Autoxidation Study of Carotane Sesquiterpenes Possessing a Non-Conjugated 1,4-Diene System

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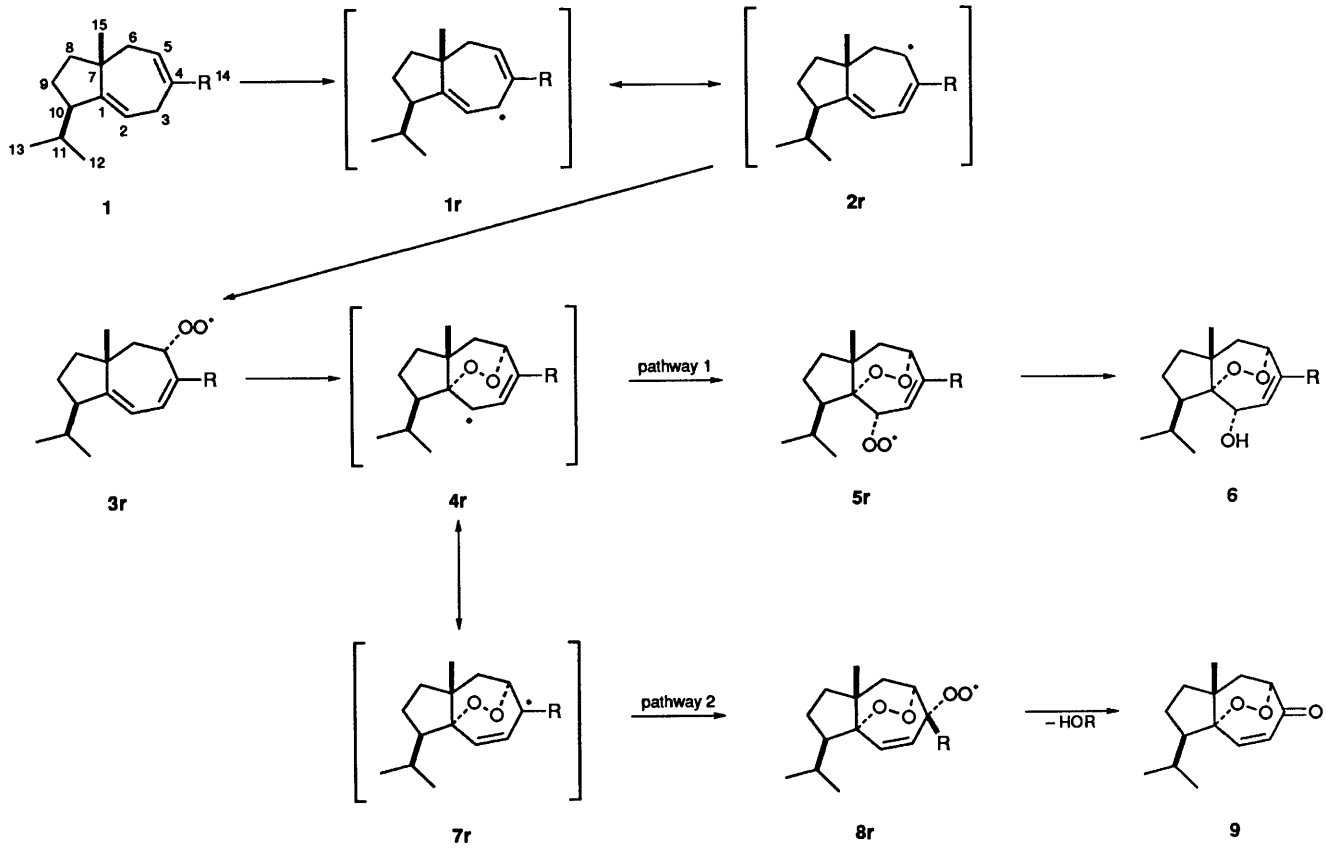
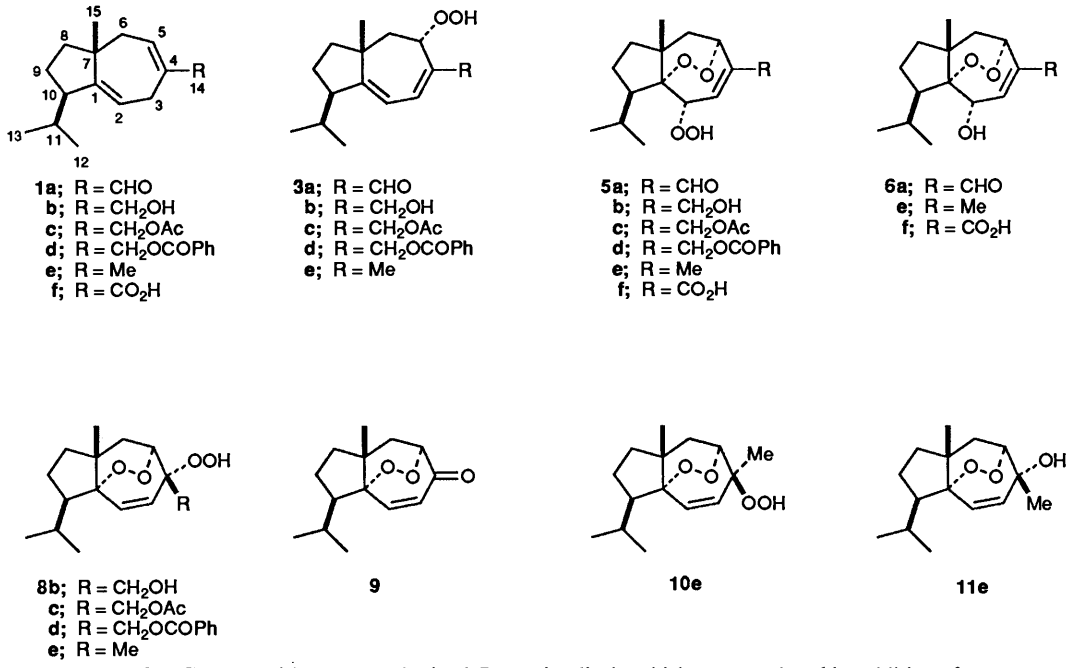
The autoxidation process of carota-1,4-diene derivatives was studied. Several intermediate compounds in the autoxidation were isolated and characterized by spectroscopic methods, and two pathways, yielding 1,5-epidioxy-2-hydroperoxy derivatives and 1,5-epidioxy-14-norcarot-2-en-4-one, respectively, were confirmed. 5-Hydroperoxy-1,3-dienes undergoing spontaneous cyclization to yield 1,5-epidioxy derivatives were isolated as key intermediates in the first oxygen-addition step, whereas 1,5-epidioxy-4-hydroperoxy derivatives were identified as precursors of 1,5-epidioxy-14-norcarot-2-en-4-one. These observations supported a unique radical-mediated peroxidation process for carota-1,4-dienes.

