Structural Influence on Radical Formation and Sensitizing Capacity of Alkylic Limonene Hydroperoxide Analogues in Allergic Contact Dermatitis

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Hydroperoxides are known to be strong contact allergens and a common cause of contact allergy. They are easily formed by the autoxidation of, for example, fragrance terpenes, compounds that are common in perfumes, cosmetics, and household products. A requirement of the immunological mechanisms of contact allergy is the formation of an immunogenic hapten−protein complex. For hydroperoxides, a radical mechanism is postulated for this formation. In our previous investigations of allylic limonene hydroperoxides, we found that the formation of carbon- and oxygen-centered radicals, as well as the sensitizing capacity, is influenced by the structure of the hydroperoxides. The aim of the present work was to further investigate the connection between structure, radical formation, and sensitizing capacity by studying alkylic analogues of the previously investigated allylic limonene hydroperoxides. The radical formation was studied in radical-trapping experiments employing 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine iron(III) chloride as an initiator and 1,1,3,3-tetramethylisoindolin-2-yloxyl as a radical trapper. We found that the investigated hydroperoxides initially form carbon- and oxygen-centered radicals that subsequently form alcohols and ketones. Trapped carbon-centered radicals and nonradical products were isolated and identified. Small changes in structure, like the omission of the endocyclic double bond or the addition of a methyl group, resulted in large differences in radical formation. The results indicate that alkoxyl radicals seem to be more important than carbon-centered radicals in the immunogenic complex formation. The sensitizing capacities were studied in the murine local lymph node assay (LLNA), and all hydroperoxides tested were found to be potent sensitizers. For two of the hydroperoxides investigated, the recently suggested thiol−ene reaction is a possible mechanism for the formation of immunogenic complexes. For the third investigated, fully saturated, hydroperoxide, the thiol−ene mechanism is not possible for immunogenic complex formation. This strongly indicates that several radical reaction pathways for immunogenic complex formation of limonene hydroperoxides are active in parallel.

Introduction

Allergic contact dermatitis (ACD)[1] is the clinical manifestation of contact allergy. It is estimated that 15−20% of the population in the Western world are allergic to one or more chemicals in their environment (*1*). A prerequisite for the immunologic mechanisms of contact allergy is that the compounds causing the contact allergy react with macromolecules in the skin (e.g., proteins). The allergenic compounds, usually small organic molecules or metal ions, are known as haptens. The reaction results in the formation of an immunogenic hapten−protein complex. This complex is specific to each hapten and recognized by the immune system, thereby starting an immunological process that ultimately leads to the development of contact allergy and ACD (*2*).

The most common mechanism for the immunogenic complex formation is the nucleophilic attack by an amino acid side chain on an electrophilic hapten (*3*). This mechanism is valid for electrophiles that participate in nonradical reactions. For urushiols (*4, 5*) and hydroperoxides (*6*−*9*), it has been proposed that the formation of immunogenic complexes can take place through a radical mechanism. In the case of urushiols, this is proposed as an alternative to a mechanism proceeding via the oxidation to quinones (*5*). The formation of radicals from hydroperoxides has been shown to cause unspecific oxidation of proteins (*10*). In theory, this unspecific oxidation could generate the immunogenic complexes of hydroperoxides. If so, hydroperoxides with different structures would form the same immunogenic complexes, and individuals sensitized to one hydroperoxide would react to other hydroperoxides (cross-reactivity). However, it has been shown that neither 1-(1-hydroperoxyl-1-methylethyl) cyclohexene (cyclohexene-hydroperoxide) (**1**, Figure 1) nor cumene hydroperoxide (**2**) cross-react with limonene-2-hydroperoxide (**3**) in guinea pigs, although they cross-react with each other (*11*). Taken together, these results show that the structure of the hydroperoxide is important for the formation of specific immunogenic complexes of hydroperoxides, and although unspecific oxidation of proteins does occur in the presence of radicals derived from hydroperoxides (*10*), there is no evidence for the formation of specific immunogenic complexes via this mechanism. Furthermore, the combination of radical-trapping experiments (*12*), cross-reactivity studies, and computational calculations (*11*) indicates that the active hapten of **1** is a mixture

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[1] Abbreviations: ACD, allergic contact dermatitis; Fe(III)TPPCl, 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine iron(III) chloride; LLNA, local lymph node assay; EC3, estimated concentration required to produce a stimulation index of 3; TMIO, 1,1,3,3-tetramethylisoindolin-2-yloxyl.

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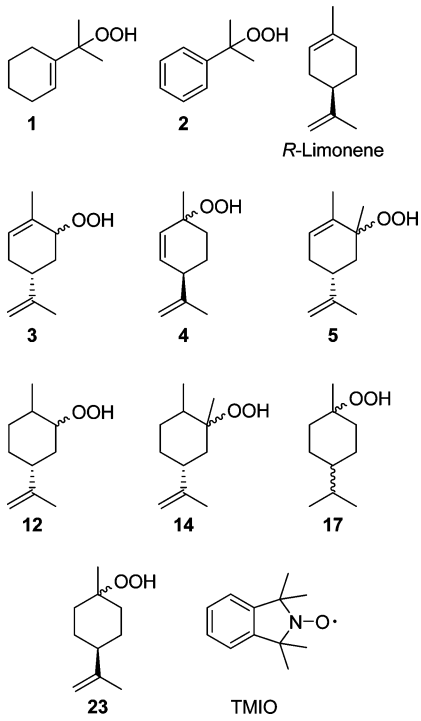


Figure 1. Structures of compounds referred to in this paper. Compounds **1** and **2** were used in a previous study (*11*). *R*-Limonene is a commonly used fragrance terpene that forms hydroperoxides **3** and **4** on air exposure (*17*). The sensitizing capacities and radical formation of allylic hydroperoxides **3**, **4**, and **5** have been previously investigated (*20*). The present study investigates alkylic hydroperoxides **12**, **14**, and **17**, using TMIO as a radical trapper. Attempted synthesis of hydroperoxide **23** was unsuccessful.

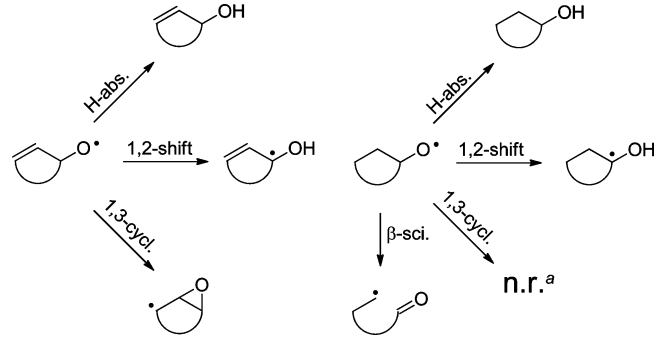
of carbon-centered and oxygen-centered radicals, whereas it is mainly an oxygen-centered alkoxyl radical in the case of **2**. This suggests the importance of both carbon- and oxygen-centered radicals in the formation of immunogenic hapten−protein complexes of hydroperoxides.

Terpenes are frequently used in household and cosmetics products due to their pleasant scent. In the presence of air and an initiator such as metal ions, heat, or ultraviolet light, these compounds are autoxidized to form a range of oxidation products (*13*−*16*). The autoxidation is facilitated by the presence of allylic hydrogens, and the primary oxidation products are allylic hydroperoxides; these have repeatedly been shown to be strong contact allergens (*15*−*18*).

R-Limonene, one of the most commonly used fragrance terpenes (*19*), is autoxidized to form hydroperoxides **3** and **4** (Figure 1). For these hydroperoxides and a synthetic analogue (**5**), we have previously shown that both carbon- and oxygen-centered radicals are available for the formation of immunogenic complexes (*20*). Alkoxyl radicals corresponding to each hydroperoxide are formed when the oxygen−oxygen bond is cleaved homolytically. These alkoxyl radicals react according to three major pathways: hydrogen abstraction, 1,2-shift, and 1,3-cyclization (Scheme 1). The preference for the different pathways is governed by the structure of the hydroperoxide. However, all of the hydroperoxides formed carbon-centered radicals through 1,3-cyclization of the initially formed alkoxyl radical involving the endocyclic double bond (*20*).

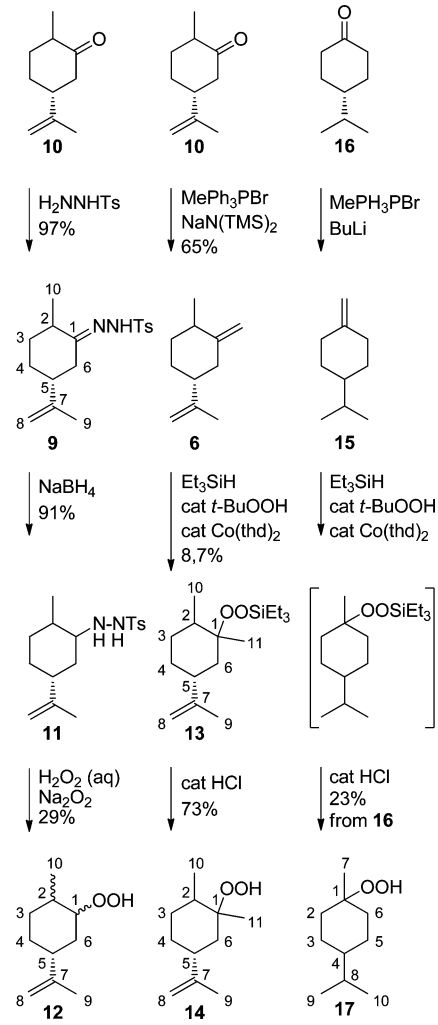
The aim of the present study was to investigate how radical formation and sensitizing capacity was influenced by the omission of the endocyclic double bond of the three previously studied allylic limonene hydroperoxides **3**−**5** (Figure 1). Removal of the endocyclic double bond will prevent the 1,3-cyclization observed in the

Scheme 1. Radical Reaction Pathways of Allylic (Right) and Alkylic (Left) Oxygen-Centered Radicals[a]



[a] n.r., no reaction.

Scheme 2. Synthetic Routes to Hydroperoxides 12, 14, and 17



previous study, whereas the hydrogen abstraction and the 1,2-shift are still possible reaction pathways (Scheme 1). A possible reaction not observed in the previous study is β-scission, which could generate cyclic ketones as well as open or expand the cyclohexane ring. The opening or expansion of the cyclohexane ring would generate carbon-centered radicals that would be available for the formation of immunogenic hapten−protein complexes.

To perform said investigations, alkylic analogues to the allylic hydroperoxides **3**−**5** were synthesized (Scheme 2) and subjected to radical-trapping studies. These were performed in the same manner as in our previous study of hydroperoxides **3**−**5** (*20*), employing Fe(III)TPPCl as a radical initiator and TMIO as a

radical trapper (Scheme 3). Trapped carbon-centered radicals and nonradical products were isolated and identified using NMR and MS. The sensitizing capacity was investigated in the murine local lymph node assay (LLNA).

