOE, Fig. 2). NOE were also useful for the complete chemical shift assignment of all H-axial and H-equatorial in the cyclohexane ring. A NOE between H-4′ (3.87 ppm) and the hydrogens H-8′$_{ax}$ (1.53 ppm) and H-6′$_{ax}$ (1.74 ppm) allowed us to attribute the H-4′ axial position for isomer **6a**. The absence of these cross-peaks in adduct **6b** is in favor of the H-4′ being on an equatorial position. A cross-peak between H-8′$_{eq}$ and the methyl group at C-9′ confirmed the position of the epoxide on the α side of the molecule for both isomers. In the case of adduct **6a** no cross-peaks were observed between H-4′ and the epoxide methyl groups. However, a NOE between H-4′ (3.92 ppm) and the methyl group at C-10′ (1.38 ppm) confirmed the equatorial position for H-4′ in adduct **6b**.

In the case of compound **7** the 500 MHz ^{1}H NMR spectrum showed a vinylic proton at 6.08 ppm, a group of signals corresponding to the scavenger aromatic protons in the 7.10–7.30 ppm region as well as a signal for a proton in position α to an alkoxyamine at 4.69 ppm. The 125 MHz ^{13}C NMR spectrum confirmed the vinylic position with a signal at 132.1 ppm (=*C*H) and the presence of a tertiary carbon at 72.8 ppm corroborated the alkoxyamine substituent. Signals at 139.6 ppm (=*C*) and at 84.1 ppm

Table 1. HMBC data of **7** showing long-range $^{1}H-^{13}C$ correlations



^{1}H position	^{13}C position
1	C-2, C-3, C-1′
3	C-1, C-2, C-1′
2′	C-2, C-3′, C-4′, C-6′
3′	C-1′, C-2′, C-5′
4′	C-2′
5′	C-3′
6′	C-2, C-2′, C-4′
4″	C-5″, C-3″a
5″	C-4″
6″	C-7″
7″	C-6″, C-7″a
8″	C-9″
9″	C-8″, C-7″a
10″	C-3″a, C-3″, C-11″
11″	C-3″, C-10″

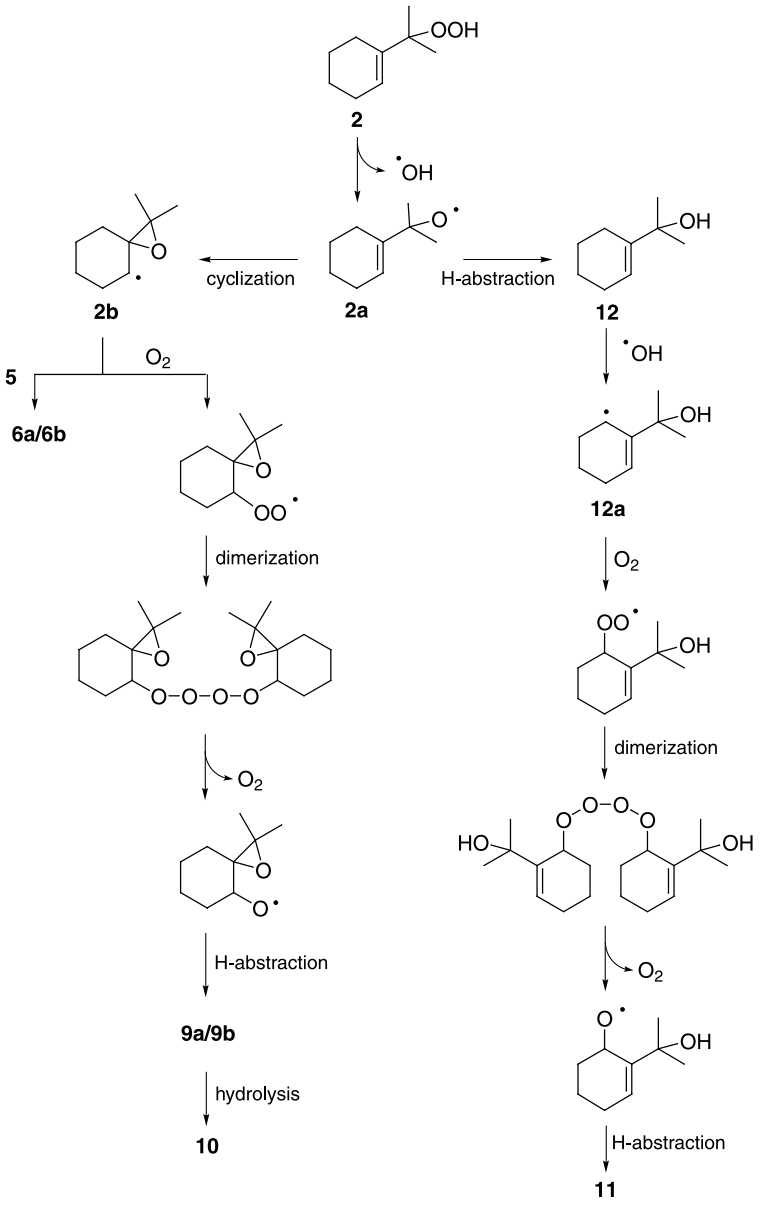
(*C*–OOH) were also present. The high-field region of the spectrum was resolved into 6 signals corresponding to 6 methyl groups. $^{1}H-^{13}C$ long-range correlations (Table 1) were consistent with a product deriving from the reaction between the radical trapping agent and the allylic carbon-centered radical in position 6′. The proton at 6.08 ppm (H-2′) was correlated via long-range coupling with carbons at 84.1 (C-2) and 72.8 ppm (C-6′). The proton at 4.69 ppm (H-6′) was correlated via long-range coupling with carbons at 84.1 (C-2) and 132.1 ppm (C-2′). Methyl protons at 1.58 (H-3) and 1.25 ppm (H-1) were correlated with the quaternary carbon at 139.6 ppm (C-1′). These results were supported by NOE experiments as protons at 4.69 (H-6′) and 6.08 ppm (H-2′) exhibited important NOE cross-peaks with the methyl groups at 1.25 (H-1) and 1.58 ppm (H-3). However, NOE experiments were not conclusive for the determination of adduct **7** conformation.

