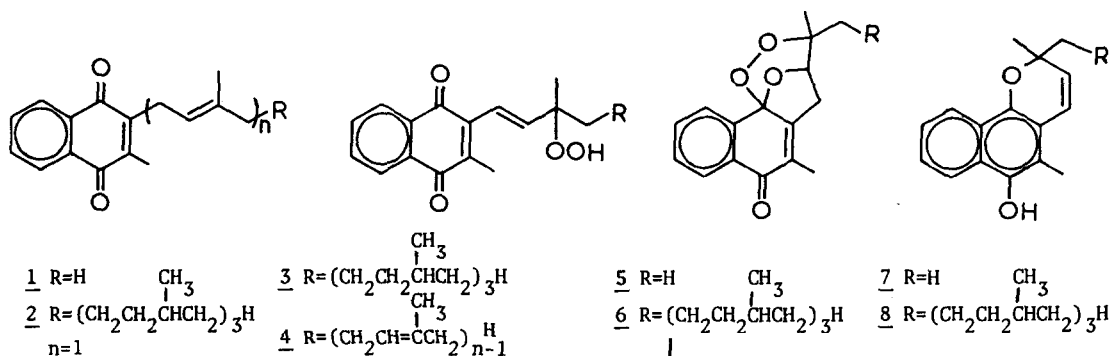# THE MECHANISM OF PHOTOOXIDATION OF THE MENAQUINONES

David Creed*, Department of Chemistry, University of Southern Mississippi, Hattiesburg,
Mississippi 39401. Harold Werbin and Tom Daniel, Molecular Biology Programs, University of
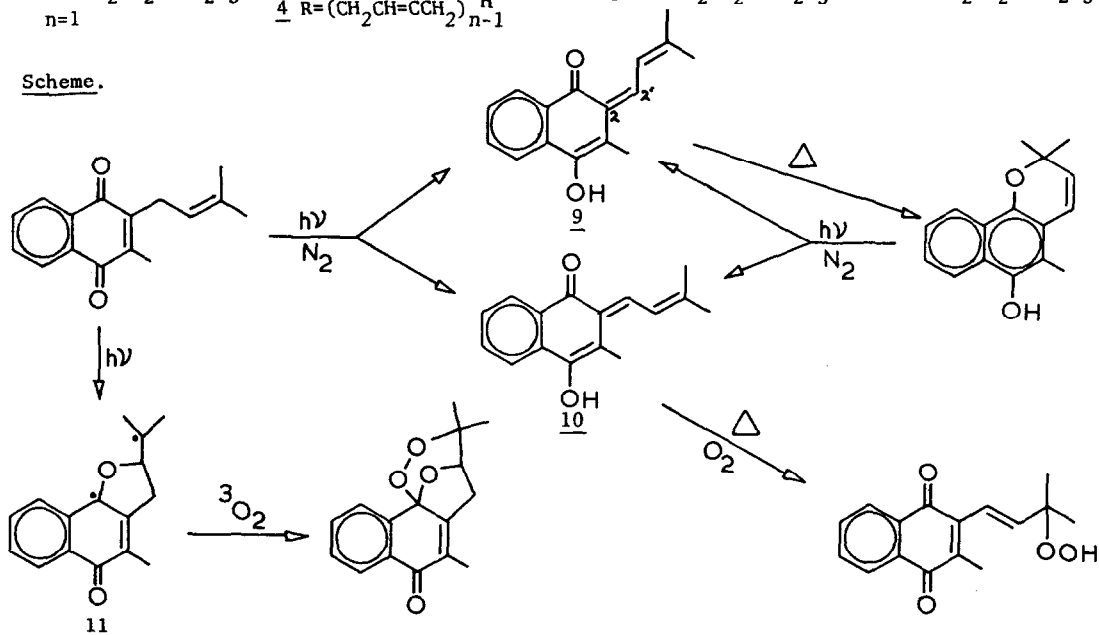Texas at Dallas, Richardson, Texas 75080.

Irradiation of menaquinone-1 under nitrogen gives rise to metastable isomeric o-quinone
methides, one of which reacts with oxygen in the dark to form the same hydroperoxide obtained
previously from direct or dye sensitized photolysis of the quinone in the presence of oxygen.