Experimental Procedures

Caution: *This study involves skin-sensitizing compounds, which must be handled with care.*

Chemicals. (2*R*,5*R*)-5-Isopropenyl-2-methylcyclohexanone [(+)-dihydrocarvone, Fluka, purum 77%], *p*-toluenesulfonhydrazide (97%), sodium borohydride (98%), hydrogen peroxide (30−35% in water), triethylsilane (97%), sodium peroxide, 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine iron(III) chloride (Fe(III)TPPCl, 97%), cobalt(II) chloride, 2,2,6,6-tetramethyl-3,5-heptanedione (≥98%), *tert*-butyl hydroperoxide (5.5 M in decane), and *N*-benzylphthalimide (99%) were purchased from Sigma Aldrich (Stockholm, Sweden). Sodium bis(trimethylsilyl)amide (2 M in THF) and *n*-butyllithium (2.5 M in hexanes) were purchased from Acros Organics (Geel, Belgium). Methyltriphenylphosphonium bromide (98%) was purchased from Lancaster (Lancashire, United Kingdom) and Sigma Aldrich. 4-Isopropylcyclohexanone (**16**) was purchased from Frinton Laboratories (Vineland, NJ), acetone was purchased from Merck (Darmstadt, Germany), and olive oil was purchased from Apoteket AB (Gothenburg, Sweden).

Instrumentation and Mode of Analysis. NMR spectroscopy was performed on a JEOL Eclipse+ 400 instrument at 400 MHz using $CDCl_3$ as the solvent. Chemical shifts (δ) are reported in ppm relative to $CHCl_3$ at 7.26 for ^1H and at 77.0 ppm for ^{13}C. ^1H and ^{13}C NMR spectra were assigned using ^{13}C distortionless enhancement by polarization transfer (DEPT), $^1H-^1H$ correlation spectroscopy (COSY), $^1H-^{13}C$ heteronuclear multiple quantum coherence (HMQC), and $^1H-^{13}C$ heteronuclear multiple bond correlation (HMBC).

Analytical HPLC was performed using two Gilson pumps model 305, a BAS UV/vis detector model UV-116 (Bioanalytical Systems, Inc., West Lafayette, IN), a CMA 200 Microsampler (CMA Microdialys AB, Stockholm, Sweden), and a Zorbax Rx-SiL column (250 mm × 4.6 mm i.d., particle size 5 μm, Agilent). The mobile phase consisted of 40% *tert*-butyl methyl ether in hexane, the flow rate was 1.0 mL/min, and the compounds were monitored at 254 nm.

Preparative HPLC was performed using a Gilson pump model 305, a Gilson UV/vis detector model 119 (Gilson Medical Electronics, Inc., Middleton, WI), and a Zorbax Rx-SiL prepHT column (250 mm × 21.2 mm i.d., particle size 7 μm, Agilent). Various concentrations of *tert*-butyl methyl ether in hexane (specified below) were used as the mobile phase, the flow rate was 21.24 mL/min, and the compounds were monitored at 205 nm.

LC/MS analyses were performed on a Hewlett-Packard 1100 HPLC/MS including a vacuum degasser, a binary pump, an autoinjector, a column thermostat, a DAD detector, and a single quadrupole mass spectrometer. The HPLC was equipped with a Zorbax SB-C18 column (150 mm × 3.0 mm i.d., particle size 3.5 μm, Agilent), and the mobile phase consisted of 0.005% pentafluoropentanoic acid, 0.1% acetic acid, and 5% acetonitrile in water (solvent A) together with 0.005% pentafluoropentanoic acid, 0.1% acetic acid, and 5% water in acetonitrile (solvent B). A linear gradient of solvent B 0−100% for 25 min was followed by 10 min of isocratic elution with 100% B; this was followed by a 3 min linear gradient 100−0% B and ended with a 3 min isocratic elution with 100% solvent A. The column temperature was 40 °C, and the flow rate was 0.40 mL/min. The mass spectrometer was equipped with an atmospheric pressure ionization electrospray (API-ES) interface used in the positive ionization mode with the following settings in the spray chamber: nebulizer pressure, 40 psig; capillary voltage, 3500 V; drying gas temperature, 350 °C; and drying gas flow rate, 10 L/min. The fragmentor voltage was set to 50−120 V, and the mass spectrometer was used in both the scan mode and the selected ion monitoring (SIM) mode. Flow injection analysis (FIA) of the hydroperoxides was performed using 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), employing an isocratic system with 50% solvent A in B. The API-ES interface was used in the positive mode, and the spray chamber settings were as follows: nebulizer pressure, 40 psig; capillary voltage, 4500 V; drying gas temperature, 150 °C; and drying gas flow rate, 12 L/min. The fragmentor voltage was set to 50 V, and the mass spectrometer was used in the scan mode.

GC analyses were performed on a Hewlett-Packard 6890 gas chromatograph equipped with an on-column injector and a flame ionization detector, using a 30 m fused silica column (HP-5; i.d. 0.25 mm, 0.25 μm film thickness) and nitrogen as the carrier gas. The column temperature was 35 °C at injection, held isothermally for 2 min, raised to 185 °C at a rate of 5 °C/min, and finally held at 185 °C for 5 min. The detector temperature was 250 °C, and 1,2,3,5-tetramethylbenzene was used as the internal standard.

Column chromatography was performed using Merck silica gel 60 (230−400 mesh ASTM), and TLC was performed using silica plated aluminum sheets (Merck, 60 F_{254} silica gel) that were developed with an anisaldehyde dip (2.1 mL of acetic acid, 5.1 mL of anisaldehyde, and 7 mL of H_2SO_4 in 186 mL of ethanol) followed by heating.

Melting points were recorded on a Büchi Melting Point B-545 and are uncorrected. Elemental analysis (EA) was performed by H. Kolbe Mikroanalytisches Laboratorium (Mülheim an der Ruhr, Germany). High-resolution mass spectra (HRMS) were obtained from the Department of Chemistry at Lund University (Lund, Sweden).

Synthesis. 1,1,3,3-Tetramethylisoindolin-2-yloxyl [TMIO (*21*)], (4*R*)-4-isopropenyl-1-methyl-2-methylenecyclohexane [**6** (*22*)], (5*R*)-5-isopropenyl-1,2-dimethyl-cyclohexane-1-ol [**7** (*23*)], and 4-isopropenyl-1-methyl-cyclohexanol [**8** (*24*)] were synthesized according to the literature.

(5*R*)-5-Isopropenyl-2-methyl-cyclohexane-1-*p*-tosylhydrazone (9). (5*R*)-5-Isopropenyl-2-methyl-cyclohexanone (**10**, 2.0 mL, 12.2 mmol) was added to a stirred solution of *p*-toluenesulfonhydrazide (2.7 g, 14 mmol) in ethanol (20 mL) at 50 °C. The reaction was followed by TLC until all of the starting material was consumed. The reaction mixture was concentrated under reduced pressure, dissolved in chloroform, and absorbed on silica. The silica was washed with ethyl acetate:hexane (1:3), and the solvent was concentrated under reduced pressure affording **9** as a yellow oil. ^1H NMR: δ 0.99−1.03 (m, 3H, H10), 1.13−1.18 (m, 1H, H3 or H4 or H6), 1.31−1.43 (m, 1H, H3 or H4 or H6), 1.62 (m, 1H, H3 or H4 or H6), 1.66 (s, 3H, H9), 1.76−1.83 (m, 1H, H3 or H4 or H6), 1.87−1.96 (m, 1H, H3 or H4 or H6), 1.96−2.01 (m, 1H, H5), 2.06−2.15 (m, 1H, H2), 2.41 (s, 3H, Ar−CH_3), 2.65−2.73 (m, 1H, H3 or H4 or H6), 4.64−4.72 (m, 2H, H8), 7.26−7.31 (m, 2H, Ar-*H*), 7.50 (br s, 1H, H16), 7.81−7.87 (m, 2H, Ar-*H*). ^{13}C NMR: δ 16.4 (C10), 20.7 (C9), 21.7 (Ar-CH_3), 31.0 (C3 or C4 or C6), 31.9 (C3 or C4 or C6), 35.3 (C3 or C4 or C6), 39.3 (C2), 45.2 (C5), 109.8 (C8), 128.4 [2C, SC(*C*H)$_2$], 129.3 [2C, (*C*H)$_2$CCH$_3$], 135.3 (Ar-*C*H$_3$), 143.9 (C11), 148.0 (C7), 163.6 (C1). MS (API-ES, 120 eV) *m*/*z* (%): 343 [M + Na] (10), 321 [M + H] (100), 166 (13), 151 (18), 107 (18). Anal. calcd for $C_{17}H_{24}$: C, 63.72; H, 7.55. Found: C, 63.96; H, 7.74. The product was used without further purification in the synthesis of **11**.

(5*R*)-5-Isopropenyl-2-methyl-cyclohexane-1-*p*-tosylhydrazine (11). Sodium borohydride (0.13 g, 3.4 mmol) was slowly added to a stirred mixture of **9** (0.54 g, 1.7 mmol) in dichloromethane (28 mL) at 0 °C under N_2. After 10 min, anhydrous methanol (7 mL) was added, and the solution was stirred at 0 °C until HPLC showed no starting material. The reaction was quenched by slow addition of water (15 mL) followed by brine (10 mL), and the aqueous phase was extracted with dichloromethane (5 × 20 mL). The organic phase was washed with brine (2 × 20 mL), dried over $MgSO_4$, and concentrated under reduced pressure affording **11** as a yellow oil that was used without further purification.

