30°) with ethyl linoleate and [1,2-^{3}H]cholesterol in 50 mM Tris–HCl buffer (pH 6.6 and 9.0) and of horseradish peroxidase (at 37°) with [4-^{14}C]cholesterol and H_2O_2 (or oxygenated buffer) in 0.1 M sodium acetate buffer (pH 5.5), analyzed by thin-layer and gas chromatographic methods developed for the purpose,[18] established that the 7-hydroperoxides III and IV were obtained as initial and chief products (III:IV ratios of 1:3 to 2:3 in 0.4–2.0% yields with lipoxygenase, 1:2 to 1:1 in 0.07–0.3% yields with peroxidase). No 5α-hydroperoxide I was detected at short times (15 min), at which time both III and IV were present, but very low levels of I were detected after about 1 hr.

Incubations without lipoxygenase, molecular oxygen, or ethyl linoleate or with heat-inactivated lipoxygenase gave essentially no detectable sterol hydroperoxides. Incubation of preformed ethyl linoleate hydroperoxides with lipoxygenase and cholesterol gave diminished amounts of sterol hydroperoxides III and IV. Incubations without peroxidase or with heat-inactivated peroxidase gave no detectable sterol hydroperoxides. Formation of sterol hydroperoxides by lipoxygenase and by peroxidase was inhibited by 1 mM propyl gallate. Peroxidase action on cholesterol was inhibited by 2 μM catalase.

The product hydroperoxides I, III, and IV were relatively stable during the enzyme incubation, but low levels of the thermal decomposition products 3β-hydroxycholest-5-en-7-one and the epimeric cholest-5-ene-3β,7-diols[16,18b] were formed slowly.[19] Incubations of the three hydroperoxides I, III, and IV with lipoxygenase and boiled lipoxygenase and with peroxidase and boiled peroxidase suggested that the sterol hydroperoxides were stable to enzymic alterations but that nonenzymic isomerization of I to III, epimerization of III, and accumulation of thermal decomposition products of I, III, and IV occurred. These nonenzymic transformations in aqueous protein dispersions thus mimicked in detail those previously demonstrated for I, III, and IV in organic solvent systems.[16,18b] However, nonenzymic isomerizations of I to III could not account for the presence of III and IV as chief early products of the action of either lipoxygenase or peroxidase on cholesterol, for the 5α-hydroperoxide I yielded III in I:III ratios of 1:1 (pH 6.6) to 1:4 (pH 9.0) with lipoxygenase and yielded III in I:III ratio of 1:6 and IV in I:IV ratio of 1:1 with peroxidase. Accordingly, the 5α-hydroperoxide I would not have escaped detection were it formed as an initial product of lipoxygenase or peroxidase action on cholesterol.

These results rule out initial formation of I and its rapid and complete isomerization to III and subsequent epimerization of III as a likely mechanism of action of soybean lipoxygenase or of horseradish peroxidase in the formation of the prominent enzymic products III and IV. Rather, our results establish that lipoxygenase and peroxidase action on cholesterol give the epimeric 7-hydroperoxides III and IV as initial and chief products in exactly the same manner as previously demonstrated in radiation-induced (radical) autoxidations of cholesterol.[1] We interpret formation by either enzyme of the epimeric 7-hydroperoxides III and IV and failure to form the 5α-hydroperoxide I as initial product as excluding participation of singlet molecular oxygen[20] and the cyclic ene mechanism from these reactions. Were singlet molecular oxygen involved it must act by enzymic processes which do not have the same stereoelectronic requirements as the cyclic ene mechanism and which afford sterol hydroperoxide products nominally expected of radical processes. Our results more reasonably support activation of substrate, possibly by generation of radical species as previously suggested.[7-9]

Extension to other enzyme systems of the use of a naturally occurring substrate such as cholesterol (in distinction to use of xenobiotic substrates[3a,4,20]) as a probe to test participation of singlet molecular oxygen may find favor.

(18) (a) L. L. Smith and F. L. Hill, *J. Chromatogr.*, **66**, 101 (1972); (b) J. I. Teng, M. J. Kulig, and L. L. Smith, *ibid.*, **75**, 108 (1973).

(19) The 7-ketone and 3β,7-diols were previously recognized as products of soybean lipoxygenase action on cholesterol; *cf.* G. Johansson, *Eur. J. Biochem.*, **21**, 68 (1971).

(20) The participation of singlet molecular oxygen in hepatic microsomal mixed function oxidase hydroxylation of xenobiotic aromatic substrates has recently been discounted; *cf.* L. A. Sternson and R. A. Wiley, *Chem.-Biol. Interactions*, **5**, 317 (1972).