Adduct **8** was found to be a cyclohexanone ring bearing at the C-2 position the radical trapping agent. The 500 MHz ^{1}H NMR spectrum indicated the loss of the compound **2** vinylic proton at 5.77 ppm, and showed signals in the aromatic region (7.00–7.20 ppm). A signal at 4.36 ppm corresponding to a proton in position α to an alkoxyamine was present. In the 125 MHz ^{13}C NMR spectrum there was no more evidence of the quaternary carbons of the starting material at 84.1 (*C*–OOH) and 139.8 ppm (=*C*) neither of the vinylic carbon at 122.4 ppm (=*C*H). However, a signal at 209.6 ppm corresponding to a carbonyl carbon atom and a signal at 89.8 ppm corresponding to a tertiary carbon in α to a carbonyl group and to the 1,1,3,3-tetramethylisoindolin moiety were present. The chemical structure of adduct **8** was corroborated by $^{1}H-^{13}C$ long-range correlations. The proton at 4.36 ppm (H-2) was correlated via long-range coupling with the quaternary carbon at 209.6 ppm (C-1) and with the methylene group at 34.8 ppm (C-3). Similarly, the protons of the methylene group at C-6 (2.37 and 2.65 ppm) were correlated via long-range coupling with the carbonyl carbon atom at C-1. The main NOE effects observed for compound **8** are shown in Fig. 2. The proton H-2 exhibited important NOE cross-peaks with protons at 1.69 ppm ($H\text{-}4_{ax}$) and 2.37 ppm ($H\text{-}6_{ax}$). Protons $H\text{-}4_{ax}$ and $H\text{-}6_{ax}$ showed also, between themselves, a NOE signal. Other relevant NOE effects were observed between H-2 and the methyl groups of the 1,1,3,3-tetramethylisoindolin moiety at 1.49 (H-8′), 1.38 (H-9′) and 1.51 ppm (H-10′).

2.1.2. Structural analysis of compounds 9a, 9b, 10 and 11. In all experiments, the polar fraction was found to be a mixture of diastereomers **9a** and **9b** and compounds **10** and **11**, in different proportions depending on the radical initiator used. Each compound was identified by comparison with reference materials obtained in a control experiment carried out in the absence of **5**.

The non-separable mixture of diastereomers **9a** and **9b** were identified as α hydroxy epoxides. Their chemical structure and conformation were established on the basis of the same arguments used for adducts **6a** and **6b**, though the 1,1,3,3-tetramethylisoindolin moiety was replaced by an hydroxyl group. Protons H-4 of both isomers (3.73 ppm and 3.83 ppm) were correlated to the quaternary carbons of the epoxide at 63.6 (C-2) and 68.9 ppm (C-3), and at 63.0 (C-2) and 66.3 ppm (C-3), respectively. Quaternary carbons of the epoxide were themselves long-range correlated with the methyl groups at 1.32 (H-9) and 1.37 ppm (H-10) for the two isomers. A NOE between the methyl at 1.37 ppm (H-10) and $H\text{-}5_{ax}$ at 1.44 ppm as a well as a NOE between the methyl at 1.32 ppm (H-9) and $H\text{-}8_{eq}$ at 1.48 ppm confirmed the pseudo-equatorial C–O bond of the epoxide for both diastereomers. A NOE between H-4 (3.73 ppm) and protons $H\text{-}6_{ax}$ (1.40 ppm) and $H\text{-}8_{ax}$ (1.33 ppm) validated the equatorial orientation of the hydroxyl group for the major isomer **9a**. The absence of these cross-peaks in the minor isomer **9b** together with the existence of a NOE signal between H-4 (3.83 ppm) and the methyl group at 1.37 ppm (H-10) is in favor of the hydroxyl group being in the axial orientation.

Compound **10** is the result of the epoxide ring opening of the α hydroxy epoxide derivative in an aqueous media. The NMR characterization data were collected in acetone-d_6 because the lack of solubility of **10** in other deuterated solvents. The 200 MHz ^{1}H NMR spectrum showed two singlets at 3.25 (OH-1) and 4.23 ppm (OH-1′), one doublet at 4.43 ppm (OH-2) and a multiplet at 3.92 ppm (H-2). The 50 MHz ^{13}C NMR spectrum presented two quaternary carbons at 73.9 (C-1′) and 76.5 ppm (C-1) and a tertiary carbon at 71.2 ppm (C-2). Hydroxyl protons in alcohols tend to be exchanging rapidly, even in non-aqueous solvents. As a result, average signals can be obtained for all hydroxyl protons present in a compound and the shape of the resonance signals can be altered becoming broad and sometimes hidden in the noise. The presence of traces of acid or water promotes these fast exchange processes. Commercial $CDCl_3$ is often both sufficiently acidic and wet with D_2O, so a complete exchange can take place accidentally in the course of a routine spectrum acquisition. A method for preventing hydroxyl exchange is to use a

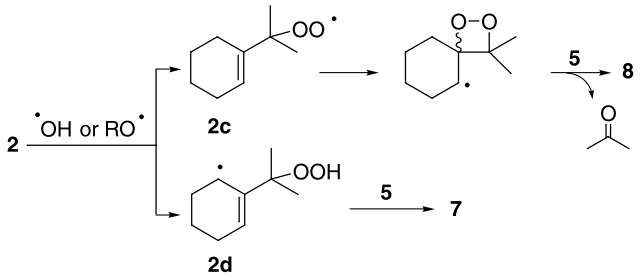


Scheme 3. Mechanistic interpretation for the obtention of **6a/6b**, **9a/9b**, **10** and **11**.

hydrogen-bonding solvent such as DMSO-d_6 or acetone-d_6. In these solvents the exchange processes are often slower. Indeed, the use of acetone-d_6 allowed us to observe isolated resonance signals for the three hydroxy groups present in compound **10**. NOE experiments were not conclusive for the stereochemistry determination of this adduct.

The 200 MHz ^{1}H spectrum of compound **11** showed the vinylic proton at 5.80 ppm (H-3) and the proton in position α to the hydroxyl group at 4.23 ppm (H-1). The 50 MHz ^{13}C NMR spectrum confirmed the vinylic position with a signal at 121.6 ppm (=*C*H) and the presence of a tertiary carbon at 72.8 ppm corroborated the hydroxyl substituent. Signals at 148.1 ppm (=*C*) and at 72.7 ppm (*C*–OH) were also present. Once again, NOE experiments were not conclusive for the conformation determination of adduct **11**.