(5*R*)-5-Isopropenyl-2-methyl-cyclohexane-1-hydroperoxide (12). Because of safety precautions, two round-bottomed flasks were each charged with **11** (3.3 g, 10 mmol) in THF (100 mL). To each flask, aqueous H_2O_2 (30%, 103 mL, 1 mol) was slowly added at 0 °C

followed by addition of Na_2O_2 (1.2 g, 15 mmol). The solutions were slowly warmed to room temperature and stirred until TLC showed no starting material. The reaction mixtures were pooled, diluted with water (200 mL), and acidified with 2 M HCl to pH 5−6, whereafter brine (30 mL) was added, and the solution was extracted with dichloromethane (3 × 200 mL). The organic phases were pooled, washed with water (2 × 200 mL), dried over Na_2SO_4, and concentrated under reduced pressure at room temperature affording **12** as a yellow oil (mixture of diastereomers). The diastereomers were enriched by two rounds of preparative HPLC purifications (isocratic, hexane/*tert*-butyl methyl ether; first round, 85:15; second round, 95:5), yielding diastereomers **12a** (0.22 g, 14%), **12b** (0.12 g, 7.8%), and **12c** (0.11 g, 7.3%). The fourth diastereomer (**12d**) could not be enriched to be a main component in any fraction using these systems. Compound **12a** (major isomer): ^{1}H NMR: δ 0.98 (d, 3H, J = 6.7 Hz, H10), 1.27−1.38 (m, 2H, H3 or H4 or H6), 1.42−1.46 (m, 1H, H3 or H4 or H6), 1.46−1.52 (m, 1H, H3 or H4 or H6), 1.69 (m, 1H, H2), 1.70 (s, 3H, H9), 1.72−1.74 (m, 1H, H3 or H4 or H6), 1.84−1.93 (m, 1H, H3 or H4 or H6), 2.18−2.26 (m, 1H, H5), 3.93−3.96 (m, 1H, H1), 4.65−4.74 (m, 2H, H8), 8.01 (s, 1H, H11). ^{13}C NMR: δ 17.0 (C10), 21.3 (C9), 25.7 (C3 or C4 or C6), 27.3 (C3 or C4 or C6), 29.5 (C3 or C4 or C6), 30.4 (C2), 39.0 (C5), 85.6 (C1), 109.0 (C8), 149.5 (C7). MS (API-ES, 50 eV) *m*/*z* (%): 193 [M + Na] (23), 171 [M + H] (86), 153 (100), 137 (16), 135 (36), 64 (47). Anal. calcd for $C_{10}H_{18}O_2$: C, 70.55; H, 10.66; O, 18.67. Found: C, 70.59; H, 10.62; O, 18.67. For the characterization data of minor isomers, see the Supporting Information.

Bis(2,2,6,6-tetramethyl-3,5-heptanedionato)cobalt(II) [Co(thd)$_2$]. Cobalt(II) chloride (2.2 g, 17 mmol) was added to a stirred solution of 2,2,6,6-tetramethyl-3,5-heptanedione (6.3 g, 34 mmol) in deionized water (30 mL) under nitrogen atmosphere at 65 °C. Sodium hydroxide (1.4 g, 34 mmol in 10 mL of H_2O) was added to the pink solution, whereupon it turned blue, and pink crystals immediately precipitated. After 3 h, the aqueous mixture was filtered, and the solid was dissolved in diethyl ether (100 mL), filtered through Celite, and concentrated under reduced pressure. The pink crude product was sublimed at 90 °C yielding Co(thd)$_2$ {6.4 g, 44%, mp 142 °C [lit. 143 °C (*25*)]} as purple crystals.

(5*R*)-5-Isopropenyl-1,2-dimethyl-cyclohexane-1-triethylsilyl peroxide (13). Triethylsilane (4.4 mL, 27.6 mmol) and *t*-butyl hydroperoxide (5.5 M in decane, 0.69 μmol, 125 μL) were added to a stirred solution of **6** (2.1 g, 13.8 mmol) in 1,2-dichloroethane (125 mL) under oxygen atmosphere. Co(thd)$_2$ (0.15 g, 0.36 mmol) was added to the clear solution at room temperature, whereupon it instantly turned dark green. The mixture was stirred for 20 min, filtered through silica, and concentrated. The crude product was purified by liquid chromatography (100% hexane) yielding **13** (0.35 g, 8.7%) as a clear oil. ^{1}H NMR: δ 0.62−0.70 [q, J = 8.06 Hz, 6H, Si(C*H*$_2$CH$_3$)$_3$], 0.90−0.93 (d, J = 6.22 Hz, 3H, H10), 0.93−0.95 (m, 1H, H$_A$6), 0.95−1.01 [t, J = 8.06 Hz, 9H, Si(CH$_2$C*H*$_3$)$_3$], 1.22 (s, 3H, H11), 1.39−1.46 (m, 4H, H2, H$_A$3, H4), 1.72 (s, 3H, H9), 1.73−1.76 (m, 1H, H$_B$3), 2.21−2.35 (m, 2H, H5 and H$_B$6), 4.66 (s, 2H, H8). ^{13}C NMR: δ 4.0 [Si(*C*H$_2$CH$_3$)$_3$], 6.9 [Si(CH$_2$*C*H$_3$)$_3$], 15.1 (C10), 21.3 (C9), 23.5 (C11), 30.9 (C4), 31.7 (C3), 39.9 (C5), 40.1 (C6), 41.1 (C2), 82.0 (C1), 107.8 (C8), 151.0 (C7). MS (API-ES, 50 eV) *m*/*z* (%): 321 [M + Na] (2), 299 [M + H] (11), 183 (49), 167 (46), 151 (31). Anal. calcd for $C_{17}H_{34}O_2Si$: C, 68.39; H, 11.48. Found: C, 68.12; H, 11.45.

(5*R*)-5-Isopropenyl-1,2-dimethyl-cyclohexane-1-hydroperoxide (14). Compound **13** (0.33 g, 11.1 mmol) was swirled in a solution of 2 drops of concentrated HCl in methanol (10 mL) for 2 min. The solution was washed with sodium bicarbonate (saturated, 50 mL) and extracted with diethyl ether (2 × 50 mL). The pooled organic phases were washed with brine (50 mL) and dried over $MgSO_4$, filtered, and concentrated under reduced pressure at room temperature. The crude product was purified by liquid chromatography (hexane/ethyl acetate 9:1) yielding **14** (0.15 g, 73%) as a clear oil. ^{1}H NMR: δ 0.92−0.96 (d, 3H, H10, J = 6.59 Hz), 1.04−1.13 (m, 1H, H$_A$6), 1.19−1.22 (m, 1H, H$_A$3), 1.29 (s, 3H, H11), 1.45−1.47 (m, 2H, H2 and H$_B$3), 1.73 (s, 3H, H9), 1.74−1.81 (m, 2H, H4), 2.18−2.28 (m, 2H, H5 and H$_A$6), 4.69 (s, 2H, H8), 6.96 (s, 1H, OO*H*). ^{13}C NMR: δ 15.0 (C10), 21.2 (C9), 23.3 (C11), 30.6 (C3), 31.8 (C4), 39.6 (C6), 40.1 (C5), 40.8 (C2), 82.7 (C1), 108.4 (C8), 150.3 (C7). MS (API-ES, 50 eV) *m*/*z* (%): 207 [M + Na] (36), 185 [M + H] (46), 167 (100), 151 (21), 149 (70), 83 (75), 64 (82). Anal. calcd for $C_{11}H_{20}$: C, 71.70; H, 10.94. Found: C, 71.66; H, 11.03.

1-Methylene-4-isopropyl-cyclohexane (15). Butyllithium (1.0 mL, 2.5 M) was added dropwise to a stirred suspension of methyltriphenylphosphonium bromide (0.9 g, 2.6 mmol) in freshly distilled THF (12 mL) under N_2 at 0 °C. After 15 min, **16** (0.3 g, 2.1 mmol) was added dropwise, and the reaction mixture was slowly warmed to room temperature. After 5.5 h, pentane (10 mL) and water (10 mL) were added, the phases were separated, the aqueous phase was extracted with pentane (10 mL), and the organic phases were pooled, washed with water (2 × 25 mL), and filtered to separate a solid byproduct. The filtrate was dried over Na_2SO_4, filtered, and concentrated under reduced pressure at 0 °C yielding a colorless oil that was used without further purification. NMR data of the crude product correspond to the literature (*22*).

4-Isopropyl-1-methyl-cyclohexane-1-hydroperoxide (17). Triethylsilane (0.5 mL, 3.2 mmol) and *t*-butyl hydroperoxide (one drop, 5.5 M in decane) were added to a stirred solution of **15** (0.2 g, 1.6 mmol) in 1,2-dichloroethane (8.9 mL) under oxygen atmosphere at room temperature. Co(thd)$_2$ (22 mg, 53 μmol) was added to the clear solution, whereupon it instantly turned dark green. The mixture was stirred for 1 h and 40 min and filtered through silica eluting with dichloromethane. The dichloromethane was evaporated, methanol (1 mL) and aqueous HCl (1 M, 1 drop) were added, and the solution was stirred at room temperature for 30 min. Dichloromethane (10 mL) and water (10 mL) were added to the reaction mixture, the phases were separated, and the aqueous phase was extracted with dichloromethane (10 mL). The organic phases were pooled, washed with water (25 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified using flash chromatography on silica gel eluting with hexane/ethyl acetate (9:1) yielding 0.87 g (23% from **16**) of the target compound. ^{1}H NMR δ 0.85 (d, J = 6.96 Hz, 6H, H9 and H10), 0.94−1.04 (m, 1H, H8), 1.21 (s, 3H, H7), 1.23−1.28 (m, 4H, H$_A$2 and H$_A$3 and H$_A$5 and H$_A$6), 1.39−1.49 (m, 3H, H$_B$3 and H4 and H$_B$5), 1.89−2.01 (m, 2H, H$_B$2 and H$_B$6). ^{13}C NMR: δ 20.0 (C9 and C10), 24.9 (C3 and C5), 25.4 (C7), 34.5 (C4), 35.5 (C2 and C6), 43.5 (C8), 80.9 (C1). MS (API-ES, 50 eV) *m*/*z* (%): 195 [M + Na] (16), 173 [M + H] (2), 155 (100), 139 (13), 83 (17), 81 (17), 74 (51), 64 (20). Anal. calcd for $C_{10}H_{20}O_2$: C, 69.72; H, 11.70. Found: C, 69.70; H, 11.88.

4-Isopropyl-1-methyl-cyclohexanol (18). Pd/C (80 mg, 5%) was added to a solution of **8** (0.38 g, 2.5 mmol) in ethanol (17 mL). The mixture was stirred at room temperature under an atmosphere of H_2 for 6 h, exposed to air overnight, filtered through Celite, and concentrated under reduced pressure. The crude product was purified by liquid chromatography (hexane/ethyl acetate 85:15) yielding two diastereomers of **18** as clear oils. Major isomer (0.14 g, 37%): ^{1}H NMR: δ 0.92 (d, J = 6.96 Hz, 6H, H9 and H10), 0.97−1.07 (m, 1H, H8), 1.25 (s, 3H, H7), 1.30−1.39 (m, 2H, H$_A$3 and H$_A$5), 1.35−1.46 (m, 2H, H$_A$2 and H$_A$6), 1.44−1.54 (m, 1H, H4), 1.54−1.62 (m, 2H, H$_B$3 and H$_B$5), 1.67−1.75 (m, 2H, H$_B$2 and H$_B$6). ^{13}C NMR: δ 20.1 (C9 and C10), 25.2 (C3 and C5), 31.5 (C7), 32.8 (C4), 39.1 (C2 and C6), 43.6 (C8), 69.3 (C1). MS (API-ES, 100 eV) *m*/*z* (%) 139 (29), 124 (14), 97 (18), 83 (100), 71 (17), 69 (18), 60 (16). HRMS (ESI) *m*/*z* calcd for $C_{10}H_{20}O$, 156.1514; found, 156.1510. For the characterization data of the minor isomer (62 mg, 16%), see the Supporting Information.