Rugosal A (2-hydroxy-1,5-epidioxycarot-3-en-14-al **6a**), first isolated from *Rosa rugosa* leaves, possesses a unique 1,5-epidioxy linkage, which is rare for a natural peroxide.[1] We have also isolated carota-1,4-dien-14-al **1a** from the leaves as a precursor of rugosal A **6a**, and have demonstrated the autoxidative conversion of diene **1a** into ene **6a** *via* 2-hydroperoxy-1,5-epidioxycarot-3-en-14-al **5a**.[2] This present study supports the radical-mediated reaction pathway shown in Scheme 1 (pathway 1). Since these radical intermediates (**1r–5r**) were labile, the corresponding hydroperoxides (**3** and **5**), with hydroperoxy groups replacing the exoperoxyl radicals, were obtained from the autoxidation mixture as stable products. To confirm that the autoxidation process occurred *via* 5-hydroperoxyl radicals, the 5-hydroperoxide intermediates **3b–e** from carota-1,4-diene derivatives **1b–e** were characterized.[3]

We have also found 1,5-epidioxy-14-norcarot-2-en-4-one **9** in a peroxide mixture from compound **1a** autoxidized in organic solvents, and we proposed a conversion scheme for enone **9** under O_2-deficient conditions.[2] In this reaction pathway, radical transformation is expected to occur first to yield the 4-exoperoxyl radical, followed by the deformylation of the C-14 atom *via* β-cleavage between C-4 and C-14 to yield the norcarotane



Scheme 1 Autoxidation process to yield 1,5-epidioxy-2-hydroxycarot-3-ene derivatives **6** and 1,5-epidioxy-14-norcarot-2-en-4-one **9** from carotenes **1**. Intermediate radicals **2r**, **4r** and **7r** could not be trapped in this study.



Structures of isolated compounds Compound **3a** was not obtained. Peroxyl radicals, which were produced by addition of oxygen to carbon radicals depicted in Scheme 1, give hydroperoxide products **3**, **5** and **8**.

derivative **9** (Scheme 1, pathway 2). However, we have not so far obtained any chemical proof of this fission process.

In this study, identification of the intermediates **3b**, **3c**, **3d**, **3e**, **5b**, **5d**, **8b**, **8c**, **8d** and **8e** using spectroscopic methods seems to validate the proposed autoxidation pathways. Here we describe the isolation and identification of these autoxidation products.

Results and Discussion

Substrates as thin films on a glass wall were heated (60 °C) to autoxidize them. The product mixture obtained by the autoxidation of carota-1,4-dien-14-ol **1b** was chromatographed on silica gel TLC plates. Two major products, 5-hydroperoxycarota-1,3-dien-14-ol **3b** and 1,5-epidioxy-14-norcarot-2-en-4-one **9**,[2] and a minor product, 1,5-epidioxy-2-hydroperoxycarot-3-en-14-ol **5b**, all of which were positive to a peroxide test (*N*,*N*-dimethyl-*p*-phenylenediamine sulfate–AcOH),[4] were isolated and identified spectroscopically. Compound **3b**, derived from a key intermediate (**3r**) in the autoxidation pathway of substrate **1** into epidioxide **6** was successfully isolated by rapid preparative TLC (PLC) (Merck Kieselgel $60F_{254}$). This labile hydroperoxide was spontaneously converted into enone **9** and some unknown derivatives in an organic solvent over a period of several hours. Other labile peroxides, including **8b**, were also obtained from the mixture, but their attempted isolation was unsuccessful.

Compound **8b**, however, precipitated as fine cubes during autoxidative degradation of compound **1b** in hexane at a low temperature. The formation of compound **8b**, $C_{15}H_{24}O_5$, suggested the oxygenation of the sesquiterpene skeleton with two O_2 molecules. The ^1H NMR spectrum of compound **8b** was similar to that of enone **9**, but the presence of a C-14 hydroxymethyl group was obvious from a pair of methylene protons [δ_H 3.92 and 3.78 (both d, *J* 12 Hz, geminal coupling)]. Two olefinic protons (δ_H 6.12 and 5.89, with *J* 12 Hz typical of a *cis* coupling) suggested the presence of a C–C double bond between C-2 and C-3. A methine proton signal at δ_H 4.62 showing vicinal and long-range couplings with a pair of methylene proton signals [δ_H 2.28 (*J* 6 Hz) and 1.89 (*J* 2 Hz)] and one of the olefinic protons [δ_H 5.89 (*J* 2 Hz)], respectively, was assigned to 5-H. The coupling patterns of these protons suggested a 1,5-epidioxy-2-ene moiety for compound **8b**. An exchangeable proton signal at δ_H 8.46 (singlet) empirically assigned to a hydroperoxy proton[2,5] was also detected. Three signals for oxygenated carbons observed at δ_C 91.7, 91.0 (both non-hydrogen bearing) and 75.9 (CH) were assignable to C-1, -4 and -5, respectively. Thus, compound **8b** was expected to have the 1,5-epidioxy-4-hydroperoxycarot-2-en-14-ol structure, and this basic structure was unambiguously confirmed for the related compounds **8d** and **8e** by C–H COSY and HMBC (heteronuclear multiple bond correlation) analyses. Peroxide **8b** was quickly degradated into enone **9** by heating (60 °C) on a glass wall or in solvents. Thus, compound **8b** was found to be a precursor of enone **9** in the thermal autoxidation.