2.1.3. Mechanistic interpretations. Several possible mechanisms for the formation of radical species from hydroperoxide **2** in the presence of different radical initiators and of 1,1,3,3-tetramethylisoindolin-2-yloxyl **5** can be invoked. Homolytic cleavage of the peroxy bond produced an alkoxyl radical **2a** that underwent a rapid cyclization to an α-oxiranylcarbinyl radical **2b** (Scheme 3). This species, trapped by **5** or by dioxygen, gave the mixture of diastereomers **6a** and **6b** and peroxyl radicals, respectively. The latter, after dimerization and loss of dioxygen, led to alkoxyl radicals that afforded the α-hydroxyepoxides **9a** and **9b** by hydrogen abstraction. Only the alkoxyl radical cyclization on the α side of **2a** was observed and the equatorial position was preferred for the radical trapping agent (**5** or dioxygen) to react (typical ratios: 83/17 for **6a/6b**, 64/36 for **9a/9b**). In the case of compounds **9a** and **9b** there was opening of the epoxide ring to form compound **10**. The formation of compound **11** was explained by the abstraction of an allylic hydrogen atom from the intermediate **12**, itself obtained from **2a** by hydrogen abstraction. The carbon-centered radical **12a**, stabilized by resonance due to the



Scheme 4. Mechanistic interpretation for the obtention of **7** and **8**.

presence of the double bond, was trapped by dioxygen affording peroxyl radicals that decay to **11** via dimerization, loss of dioxygen and then hydrogen abstraction. No products of β-scission of **2a** were detected during the experiments.

The **2** peroxyl-hydrogen bond could be cleaved homolytically[17,18] to give the peroxyl radical **2c** (Scheme 4). Indeed, in the experimental conditions used the hydroxyl (HO·), and possibly alkoxyl radicals, could easily perform this hydrogen abstraction. The peroxyl radical **2c** could rearrange, via intramolecular addition to the double bond, leading to a carbon-centered radical with a dioxetane structure. The latter cleaved[19] to form acetone and a carbonylic compound, and the alkyl radical was trapped by **5** to afford compound **8**. Finally, allylic hydrogen abstraction from **2** gave the alkyl radical **2d** which was trapped by **5** to afford **7**.

The isolated products were obtained in different yields depending on the method used to induce the radical generation (thermally, photochemically or by use of a transition metal ion). As shown in Table 2, in the presence of TPP–Fe^{3} the decomposition of **2** was faster, and only adducts resulting from trapping of alkyl radicals derived from **2a** cyclization were observed. When the radical inducer was TPP–Fe^{3} an electron transfer process was probably involved, leading mostly to alkoxyl radicals and hydroxyl ions HO^{-}, and not HO· radicals. This could account for the absence of products **7**, **8** and **11**. The mixture **6a/6b** was obtained with a rather high yield (21%), together with **9a/9b** (6%) and **10** (3%).

Table 2. Yields of the radical trapping adducts obtained according to the radical inducer used

Radical inducer	Time[a]	Products yield (%)					
		6a/6b[b]	**7**	**8**	**9a/9b**[b]	**10**	**11**
Heat[c,d]	3 days	4 (80/20)	1	7	3 (64/36)	6	6
Heat[c,e]	6 days	3 (91/9)	–	4	4 (60/40)	7	10
Heat[f]	3 days	–	–	–	2 (81/19)	12	6
hν	3 weeks	1 (83/17)	–	6	3 (67/33)	6	8
TPP–Fe^{3+}	3 h	21 (80/20)	–	–	6 (65/35)	3	–

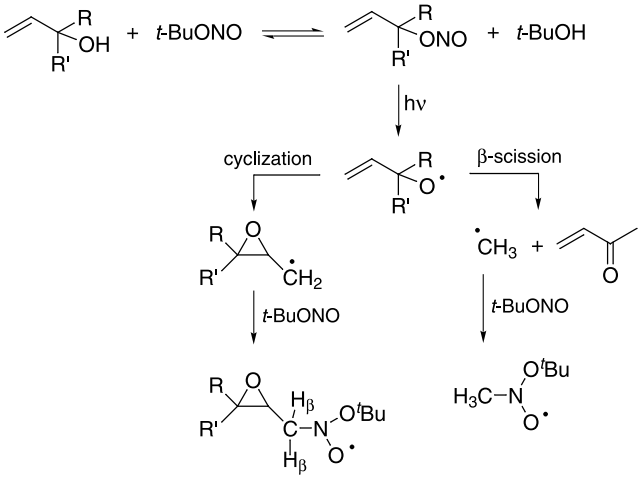
[a] The reactions were followed by TLC and stopped after complete disappearance of **2**.
[b] Ratio in brackets.
[c] Thermal cleavage was carried out with the system protected and unprotected against daylight, to evaluate if the generation of radicals was exclusively heat dependent.
[d] Reflux; system unprotected against daylight.
[e] Reflux; system protected against daylight.
[f] Control experiment carried out in the absence of **5**, under reflux and with the system unprotected against daylight.

Thermal and photochemical decomposition of **2** afforded a small yield of **6a/6b** (1–4%) and of **9a/9b** (3–4%), but a slightly higher yield of **10** (6–7%). Furthermore, products resulting from the cyclization of the peroxyl radical **2c** (**8**, 4–7%), as well as from the allylic radicals **2d** (**7**, 1%) and **12a** (**11**, 6–10%) were detected. The overall yield of trapped radicals was very similar for both, the thermal (28%) and photochemical (24%) reactions. However, the photochemically induced reaction was slower than the thermally induced one. The influence of daylight was also evidenced when heating to induce the radical formation: the reaction was less efficient when the system was protected against daylight, with a marked increase in reaction time. Compound **7** (1%) was only obtained when a combination of heating and daylight was used. A complementary reaction in the absence of **5** was carried out. Obviously in this case, only compounds resulting from the decay of the dioxygen trapped alkyl radicals were obtained. Among these, **10** was isolated in a 12% yield. The formation of other compounds in a very low yield, which could not be isolated and characterized is not excluded.