Chemical Radical-Trapping Experiments with TMIO, General Procedure. Fe(III)TPPCl (1 equiv) was added to a solution of hydroperoxide (1 equiv) and TMIO (2 equiv) in acetonitrile/water (1:1). The reaction was stirred at room temperature until TLC showed no starting material. The reaction mixture was filtered to remove excess Fe(III)TPPCl, and the residue was washed three times with hexane. The phases were separated, the aqueous phase was extracted three times with hexane, and the organic phases were

pooled, dried over $MgSO_4$, and concentrated under reduced pressure. The crude products were purified using chromatography on silica gel eluting with various mixtures of solvents specified below. The isolated products were characterized and quantified using 1D and 2D NMR, LC/MS, GC/FID, and EA.

Trapping Experiments with 12. Starting from 0.31 g (1.8 mmol) of **12**, two products were isolated using liquid chromatography on silica gel eluting with stepwise gradient hexane/diethyl ether (1:1), hexane/ethyl acetate (1:1), hexane/methanol (9:1). The products isolated were dihydrocarvone (**10**, 0.11 g, 41%) and dihydrocarveol (**19**, 8.93 mg, 3.2%). ^{1}H and ^{13}C NMR data agreed with authentic samples.

Trapping Experiments with 14. Starting from 0.29 g (1.6 mmol) of **14**, three products were isolated using liquid chromatography on silica gel eluting with stepwise gradient hexane/diethyl ether (1:1), hexane/ethyl acetate (1:1), hexane/methanol (9:1). The products isolated were compounds **20**, **21**, and **7**. For 4′-isopropenyl-7′-(1,1,3,3-tetramethylisoindolyl-2-oxy)-octa-2′-one (**20**, 0.26 g, 47%, mixture of diastereomers 50:50): ^{1}H NMR: δ 1.20−1.24 (m, 3H, H8′), 1.26−1.32 (m, 4H, H_A5′ and H10 or H11 or H12 or H13), 1.32−1.38 (m, 4H, H_B5′ and H10 or H11 or H12 or H13), 1.40−1.54 (m, 6H, H10 and/or H11 and/or H12 and/or H13), 1.63−1.66 (m, 2H, H6′), 1.67 (s, 3H, H11′), 2.12 (s, 3H, H1′), 2.46−2.56 (m, 2H, H3′), 2.58−2.66 (m, 1H, H4′), 3.83−3.89 (m, 1H, H7′), 4.72−4.80 (m, 2H, H10′), 7.06−7.12 (m, 2H, H7 and H4), 7.18−7.24 (m, 2H, H5 and H6). ^{13}C NMR: δ 18.8/18.9 (C11′), 19.9/20.0 (C8′), 25.3/25.5 (2C, C10 or C11 or C12 or C13), 29.2/29.3 (C5′), 30.2/30.4 (C1′), 30.7 (2C, C10 or C11 or C12 or C13), 33.66/33.72 (C6′), 43.05/43.14 (C4′), 48.6 (C3′), 67.3 (C1 or C3), 67.7 (C1 or C3), 78.6/78.8 (C7′), 112.17/112.21 (C10′), 121.6 (C4 and C7), 127.2 (C5 and C6), 145.47/145.54 (C9′), 146.47/146.54 (C8 and C9), 208.3 (C2′). MS (API-ES, 120 eV) *m*/*z* (%): 380 [M + Na] (1), 359 (25), 358 [M + H] (100), 192 (30), 161 (6), 160 (48). Anal. calcd for $C_{23}H_{35}NO_2$: C, 77.27; H, 9.87. Found: C, 77.72; H, 9.73. For 4-isopropenyl-octa-2,7-dione (**21**, 40.4 mg, 14%), major isomer: ^{1}H NMR: δ 1.55−1.77 (m, 2H, H5), 1.66 (s, 3H, H11), 2.15 (s, 6H, H1 and H8), 2.35−2.43 (m, 2H, H6), 2.43−2.57 (m, 2H, H3), 2.59−2.67 (m, 1H, H4), 4.74−4.84 (m, 2H, H10). ^{13}C NMR: δ 18.7 (C11), 26.6 (C5), 30.3 (C1 or C8), 30.4 (C1 or C8), 41.4 (C6), 42.1 (C4), 48.4 (C3), 112.9 (C10), 145.9 (C9), 207.9 (C2), 208.7 (C7). MS (API-ES, 70 eV) *m*/*z* (%): 205 [M + Na] (64), 183 [M + H] (75), 165 (83), 123 (57), 107 (100). Anal. calcd for $C_{11}H_{18}O_2$: C, 72.49; H, 9.95. Found: C, 72.11; H, 10.18. Compound **7** (10.1 mg, 3.8%), ^{1}H and ^{13}C NMR data agreed with the literature (*23*).

Trapping Experiments with 17. Starting from 0.13 g (0.76 mmol) of **17**, two products were isolated using flash chromatography on silica gel eluting with hexane/ethyl acetate (9:1). 3′-Isopropyl-1′-(1,1,3,3-tetramethylisoindolyl-2-oxy)-heptan-6′-one (**22**, 39 mg, 15%): ^{1}H NMR: δ 0.91 (d, J = 7.0 Hz, 6H, H9′ and H10′), 1.46 (br s, 12H, H10 and H11 and H12 and H13), 1.45−1.57 (m, 3H, H_A2′ and H_A4′ and H3′ or H8′), 1.60−1.80 (m, 3H, H_B2′ and H_B4′ and H3′ or H8′), 2.17 (s, 3H, H7′), 2.41−2.57 (m, 2H, H5′), 3.90−4.00 (m, 2H, H1′), 7.09−7.14 (m, 2H, H4 and H7), 7.21−7.24 (m, 2H, H5 and H6). ^{13}C NMR: δ 19.0 (C9′ or C10′), 19.2 (C9′ or C10′), 25.0 (C4′), 25.5 (2C, C10 and C13 or C11 and C12), 29.6 (C3′), 29.9 (C2′), 30.1 (C7′), 30.5 (2C, C10 and C13 or C11 and C12), 40.6 (C8′), 42.2 (C5′), 67.2 (C1 and C3), 76.3 (C1′), 121.6 (C4 and C7), 127.3 (C5 and C6), 145.3 (C8 and C9), 209.4 (C6′). MS (API-ES, 120 eV) *m*/*z* (%): 346 [M + H] (100), 160 (36). Anal. calcd for $C_{22}H_{35}NO_2$: C, 76.92; H, 9.68. Found: C, 77.02; H, 10.47. 4-Isopropenyl-1-methyl-cyclohexane-1-ol (**18**: major isomer, 16 mg, 13%; minor isomer, 14 mg, 11%), ^{1}H and ^{13}C NMR data agreed with the synthesized reference.

Sensitization Experiments in Mice. The sensitizing potency of the hydroperoxides was investigated using the LLNA (*26*) as previously reported (*20*). All animal procedures were approved by the local ethics committee. Each hydroperoxide was tested in five different concentrations (Table 1), using mice in groups of three.

Table 1. LLNA Responses for Compounds 3−5, 12, 14, and 17[a]

compd and test concn (% w/v)	[^{3}H]thymidine incorp (dpm/lymph node)	SI	EC3 value	
			M	% w/v
3[b]			0.049	0.83
4[b]			0.019	0.33
5[b]			0.071	1.29
12			0.065	1.1
control	702			
0.1	902	1.3		
0.5	1010	1.4		
1.0	1910	2.7		
2.5	4724	6.7		
7.5	9908	14		
14			0.037	0.68
control	509			
0.1	734	1.4		
0.5	1036	2.0		
1.0	2378	4.7		
2.5	7092	14		
7.5	6848	14		
17			0.020	0.35
control	226			
0.1	217	1.0		
0.5	968	4.3		
1.0	1643	7.3		
2.5	1812	8.0		
7.5	3927	17		

[a] The local lymph node experiments were performed as previously reported (*20*). The SIs correspond to the increase in thymidine incorporation of treated groups relative to vehicle-treated controls. EC3 values (the estimated concentration required to induce an SI of 3) were calculated using linear interpolation (*40*). [b] Compunds previously investigated (*20*); EC3 values are included here for reference.

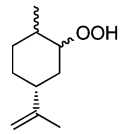
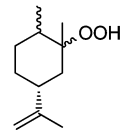
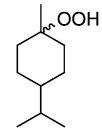
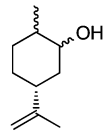
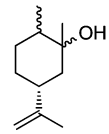
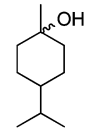
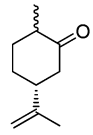
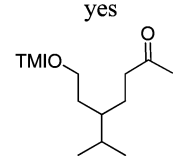
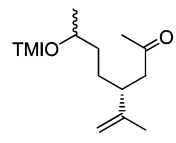
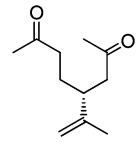
Results and Discussion

The aim of the present study was to investigate how the removal of the endocyclic double bond of the previously investigated allylic hydroperoxides **3**−**5** (*20*) influenced the radical formation and sensitizing capacities. Thus, the alkylic hydroperoxides **12**, **14**, and **17** have been synthesized and subjected to radical-trapping experiments as well as sensitizing studies. The radical-trapping experiments utilized Fe(III)TPPCl as a radical initiator and TMIO as a radical trapper (Scheme 3). The results show that small changes in structure, like the omission of the endocyclic double bond or the addition of a methyl group, resulted in large differences in radical formation. All of the investigated hydroperoxides formed large amounts of radicals; however, the identity of the radicals was influenced by the structure of the parent hydroperoxide. The results also indicate that alkoxyl radicals seem to be more important than carbon-centered radicals in the immunogenic complex formation. The sensitizing capacities were investigated in the murine LLNA, where all hydroperoxides were found to be potent sensitizers.

Synthesis. Compound **10** was reacted with hydrazintosylate to afford hydrazone **9** that was reduced with sodium borohydride to hydrazine **11** (Scheme 2). Attempts to purify **11** using liquid chromatography on silica gel resulted in decomposition. Because of this instability, no satisfactory NMR spectra could be obtained, and **11** was used without further purification. Treatment with aqueous hydrogen peroxide yielded four diastereomers of hydroperoxide **12**, three of which could be enriched as major products through consecutive preparative HPLC.