Compound **3b** was also labile. 14-Acetoxycarota-1,4-diene **1c**, derived from the alcohol **1b**, was therefore autoxidized to obtain a more stable 5-hydroperoxy-1,3-diene product, and subsequently a major product **3c**, $C_{17}H_{26}O_4$, was isolated by PLC as a comparatively stable syrup (*e.g.*, 0.9 mg from 3.0 mg of **1c**). The UV λ_{max}(MeOH) at 262 nm and two olefinic protons [δ_H 5.97 (d, *J* 8 Hz, 3-H) and 5.34 (dd, *J* 8 and 2 Hz, 2-H)], showing a vicinal coupling of olefinic protons (=CH–CH=) distinguishable from that of *cis* coupling (–CH=CH–, *J* 12 Hz for compounds **9** and **8b**), confirmed its 1,3-diene structure. The exchangeable proton signal at δ_H 8.20 (singlet) was assigned to the C-5 hydroperoxide proton.[2,5] A methine proton signal at δ_H 4.59 (br ddd, *J* 12, 6 and 2 Hz, 5-H) coupled with a pair of C-6 methylene proton signals at δ_H 2.41 (dd, *J* 13 and 12 Hz) and 2.10 (dd, *J* 13 and 6 Hz) confirmed the position of the 5-hydroperoxide group. The carbon signals assignable to two olefinic bonds were at δ_C 162.1, 114.2 and 134.0, and the remaining one overlapped with the deuteriated benzene peaks [δ_C ~128, the relevant signal was confirmed by the ^{13}C NMR spectrum of 14-benzoyloxy-5-hydroperoxy derivative **3d** measured in $CDCl_3$ (δ_C 128.4)]. An oxygenated sp^3 carbon signal at δ_C 82.4 was appropriate for the hydroperoxylated C-5.[2,5] Thus, it was confirmed that 5-hydroperoxyl radical **3r** was an intermediate in the peroxidation (Scheme 1). After being left in deuteriated benzene, compound **3c** was gradually converted into enone **9**.

Another major product, **8c**, exhibiting a similar signal pattern to that of the alcohol **8b** in the ^1H NMR spectrum, was also

obtained. As compound **8c** converted spontaneously into enone **9** during the isolation process on TLC as did alcohol **8b**, 1,5-epidioxy-4-hydroperoxy substitution seemed to be necessary for precursors to enone **9**, as we have previously proposed.[2]

14-Benzoyloxy derivative **1d**, prepared to enable us to monitor autoxidation products by UV spectroscopy, was autoxidized under the same conditions as was acetate **1c**. Accordingly, a stable solid (**8d**) was obtained as a major product (33%) together with compounds **3d** (6%), **5d** (5%), and **9** (trace). Compound **8d** was comparatively thermostable; however, when it was subjected to acid-catalysed peroxide rearrangement,[6] a small amount of compound **9** was formed.

Stereochemistry of benzoate **8d** at C-4 was elucidated as *S* by NOE measurements between 15-H_3 and 14-H_2. Since the radical intermediate **7r** had a C-4 radical carbon with a delocalized π-electron bond,[7] the conformation of radical **7r** afforded the opportunity for back-side attack in the second $^3O_2$ addition on the C-4 carbon to yield the exoperoxyl radical **8r**. It is likely that the front-side attack is blocked by the close proximity of the C-15 methyl group to the C-4 carbon.

Intermediate 5-hydroperoxide **3d** was also more stable than acetate **3c**, though it was still converted spontaneously into two types of 1,5-epidioxyhydroperoxy derivative (compounds **5d** and **8d**) when it was left in deuteriochloroform for a week. Compound **1d** thus showed conversion into both 4-hydroperoxy and 2-hydroperoxy 1,5-epidioxides (**5d** and **8d**) *via* the 5-hydroperoxy intermediate.

Autoxidation products of carota-1,4-diene (**1e**, non-oxygenated at C-14) prepared from (+)-carotol[8] were also investigated. From the product mixture, compounds **3e**, **8e** (31% yield), 4-hydroperoxide **10e**, and alcohol **11e** were identified. When the isolated hydroperoxide **3e** was left in deuteriochloroform overnight, it was spontaneously converted into epidioxides **8e** (>90% yield) and **10e**.

The autoxidation process in non-conjugated carota-1,4-diene derivatives is suggestive of the transient occurrence of allyl radical mesomerization between the secondary radical **4r** and the tertiary radical **7r**.[9] This radical mesomerization is, however, insignificant in compound **1a** and in carota-1,4-dien-14-oic acid **1f** having a conjugation system on C-4, -5 and -14. Compounds **1b–e** without a conjugation system on C-14 all underwent delocalization of an unpaired electron to result in formation of the corresponding postulated intermediates **7r**.

The present study on autoxidation of carota-1,4-dienes thus gave some direct evidence for exoperoxyl radical-mediated cyclization resulting in 1,5-epidioxide formation from cyclic 1,4-dienes.[10] Since we have found that 1,5-epidioxy-2-hydroxycarot-3-ene **6e** occurs in greater amounts than those of the C-4 oxygenated derivatives **8e** and **11e** in glandular trichome exudates of *R. rugosa* leaves,[11] oxidation of carota-1,4-diene derivatives in the exudate may be brought about through an alternative pathway.

Experimental

General.—^{1}H and ^{13}C NMR spectra were measured on a JEOL EX270 spectrometer (270/68 MHz), and some 2-dimensional data were taken by a Bruker AM500 (500/125 MHz). *J*-Values are given in Hz. EI-MS and EI-HRMS were measured on a JEOL JMS DX300 (70 eV), and FI-MS, FD-MS, and FI-HRMS were measured on a JEOL JMS 015G-2. TLC plates (Merck Kieselgel 60F_{254}, 0.25 mm) were used for PLC. M.p.s were measured on a Micro Melting Point Apparatus (Yanaco) and are uncorrected. Derivatives **1b–d**, **3b–c**, **5b–e**, **8b–e**, **10e** and **11e** are new compounds.