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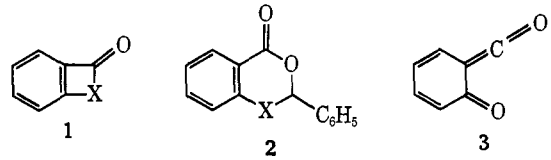
Benzpropiolactone[1]

Sir:

Benzpropiolactone (**1a**) has been of interest for some time as a possible intermediate (or by-product) in the decomposition of benzenediazonium-2-carboxylate to benzyne.[2,3] Thiobenzpropiolactone has been generated at 77°K by photochemical elimination of benzaldehyde from **2b**.[4] Attempts to generate **1a** by ir-



1 2 3

a, X = O
b, X = S

radiation of **2a** gave instead the ketoketene (**3**).[4] Dvořák, Kolc, and Michl have recently observed the ultraviolet spectrum of the same ketoketene at 77°K in the irradiation of phthaloyl peroxide.[5] Horner,[6] Wittig,[7] and Jones[8] have previously shown that phthaloyl peroxide (Figure 1) can serve as a photochemical precursor for benzyne at room temperature, and DeCamp[9] has shown by trapping experiments that loss of carbon dioxide can occur in stepwise fashion. We wish to record the observation of benzpropiolactone.

(1) Photochemical Transformations. XLIX.
(2) R. W. Hoffmann, "Dehydrobenzene and Cycloalkynes," Academic Press, New York, N. Y., 1967, pp 76, 237, and references cited.
(3) R. R. Schmidt and W. Schneider, *Tetrahedron Lett.*, 5095 (1970).
(4) O. L. Chapman and C. L. McIntosh, *J. Amer. Chem. Soc.*, **92**, 7005 (1970).
(5) V. Dvořák, J. Kolc, and J. Michl, *Tetrahedron Lett.*, 3443 (1972).
(6) L. Horner and H. Brüggemann, *Justus Liebigs Ann. Chem.*, **635**, 27 (1960).
(7) G. Wittig and H. F. Ebel, *ibid.*, **650**, 20 (1961).
(8) M. Jones, Jr., and M. R. DeCamp, *J. Org. Chem.*, **36**, 1536 (1971).
(9) M. R. DeCamp, Ph.D. Thesis, Princeton University, 1972.

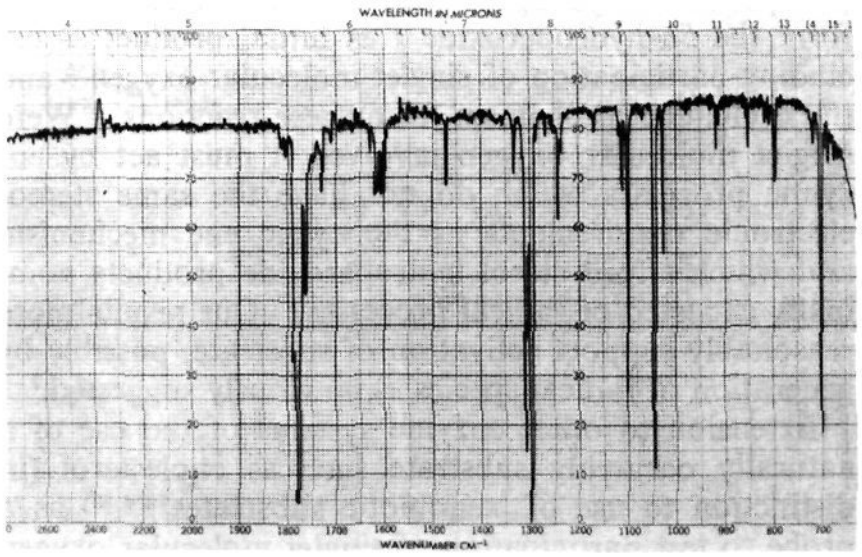


Figure 1. Phthaloyl peroxide matrix isolated in argon at 8°K before irradiation.

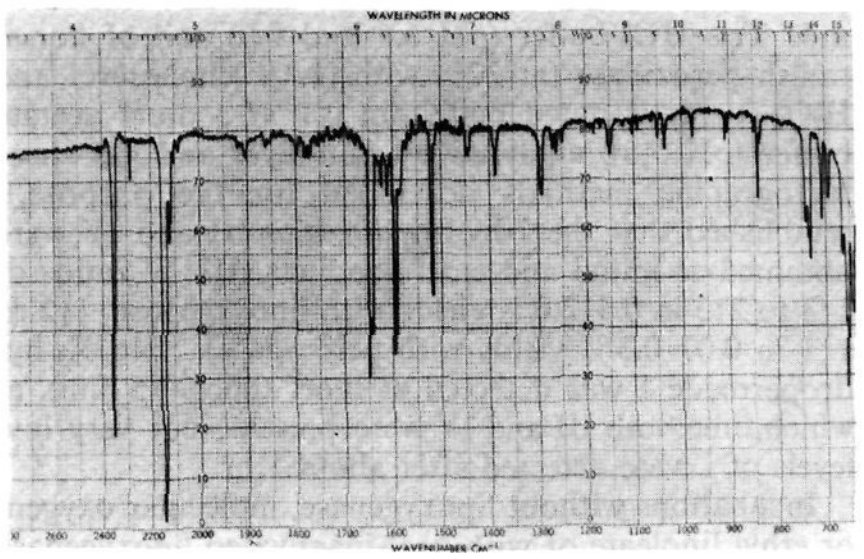


Figure 3. Phthaloyl peroxide matrix isolated in argon at 8°K after irradiation with light of wavelength >3150 A.

