transformations and also as chiral substrate samples to investigate stereochemical specificity of such γ-carbon processing enzymes as cystathionine γ-synthetase[6] and threonine synthase.[7] Finally, we have determined for the first time the stereochemical outcome of catalytic action of homoserine dehydrogenase, a central enzyme in biosynthesis of several (e.g., methionine, threonine) of the common α-amino acids.

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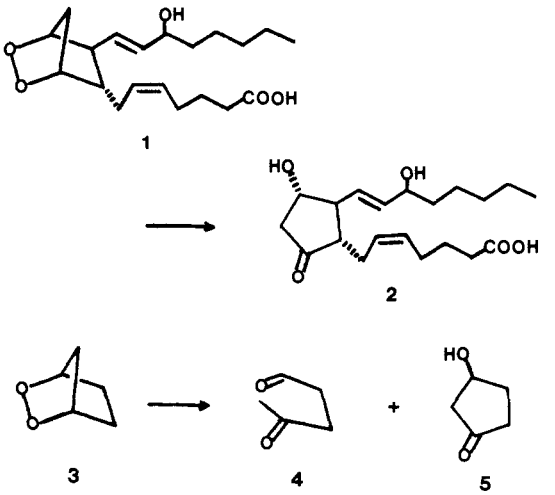
Prostaglandin Endoperoxides. 11. Mechanism of Amine-Catalyzed Fragmentation of 2,3-Dioxabicyclo[2.2.1]heptane[1]

Sir:

Disproportionation of prostaglandin endoperoxides (e.g., **1** → **2**) is a key step in the biosynthesis of D and E prostaglandins.[2] The fragmentation of 2,3-dioxabicyclo[2.2.1]heptane (**3**)[3] to levulinaldehyde (**4**)[4] which invariably accompanies disproportionation to 3-hydroxycyclopentanone (**5**) is fascinating since natural derivatives of **4** from **1** remain unknown. This paradox inspired us to examine carefully the mechanism of amine-catalyzed decomposition of **3.** We now report that amine catalysis of fragmentation (**3** → **4**) and disproportionation (**3** → **5**) are closely related mechanistically. Rate-determining cleavage of a bridgehead C-H bond generates a keto alkoxide which partitions between retro-aldol cleavage leading to **4** and protonation giving **5.**



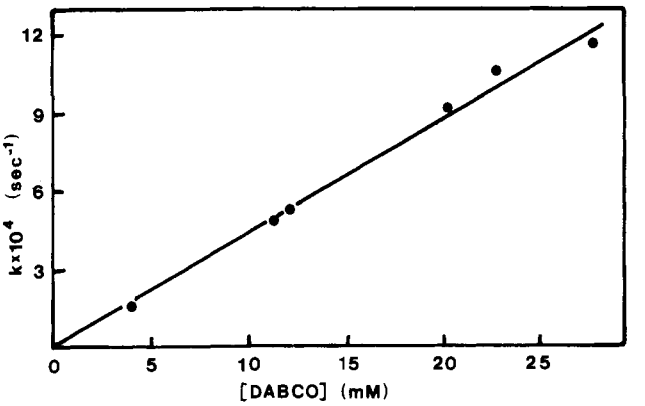


Figure 1. Correlation of pseudo-first-order rate constant for appearance of **4** with concentration of 1,4-diazabicyclo[2.2.2]octane.