Preparation of Substrate (7R,10R)-*Carota*-1,4-*dien*-14-*ol* **1b**.—Throughout this series, (7*R*,10*R*)-carota-1,4-dien-14-al **1a**, previously isolated from *R. rugosa* leaves, was used. An excess of $NaBH_4$ (~200 mg) was added to a solution of compound **1a** (232.6 mg) in 50% EtOH–$CHCl_3$ (6 cm^3), and the mixture was stirred overnight at room temperature. The concentrated reaction mixture was partitioned between distilled water (100 cm^3) and EtOAc (50 cm^3), and the organic layer was dried over Na_2SO_4 followed by concentration. The product mixture was directly subjected to PLC [R_f 0.48, in hexane–ethyl acetate (4:1)] to yield compound **1b** as a syrup (185.3 mg, 79%); EI-HRMS (Found: M^+, 220.1794. $C_{15}H_{24}O$ requires M, 220.1827); FIMS *m/z* 220 (M^+, 100%); EIMS *m/z* 220 (M^+, 3.4%), 202 ($M^+ - H_2O$, 14), 189 (6.5), 177 (38), 159 (53), 131 (32), 117 (36), 105 (54), 91 (81), 81 (72), 79 (55), 67 (38), 55 (44) and 41 (100); δ_H(270 MHz; C_6D_6; HH-COSY) 5.60 (br d, *J* 8, 5-H), 5.21 (ddd, *J* 6, 4 and 2, 2-H), 3.72 (br s, 14-H_2), 2.90 (br d, *J* 22, 3-H^a), 2.67 (ddd, *J* 22, 6 and 2, 3-H^b), 2.39 (m, 10-H), 2.33 (br d, *J* 16, 6-H^a), 2.02 (dd, *J* 16 and 8, 6-H^b), 1.81 (sept d, *J* 7 and 5, 11-H), 1.09 (s, 15-H_3), 0.95 (d, *J* 7, 12-H_3) and 0.83 (d, *J* 7, 13-H_3); δ_C(68 MHz; C_6D_6; DEPT and CH-COSY) 152.5 (C-1), 138.1 (C-4), 123.4 (CH-5), 116.7 (CH-2), 69.5 (CH_2-14), 52.9 (CH-10), 45.7 (C-7), 41.9 (CH_2-8), 40.8 (CH_2-6), 30.8 (CH_2-3), 29.7 (CH-11), 24.1 (Me-15), 23.8 (CH_2-9), 22.0 (Me-12) and 17.3 (Me-13).

Autoxidation of Compound **1b**.—Compound **1b** (6.4 mg) as a thin film on a glass wall was heated at 60 °C for 2 h in the dark. The reaction mixture was then collected by washing with EtOAc, and was chromatographed on PLC plates [hexane–EtOAc (1:1); *cf.* **1b** R_f 0.69]. Compounds **9** (2.9 mg, 42%), **3b** (0.7 mg, 10%) and **5b** (0.9 mg, 11%) were detected at R_f 0.74, 0.43 and 0.08, respectively. From a hexane solution of compound **1b** [~80 mg/4 cm^3, containing β-carotene (~0.5 mg)] stored at 4 °C in the dark for 3 weeks there precipitated cubes of compound **8b** [~5 mg, R_f 0.26 in hexane–EtOAc (1:1)].

5-*Hydroperoxycarota*-1,3-*diene*-14-*ol* **3b**. A syrup; vanillin–sulfuric acid test: bluish purple; *N,N*-dimethyl-*p*-phenylenediamine sulfate test: positive (pink); λ_{max}(MeOH)/nm 264; δ_H(270 MHz; $CDCl_3$; HH-COSY) 6.10 (br d, *J* 8, 3-H), 5.49 (dd, *J* 8 and 2, 2-H), 4.66 (br ddd, *J* 12, 6 and 2, 5-H), 4.41 (d, *J* 12, 14-H^a), 4.16 (d, *J* 12, 14-H^b), 2.60 (m, 10-H), 0.98 (d, *J* 7, 12-H_3), 0.97 (s, 15-H_3) and 0.76 (d, *J* 7, 13-H_3).

1,5-*Epidioxy*-2-*hydroperoxycarot*-3-*en*-14-*ol* **5b**. A syrup; vanillin–sulfuric acid test: greyish light blue; *N,N*-dimethyl-*p*-phenylenediamine sulfate test: positive (pink); δ_H(270 MHz; $CDCl_3$) 6.06 (d, *J* 6, 3-H), 4.78 (d, *J* 6, 2-H), 4.67 (m, 5-H), 4.19 (br s, 14-H_2), 2.57 (sept d, *J* 7 and 5, 11-H), 2.20 (dd, *J* 14 and 5, 6-H^a), 0.98 (d, *J* 7, 12-H_3), 0.97 (s, 15-H_3) and 0.92 (d, *J* 7, 13-H_3).

1,5-*Epidioxy*-4-*hydroperoxycarot*-2-*en*-14-*ol* **8b**. Cubes from hexane, m.p. 131–131.5 °C; vanillin–sulfuric acid test: greyish brown; *N,N*-dimethyl-*p*-phenylenediamine sulfate test: positive (pink); FIMS *m/z* 284 (M^+, 69%), 268 (29), 237 (53), 236 (58), 220 (76) and 78 (100); FI-HRMS (Found: M^+, 284.1630. $C_{15}H_{24}O_5$ requires M, 284.1624); δ_H(270 MHz; $CDCl_3$) 8.46 (s, 4-OOH), 6.12 (d, *J* 12, 2-H), 5.89 (dd, *J* 12 and 2, 3-H), 4.62 (ddd, *J* 6, 2 and 2, 5-H), 3.92 (d, *J* 12, 14-H^a), 3.78 (d, *J* 12, 14-H^b), 2.28 (dd, *J* 15 and 6, 6-H^a), 1.89 (dd, *J* 15 and 2, 6-H^b), 0.97 (d, *J* 6, 12-H_3), 0.90 (s, 15-H_3) and 0.88 (d, *J* 6, 13-H_3); δ_C(68 MHz; $CDCl_3$) 132.6 (CH-2), 127.8 (CH-3), 91.7 (C-1), 91.0 (C-4), 75.9 (CH-5), 63.6 (CH_2-14), 54.4 (CH-10), 43.3 (C-7), 39.7 (CH_2-8), 35.0 (CH_2-6), 28.9 (CH-11), 25.0 (Me-15), 24.5 (CH_2-9), 23.4 (Me-12) and 19.3 (Me-13).

1,5-*Epidioxy*-14-*norcarot*-2-*en*-4-*one* **9**. A syrup; vanillin–sulfuric acid test; pinkish brown; *N,N*-dimethyl-*p*-phenylenediamine sulfate test; positive (pink); λ_{max}(MeOH)/nm 227; FDMS *m/z* 237 (M^+ + 1, 100%), 236 (M^+, 98) and 110 (24); FI-HRMS (Found: M^+, 236.1440. $C_{14}H_{20}O_3$ requires M,

236.1413); δ_H(270 MHz; $CDCl_3$; HH-COSY) 6.85 (d, *J* 12, 2-H), 6.54 (dd, *J* 12 and 2, 3-H), 4.57 (ddd, *J* 5, 3 and 2, 5-H), 2.18 (dd, *J* 14 and 5, 6-H^a), 1.91 (dd, *J* 14 and 3, 6-H^b), 1.03 (d, *J* 6, 12-H_3), 0.95 (d, *J* 6, 13-H_3) and 0.72 (s, 15-H_3); δ_C(125 MHz; $CDCl_3$; DEPT and CH-COSY) 204.1 (C-4), 142.6 (CH-2), 135.5 (CH-3), 93.0 (C-1), 82.6 (CH-5), 55.0 (CH-10), 44.7 (C-7), 39.5 (CH_2-8), 36.5 (CH_2-6), 28.8 (CH-11), 25.2 (Me-15), 24.7 (CH_2-9), 23.1 (Me-12) and 19.9 (Me-13).