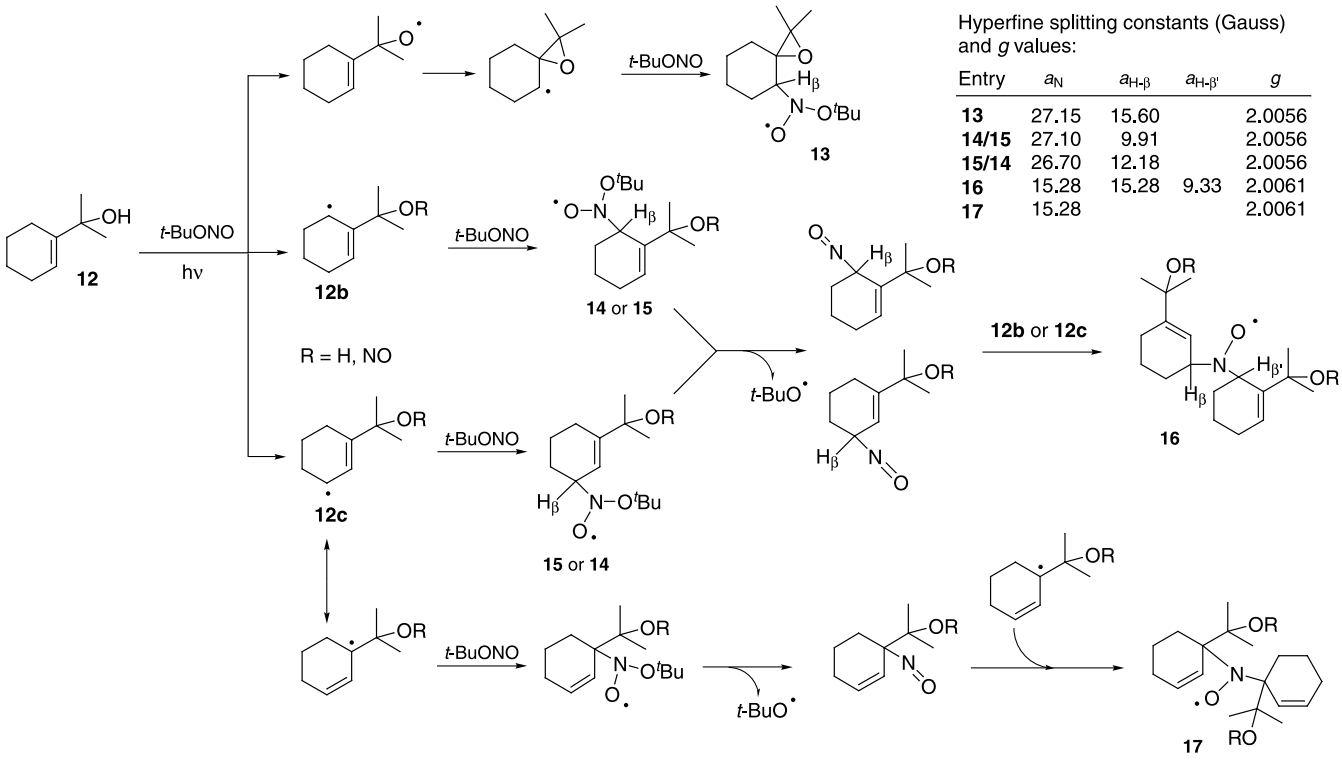
2.2. ESR experiments

As described in preliminary communications, a spin trapping ESR technique can be employed to characterize oxiranylcarbinyl radicals as discrete reaction intermediates, formed via cyclization of allyloxyl radicals.[20,21] The technique is based on the known ability of alkyl nitrites, in particular of *tert*-butyl nitrite (*t*-BuONO), to act as spin trap for short-lived alkyl radicals.[22] Briefly, the allyloxyl radical is generated by photolysis of the corresponding nitrite[23] prepared via the facile exchange reaction between the parent alcohol[24] and *t*-BuONO (Scheme 5). The alkyl radicals formed by rapid decay of the allyloxyl radical are trapped by *t*-BuONO, in excess in the reaction medium, to give fairly stable nitroxides detectable by ESR.

In order to get more detailed information on the evolution of the allyloxyl radical derived from **2** the described ESR technique was used and applied to the parent alcohol **12**. Indeed, photolysis of the **12** corresponding nitrite afforded the same allyloxyl radical **2a** obtained by homolytic



Scheme 5. Formation of stable nitroxide compounds by *t*-BuONO spin trapping of alkyl radicals.



Scheme 6. Nitroxides obtained from alcohol **12** and *t*-BuONO.

cleavage of the peroxide bond of **2**. Moreover, hydroperoxides are often unstable species and difficult to handle. To work with the parent stable tertiary alcohol was therefore an attractive alternative for our study.

The experiments were conducted under continuous flow conditions (0.02–0.1 mL/min) on acetonitrile solutions of **12** (70 mM) in the presence of an excess of *t*-BuONO (115 mM), which were photolysed ($\lambda > 250$ nm) directly in the ESR spectrometer cavity at different temperatures in the range 223–243 K. The analysis of all obtained ESR spectra allowed the identification of five different radical species, **13**–**17** (Scheme 6).

The characteristic hyperfine splitting constants of nitrogen atoms (a_N), β-protons ($a_{H\text{-}\beta}$, $a_{H\text{-}\beta}{}'$) and *g* values, identified these species as alkyl alkoxy nitroxides formed by trapping of alkyl radicals by *t*-BuONO. Their structure was also confirmed by means of computer simulations. Typical ESR spectra are shown in Fig. 3.

Nitroxide **13** was formed by *t*-BuONO trapping of an oxiranylcarbinyl radical, formed via cyclization of the allyloxyl radical derived from photolysis of the **12** nitrite. Nitroxides **14** and **15**, also if not unambiguously attributed, were formed by *t*-BuONO trapping of allylic radicals, obtained by hydrogen abstraction. Nitroxides derived from the coupling of species generated in situ were also observed. In fact, the loss of the *tert*-butoxide group from **15** and/or **14** afforded a nitroso derivative, which could act as a spin trap of a carbon-centered allylic radical to give nitroxide **16**. Similarly, **17** was obtained after coupling of a rearranged allylic radical with a second nitroso derivative, itself formed by cleavage of the *tert*-butoxide group of a nitroxide intermediate. Radicals **16** and **17** were formed in very low yield. They could only be detected when stopping the irradiation and under static conditions (Fig. 3b).