Extensive efforts to transform alcohol **7** (Table 2) directly to hydroperoxide **14** via substitution were unsuccessful. In general, no reaction was observed, or products formed and identified originated from the elimination of water (4-isopropenyl-1,2-dimethyl-cyclohexene) or addition to the isoprene unit, typically in a Markovnikov manner. Further attempts to substitute the

Table 2. Relationship between Structure, Radical Formation, and Products Isolated in the Radical-Trapping Experiments with Hydroperoxides 12, 14, and 17

	 **12**	 **14**	 **17**
pathway	sec[a].	tert.	tert.
i) H-abstraction			
O•[b]	yes	yes	yes
products	 **19** 3.2%	 **7** 3.8%	 **18** 24%
ii) 1,2-shift			
C•[c]	yes	n.d.[d]	n.d.
products	 **10** 41%	n.d.	n.d.
iii) β-scission			
C• prim.[e]	n.d.	n.d.	yes
products	n.d.	n.d.	 **22** 15%
C• sec.[e]	n.d.	yes	n.d.
products	n.d.	 **20** 47%  **21** 14%	n.d.

[a] Secondary or tertiary hydroperoxide. [b] Oxygen-centered radicals formed by oxygen−oxygen bond cleavage (pathway i). [c] Carbon-centered radicals formed by 1,2-shift (pathway ii). [d] n.d., not detected. [e] Primary or secondary carbon-centered radical formed by β-scission (pathway iii).

hydroxyl into a chloride, bromide, mesylate, or tosylate leaving group were also unsuccessful, again resulting in elimination of water or addition to the isoprene unit. Instead, the peroxyl functionality was introduced as a silyl peroxide via a cobalt-catalyzed addition to an alkene. The appropriate alkene **6** was synthesized from **10** using standard Wittig conditions (Scheme 2). The cobalt catalyst [$Co(thd)_2$] was prepared by treating cobalt(II) chloride with 2,2,6,6-tetramethyl-3,5-heptanedione and sodium hydroxide in water. $Co(thd)_2$ precipitated and was purified by sublimation to give purple crystals. Treatment of alkene **6** with triethylsilane, *tert*-butyl hydroperoxide, and $Co(thd)_2$ under oxygen atmosphere furnished silyl peroxide **13** as well as the byproduct caused by peroxidation of the isopropenyl group. Compound **13** was desilylated by treatment with acidic methanol yielding hydroperoxide **14** (Scheme 2).

Attempted synthesis of hydroperoxide **23** (Figure 1) by silyl peroxidation of the corresponding dialkene with the cobalt-catalyzed method used for hydroperoxide **14** was abandoned

due to very low yields of the correct silyl peroxide. The formation of the isopropenyl double bond via tosylate elimination was unsuccessful due to the incompatibility of the previously installed silyl peroxide with the elimination conditions. In view of the proposed mechanism for immunogenic complex formation of unsaturated hydroperoxides (*27*), it was interesting to investigate the sensitizing capacity of a fully saturated hydroperoxide. Furthermore, no reactions including the isopropenyl unit had been observed in the trapping experiments with hydroperoxides **3**−**5** (*20*). Therefore, it was decided to synthesize the fully saturated analogue of hydroperoxide **23**, that is, hydroperoxide **17** (Figure 1). To do this, alkene **15** was synthesized from ketone **16** using standard Wittig conditions (Scheme 2). Compound **15** was then treated with triethylsilane, *tert*-butyl hydroperoxide, and $Co(thd)_2$ under oxygen atmosphere yielding silyl peroxide **24** that was subsequently desilylated with acidic methanol to furnish hydroperoxide **17**.

Alcohol **18** (Table 2) was synthesized as a reference compound starting from limonene oxide that was transformed into β-terpineol using lithium aluminum hydride. β-Terpineol was reduced to the fully saturated alcohol **18** by treatment with palladium on carbon.

Sensitizing Capacity of the Hydroperoxides. The LLNA was used to determine the sensitizing capacity of hydroperoxides **12**, **14**, and **17** (Figure 1). Each hydroperoxide was tested at five different concentrations along with a control group that was tested with the vehicle alone. Compound **12** had an EC3 value of 0.065 M (1.1% w/v), **14** had an EC3 value of 0.037 M (0.68% w/v), and **17** had an EC3 value of 0.020 M (0.35% w/v) (Table 1). Thus, **12**, **14**, and **17** are potent sensitizers, and the found EC3 values are in agreement with the values of several other previously tested hydroperoxides (*8, 9, 14, 15, 20*). This suggests that the sensitizing capacities of the hydroperoxides are mainly correlated to the hydroperoxide group and depend less on the structure of the compound.

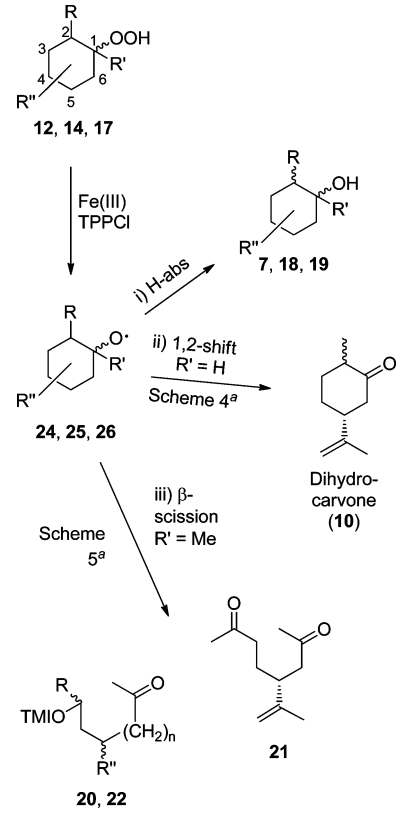
Chemical-Trapping Experiments with TMIO. Trapping experiments with hydroperoxides **12**, **14**, and **17** were performed to identify and quantify the major products formed. The hydroperoxides were allowed to react in a 1:1 mixture of acetonitrile/water using Fe(III)TPPCl as an initiator, cleaving the oxygen−oxygen bond of the hydroperoxide group homolytically. TMIO was included in the experiments to trap carbon-centered radicals. The outcome of the trapping experiments was analyzed by NMR, GC/FID, and LC/MS.

Quantification of the products formed from hydroperoxides **12** and **14** was performed using GC/FID. Hydroperoxide **14**, TMIO, and the expected products **7**, **10**, and **19** (Table 2) were calibrated against an internal standard. Hydroperoxide **12** partly degraded to **19** on the GC column and was therefore not quantified. However, no traces of unreacted hydroperoxide **12** were found in the reaction mixture, according to NMR and LC/MS analyses.

The products identified in the trapping experiment with **12** were **19** (3.2%) and **10** (41%; Table 2). The products identified in the trapping experiment with **14** were alcohol **7** (3.8%), diketone **21** (14%), and TMIO adduct **20** (47%). The products identified in the trapping experiment with **17** were alcohol **18** (24%) and TMIO adduct **22** (15%).

The homolytic cleavage of the oxygen−oxygen bond yields alkoxyl radicals (**24**−**26**) corresponding to each hydroperoxide. The alkoxyl radicals react according to three major pathways (Scheme 3): (i) hydrogen abstraction yielding the analogous alcohols; (ii) a 1,2-shift generating a carbon-centered α-hydroxyl radical, followed by trapping by TMIO

Scheme 3. Mechanistic Proposal That Accounts for the Products Isolated in the Trapping Experiments[a]



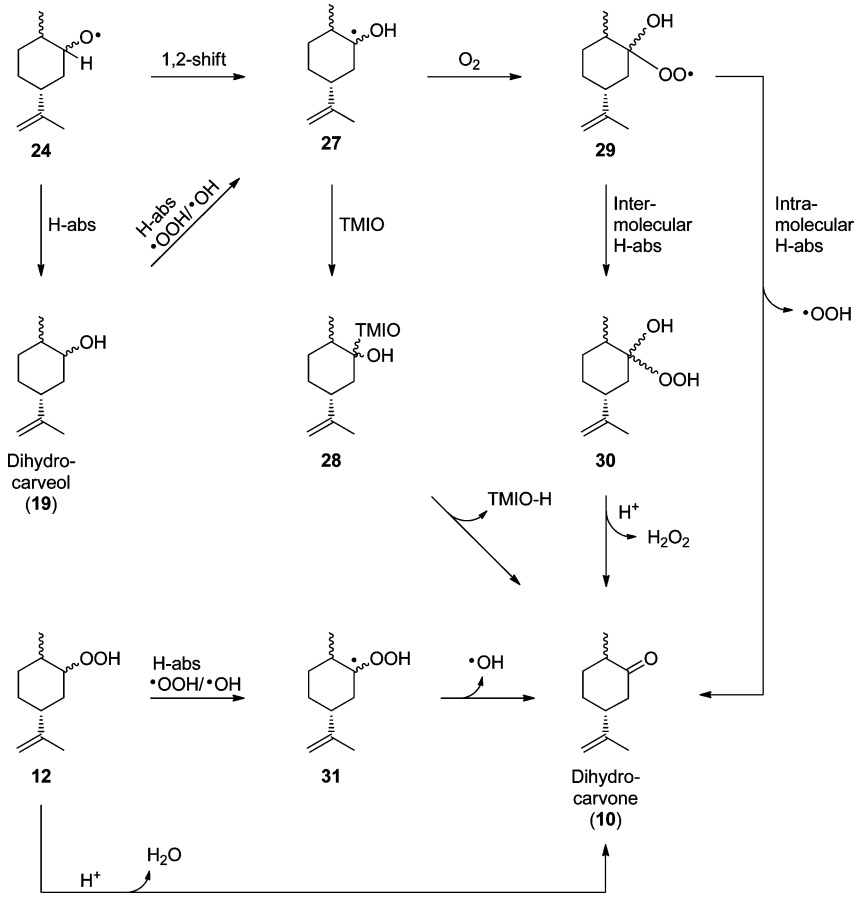
[a] Compound **12**: R = Me, R′ = H, and R″ = isopropenyl in position 5. Compound **14**: R = Me, R′ = Me, and R″ = isopropenyl in position 5. Compound **17**: R = H, R′ = Me, and R″ = isopropyl in position 4. Compound **20**: n = 1, R = Me, and R″ = isopropenyl. Compound **22**: n = 2, R = H, and R″ = isopropyl. [a]See the specified scheme for mechanistic details.

or dioxygen and subsequent expulsion of TMIO-H or hydrogen peroxide furnishing the carbonyl group in **10** (vide infra, Scheme 4); and (iii) β-scission generating primary or secondary carbon-centered alkyl radicals, followed by trapping by TMIO yielding adducts **20** and **22** or by dioxygen yielding a peroxyl radical that reacts further to give carbonyl compound **21** (vide infra, Scheme 5). The preferred reaction pathways together with formed radicals and products for each hydroperoxide are summarized in Table 2.