Isoprenoid electron-transport quinones are of considerable photobiological interest[1] as
potential cellular targets for near-uv radiation. A recent report[2] indicates a high quantum
efficiency (0.26) for photolysis of menaquinone-8, 1 (n = 8), in E. Coli B/r although photo-
products were not isolated. Ohmae and Katsui[3] first reported that irradiation of Vitamin K_1,
2, in benzene in the presence of O_2 gave the hydroperoxide 3. Snyder and Rapoport[4] later
showed that 3 and 4 (n = 1) could be obtained by both direct and dye sensitized photooxidation
of 2 and 1 (n = 1) respectively. Paradoxically however they also reported that menaquinone-9,
1 (n = 9), a quinone with nine unsaturated isoprene units in its side chain, gave the hydro-
peroxide 4 (n = 9) from preferential oxidation of the first isoprene unit upon direct irradia-
tion in the presence of O_2. No products were isolated from dye sensitized photooxidation of
1 (n = 9) presumably because of random oxidation of other isoprene units in the side chain by
singlet O_2. Snyder and Rapoport[4] concluded that direct photooxidation of menaquinones and
Vitamin K_1 occurred via a quinone-oxygen complex whose structure ensured preferential reaction
at the isoprene unit closest to the quinone ring. Wilson et al.[5], have recently isolated
stable trioxanes 5 and 6 from irradiation of 1 (n = 1) and 2 under high pressures of oxygen at
-30°C in $CFCl_3$. Not unreasonably they suggested that biradical precursors of 5 and 6 are also
precursors to the hydroperoxides 3 and 4. We now present evidence for an alternate mechanism
involving reaction of O_2 in the dark with metastable ground state intermediates formed upon
irradiation of the quinone under N_2.

Irradiation (365 nm) of menaquinone-1, 1 (n = 1), or Vitamin K_1, 2, under N_2 in 95%
ethanol at ambient temperature results in a dramatic color change from yellow to orange. Upon
standing for two hours under N_2 until the orange color discharged and then work-up (dim red
light, solvent evaporation at <40°C, preparative tlc on silica gel) the known[6] chromenols 7
or 8 were isolated. However if immediately after the light source is turned off O_2 is bubbled

1 R=H $\quad$ CH$_3$
2 R=(CH$_2$CH$_2$CHCH$_2$)$_3$H
$\quad$ n=1

3 R=(CH$_2$CH$_2$CHCH$_2$)$_3$H
4 R=(CH$_2$CH=CCH$_2$)$_{n-1}$H

5 R=H $\quad$ CH$_3$
6 R=(CH$_2$CH$_2$CHCH$_2$)$_3$H

7 R=H $\quad$ CH$_3$
8 R=(CH$_2$CH$_2$CHCH$_2$)$_3$H

Scheme.

through the irradiated mixture in the dark for 15-30 mins. and then the reaction mixture is worked up the hydroperoxides **4** (n = 1) and **3** can be isolated[4] together with reduced yields of chromenols **7** and **8**. Bubbling O$_2$ in the dark through **1** (n = 1), **2**, **7** or **8** does not lead to hydroperoxide formation. However if the chromenol **7** is irradiated under N$_2$ an orange color is observed. Work-up of N$_2$ bubbled solutions gives only **7** and traces of several uncharacterized photoproducts. No significant amount of **1** (n = 1) could be observed in this experiment. However O$_2$ bubbling in the dark following irradiation under N$_2$ of **7** followed by work-up gives hydroperoxide **4** (n = 1). There is no doubt that an intermediate formed by irradiation under N$_2$ of either menaquinones or their chromenol photoproducts reacts with O$_2$ in the dark to give hydroperoxides such as **3** and **4** (n = 1).
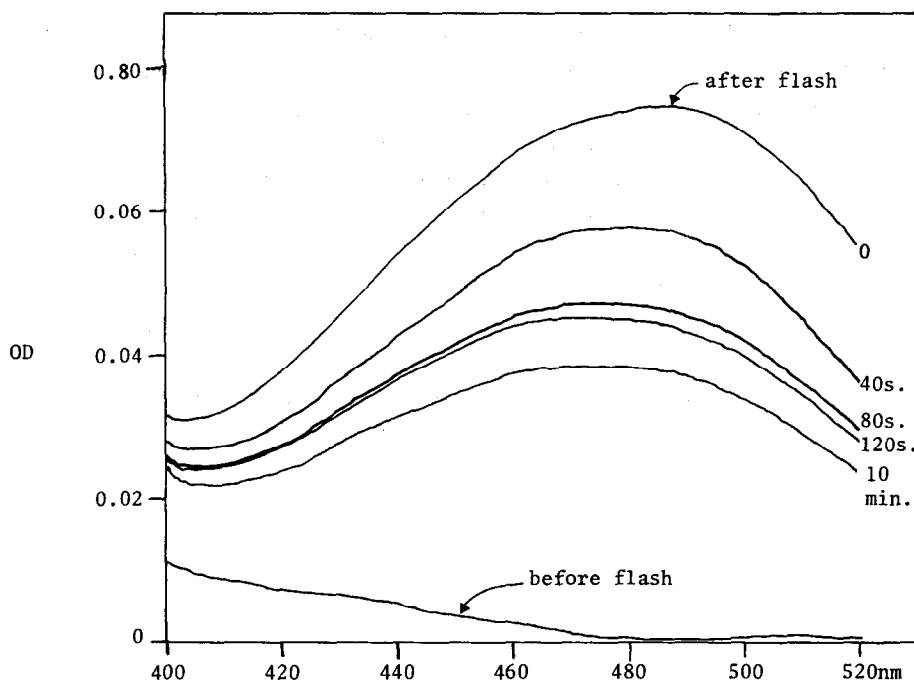
Figure. Absorption spectra of transients generated by flash photolysis of **1** (n = 1) in 95% ethanol. (times after the flash are indicated).