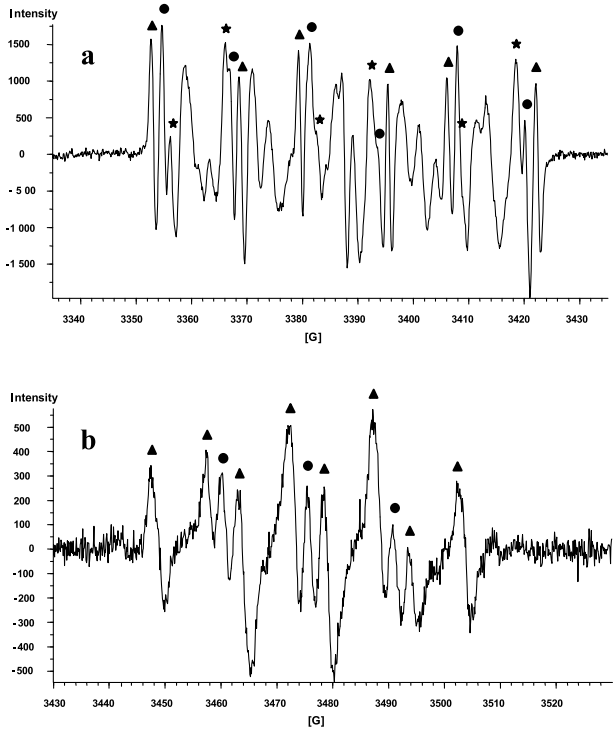


Figure 3. (a) ESR spectrum obtained in the photolysis of an acetonitrile solution of **12**, 70 mM, and *t*-BuONO, 115 mM, at 223 K (flow rate 0.1 mL/min), showing radicals **13** (▲), **14** (★) and **15** (●). (b) ESR spectrum obtained after stopping the irradiation and under static conditions, at 233 K, showing radicals **16** (▲) and **17** (●).

The a_N hyperfine splitting constant values for **13**–**15** were characteristic of an R-N(O·)-O^{t}Bu nitroxide–type (26–28 Gauss), whilst those of **16** and **17** (a_N=15–16 Gauss) of an R-N(O·)-R′ nitroxide-type. The *g* values corresponded as well to the values generally observed for the R-N(O·)-O^{t}Bu and R-N(O·)-R′ nitroxide-types, i. e. 2.0056 and 2.0061 respectively. Nevertheless, the $a_{H\text{-}\beta}$ hyperfine splitting constants (9.3–15.6 Gauss) for these nitroxides were higher than those observed in the oxiranylcarbinyl radicals formed by cyclization of linear allyloxyl radicals (5.2–9.9 Gauss).[21] This difference can be explained by the conformation of the cyclic system under study, which shows a more rigid structure than that of the linear systems studied previously.

ESR studies confirmed the formation of oxiranylcarbinyl radicals, via cyclization of the parent allyloxyl radicals, as well as the allylic hydrogen abstraction, as evidenced by the trapping of the corresponding allylic radicals. Moreover, hydrogen abstraction from different positions was observed. However, no adducts formed by trapping of alkyl radicals deriving from a β-scission process were observed.

3. Conclusion

The results of our experiments, obtained using a combination of radical trapping and ESR studies, confirmed the formation of carbon-centered reactive radicals derived from the allergenic cyclic tertiary allylic hydroperoxide **2**. The fact that radicals derived from **2** could be trapped does not prove that they are able to form adducts with skin proteins in vivo, but the in situ generation of such reactive radicals in the epidermis could lead to the formation of antigenic structures, the first step of the ACD mechanism. Most known allergens are low molecular weight electrophiles, or molecules that are metabolized into electrophiles, that are able to react with the side chains of amino acids containing nucleophilic groups, such as lysine, cysteine, histidine, methionine, and tyrosine. In this respect, the formation of radical intermediates could lead to the modification of other amino acids or to the formation of carbon–carbon bonds which are not usually considered in the mechanism of ACD, giving new insights into the mechanism of hapten–protein interactions. Further studies concerning the reactivity of **2** with amino acids and with skin proteins will now be undertaken. Studies concerning the formation and the identification of reactive radicals derived from 15-hydroperoxyabietic acid **1**, containing a bis-allylic tertiary hydroperoxide, as well as their reactivity, are already in progress.

4. Experimental

CAUTION: skin contact with hydroperoxides must be avoided. Since these compounds are skin sensitizing substances, they must be handled with care.

4.1. Chemicals and reagents

The allylic hydroperoxide 1-(1-hydroperoxy-1-methylethyl)cyclohexene **2** and its former allylic alcohol 1-(1-hydroxy-1-methylethyl)cyclohexene **12** were prepared using the procedure reported by Lepoittevin and Karlberg.[7] The free radical-trapping agent 1,1,3,3-tetramethylisoindolin-2-yloxyl **5** was synthesized using a method previously reported in the literature, based on the reaction of *N*-benzylphthalimide with methylmagnesium bromide in refluxing toluene, followed by hydrogenolysis and oxidation.[12] The *meso*-tetraphenylporphyrin iron(III) chloride complex (TPP–Fe^{3+}) was purchased from Fluka (St Quentin Fallavier, France). The *tert*-butyl nitrite (*t*-BuONO) was prepared following the standard procedure reported in the literature and distilled under vacuum before use.[25] Spectrophotometric grade acetonitrile (99.8%), ethyl acetate (99.8%), and dichloromethane (99.5%) were purchased from Carlo Erba (Val de Reuil, France). Hexane (99%) was obtained from SDS (Peypin, France). All solvents were used as delivered. Aqueous solutions were prepared with deionized water. All other chemicals were obtained from Aldrich Chemical Co. (St Quentin Fallavier, France) and used without further purification. 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 (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) or on aluminum oxide (Merck 90, standardized).

4.2. Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200 and AM500 MHz spectrometers. The 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 ^{1}H and 77.00 ppm for ^{13}C; acetone-d_6, 2.05 ppm for ^{1}H and 207.07 and 30.92 ppm for ^{13}C). Multiplicities are indicated by s (singlet), d (doublet), t (triplet), and m (multiplet). ^{1}H coupling constants (*J*) were obtained by first-order analysis and are reported in Hz. The different types of carbon in the structures have been identified by the DEPT-135 technique. The two-dimensional phase-sensitive ^{1}H–^{1}H COSY (correlation spectroscopy) spectra were collected using a standard pulse sequence with a 254 ms acquisition time, a sweep width of 12.25 ppm, 512 complete t_1 data points, and 8 scans per increment. The two-dimensional phase-sensitive ^{1}H–^{1}H NOESY (nuclear Overhauser effect spectroscopy) spectra were collected using an 800 ms mixing time and a 254 ms acquisition time, a sweep width of 12.25 ppm, 512 complete t_1 data points, and 16 scans per increment. The two-dimensional ^{1}H–^{13}C HSQC (heteronuclear single-quantum correlation) spectra and ^{1}H–^{13}C HMBC (heteronuclear multiple-bond correlation) spectra were collected using a standard pulse sequence, with a 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 209.23 ppm. The data were processed using XWINNMR software. Infrared spectra were obtained on a Perkin–Elmer FT-IR 1600 spectrometer; peaks are reported in reciprocal centimeters. Melting points were determined on a Büchi Tottoli 510 apparatus and are

uncorrected. Elementary analyses were performed by the microanalytical laboratory of the CRM (Centre de Recherche sur les Macromolécules), Strasbourg, France. Electron spin resonance (ESR) spectra were recorded on a Bruker ESP 300E spectrometer equipped with a X-band microwave bridge, 100 KHz modulation, and a variable temperature apparatus (B-VT 2000). Irradiation of the solutions inside the ESR cavity was performed with an Oriel 66901-M high-pressure mercury lamp. Hyperfine splitting assignments were obtained by means of computer simulation.