Preparation of (7R,10R)-14-*Acetoxycarota*-1,4-*diene* **1c**.—Compound **1b** (92.3 mg) was dissolved in pyridine–Ac_2O (1:1) and the solution was kept at 60 °C for 2 h. The reaction mixture was diluted with an excess of toluene and was then evaporated under reduced pressure, and subjected to PLC [hexane–EtOAc (10:1)]. Subsequently, the product **1c** was obtained as a syrup (97.6 mg, 89%), R_f 0.65 [hexane–EtOAc (10:1)]; FIMS *m/z* 262 (M^+, 100%), 220 (18) and 202 (15); EIMS *m/z* 202 (22%), 187 (14), 159 (100), 131 (23), 117 (33), 105 (29), 91 (21) and 43 (45); δ_H(270 MHz; $CDCl_3$; HH-COSY) 5.77 (br d, *J* 8, 5-H), 5.22 (ddd, *J* 6, 3 and 3, 2-H), 4.43 (br s, 14-H_2), 2.98 (br d, *J* 22, 3-H^a), 2.83 (br dd, *J* 22 and 3, 3-H^b), 2.43 (m, 10-H), 2.32 (br d, *J* 17, 6-H^a), 2.10 (dd, *J* 17 and 8, 6-H^b), 2.07 (s, 14-OAc), 1.85 (sept d, *J* 7 and 5, 11-H), 1.04 (s, 15-H_3), 0.94 (d, *J* 7, 12-H_3) and 0.79 (d, *J* 7, 13-H_3); δ_C(68 MHz; $CDCl_3$; DEPT and CH-COSY) 171.0 (14-O*C*OMe), 153.1 (C-1), 132.5 (C-4), 127.9 (CH-5), 115.5 (CH-2), 71.2 (CH_2-14), 52.6 (CH-10), 45.4 (C-7), 41.5 (CH_2-8), 40.6 (CH_2-6), 30.8 (CH_2-3), 29.1 (CH-11), 23.7 (Me-15), 23.5 (CH_2-9), 21.8 (Me-12), 21.1 (14-OCO*Me*) and 17.0 (Me-13).

Autoxidation of Compound **1c**.—A thin film of compound **1c** (39.0 mg, from crude **1a**) on a glass wall heated at 60 °C for 2 h in the dark yielded two products showing UV-quenching spots on silica gel $60F_{254}$ plates with positive responses to peroxide reagent (*N,N*-dimethyl-*p*-phenylenediamine sulfate reagent).[4] 14-Acetoxy-5-hydroperoxycarota-1,3-diene **3c** [2.3 mg as a syrup, R_f 0.27, in hexane–EtOAc (6:1) *cf.* **1c**, R_f 0.69] and compound **9** (4.1 mg, R_f 0.45) were isolated by PLC. Compound **8c** (5.6 mg, R_f 0.09) was also isolated as a major product, whereas compound **5c** (R_f 0.07) was obtained as a small amount of impure syrup, but its attempted purification was unsuccessful.

14-*Acetoxy*-5-*hydroperoxycarota*-1,4-*diene* **3c**. *N,N*-Dimethyl-*p*-phenylenediamine sulfate test: positive (pink); FDMS *m/z* 294 (M^+, 100%); FI-HRMS (Found: M^+, 294.1842. $C_{17}H_{26}O_4$ requires M, 294.1855); λ_{max}(MeOH)/nm 262; ν_{max}(CCl_4; 0.3 mmol dm^{-3})/cm^{-1} 3552 (OOH), 2960 and 1745; δ_H(270 MHz; C_6D_6; HH-COSY) 8.20 (s, 5-OOH), 5.97 (br d, *J* 8, 3-H), 5.34 (dd, *J* 8 and 2, 2-H), 4.84 (d, *J* 12, 14-H^a), 4.71 (d, *J* 12, 14-H^b), 4.59 (br ddd, *J* 12, 6 and 2, 5-H), 2.41 (dd, *J* 13 and 12, 6-H^a), 2.36 (br m, 10-H), 2.10 (dd, *J* 13 and 6, 6-H^b), 1.62 (s, 14-OAc), 0.85 (d, *J* 7, 12-H_3), 0.79 (s, 15-H_3) and 0.70 (d, *J* 7, 13-H_3); δ_C(68 MHz; C_6D_6) 170.6 (14-O*C*OMe), 162.1 (C-1), 134.0 (C-4), 114.2 (CH-2), 82.4 (CH-5), 68.3 (CH_2-14), 52.1 (CH-10), 42.9 (C-7), 41.2 (CH_2-6), 40.2 (CH_2-8), 29.5 (CH-11), 22.7 (CH_2-9), 21.8 (Me-12), 20.6 (14-OCO*Me*), 20.3 (Me-15) and 16.6 (Me-13). The C-3 carbon signal overlapped with the solvent peak.

14-*Acetoxy*-1,5-*epidioxy*-4-*hydroperoxycarot*-2-*ene* **8c**. A syrup; vanillin–sulfuric acid test: yellowish brown; *N,N*-dimethyl-*p*-phenylenediamine sulfate test: positive (pink); δ_H(270 MHz; $CDCl_3$) 9.12 (s, 4-OOH), 6.12 (d, *J* 12, 2-H), 5.96 (dd, *J* 12 and 3, 3-H), 4.56 (d, *J* 13, 14-H^a), 4.45 (ddd, *J* 6, 2 and 2, 5-H), 4.28 (d, *J* 13, 14-H^b), 2.32 (dd, *J* 15 and 6, 6-H^a), 2.16 (s, 14-OAc), 1.91 (dd, *J* 15 and 2, 6-H^b), 0.96 (d, *J* 6, 12-H_3), 0.93 (s, 15-H_3) and 0.88 (d, *J* 6, 13-H_3); δ_C(68 MHz; $CDCl_3$) 172.3 (14-O*C*OMe), 132.0 (CH-2), 127.4 (CH-3), 90.7 (C-1), 90.3 (C-4), 74.8 (CH-5), 62.0 (CH_2-14), 54.1 (CH-10), 43.3 (C-7), 39.8 (CH_2-8), 35.6 (CH_2-6), 28.7 (CH-11), 24.8 (Me-15), 24.0 (CH_2-9), 23.3 (Me-12), 20.9 (14-OCO*Me*) and 19.1 (Me-13). An NOE between 15-H_3 and 14-H_2 indicated the stereostructure as 1*S*,4*S*,5*S*,7*R*,10*R*.