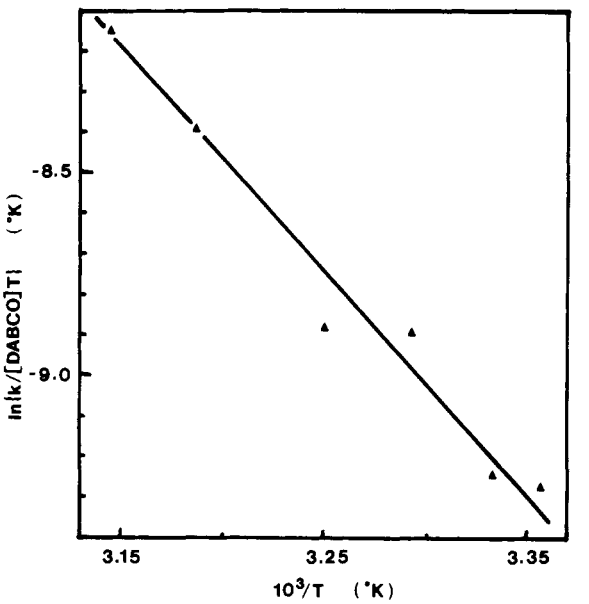


Figure 2. Temperature dependence of the pseudo-first-order rate constant (k) for appearance of **4.**

Decomposition of **3** in benzene solution in the presence of catalytic amounts of 1,4-diazabicyclo[2.2.2]octane (Dabco) was monitored by ^1H FT NMR. At 30.0 °C **4** and **5** are formed in 77-78 and 22-23% yields, respectively, over a range of catalyst concentrations from 4 to 28 mM. Over this range, the pseudo-first-order rate of appearance[5] of **4** is linearly correlated with catalyst concentration (Figure 1).

Rate constants were determined at various temperatures between 24.8 and 45.0 °C with 0.010 M Dabco and a 1.0 M initial concentration of **3.** These data show an excellent linear correlation of ln $(k/[\text{Dabco}]T)$ with $1/T$ where k is the observed pseudo-first-order rate constant for appearance of **4** (Figure 2). Activation parameters calculated from the rate constants listed in Table I are $\Delta H^{\ddagger} = 10.6 \pm 0.9$ kcal mol^{-1} and $\Delta S^{\ddagger} = -30 \pm 3$ eu.

Unimolecular thermal decomposition of **3** in nonpolar solvents, which gives 4,5-epoxypentanal almost exclusively, shows a considerably higher $\Delta H^{\ddagger} = 20.7 \pm 1.8$ kcal mol^{-1}.[1c] The large negative entropy of activation observed for the catalyzed fragmentation is consistent with a highly organized bimolecular transition state involving endoperoxide **3** and a molecule of catalyst.

Three different mechanistic types known for amine catalysis

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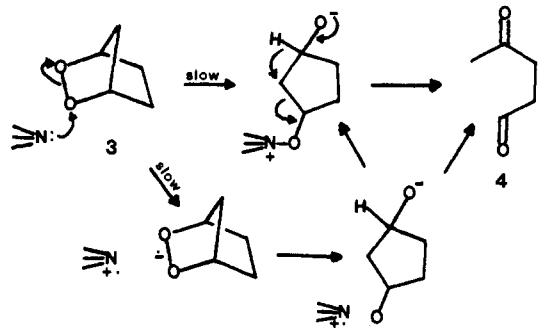
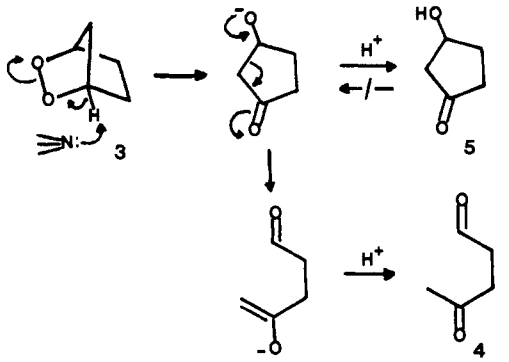
Scheme I

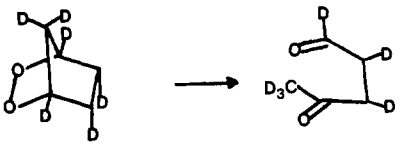


Scheme II



of peroxide decompositions could accommodate the observed kinetic and thermodynamic behavior. Rate-determining nucleophilic cleavage of the peroxide bond by the amine catalyst[6] might generate **4** by subsequent decomposition of a zwitterionic intermediate (Scheme I). Rate-limiting one-electron transfer from the amine to peroxide[7] might generate **4** by subsequent decomposition of the reduced peroxide, again possibly via a zwitterionic intermediate (Scheme I). Alternatively, rate-determining abstraction of a bridgehead proton by the amine catalyst with concomitant cleavage of the peroxide bond[8] might generate a keto alkoxide which affords **4** via retro-aldol cleavage (Scheme II). Protonation of the keto alkoxide before cleavage would produce the expected[8] disproportionation product **5**.