4.3. Radical trapping experiments

4.3.1. Generation of radicals by thermal cleavage. (a) Allylic hydroperoxide **2** (300 mg, 1.92 mmol) was dissolved in a 1:1 (v/v) mixture of H_2O/CH_3CN (50 mL), together with the nitroxide **5** (731 mg, 3.84 mmol). The solution was heated to reflux, unprotected against daylight. The reaction was followed by TLC (hexane–AcOEt 9:1) until complete disappearance of the hydroperoxide. After 72 h stirring under reflux, the solution was allowed to cool down to room temperature and concentrated under reduced pressure. The crude product was fractionated into polar and non-polar compounds by silica gel column chromatography (hexane–AcOEt 95:5 until complete elution of **5**, then hexane–AcOEt 5:5). Further purification of the non-polar fraction was carried out by column chromatography on silica gel (hexane–AcOEt 95:5) to give a non-separable mixture of two diastereomers, **6a** and **6b** (80/20 ratio), as a white solid (25.7 mg, 4%), and compounds **7** (8.1 mg, 1%) and **8** (40.2 mg, 7%), also as white solids. The polar fraction was purified by column chromatography on silica gel (hexane–AcOEt 7:3, then hexane–AcOEt 5:5) and was found to contain a non-separable mixture of two diastereomers, **9a** and **9b** (64/36 ratio), as a colorless oil (9.6 mg, 3%), compound **10** (20.3 mg, 6%) as a white solid and compound **11** (17.5 mg, 6%) as a colorless oil.

(b) To a solution of hydroperoxide **2** (300 mg, 1.92 mmol) in a mixture of H_2O/CH_3CN 1:1 (v/v) (50 mL), was added the nitroxide **5** (731 mg, 3.84 mmol). The solution was protected against daylight with aluminum folder and heated to reflux. The reaction was followed by TLC (hexane–AcOEt 9:1). Complete disappearance of the hydroperoxide was observed after 6 days stirring under reflux. The solution was then allowed to cool down to room temperature and was concentrated under reduced pressure. The crude product was fractionated into polar and non-polar compounds by silica gel column chromatography (hexane–AcOEt 95:5 until complete elution of **5**, then hexane–AcOEt 5:5). Further purification of the non-polar fraction was carried out by column chromatography on silica gel (hexane–AcOEt 95:5) to give a non-separable mixture of diastereomers **6a** and **6b** (91/9 ratio) as a white solid (16.8 mg, 3%), and compound **8** (23.2 mg, 4%), also as a white solid. The polar fraction was purified by column chromatography on silica gel (hexane–AcOEt 7:3, then hexane–AcOEt 5:5) and was found to contain a non-separable mixture of diastereomers **9a** and **9b** (60/40 ratio) as a colorless oil (12.4 mg, 4%), compound **10** (22.2 mg, 7%) as a white solid and compound **11** (29 mg, 10%) as a colorless oil.

4.3.2. Generation of radicals by photochemical cleavage. Hydroperoxide **2** (300 mg, 1.92 mmol) was dissolved in a 1:1 (v/v) mixture of H_2O/CH_3CN (50 mL). The nitroxide **5** (731 mg, 3.84 mmol) was added to the solution and the stirred reaction mixture was irradiated for 3 weeks with a 150 W tungsten filament lamp at a distance of 30 cm. The evolution of the reaction was followed by TLC (hexane–AcOEt 9:1) until complete disappearance of the hydroperoxide. The mixture was concentrated under reduced pressure and fractionated into polar and non-polar compounds by silica gel column chromatography (hexane–AcOEt 95:5 until complete elution of **5**, then hexane–AcOEt 5:5). Purification of the non-polar fraction was carried out by column chromatography on silica gel (hexane–AcOEt 95:5) to give a non-separable mixture of diastereomers **6a** and **6b** (83/17 ratio) as a white solid (7.6 mg, 1%), and compound **8** (34.3 mg, 6%), also as a white solid. The polar fraction was purified by column chromatography on silica gel (hexane–AcOEt 7:3, then hexane–AcOEt 5:5) to give a non-separable mixture of diastereomers **9a** and **9b** (67/33 ratio) as a colorless oil (8.2 mg, 3%), compound **10** (19.8 mg, 6%) as a white solid and compound **11** (24.2 mg, 8%) as a colorless oil.

4.3.3. Generation of radicals by reduction from TPP–Fe^{3+}. To a solution of hydroperoxide **2** (300 mg, 1.92 mmol) in a 1:1 (v/v) mixture of H_2O/CH_3CN (50 mL) were added **5** (731 mg, 3.84 mmol) and TPP–Fe^{3+} (1.5 g, 2.1 mmol). The reaction mixture was stirred at room temperature for 3 h (complete disappearance of **2** as shown by TLC; hexane–AcOEt 9:1). The mixture was concentrated under reduced pressure, taken up with the minimum amount of CH_2Cl_2, and purified by column chromatography on silica gel (hexane–AcOEt 8:2) in order to eliminate the excess of TPP–Fe^{3+}. Further column chromatography on silica gel (hexane–AcOEt 9:1) allowed the fractionation of the crude product into polar and non-polar compounds. The non-polar fraction was purified by column chromatography on aluminum oxide (hexane–AcOEt 97:3) to give exclusively a non-separable mixture of diastereomers **6a** and **6b** (80/20 ratio) as a white solid (130 mg, 21%). The polar fraction was purified by column chromatography on silica gel (hexane–AcOEt 7:3, then hexane–AcOEt 5:5) to give a non-separable mixture of diastereomers **9a** and **9b** (65/35 ratio) as a colorless oil (19.3 mg, 6%), and compound **10** (9.4 mg, 3%) as a white solid.