The products isolated from the trapping experiment with hydroperoxide **12** indicate a preference of the initially formed alkoxyl radical (**24**) to undergo a 1,2-shift, eventually resulting in **10** as the major product (Scheme 3). Alkoxyl radical **24** can abstract a hydrogen atom and form **19** or undergo a 1,2-shift to form the carbon-centered radical **27** (Scheme 4). Being a carbon-centered radical, **27** is most likely trapped by TMIO, resulting in adduct **28**. However, adduct **28** was not detected. It is believed that it decomposes to **10** and TMIO-H. The addition of dioxygen to **27** yields peroxyl radical **29**, which can abstract a hydrogen atom and form **30**. However, hydroperoxide **30** was not detected; it is believed that it decomposes to **10** and H_2O_2 through a general acid-catalyzed fragmentation. The yields of **10** and **19** are further affected by the actions of hydroxyl and hydroperoxyl radicals on hydroperoxide **12** and alcohol **19** producing carbon-centered radicals **27** and **31**, which ultimately form **10**.

The products isolated from the trapping experiments with hydroperoxide **14** clearly indicate a preference for alkoxyl radical **25** reacting according to the β-scission pathway, resulting

Scheme 4. Mechanistic Proposal for the Formation of 10 in the Trapping Experiment with Hydroperoxide 12



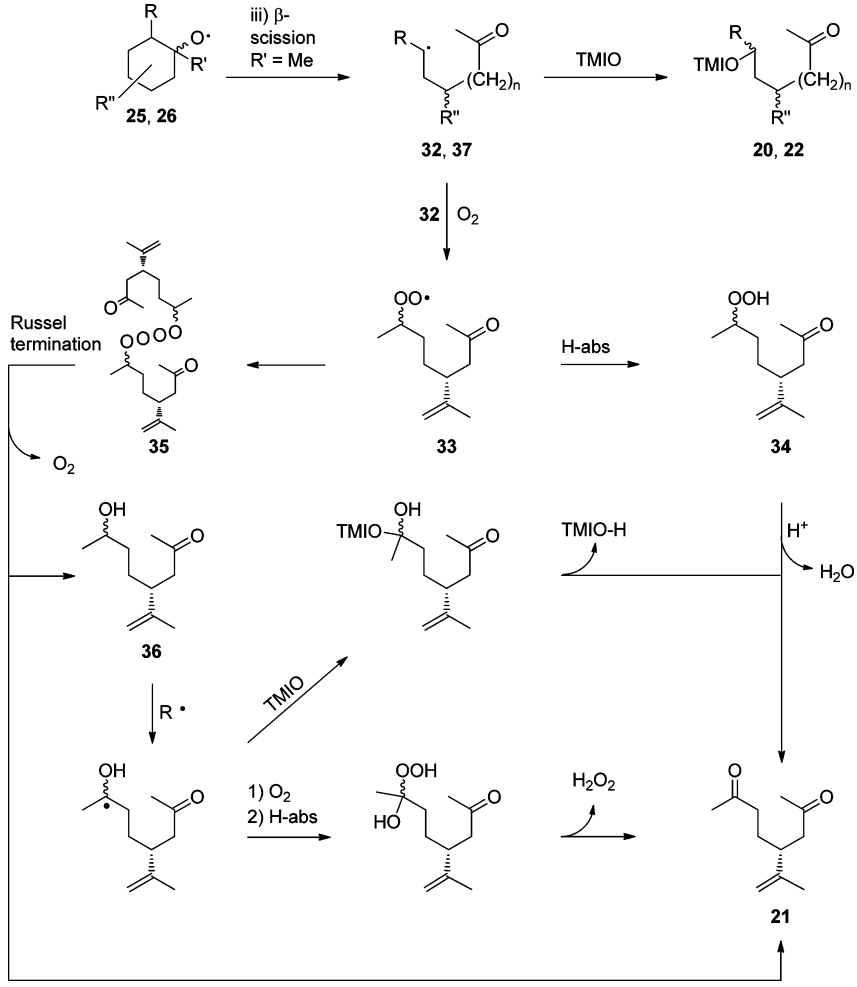
in TMIO adduct **20** and diketone **21** as the major products. Alkoxyl radical **25** is formed by homolytic cleavage of **14**. It can abstract a hydrogen atom and form alcohol **7** or react via a β-scission pathway (Scheme 3). Three β-scissions are possible for radical **25**. β-Scission of the methyl group would generate **10**; however, no evidence for the formation of **10** was found. β-Scission of one of the bonds in the cyclohexane ring would result in ring opening and generate either a primary or a secondary carbon-centered radical. No TMIO adduct corresponding to the primary carbon-centered radical was detected. The preferred β-scission generates the secondary carbon radical **32**, which is trapped by TMIO yielding adduct **20** (Scheme 5). The addition of dioxygen to **32** yields peroxyl radical **33**, which abstracts a hydrogen atom to form hydroperoxide **34** or dimerizes to tetroxide **35**. Hydroperoxide **34** can form diketone **21** via acid-catalyzed loss of water, whereas dimer **35** decomposes into diketone **21** and alcohol **36** via the Russell mechanism (*28*). Alcohol **36** could potentially be transformed into ketone **21** by hydrogen abstraction followed by trapping, either by TMIO or dioxygen. This would be followed by an expulsion of TMIO-H or hydrogen peroxide, respectively, forming diketone **21**. As alcohol **36** is not detected, dimer **35** is either not formed, or **36** is consumed in the reaction.

Homolytic cleavage of hydroperoxide **17** generates alkoxyl radical **26** that can abstract a hydrogen atom and form alcohol **18** (Table 2) or undergo β-scission, which results in opening of the cyclohexane ring. There are two possible ring-opening β-scissions, both resulting in carbon-centered radical **37**, which is subsequently trapped by TMIO, forming adduct **22** (Scheme 5). β-Scission of the methyl group would generate **16** (Scheme 2), but no evidence for ketone formation was found. The isolation and identification of alcohol **18** as the major product indicate that hydrogen abstraction is the favored reaction pathway in the trapping experiment with **17**.

Structural Analysis of Carbon Radical Adducts and Nonradical Products from the Trapping Experiments. The TMIO adducts and nonradical products from the radical-trapping experiments were identified using a combination of NMR and LC/MS. Liquid chromatography confirmed the purity of the samples, and mass spectroscopy identified ion masses corresponding to $[M + H]^+$ or $[M + Na]^+$. In the case of the TMIO adducts, fragmentation patterns indicated the presence of TMIO as well as a terpene residue. NMR shifts for individual ^1H and ^{13}C signals corresponded to previously characterized adducts and known structural elements of the present hydroperoxides. The structures of the terpene residues and the nonradical products were elucidated using a combination of 1D and 2D NMR experiments.

The structure of TMIO adduct **20** (Figure 2) was confirmed by 2D NMR correlations. The integrity of the TMIO unit was established by ^2J(C, H) correlations between the hydrogens in positions 10−13 and the quaternary carbons in positions 1, 3, 8, and 9 as well as ^3J(H, H), ^2J(C, H), and ^3J(C, H) couplings between the aromatic hydrogens in positions 4−7 and the aromatic carbons in positions 4−9. The preservation of the isopropenyl unit was confirmed by ^4J(H, H) and ^3J(C, H) correlations between the hydrogens in position 10′ and the hydrogens and the carbon, respectively, in position 11′. Furthermore, the hydrogens in position 10′ displayed a ^2J(C, H) coupling to the carbon in position 9′. Both carbon and hydrogens in position 10′ displayed ^3J(C, H) couplings to the carbon and the hydrogen in position 4′; this confirms the attachment of the isopropenyl group to the alkane chain. The carbonyl group was identified by its characteristic carbon shift and the ^2J(C, H)

Scheme 5. Mechanistic Proposal for the Formation of Ketone 21 in the Trapping Experiment with Hydroperoxide 14[a]



[a] Compound **20**: $n = 1$, R = Me, and R″ = isopropenyl. Compound **22**: $n = 2$, R = H, and R″ = isopropyl. Compound **32**: $n = 1$, R = Me, and R″ = isopropenyl. Compound **26**: $n = 2$, R = H, and R″ = isopropyl.

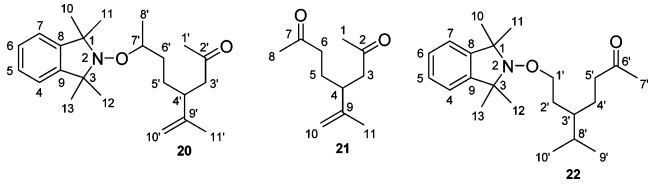


Figure 2. Products derived from carbon-centered radicals and isolated in the radical-trapping experiments.

couplings to the adjacent hydrogens in positions 1′ and 3′. The attachment and position of TMIO were identified by the typical shift of the carbon in position 7′ and the ^2J(C, H) correlation between the hydrogens in position 8′ and the carbon in position 7′ as well as the ^3J(H, H) couplings between the hydrogens in positions 6′ and 8′ with the hydrogen in position 7′.

The identity of product **21** (Figure 2) was supported by 2D NMR correlations. The integrity of the isopropenyl unit was confirmed by ^4J(H, H) and ^3J(C, H) correlations between the hydrogens in position 10 and the hydrogens and the carbon, in position 11. ^3J(C, H) correlations between the hydrogens in position 10 and the carbon in position 4 as well as between the carbon in position 11 and the hydrogen in position 4 confirm the attachment of the isopropenyl group to the alkane chain. The position of the isopropenyl group on the alkane chain was identified by the ^3J(H, H) couplings between the hydrogens in position 5 and positions 4 and 6, respectively, and the ^2J(C, H) coupling connecting the hydrogens in position 3 to the carbon in position 4. The carbonyl groups and the acetyl structural fragments were identified by their characteristic carbon shifts and the ^2J(C, H) couplings between the hydrogens in positions 1 and 8 to the carbons in positions 2 and 7, respectively. The attachment of the carbonyls to the alkane chain was evident from the ^2J(C, H) couplings to the hydrogens in positions 3 and 6, respectively.

TMIO adduct **22** (Figure 2) was identified by 2D NMR correlations. The preservation of the TMIO unit was confirmed by the ^2J(C, H) couplings between the hydrogens in positions 10−13 and the carbons in positions 1 and 3, as well as the ^2J(C, H) and ^3J(C, H) couplings of the aromatic hydrogens in positions 4−7 to the quaternary carbons in positions 8 and 9. The connection of the TMIO unit to the alkane chain was confirmed by the characteristic shift of the carbon in position 1′. The integrity of the isopropyl group was confirmed by the ^3J(C, H) couplings of the hydrogens in positions 9′ and 10′ to the carbon in position 8′. The attachment and position of the isopropyl group to the alkane chain were evident from the ^2J(C, H) coupling between the carbon in position 8′ and the hydrogen in position 3′. The identity and position of the acetyl group were confirmed by the typical shift of the carbonyl carbon and the ^2J(C, H) couplings of the carbonyl carbon to the hydrogens in positions 5′ and 7′, respectively.