In 1970 Leary and Porter[7] reported that flash photolysis of Vitamin K_1, **2**, in ethanol afforded a long-lived ($\tau_{1/2} \simeq 30$ min.) species with $\lambda_{max} = 475$ nm and complex decay kinetics. They suggested this species is an o-quinone methide precursor of chromenol **8**. Similar species were later observed upon flash photolysis of plastoquinones[8] and tocoquinone-1[9]. We find that flash photolysis of 2×10^{-4}M **1** (n = 1) in ethanol under N_2 (Figure) indeed produces an orange intermediate but its λ_{max} is time dependent and its decay is distinctly biphasic indicating the formation of two transient species. Immediately after the flash absorption with $\lambda_{max} = 485 \pm 5$ nm is observed. A reduction in absorption intensity occurs in $\simeq 1$ min. together with a shift to $\lambda_{max} = 475 \pm 5$ nm. The initial fast decay of the 485 nm species is first order with $\tau = 15 \pm 2$ sec. under either N_2, air, or O_2. Decay of the 475 nm species is much slower, O_2 dependent, and approximately first order with $\tau = 156$ min. (N_2), 33 min. (air), and 21 min. (O_2). Flash photolysis of 2×10^{-4}M chromenol **7** under the same conditions results in similar spectral shifts and biphasic decay with $\tau = 17 \pm 3$ sec. and $\simeq 7$ min. The fast decaying transient from **7** with $\lambda_{max} = 485$ nm is presumably the same as that obtained from **1** (n = 1). The slower decay with $\lambda_{max} = 475$ nm initially appeared to be different but its lifetime is very dependent on [**7**]. For 10^{-3}M, 4.0×10^{-4}M, 2.0×10^{-4}M, and 4.0×10^{-5}M **7**, τ values for the slow decay are 0.8, 2.2, 7.0 and 22 min. respectively. Extrapolated to [**7**] = 0, τ is identical within experimental error to τ for the species from **1** (n = 1).

We suggest (Scheme) that the faster decaying species is $\underline{9}$ and the slower decaying species is $\underline{10}$, geometrical isomers about the 2,2' formal double bond, of the o-quinone methide observed by Leary and Porter[7]. The fast O_2 independent decay of $\underline{9}$ is attributed to bond rotation about the 2',3' formal single bond followed by ring closure to the chromenol $\underline{7}$. The slow O_2 dependent decay of $\underline{10}$ affords hydroperoxide, $\underline{4}$ (n = 1). We do not know whether the latter process is a direct addition of ground state O_2 to $\underline{10}$ or perhaps more likely involves a free radical chain mechanism. We note that 2×10^{-4}M 4-methoxyphenol increases τ for $\underline{10}$ in air saturated ethanol to the N_2 saturated value supporting the latter suggestion. Formation of trioxanes such as $\underline{5}$ and $\underline{6}$, an analogous trioxane[1] from plastoquinone-1, and the related inter-molecular quinone-olefin-O_2 adducts[10] probably involves O_2 interception of triplet biradicals such as $\underline{11}$. Whether there is any involvement of singlet oxygen in the direct photooxidation of menaquinones remains to be established. It is clear however that menaquinone hydroperoxides can be generated without the simultaneous participation of light and molecular oxygen.

1. D. Creed, H. Werbin, and E. T. Strom, J. Am. Chem. Soc., 93, 502 (1971).

2. J. J. Madden, D. T. Boatwright, and J. Jagger, Photochem. Photobiol., in press.

3. M. Ohmae and G. Katsui, Vitamins (Japan), 35, 116 (1967).

4. C. D. Snyder and H. Rapoport, J. Am. Chem. Soc., 91, 731 (1969)

5. R. M. Wilson, T. F. Walsh, and S. K. Gee, Tet. Lett., 21, 3459 (1980).

6. S. Fujisawa, S. Kawabata, and R. Yamamoto, Yakugaku Zasshi, 87, 1451 (1967).

7. G. Leary and G. Porter, J. Chem. Soc., (A), 2273 (1970).

8. D. Creed, B. J. Hales, and G. Porter, Proc. Roy. Soc., (A), 334, 505 (1973).

9. G. Leary, J. Chem. Soc., (A), 2248 (1971).

10. R. M. Wilson, S. W. Wunderly, J. G. Kalmbacher, and W. Brabender, Ann. N.Y. Acad. Sci., 267, 201 (1976) and references therein.