4.3.4. Radical trapping experiment in the absence of 5. Allylic hydroperoxide **2** (300 mg, 1.92 mmol) was dissolved in a 1:1 (v/v) mixture of H_2O/CH_3CN (50 mL). The solution was heated to reflux without protection against daylight. The reaction was followed by TLC (hexane–AcOEt 7:3) until complete disappearance of the hydroperoxide. After 72 h stirring under reflux, the solution was allowed to cool down to room temperature and concentrated under reduced pressure. Column chromatography of the crude product over silica gel (hexane–AcOEt 7:3, then hexane–AcOEt 5:5) gave, as a colorless oil, a non-separable mixture of diastereomers **9a** and **9b**, in a 81/19 ratio (6.5 mg, 2%), together with compound **10** (39.5 mg, 12%) as a white solid, and compound **11** (16.6 mg, 6%) as a colorless oil.

4.4. Characterization data for radical trapping adducts

4.4.1. Non-separable mixture of diastereomers 6a and 6b. White solid; mp 86–88°C; IR ($CHCl_3$) ν 2974 (=C–H), 2935 (C–H), 1003 cm^{-1} (C–O–C). Anal. Calcd for $C_{21}H_{31}NO_2$: C, 76.60; H, 9.50; N, 4.30. Found: C, 76.49; H, 9.59; N, 4.19.

2-(2′,2′-Dimethyl-1′-oxa-spiro[2.5]oct-4′(S)-yloxy)-1,1,3,3-tetramethyl-2,3-dihydro-1H-isoindole (6a). Major isomer (80%, ^1H integration); ^1H NMR ($CDCl_3$, 500 MHz): δ 1.33 (s, 3H, H-10′), 1.42 (s, 6H, H-9 and H-11), 1.43 (s, 3H, H-9′), 1.45 (s, 3H, H-8), 1.53 (m, 4H, H-10 and H-$8'_{ax}$), 1.56 (m, 1H, H-$6'_{eq}$), 1.61 (m, 1H, H-$7'_{eq}$), 1.74 (m, 2H, H-$5'_{ax}$ and H-$6'_{ax}$), 1.77 (m, 1H, H-$7'_{ax}$), 2.10 (m, 1H, H-$5'_{eq}$), 2.20 (m, 1H, H-$8'_{eq}$), 3.87 (m, 1H, H-4′), 7.10 (ddd, J=6.4, 2.3 and 1.2 Hz, 2H, H-4 and H-7), 7.23 (dt, J=6.3 and 2.3 Hz, 2H, H-5 and H-6); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.6 (C-6′), 20.9 (C-10′), 22.2 (C-9′), 23.6 (C-7′), 25.4 (C-9 and C-11), 25.9 (C-8′), 29.1 (C-8), 29.8 (C-5′), 30.3 (C-10), 65.1 (C-1), 66.9 (C-3), 67.6 (C-2′), 68.1 (C-3′), 79.1 (C-4′), 121.5 (C-4 and C-7), 127.2 (C-5 and C-6), 145.1 (C-3a and C-7a).

2-(2′,2′-Dimethyl-1′-oxa-spiro[2.5]oct-4′(R)-yloxy)-1,1,3,3-tetramethyl-2,3-dihydro-1H-isoindole (6b). Minor isomer (20%, ^1H integration); ^1H NMR ($CDCl_3$, 500 MHz): δ 1.32 (m, 2H, H-$5'_{ax}$ and H-$6'_{ax}$), 1.36 (m, 1H, H-$6'_{eq}$), 1.37 (s, 3H, H-9′), 1.38 (s, 3H, H-10′), 1.40 (s, 9H, H-8, H-9 and H-11), 1.44 (m, 1H, H-$8'_{ax}$), 1.56 (s, 3H, H-10), 1.59 (m, 1H, H-$7'_{ax}$), 1.86 (m, 1H, H-$7'_{eq}$), 2.23 (m, 1H, H-$8'_{eq}$), 2.32 (m, 1H, H-$5'_{eq}$), 3.92 (m, 1H, H-4′), 7.10 (ddd, J=6.4, 2.3 and 1.2 Hz, 2H, H-4 and H-7), 7.23 (dt, J=6.3 and 2.3 Hz, 2H, H-5 and H-6); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.0 (C-6′), 20.4 (C-9′), 20.9 (C-10′), 25.2 (C-8), 25.3 (C-11), 25.4 (C-7′), 25.6 (C-9), 26.7 (C-8′), 30.0 (C-10), 31.0 (C-5′), 65.1 (C-1), 66.9 (C-3), 67.3 (C-2′), 68.3 (C-3′), 82.9 (C-4′), 121.5 (C-4 and C-7), 127.2 (C-5 and C-6), 145.8 (C-3a and C-7a).

4.4.2. 2-[6′-(1″,1″,3″,3″-Tetramethyl-1″,3″-dihydro-isoindol-2″-yloxy)-cyclohex-1′-enyl]-prop-2-yl-hydroperoxide (7). White solid; mp 96–98°C; ^1H NMR ($CDCl_3$, 500 MHz): δ 1.22 (m, 1H, H-$5'_{eq}$), 1.25 (s, 3H, H-1), 1.58 (m, 7H, H-3, H-$4'_{ax}$ and H-8″), 1.60 (s, 6H, H-9″ and H-11″), 1.91 (m, 4H, H-$4'_{eq}$ and H-10″), 2.07 (m, 1H, H-$3'_{peq}$), 2.31 (m, 1H, H-$3'_{pax}$), 2.82 (m, 1H, H-$5'_{ax}$), 4.69 (m, 1H, H-6′), 6.08 (t, J=3.8 Hz, 1H, H-2′), 7.09 (ddd, J=6.4, 2.3 and 1.2 Hz, 2H, H-4″ and H-7″), 7.24 (dt, J=6.4 and 2.3 Hz, 2H, H-5″ and H-6″); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.1 (C-4′), 24.4 (C-3 and C-8″), 25.7 (C-1, C-3′ and C-9″), 26.0 (C-5′), 29.3 (C-10″), 29.4 (C-11″), 68.3 (C-1″ and C-3″), 72.8 (C-6′), 84.1 (C-2), 121.5 (C-4″ and C-7″), 127.2 (C-5″ and C-6″), 132.1 (C-2′), 139.6 (C-1′), 143.0 (C-3″a), 144.5 (C-7″a); IR ($CHCl_3$) ν 3287 (O–O–H), 2974 (=C–H), 2927 cm^{-1} (C–H); Anal. Calcd for $C_{21}H_{31}NO_3$: C, 73.00; H, 9.00; N, 4.10. Found: C, 73.15; H, 9.10; N, 3.80.