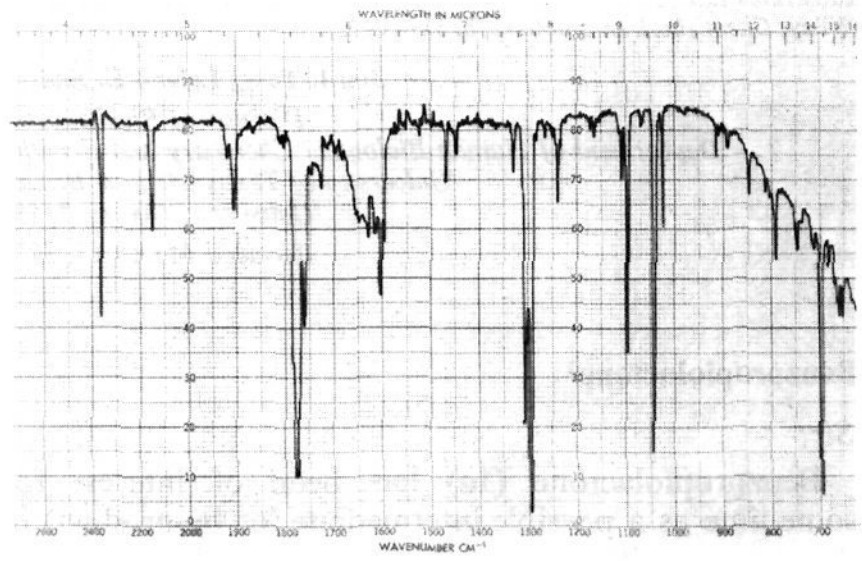
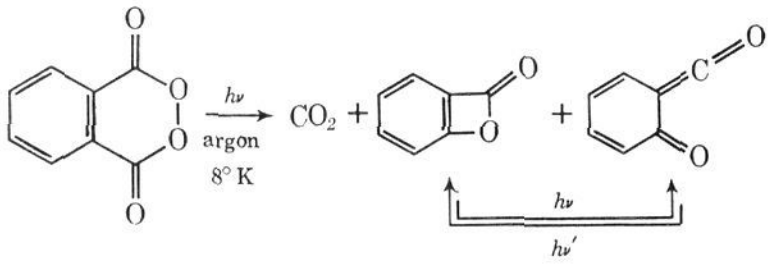


Figure 2. Phthaloyl peroxide matrix isolated in argon at 8°K after irradiation with light of wavelength >3400 Å.

Irradiation (>3400 Å) of phthaloyl peroxide matrix isolated in argon at 8°K gives rise to bands (Figure 2) characteristic of carbon dioxide (2340, 665 cm^{-1}), the ketoketene (2139, 1650 cm^{-1}), and a new species with carbonyl absorption at 1904 cm^{-1}. All three products



are primary products within the limits of our detection. The ratio of the ketoketene to the new species depends strongly on the wavelength of the light used in the irradiation. Long wavelength (>3400 Å) light favors the 1904-cm^{-1} species and shorter wavelength (>3150 Å) light favors the ketoketene. The wavelength dependence is a consequence of the photochemical interconversion of the ketoketene and the 1904-cm^{-1} species. Light of sufficiently short wavelength converts essentially all of the 1904-cm^{-1} species to the ketoketene. Conversely, irradiation of the ketoketene with long wavelength light regenerates the 1904-cm^{-1} species. Irradiation (>3150 Å) of phthaloyl peroxide in a matrix which contains methanol gives the ketoketene (Figure 3). Warming causes concurrent loss of the ketoketene bands and appearance of bands characteristic of methyl salicylate. This observation firmly identifies the ketoketene. The photochemical interconversion of the ketoketene and the 1904-cm^{-1} species leads us to conclude that the 1904-cm^{-1} species is the elusive benzpropiolactone (**1a**). Attempts to define the thermal chemistry of benzpropiolactone have been uniformly unsuccessful.

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Intramolecular Oxidative Coupling of Monophenolic Benzylisoquinolines. Quinonoid Oxoaporphines[1]

Sir:

Oxidative cyclization of benzyltetrahydroisoquinolines has long been considered as a biogenetic route to aporphine alkaloids.[2] In 1957, Barton and Cohen[3] suggested that phenolic oxidation of diphenolic benzyltetrahydroisoquinoline precursors may generate the bond between the aporphine rings A and D, and this proposal has been amply supported by the results of numerous subsequent biosynthetic and synthetic studies.[4,5] We wish to report herewith a second oxidative route, efficient intramolecular coupling of monophen-

(1) This investigation was supported by a grant from the National Cancer Institute (CA-12059).
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(4) A. R. Battersby in "Oxidative Coupling of Phenols," W. I. Taylor and A. R. Battersby, Ed., Marcel Dekker, New York, N. Y., 1967.
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