Preparation of (7R,10R)-14-*Benzoyloxycarota*-1,4-*diene* **1d**.—Compound **1b** (93.0 mg) was mixed with benzoic anhydride–Et_3N (200 mg/2 cm^3) and kept at 70 °C for 2 h. The reaction mixture was then applied to TLC [hexane–EtOAc (10:1)], and pure compound **1d** (R_f 0.68) (90.7 mg, 66%) was obtained as a syrup. Vanillin–sulfuric acid test: purple; λ_{max}(MeOH)/nm 267, 273 and 280; FIMS *m/z* 324 (M^+, 100%); FI-HRMS (Found: M^+, 324.2090. $C_{22}H_{28}O_2$ requires M, 324.2090); δ_H(270 MHz; $CDCl_3$; HH-COSY) 8.05 (dd, *J* 7 and 1, 2′- and 6′-H), 7.54 (br dd, *J* 7 and 7, 4′-H), 7.41 (br dd, *J* 7 and 7, 3′- and 5′-H), 5.84 (br d, *J* 7, 5-H), 5.24 (ddd, *J* 6, 3 and 3, 2-H), 4.67 (br s, 14-H_2), 3.08 (br d, *J* 22, 3-H^a), 2.92 (dd, *J* 22 and 4, 3-H^b), 2.43 (br m, 10-H), 2.35 (br d, *J* 16, 6-H^a), 2.14 (dd, *J* 16 and 7, 6-H^b), 1.84 (sept d, *J* 7 and 6, 11-H), 1.06 (s, 15-H_3), 0.93 (d, *J* 7, 12-H_3) and 0.78 (d, *J* 7, 13-H_3); δ_C(68 MHz; $CDCl_3$; DEPT and CH-COSY) 166.4 (*C*OPh), 153.0 (C-1), 132.8 (CH-4′), 132.5 (C-4), 130.4 (C-1′), 129.5 (CH-2′ and -6′), 128.4 (CH-3′ and -5′), 127.9 (CH-5), 115.5 (CH-2), 71.5 (CH_2-14), 52.5 (CH-10), 45.4 (C-7), 41.4 (CH_2-8), 40.5 (CH_2-6), 30.9 (CH_2-3), 29.1 (CH-11), 23.7 (Me-15), 23.4 (CH_2-9), 21.7 (Me-12) and 17.0 (Me-13).

Autoxidation of Compound **1d**.—Compound **1d** (26.7 mg) as a thin film was kept at 60 °C for 2 h. From the reaction mixture, compounds **8d** (10.6 mg, 33%), **3d** (1.9 mg, 6%), **5d** (1.6 mg, 5%) and unchanged **1d** (14.8 mg, 55% recovery) were obtained by TLC. Some trace compounds were also detected.

14-*Benzoyloxy*-5-*hydroperoxycarota*-1,4-*diene* **3d**. A syrup; R_f 0.38 [hexane–EtOAc (4:1), *cf.* **1d**, 0.78]; *N,N*-dimethyl-*p*-phenylenediamine sulfate test: positive (pink); vanillin–sulfuric acid test: brownish purple; FIMS *m/z* 356 (M^+, 56%), 338 ($M^+ - H_2O$, 37), 237 (30), 236 (100), 234 (46), 220 (45) and 122 (56); FI-HRMS (Found: M^+, 356.1987. $C_{22}H_{28}O_4$ requires M, 356.1988); λ_{max}(MeOH)/nm 263, 267 and 394; δ_H(270 MHz; $CDCl_3$; HH-COSY) 8.66 (s, 5-OOH), 8.06 (br d, *J* 7, 2′-and 6′-H), 7.58 (br dd, *J* 7 and 7, 4′-H), 7.45 (br dd, *J* 7 and 7, 3′-and 5′-H), 6.20 (br d, *J* 8, 3-H), 5.10 (dd, *J* 8 and 2, 2-H), 5.05 (d, *J* 12, 14-H^a), 4.95 (d, *J* 12, 14-H^b), 4.70 (br dd, *J* 12 and 6, 5-H), 2.62 (br m, 10-H), 2.33 (dd, *J* 13 and 12, 6-H^a), 2.14 (dd, *J* 13 and 6, 6-H^b), 1.96 (sept d, *J* 7 and 5, 11-H), 0.99 (s, 15-H_3), 0.96 (d, *J* 7, 12-H_3) and 0.76 (d, *J* 7, 13-H_3); δ_C(68 MHz; $CDCl_3$; DEPT) 166.8 (*C*OPh), 162.6 (C-1), 133.2 (C-4′), 132.5 (C-4), 130.1 (C-1′), 129.6 (CH-2′ and -6′), 128.5 (CH-3′ and -5′), 128.4 (CH-3), 113.5 (CH-2), 82.2 (CH-5), 68.1 (CH_2-14), 51.9 (CH-10), 42.6 (C-7), 40.9 (CH_2-6), 39.6 (CH_2-8), 29.2 (CH-11), 22.4 (CH_2-9), 21.6 (Me-15), 20.0 (Me-12) and 16.4 (Me-13).