4.4.3. 2-(1′,1′,3′,3′-Tetramethyl-1′,3′-dihydro-isoindol-2′-yloxy)-cyclohexanone (8). White solid; mp 76–78°C; ^1H NMR ($CDCl_3$, 500 MHz): δ 1.38 (s, 3H, H-9′), 1.41 (s, 3H, H-11′), 1.49 (s, 3H, H-8′), 1.51 (s, 3H, H-10′), 1.69 (m, 1H, H-4_{ax}), 1.78 (m, 1H, H-5_{ax}), 1.92 (m, 1H, H-5_{eq}), 1.97 (m, 2H, H-3_{ax} and H-4_{eq}), 2.34 (m, 1H, H-3_{eq}), 2.37 (m, 1H, H-6_{ax}), 2.65 (m, 1H, H-6_{eq}), 4.36 (ddd, J=9.5, 5.2 and 1.2 Hz, 1H, H-2), 7.09 (ddd, J=6.4, 2.3 and 1.2 Hz, 2H, H-4′ and H-7′), 7.23 (dt, J=6.4 and 2.3 Hz, 2H, H-5′ and H-6′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 23.2 (C-4), 25.1 (C-8′), 25.4 (C-9′), 27.7 (C-5), 29.8 (C-11′), 30.2 (C-10′), 34.8 (C-3), 41.2 (C-6), 67.1 (C-3′), 67.7 (C-1′), 89.8 (C-2), 121.6 (C-4′ and C-7′), 127.3 (C-5′ and C-6′), 143.8 (C-3′a), 145.0 (C-7′a), 209.6 (C-1); IR ($CHCl_3$) ν 2934 (=C–H), 2927 (C–H), 1721 cm^{-1} (C=O); Anal. Calcd for $C_{18}H_{25}NO_2$: C, 75.20; H, 8.80; N, 4.90. Found: C, 74.97; H, 8.86; N, 4.59.

4.4.4. Non-separable mixture of diastereomers 9a and 9b. Colorless oil; IR (film) ν 3450 (O–H), 2970 cm^{-1} (C–H); Anal. Calcd for $C_9H_{16}O_2$: C, 69.19; H, 10.32. Found: C, 69.17; H, 10.43.

2,2-Dimethyl-1-oxa-spiro[2.5]octan-4(S)-ol (9a). Major isomer (64%, ^1H integration); ^1H NMR ($CDCl_3$, 500 MHz): δ 1.29 (m, 1H, H-7_{ax}), 1.32 (s, 3H, H-9), 1.33 (m, 1H, H-8_{ax}), 1.37 (s, 3H, H-10), 1.40 (m, 1H, H-6_{ax}), 1.44 (m, 1H, H-5_{ax}), 1.48 (m, 1H, H-8_{eq}), 1.82 (m, 2H, H-5_{eq} and H-7_{eq}), 2.17 (m, 1H, H-6_{eq}), 3.73 (m, 1H, H-4_{ax}); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.9 (C-9), 21.1 (C-10), 24.6 (C-8), 25.1 (C-6), 25.3 (C-7), 31.5 (C-5), 63.6 (C-2), 68.9 (C-3), 69.5 (C-4).

2,2-Dimethyl-1-oxa-spiro[2.5]octan-4(R)-ol (9b). Minor isomer (36%, ^1H integration); ^1H NMR ($CDCl_3$, 500 MHz): δ 1.29 (m, 1H, H-7_{ax}), 1.32 (s, 3H, H-9), 1.33 (m, 1H, H-8_{ax}), 1.37 (s, 3H, H-10), 1.44 (m, 1H, H-5_{ax}), 1.48 (m, 1H, H-8_{eq}), 1.53 (m, 1H, H-6_{ax}), 1.70 (m, 1H, H-6_{eq}), 1.80 (m, 1H, H-7_{eq}), 1.98 (m, 1H, H-5_{eq}), 3.83 (m, 1H, H-4_{eq}); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.8 (C-9), 21.0 (C-10), 23.4 (C-6), 24.6 (C-8), 25.3 (C-7), 32.2 (C-5), 63.0 (C-2), 66.3 (C-3), 69.3 (C-4).

4.4.5. 1-(1′-Hydroxy-1′-methyl-ethyl)-cyclohexane-1,2-diol (10). White solid; mp 150–152°C; ^1H NMR (acetone-d_6, 200 MHz): δ 1.14 (s, 3H, H-3′), 1.28 (s, 3H, H-2′), 1.29 (m, 4H, H-4 and H-5), 1.40 (m, 2H, H-6), 1.44 (m, 2H, H-3), 3.25 (s, 1H, OH-1), 3.92 (m, 1H, H-2), 4.23 (s, 1H, OH-1′), 4.43 (d, J=2.2 Hz, 1H, OH-2); ^{13}C NMR (acetone-d_6, 50 MHz): δ 19.7 (C-5), 21.7 (C-4), 24.6 (C-3′), 25.4 (C-2′), 26.2 (C-3), 30.5 (C-6), 71.2 (C-2), 73.9 (C-1′), 76.5 (C-1). IR (DMSO) ν 3440 (O–H), 2970 cm^{-1} (C–H). Anal. Calcd for $C_9H_{18}O_3$: C, 62.00; H, 10.40. Found: C, 62.40; H, 10.28.

4.4.6. 2-(1′-Hydroxy-1′-methyl-ethyl)-2-cyclohexen-1-ol (11). Colorless oil; CAS registry number [220589-54-8]; ^1H NMR ($CDCl_3$, 200 MHz): δ 1.35 (s, 6H, H-2′ and H-3′), 1.65 (m, 2H, H-5), 1.80 (m, 2H, H-6), 1.96 (m, 2H, H-4), 4.23 (m, 1H, H-1), 5.80 (m, 1H, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 24.6 (C-5), 28.9 (C-2′ and C-3′), 29.7 (C-4), 31.8 (C-6), 66.1 (C-1), 72.7 (C-1′), 121.6 (C-3), 148.1 (C-2); IR (film) ν 3357 (O–H), 2927 (=C–H), 2854 cm^{-1} (C–H).

4.5. ESR experiments

An oxygen-free acetonitrile solution (40 mL) containing allylic alcohol **12** (70 mM) and *t*-BuONO in excess

(115 mM) was continuously flowed (flow rate 0.02–0.1 mL/min) through a flat quartz cell (0.3 mm optical path length) inside the ESR cavity and directly irradiated, at low temperature (typically between 223 and 243 K), with the unfiltered output of an Oriel 66901-M high-pressure mercury lamp. The solutions were deaerated prior to use by purging with N_2-gas for 50–60 min. The ESR hyperfine splitting constants of the observed nitroxides are shown in Scheme 6.

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