In the ^{13}C NMR experiment with TMIO adduct **22** at room temperature, no signals corresponding to the methyl groups in

positions 10−13 were detected. To investigate if this was due to inversion of the nitrogen configuration, a new set of experiments was run at lowered temperatures. At −50 °C, two new signals were displayed; at −10 °C, these signals were broadened; at 10 °C, they were very broad; and at 25 °C, they were missing. This confirmed our hypothesis.

Radical Formation Pathways. The structures of the alkylic hydroperoxides clearly influence which reaction pathways will dominate the radical formation and thereby the products identified in the radical-trapping experiments (Table 2). In our previous study on the corresponding allylic hydroperoxides (*20*), products identified were attributed to three reaction pathways: hydrogen abstraction, 1,2-shift, and 1,3-cyclization (Scheme 1). In the present study, products identified can be attributed to hydrogen abstraction (alcohols **7**, **18**, and **19**, Scheme 3) and 1,2-shift (**10**) but not to the 1,3-cyclization. Instead, products formed by β-scission (TMIO adducts **20** and **22** and ketone **21**) were identified. The lack of cyclization products is explained by the lack of the endocyclic double bond. The high yields of **10** and β-scission products compared with the yields of alcohols can be explained by the rate difference between intra- and intermolecular reactions. There are two possible intramolecular reaction mechanisms: 1,2-shift and β-scission. The 1,2-shift requires a hydrogen on the hydroperoxide-bearing carbon. Thus, no products from this pathway were detected from the tertiary hydroperoxides; instead, β-scission products were identified. The lower yield of alcohol and higher yield of β-scission products in the trapping experiment with **14**, as compared with the trapping experiment with **17**, indicate that the secondary carbon-centered radical originating from **14** is more easily formed than the primary carbon-centered radical originating from **17**. This is in accordance with the general stability of carbon-centered radicals.

In summary, the structure of the hydroperoxides and the rates of the available reaction pathways will combine to dictate the identity and quantity of the radicals formed (Table 2). These radicals are potentially available for formation of immunogenic hapten−protein complexes.

Immunogenic Complex Formation. The exact mechanism of immunogenic complex formation of hydroperoxides is not known. It is postulated that it takes place via a radical mechanism (*6*−*9*), but only one specific mechanism have been proposed (*27*). As this mechanism only accounts for olefinic hydroperoxides, it is likely that other radical mechanisms are active in parallel. Their individual activity will depend on the identity of the formed radicals. The activity of different radical mechanisms is likely to influence the sensitizing capacity of the parent hydroperoxide. Thus, the identities of the formed radicals are important for the sensitizing capacity of the hydroperoxide. Furthermore, there is a threshold for the immunogenic response (*29*), meaning that a minimum number of immunogenic complexes need to be formed to trigger the response. This makes the amounts of radicals formed important, as more radicals potentially mean a higher amount of immunogenic complexes. In the radical-trapping experiments, the amount of alcohol indicates the amount of alkoxyl radicals available for immunogenic complex formation, whereas the amount of TMIO adducts indicate the amount of carbon-centered radicals available for immunogenic complex formation.

Hydroperoxide **12** does not form any TMIO adducts in the radical-trapping experiment. Instead, large amounts of **10** and minor amounts of **19** (Table 2) were identified. Compound **10** is a known nonsensitizer (*30*), and **19** is very unlikely to be a sensitizer as other aliphatic alcohols tested have been nonsensitizers (*31, 32*). Thus, the formation of these compounds cannot account for the high sensitizing capacity of hydroperoxide **12**. This implies the importance of radicals in immunogenic complex formation of hydroperoxides. As only small amounts of alcohol and no TMIO adducts are detected in the trapping experiments, the alkoxyl radicals are likely to be important in the immunogenic complex formation.

In the radical-trapping experiment with **14**, the high yields of **20** and **21** indicate the formation of large amounts of carbon-centered radicals (Table 2). Only a small amount of the corresponding alcohol is formed, indicating that only a small amount of alkoxyl are radicals available for the formation of immunogenic complexes. As both **12** and **14** are potent sensitizers, carbon-centered radicals may be less important than oxygen-centered radicals in the immunogenic complex formation. Ketone **21** is unlikely to be responsible for the sensitizing capacity of hydroperoxide **14**. The reactive groups of **21** are two carbonyl groups. The sensitizing potential of a carbonyl group is dependent on the electron deficiency of the carbony1 atom (*33*). In analogy with **10**, the substituents on both of the carbonyl groups of ketone **21** are electron-donating alkyl groups; this makes it unlikely that the electron deficiency of the carbonyl carbons is pronounced enough to generate a potent sensitizer.

In the radical-trapping experiment with **17**, a large amount of alcohol is formed together with a somewhat lower amount of TMIO adducts (Table 2). Thus, a large amount of alkoxyl radicals are potentially available for immunogenic complex formation, together with a slightly lower amount of carbon-centered radicals. In our previous studies of allylic hydroperoxides we found that hydroperoxide **4** had a significantly higher sensitizing capacity than hydroperoxides **3** and **5** (*34*). These results were obtained using a modified LLNA comprising nonpooled lymph nodes and statistical evaluation. A similar study has not been performed on hydroperoxides **12**, **14**, and **17**. However, there are major similarities in the radical formation and the sensitizing capacities of hydroperoxides **4** and **17**. Thus, it is possible that hydroperoxide **17** has a higher sensitizing capacity than hydroperoxides **12** and **14**. If so, this would be an indication of both the importance of the amount of radicals formed and the potency of the alkoxyl radicals.

We have recently presented results that suggest the thiol−ene radical reaction as a possible mechanism for the formation of immunogenic hapten−protein complexes from olefinic hydroperoxides (*27*). In the proposed mechanism, a thiyl radical is formed from the thiol group of cysteine, probably by hydrogen abstraction. Thereafter, the thiyl radical adds to double bonds of unsaturated compounds derived from the hydroperoxide. We have identified products resulting from the addition of the thiol group of cysteine and GSH over the double bonds of carvone and carveol. These products were formed in a system that initially contained only **3**, Fe(III)TPPCl, and cysteine or GSH. No adducts were detected when Fe(III)TPPCl was omitted from the reaction mixture, indicating a radical mechanism and the importance of radical formation.

Numerous radicals are formed in the proposed mechanisms of this work as well as in our previous investigation (*20*). Any of these radicals could potentially abstract a hydrogen atom from the thiol group of cysteine, thereby initiating the thiol−ene reaction. This increases the number of possible initiations, while simultaneously being integrated in the formation of nonradical unsaturated compounds (except for **17**, vide infra). Thus, the thiol−ene reaction is a possible mechanism for the formation of immunogenic hapten−protein complexes of hydroperoxides **12** and **14**.

The formation of thiyl radicals is a key step in the proposed thiol−ene mechanism. The thiyl radicals are probably formed by hydrogen abstraction, which might explain why the alkoxyl radicals seem to have a higher impact on the sensitizing capacity than the carbon-centered radicals. On the basis of bond dissociation energies, the formation of an oxygen−hydrogen bond is energetically favored over the formation of a carbon−hydrogen bond (*35*). This might result in more thiyl radicals formed for a given amount of alkoxyl radicals as compared with the same amount of carbon-centered radicals.

Being fully saturated, hydroperoxide **17** cannot form immunogenic complexes via the thiol−ene mechanism proposed for olefinic hydroperoxides. However, several other radical mechanisms might still be possible, for example, the direct addition of alkoxyl radicals to amino acid side chains or amino acid radicals. Hydrogen abstraction from fully saturated positions on compounds derived from **17** would give carbon-centered radicals that could possibly react with amino acid radicals in termination reactions. The high sensitizing capacity of **17**, together with its incompatibility with the thiol−ene reaction mechanism, strongly indicates that several radical reaction pathways are available for the radical immunogenic complex formation of hydroperoxides.

If the alkyl groups attached to the hydroperoxide-bearing carbon of a hydroperoxide are strongly electron donating and have the properties necessary to stabilize a plus charge on that carbon, a carbocation can be formed from the hydroperoxide under acidic conditions (*36, 37*). This carbocation would be available for S_N1 attack by amino acid side chains. However, no reports of alkylic hydroperoxides reacting in this fashion have been found in the literature. Several nonradical products were formed from the hydroperoxides in the radical-trapping experiments in both this and our previous study of allylic hydroperoxides (*20*). In theory, these products could be attacked by amino acid side chains in nucleophilic−electrophilic reactions. Nevertheless, none of these products have a known or suspected sensitizing capacity comparable with the hydroperoxides investigated and are therefore unlikely to be responsible for the high sensitizing capacity of the hydroperoxides. Independent of structure, all of the hydroperoxides in this as well as in our previous study (*20*) form large amounts of radicals and are potent sensitizers. Taken together, we find strong indications of a radical mechanism for the immunogenic hapten−protein complex formation of hydroperoxides.

All hydroperoxides tested have been potent sensitizers with small differences in their sensitizing capacities (see refs *8*, *9*, *14*, *15*, and *20* and this work). This may be explained by the depletion of local antioxidant reserves by the formation of high amounts of radicals in the skin (*38, 39*). Furthermore, this depletion may facilitate the formation of immunogenic complexes via a radical mechanism (*27*). This offers an explanation to why all hydroperoxides are strong sensitizers and have the ability to form specific antigens (*11*).

Conclusion

We conclude that small changes in structure, like the omission of the endocyclic double bond or the addition of a methyl group, result in large differences in radical formation of limonene hydroperoxides and their analogues. Furthermore, our results indicate that alkoxyl radicals seem to be more important in the immunogenic complex formation than carbon-centered radicals. All hydroperoxides investigated formed high amounts of radicals and were potent sensitizers. A possible explanation for the high sensitizing capacities of hydroperoxides is the weakening of the antioxidant defenses that is caused by the formation of high amounts of radicals in the skin (*38, 39*). This mode of action is compatible with the formation of specific antigens of hydroperoxides (*11*). For two of the hydroperoxides investigated in the present study, the formation of radicals and the simultaneous presence of unsaturated terpenoids make the recently suggested thiol−ene reaction (*27*) a possible mechanism for the formation of immunogenic complexes. The thiol−ene mechanism is not possible for the fully saturated hydroperoxide that is also investigated in this study. This hydroperoxide is a potent sensitizer and forms large amounts of radicals, results that strongly indicate that more than one radical reaction pathway for immunogenic complex formation of hydroperoxides is active.