14-*Benzoyloxy*-1,5-*epidioxy*-2-*hydroperoxycarot*-3-*ene* **5d**. A solid; R_f 0.17 [hexane–EtOAc (4:1)]; vanillin–sulfuric acid test: purplish brown; *N,N*-dimethyl-*p*-phenylenediamine sulfate test: positive (pink); FDMS *m/z* 389 ($M^+ + 1$, 30%), 388 (M^+, 16), 372 (43), 371 ($M^+ - OH$, 100), 355 ($M^+ - OOH$, 40) and 105 (42); δ_H(270 MHz; $CDCl_3$; HH-COSY) 8.76 (s, 2-OOH), 8.04 (br d, *J* 7, 2′- and 6′-H), 7.60 (br dd, *J* 7, 4′-H), 7.47 (br dd, *J* 7 and 7, 3′- and 5′-H), 6.23 (br d, *J* 7, 3-H), 4.89 (br s, 14-H_2), 4.80 (d, *J* 6, 2-H), 4.70 (dd, *J* 5 and 2, 5-H), 2.56 (sept d, *J* 7 and 2, 11-H), 2.24 (dd, *J* 14 and 5, 6-H^a), 2.05 (m, 10-H), 1.93 (dd, *J* 14 and 2, 6-H^b), 0.98 (d, *J* 7, 12-H_3), 0.97 (s, 15-H_3) and 0.92 (d, *J* 7, 13-H_3); δ_C(68 MHz; $CDCl_3$; DEPT) 166.0 (*C*OPh), 144.1 (C-4), 133.4 (C-4′), 130.0 (C-1′), 129.7 (CH-2′ and -6′), 128.6 (CH-3′ and -5′), 126.3 (CH-3), 95.9 (C-1), 81.6 (CH-2), 74.7 (CH-5), 66.1 (CH_2-14), 54.4 (CH-10), 41.4 (CH_2-6), 40.4 (C-7), 39.9 (CH_2-8), 26.1 (CH-11), 24.8 (Me-15), 23.3 (Me-12), 20.1 (CH_2-9) and 17.9 (Me-13).

14-*Benzoyloxy*-1,5-*epidioxy*-4-*hydroperoxycarot*-2-*ene* **8d**.

Amorphous solid from hexane, m.p. 150–150.5 °C; R_f 0.21 [hexane–EtOAc (4:1)]; vanillin–sulfuric acid test: greyish purple; *N,N*-dimethyl-*p*-phenylenediamine sulfate test: positive (pink); FDMS *m/z* 389 ($M^+ + 1$, 85%), 388 (M, 100), 372 (71), 371 ($M^+ - OH$, 95), 355 ($M^+ - OOH$, 42) and 235 (23); δ_H(270 MHz; $CDCl_3$) 9.33 (s, 4-OOH), 8.06 (br d, *J* 7, 2′-6′-H), 7.61 (br dd, *J* 7 and 7, 4′-H), 7.47 (br dd, *J* 7 and 7, 3′- and 5′-H), 6.15 (d, *J* 12, 2-H), 6.09 (dd, *J* 12 and 3, 3-H), 4.81 (d, *J* 13, 14-H^a), 4.56 (ddd, *J* 6, 3 and 2, 5-H), 4.55 (d, *J* 13, 14-H^b), 2.38 (dd, *J* 15 and 6, 6-H^a), 2.02 (dd, *J* 15 and 2, 6-H^b), 1.41 (m, 9-H^b), 0.98 (s, 15-H_3), 0.98 (d, *J* 6, 12-H_3) and 0.89 (d, *J* 6, 13-H_3); δ_C(68 MHz; $CDCl_3$; DEPT and CH-COSY) 167.4 (*C*OPh), 133.7 (CH-4′), 132.0 (CH-2), 129.9 (CH-2′ and -6′), 129.1 (C-1′), 128.6 (CH-3′ and -5′), 127.4 (CH-3), 90.7 (C-1), 90.5 (C-4), 76.5 (CH-5), 62.4 (CH_2-14), 54.1 (CH-10), 43.3 (C-7), 39.8 (CH_2-8), 35.7 (CH_2-6), 28.7 (CH-11), 24.8 (Me-15), 24.0 (CH_2-9), 23.3 (Me-12) and 19.1 (Me-13). When the C-15 methyl signal was irradiated, a clear NOE was observed on 14-H^b (2%). This suggested that compound **8d** possessed the 1*S*,4*S*,5*S*,7*R*,10*R* absolute configuration.

Autoxidation of Carota-1,4-*diene* **1e**.—Compound **1e** was prepared from carotol acetate as described in one of our previous papers.[8] A thin film of diene **1e** (35.6 mg) was kept at 60 °C for 2 h, and the reaction mixture thus obtained was then subjected to PLC [hexane–EtOAc (4:1)]. Four products, **3e** (0.8 mg, 2%), **8e** (14.4 mg, 31%), **10e** (2.5 mg, 5%) and **11e** (2.4 mg, 5%), were obtained. No signals assignable to the protons of compounds **6e** or **5e** were detected, although minor products in the autoxidation mixture were carefully investigated by ^1H NMR spectroscopy.

(5S,7R,10R)-5-*Hydroperoxycarota*-1,3-*diene* **3e**. A syrup; R_f 0.47 [hexane–EtOAc (4:1)]; vanillin–sulfuric acid test: greyish brown; *N,N*-dimethyl-*p*-phenylenediamine sulfate test: positive (pink); λ_{max}(MeOH)/nm 266; FIMS *m/z* 237 ($M^+ + 1$, 36%), 236 (M^+, 100), 220 (34), 219 (37) and 218 (92); FI-HRMS (Found: M^+, 236.1780. $C_{15}H_{24}O_2$ requires M, 236.1777); δ_H(270 MHz; $CDCl_3$) 7.44 (s, 5-OOH), 5.82 (br d, *J* 8, 3-H), 5.40 (dd, *J* 8 and 2, 2-H), 4.49 (br dd, *J* 12 and 6, 5-H), 2.59 (br m, 10-H), 2.17 (dd, *J* 13 and 12, 6-H^a), 2.09 (dd, *J* 13 and 6, 6-H^b), 1.91 (br s, 14-H_3), 0.96 (s, 15-H_3), 0.95 (d, *J* 7, 12-H_3) and 0.76 (d, *J* 7, 13-H_3).

