likely that it is this close approach which is responsible for the appreciable rise in energy found by Genson and Christoffersen in the region of the gauche forms. It appears then probable that a gain in stability could be obtained for the gauche form by twisting the cationic head ($N^+(CH_3)_3$) out of the standard conformation adopted for it in this (and in all other) calculation, which corresponds to $\tau_3 = 180°$. In fact, as shown in the last line of Table II, a rotation of 20° of the cationic head ($\tau_3 = 160°$) stabilizes the gauche form ($\tau_1 = 180°$, $\tau_2 = 60°$) sufficiently to make it more stable than the trans form.

It seems therefore that: (1) there may be some essential disagreement between the molecular fragment SCF procedure and the standard *ab initio* SCF procedure, particularly with regard to the importance of the nonbonded repulsive interaction, and (2) that a standard *ab initio* SCF procedure predicts the gauche conformation of acetylcholine as the most stable one.

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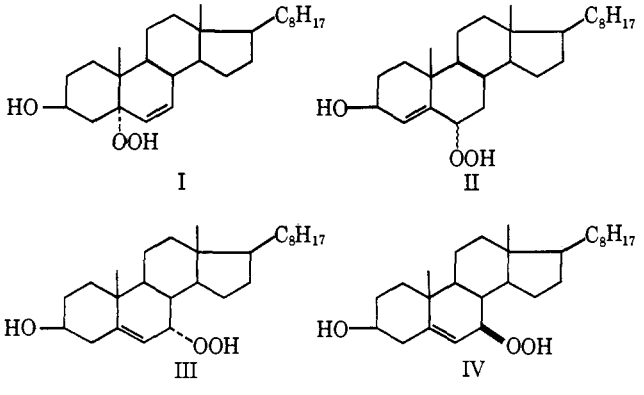
Sterol Metabolism. XXIV. On the Unlikely Participation of Singlet Molecular Oxygen in Several Enzyme Oxygenations[1]

Sir:

Interest in the possible participation of excited-state singlet molecular oxygen in enzyme reactions[2] includes suggestions that singlet molecular oxygen be involved in the action of the dioxygenases quercetinase from *Aspergillus flavus*,[3] soybean lipoxygenase,[4] and horseradish peroxidase.[5] Experimental work supporting the suggested utilization of singlet molecular oxygen rests on the principle of identity of products and similarity of products distribution between the suspect reaction and reactions (chiefly photosensitized oxygenations[6]) in which singlet molecular oxygen has been implicated. The suggestion is in contrast to previously expressed mechanism concepts in which radical processes have been posited for lipoxygenase[7] and for peroxidase.[8] Evidence for participation of free radicals in the action of lipoxygenase[9] and stereospecificity studies of hydrogen abstraction[10] appear not to support a singlet molecular oxygen mechanism for lipoxygenase action, and other reservations on the matter have been reported.[11] However, the recognized complexity of soybean lipoxygenase (isoenzymes,[12] hydroperoxide isomerases,[13] associated carotene oxidase[14]) potentially compromises prior work.

We sought to examine the mechanism of action of soybean lipoxygenase and horseradish peroxidase in regard to possible participation of singlet molecular oxygen using cholesterol as a substrate for which different products are obtained depending on whether excited-state singlet or ground-state triplet molecular oxygen is involved. Photosensitized oxidations of cholesterol in which singlet molecular oxygen is implicated yield 3β-hydroxy-5α-cholest-6-ene 5-hydroperoxide (I) as the major product, accompanied by small amounts of the epimeric 3β-hydroxycholest-4-ene 6-hydroperoxides (II) but with no detectable formation of cholesterol 7α-hydroperoxide (III) or cholesterol 7β-hydroperoxide (IV).[15] Furthermore, radical-induced autoxidations of cholesterol (interpreted as involving radical processes and ground-state molecular oxygen) provide the 7β-hydroperoxide IV as the major product, accompanied by small amounts of the 7α-hydroperoxide III but with no detectable Δ⁶-5α-hydroperoxide I.[1] Isomerization of I to III or epimerization of III to IV[16] did not occur, and an absolute differentiation by product nature between excited-state and ground-state molecular oxygen oxidations obtained.[17]



Incubations for 2 hr of soybean lipoxygenase (at

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30°) with ethyl linoleate and [1,2-^{3}H]cholesterol in 50 m*M* 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 m*M* 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.

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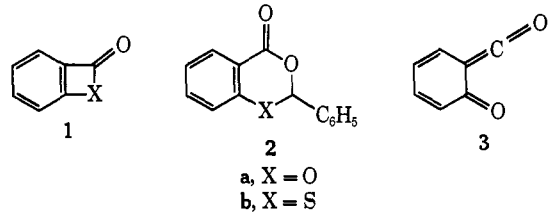
Benzpropiolactone[1]

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 irradiation 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 2 3

a, X = O
b, X = S

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