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Supporting Information Available: ^{1}H and ^{13}C NMR data for the minor isomers of (5*R*)-5-isopropenyl-2-methyl-cyclohexane-1-hydroperoxide (**12b**−**d**) and full characterization data for the minor isomer of **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Nielsen, N. H., Linneberg, A., Menné, T., Madsen, F., Frölund, L., Dirksen, A., and Jörgensen, T. (2001) Allergic contact sensitization in an adult danish population: Two cross-sectional surveys eight years apart (the Copenhagen allergy study). *Acta Derm.-Venereol. 81*, 31–34.

(2) Rustemeyer, T., van Hoogstraten, I. M. V., von Blomberg, B. M. E., and Scheper, R. J. (2006) Mechanisms in allergic contact dermatitis. In *Contact Dermatitis* (Frosch, P. J., Menné, T., and Lepoittevin, J. P., Eds.) pp 11−43, Springer-Verlag, Berlin, Germany.

(3) Karlberg, A. T., Bergström, M. A., Börje, A., Luthman, K., and Nilsson, J. L. G. (2008) Allergic contact dermatitis−Formation, structural requirements, and reactivity of skin sensitizers. *Chem. Res. Toxicol. 21*, 53–69.

(4) Schmidt, R. J., Khan, L., and Chung, L. Y. (1990) Are free radicals and not quinones the haptenic species derived from urushiols and other contact allergenic mono- and dihydric alkylbenzenes? The significance of NADH, glutathione, and redox cycling in the skin. *Arch. Dermatol. Res. 282*, 56–64.

(5) Xia, Z. Y., Miyakoshi, T., and Yoshida, T. (2004) Lipoxygenase-catalyzed polymerization of phenolic lipids suggests a new mechanism for allergic contact dermatitis induced by urushiol and its analogs. *Biochem. Biophys. Res. Commun. 315*, 704–709.

(6) Gäfvert, E., Shao, L. P., Karlberg, A. T., Nilsson, U., and Nilsson, J. L. G. (1994) Contact allergy to resin acid hydroperoxides−Hapten binding via free-radicals and epoxides. *Chem. Res. Toxicol. 7*, 260–266.

(7) Lepoittevin, J. P., and Karlberg, A. T. (1994) Interactions of allergenic hydroperoxides with proteins: A radical mechanism? *Chem. Res. Toxicol. 7*, 130–133.

(8) Mutterer, V., Giménez-Arnau, E., Karlberg, A. T., and Lepoittevin, J. P. (2000) Synthesis and allergenic potential of a 15-hydroperoxyabietic acid-like model: Trapping of radical intermediates. *Chem. Res. Toxicol. 13*, 1028–1036.

(9) Bezard, M., Karlberg, A. T., Montelius, J., and Lepoittevin, J. P. (1997) Skin sensitization to linalyl hydroperoxide: Support for radical intermediates. *Chem. Res. Toxicol. 10*, 987–993.

(10) Redeby, T., Nilsson, U., Altamore, T. M., Ilag, L., Ambrosi, A., Broo, K., Börje, A., and Karlberg, A. T. (2010) Specific adducts formed through a radical reaction between peptides and contact allergenic hydroperoxides. *Chem. Res. Toxicol. 23*, 203–210.

(11) Christensson, J. B., Matura, M., Bäcktorp, C., Börje, A., Nilsson, J. L. G., and Karlberg, A. T. (2006) Hydroperoxides form specific antigens in contact allergy. *Contact Dermatitis 55*, 230–237.

(12) Giménez-Arnau, E., Haberkorn, L., Grossi, L., and Lepoittevin, J. P. (2002) Identification of alkyl radicals derived from an allergenic cyclic

tertiary allylic hydroperoxide by combined use of radical trapping and ESR studies. *Tetrahedron 58*, 5535–5545.
(13) Nilsson, U., Bergh, M., Shao, L. P., and Karlberg, A. T. (1996) Analysis of contact allergenic compounds in oxidized d-limonene. *Chromatographia 42*, 199–205.
(14) Sköld, M., Börje, A., Harambasic, E., and Karlberg, A. T. (2004) Contact allergens formed on air exposure of linalool. Identification and quantification of primary and secondary oxidation products and the effect on skin sensitization. *Chem. Res. Toxicol. 17*, 1697–1705.
(15) Hagvall, L., Bäcktorp, C., Svensson, S., Nyman, G., Börje, A., and Karlberg, A. T. (2007) Fragrance compound geraniol forms contact allergens on air exposure. *Chem. Res. Toxicol. 20*, 807–814.
(16) Sköld, M., Hagvall, L., and Karlberg, A. T. (2008) Autoxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens. *Contact Dermatitis 58*, 9–14.
(17) Karlberg, A. T., Shao, L. P., Nilsson, U., Gäfvert, E., and Nilsson, J. L. G. (1994) Hydroperoxides in oxidized D-limonene identified as potent contact allergens. *Arch. Dermatol. Res. 286*, 97–103.
(18) Sköld, M., Börje, A., Matura, M., and Karlberg, A. T. (2002) Studies on the autoxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. *Contact Dermatitis 46*, 267–272.
(19) Groot, A. C., and Frosch, P. J. (1997) Adverse reactions to fragrances. A clinical review. *Contact Dermatitis 36*, 57–86.
(20) Johansson, S. G. H., Giménez-Arnau, E., Grøtli, M., Karlberg, A.-T., and Börje, A. (2008) Carbon- and oxygen-centered radicals are equally important haptens of allylic hydroperoxides in allergic contact dermatitis. *Chem. Res. Toxicol. 21*, 1536–1547.
(21) Griffiths, P. G., Moad, G., Rizzardo, E., and Solomon, D. H. (1983) Synthesis of the radical scavenger 1,1,3,3-tetramethylisoindolin-2-yloxyl. *Aust. J. Chem. 36*, 397–401.
(22) Bergström, M. A., Luthman, K., Nilsson, J. L. G., and Karlberg, A. T. (2006) Conjugated dienes as prohaptens in contact allergy: In vivo and in vitro studies of structure−activity relationships, sensitizing capacity, and metabolic activation. *Chem. Res. Toxicol. 19*, 760–769.
(23) Pyun, H. J., Coates, R. M., Wagschal, K. C., McGeady, P., and Croteau, R. B. (1993) Regiospecificity and isotope effects associated with the methyl-methylene eliminations in the enzyme-catalyzed biosynthesis of (*R*)- and (*S*)-limonene. *J. Org. Chem. 58*, 3998–4009.
(24) Gurudutt, K. N., Rao, S., and Srinivas, P. (1992) Stereospecific synthesis of trans-beta terpineol. *Flavour Fragrance J. 7*, 343–345.
(25) Hammond, G. S., Wu, C. H. S., and Nonhebel, D. C. (1963) Chelates of β-diketones. V. Preparation and properties of chelates containing sterically hindered ligands. *Inorg. Chem. 2*, 73–76.
(26) Kimber, I., Hilton, J., Dearman, R. J., Gerberick, G. F., Ryan, C. A., Basketter, D. A., Scholes, E. W., Ladics, G. S., Loveless, S. E., House, R. V., and Guy, A. (1995) An international evaluation of the murine local lymph node assay and comparison of modified procedures. *Toxicology 103*, 63–73.
(27) Johansson, S. G. H., Redeby, T., Altamore, T. M., Nilsson, U., and Börje, A. (2009) Mechanistic proposal for the formation of specific immunogenic complexes via a radical pathway: A key step in allergic contact dermatitis to olefinic hydroperoxides. *Chem. Res. Toxicol. 22*, 1774–1781.
(28) Russell, G. A. (1957) Deuterium-isotope effects in the autoxidation of aralkyl hydrocarbons. Mechanism of the interaction of peroxy radicals. *J. Am. Chem. Soc. 79*, 3871–3877.
(29) Basketter, D. A., Evans, P., Gerberick, G. F., and Kimber, I. A. N. (2002) Factors affecting thresholds in allergic contact dermatitis: Safety and regulatory considerations. *Contact Dermatitis 47*, 1–6.
(30) Nilsson, A. M., Gäfvert, E., Salvador, L., Luthman, K., Bruze, M., Gruvberger, B., Nilsson, J. L., and Karlberg, A. T. (2001) Mechanism of the antigen formation of carvone and related alpha, beta-unsaturated ketones. *Contact Dermatitis 44*, 347–356.
(31) Basketter, D. A., Gerberick, G. F., and Kimber, I. (1998) Strategies for identifying false positive responses in predictive skin sensitization tests. *Food Chem. Toxicol. 36*, 327–333.
(32) Ryan, C. A., Gerberick, G. F., Cruse, L. W., Basketter, D. A., Lea, L., Blaikie, L., Dearman, R. J., Warbrick, E. V., and Kimber, I. (2000) Activity of human contact allergens in the murine local lymph node assay. *Contact Dermatitis 43*, 95–102.
(33) Aptula, A. O., Patlewicz, G., and Roberts, D. W. (2005) Skin sensitization: Reaction mechanistic applicability domains for structure-activity relationships. *Chem. Res. Toxicol. 18*, 1420–1426.
(34) Christensson, J. B., Johansson, S. G. H., Hagvall, L., Jonsson, C., Börje, A., and Karlberg, A.-T. (2008) Limonene hydroperoxide analogues differ in allergenic activity. *Contact Dermatitis 59*, 344–352.
(35) McMurry, J. (2004) Bond dissociation enthalpies table. *Organic Chemistry*, p 154, Brooks/Cole−Thomson Learning, Belmont.
(36) Davies, A. G., Foster, R. V., and Nery, R. (1954) Organic peroxides. Part IV. Alkyl oxygen heterolysis in organic peroxides. *J. Chem. Soc.* 2204–2209.
(37) Taljaard, B., Goosen, A., and Mccleland, C. W. (1987) Synthesis of hydrogen peroxide: Acid-catalyzed decomposition of 9-hydroperoxy-9-phenylxanthene and its derivatives. *S. Afr. J. Chem. 40*, 139–145.
(38) Shvedova, A. A., Kisin, E. R., Murray, A. R., Kommineni, C., Castranova, V., Mason, R. P., Kadiiska, M. B., and Gunther, M. R. (2002) Antioxidant balance and free radical generation in vitamin E-deficient mice after dermal exposure to cumene hydroperoxide. *Chem. Res. Toxicol. 15*, 1451–1459.
(39) Murray, A. R., Kisin, E. R., Kommineni, C., Vallyathan, V., Castranova, V., and Shvedova, A. A. (2007) Pro/antioxidant status and AP-1 transcription factor in murine skin following topical exposure to cumene hydroperoxide. *Carcinogenesis 28*, 1582–1588.
(40) Basketter, D. A., Lea, L. J., Dickens, A., Briggs, D., Pate, I., Dearman, R. J., and Kimber, I. (1999) A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. *J. Appl. Toxicol. 19*, 261–266.

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