(1S,4R,5S,7R,10R)-1,5-*Epidioxy*-4-*hydroperoxycarot*-2-*ene* **8e**. A syrup; R_f 0.21 [hexane–EtOAc (4:1), *cf.* **1e**, R_f 0.97]; vanillin–sulfuric acid test: purplish brown; *N,N*-dimethyl-*p*-phenylenediamine sulfate test: positive (pink); FIMS *m/z* 269 ($M^+ + 1$, 31%), 268 (M^+, 100), 252 (87), 250 ($M^+ - H_2O$, 29), 236 (39) and 234 (33); FI-HRMS (Found: M^+, 268.1690. $C_{15}H_{24}O_4$ requires M, 268.1674); δ_H(270 MHz; $CDCl_3$; HH-COSY) 8.62 (br s, 4-OOH), 6.02 (d, *J* 12, 2-H), 5.86 (dd, *J* 12 and 2, 3-H), 4.44 (ddd, *J* 6, 2 and 2, 5-H), 2.22 (dd, *J* 15 and 6, 6-H^a), 1.90 (dd, *J* 15 and 2, 6-H^b), 1.34 (s, 14-H_3), 0.97 (d, *J* 6, 12-H_3), 0.89 (s, 15-H_3) and 0.88 (d, *J* 6, 13-H_3); δ_C(68 MHz; $CDCl_3$; DEPT and CH-COSY) 133.0 (CH-2), 129.8 (CH-3), 91.1 (C-1), 89.1 (C-4), 80.2 (CH-5), 54.5 (CH-10), 43.2 (C-7), 39.7 (CH_2-8), 35.7 (CH_2-6), 28.9 (CH-11), 25.1 (Me-15), 24.4 (CH_2-9), 23.4 (Me-12), 20.0 (Me-14) and 19.4 (Me-13). When 14-H_3 was irradiated, NOEs on 15-H_3 (4.5%), 6-H^b (3%), 5-H (2%) and 3-H (2.5%) were observed, while irradiation of 15-H_3 showed NOEs on 6-H^b (2%), 2-H (0.5%) and 14-H_3 (4%). Thus, the stereostructure of compound **8e** as (1*S*,4*R*,5*S*,7*R*,10*R*) was confirmed.

Compound **8e** (14.4 mg) was treated with a solution of triphenylphosphine (10 mg) in 50% EtOAc–$CHCl_3$ (3 cm^3) for 3 h,[12] and 1,5-epidioxy-4-hydroxycarot-2-ene **11e** (8.0 mg, 59%) was obtained. (1*S*,4*R*,5*S*,7*R*,10*R*)-1,5-Epidioxy-4-hydroxycarot-2-ene **11e** was a syrup, R_f 0.26 [hexane–EtOAc (4:1)]; vanillin–sulfuric acid test: brown; *N,N*-dimethyl-*p*-phenylenediamine sulfate test: positive (pink); δ_H(270 MHz; $CDCl_3$; HH-COSY) 5.83 (dd, *J* 11 and 2, 3-H), 5.74 (d, *J* 11, 2-H), 4.03 (ddd, *J* 6, 2 and 2, 5-H), 2.95 (br s, 4-OH), 2.19 (dd, *J* 15 and 6, 6-H^a), 1.94 (dd, *J* 15 and 2, 6-H^b), 1.28 (s, 14-H_3), 0.97 (d, *J* 6, 12-H_3), 0.88 (d, *J* 6, 13-H_3) and 0.85 (s, 15-H_3); δ_C(68/125 MHz; $CDCl_3$; DEPT, CH-COSY and HMBC) 137.8 (CH-3), 125.2 (CH-2), 91.1 (C-1), 82.5 (CH-5), 54.5 (CH-10), 42.7 (C-7), 39.7 (CH_2-8), 35.2 (CH_2-6), 28.9 (CH-11), 25.3 (Me-15), 24.6 (CH_2-9), 23.4 (Me-12), 22.2 (Me-14) and 19.5 (Me-13). The C-4 carbon signal overlapped with solvent peaks; however, this signal was visible in C_6D_6 at δ_C 77.3 (C). Compound **11e** isolated from the autoxidation mixture of compound **1e** agreed in its spectroscopic properties with those of the reduction derivative from compound **8e**.

(1S,4S,5S,7R,10R)-1,5-*Epidioxy*-4-*hydroperoxycarot*-2-*ene* **10e**. A syrup; R_f 0.16 [hexane–EtOAc (4:1)]; *N,N*-dimethyl-*p*-phenylenediamine sulfate test: positive (pink); vanillin–sulfuric acid test: bluish purple; FDMS *m/z* 268 (M^+, 72%), 252 (83), 250 (100), 236 (99), 234 (87) and 218 (63); δ_H(270 MHz; $CDCl_3$) 7.41 (s, 4-OOH), 5.94 (d, *J* 11, 2-H), 5.69 (dd, *J* 11 and 2, 3-H), 4.32 (ddd, *J* 6, 2 and 2, 5-H), 2.28 (dd, *J* 14 and 2, 6-H^a), 2.07 (dd, *J* 14 and 6, 6-H^b), 1.56 (s, 14-H_3), 0.96 (d, *J* 6, 12-H_3), 0.95 (s, 15-H_3) and 0.88 (d, *J* 6, 13-H_3).

Conversion of 5-*Hydroperoxycarota*-1,3-*diene Derivatives* **3** *into* 1,5-*Epidioxy Derivatives*.—Compound **3d** (2.3 mg) kept in deuteriochloroform at 4 °C for a week spontaneously converted into compound **8d** (~40% from intensity of proton peaks of 15- and 13-methyl groups) and compound **5d** (~10% from C-14 methylene peaks with 2′- and 6′-H aromatic protons as standard peak). About 50% of the starting material remained unchanged. Air exposure of compound **3d** on dried silica gel TLC plates showed its more drastic oxidative decomposition (5–60 min), and the major oxidative product was **5d**.

Compound **3e** also showed a self-conversion into 1,5-epidioxy derivatives. Compound **3e** was more labile than **3d** and it disappeared completely in deuteriochloroform within 2 days. More than 90% of the self-conversion product was compound **8e**, and its epimer **10e** was also detected as a minor product (~5%). Any signals assignable to 2-hydroperoxy-1,5-epidioxide **5e** were not observed.

β-*Cleavage of* 1,5-*Epidioxy*-4-*hydroperoxycarot*-2-*ene Derivatives* **8**.—Compound **8b** (0.9 mg) was redissolved in EtOAc to make a thin film on a glass wall, and this was heated at 60 °C for 1 h. Most of the substrate **8b** remained unchanged but 18% of the 4-hydroperoxide cleaved to yield enone **9** (from intensity of their C-15 methyl peaks). By thermal degradation at 60 °C for 3 h in deuteriochloroform (~1 mg/0.4 cm^3), about half of substrate **8b** was converted into enone **9**. On the other hand, compound **8d** was thoroughly unchanged under the degradation conditions. When compound **8d** (25.1 mg) was dissolved in *p*-TsOH–dioxane (2 mg, 2 cm^3) and kept at 65 °C overnight, however, a small amount of a single product, identified as enone **9** (0.8 mg), was obtained together with benzoic acid (0.8 mg); most of the starting material **8d** remained unchanged (11.0 mg).

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