To differentiate between these alternative mechanisms, a deuterated analogue **3**-d_6 was prepared[9] and rates of appearance[10] of **4** and **4**-d_6 in the presence of identical solutions of



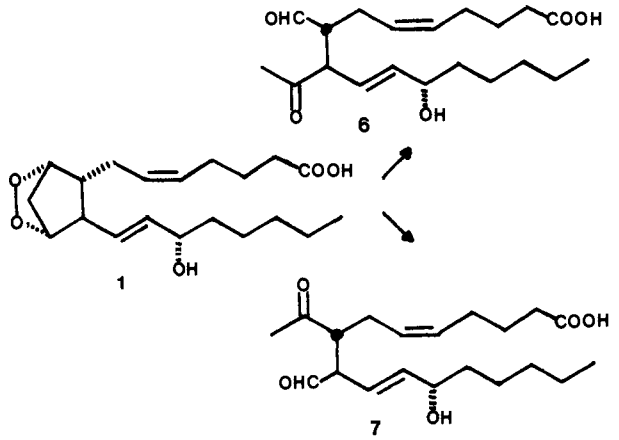
0.015 M Dabco were monitored by ^1H and ^2H NMR, respectively. A ratio of $k_H/k_D = 8$ was found between the rate constants for fragmentation of **3** and **3**-d_6. The large deuterium isotope effect supports the mechanism of Scheme II, involving rate-determining cleavage of a bridgehead C–H bond, but not the mechanisms of Scheme I. An isotope effect of similar magnitude ($k_H/k_D = 6$) was found previously for disproportionation of *tert*-butyl α-phenethyl peroxide catalyzed by piperidine.[8] The **3** → **5** disproportionation must therefore be closely related mechanistically to the **3** → **4** fragmentation as indicated in Scheme II. Since Dabco does not catalyze fragmentation of **5** to give **4** either protonation of the keto alkoxide is not reversible under the reaction conditions or the keto alkoxide generated from **3** is vibrationally excited. This could explain the difference in the proclivities toward retro-aldol cleavage of a keto alkoxide generated from the highly strained bicyclic peroxide **3** and one generated by deprotonation of **5**.

Table I. Rate Constants for Amine-Catalyzed Fragmentation of **3** to **4**

amine	reaction temp, °C	second-order rate constant $k_2 \times 10^2$ M^{-1} s^{-1}
Dabco	24.8	2.80 ± 0.04
Dabco	27.0	2.91 ± 0.03
Dabco	30.6	4.19 ± 0.07
Dabco	34.6	4.26 ± 0.01
Dabco	40.8	7.11 ± 0.15
Dabco	45.0	9.21 ± 0.22
imidazole	45.0	0.11 ± 0.002
N-methylimidazole	45.0	0.03 ± 0.001

The behavior of imidazoles also supports the hypothesis that amines act as basic rather than nucleophilic catalysts in promoting decomposition of **3**. Imidazoles are weaker bases but stronger nucleophiles than Dabco.[11] Imidazoles are much less effective catalysts than Dabco (Table I). Interestingly, imidazole-catalyzed decomposition of **3** at 45 °C affords a 53:47 ratio of **4** and **5** in contrast to the 82:18 ratio formed with Dabco or the 78:22 ratio formed with *N*-methylimidazole as catalyst. The keto alkoxide intermediate is apparently protonated more efficiently by the conjugate acid of the protic amine imidazole than by the conjugate acid of the aprotic amines Dabco or *N*-methylimidazole.

From our studies on model endoperoxide **3**, it seems reasonable to conclude that any disproportionation of a prostaglandin endoperoxide (e.g., **1** → **2**) *via bridgehead proton abstraction leading to a keto alkoxide intermediate* will tend to generate fragmentation products (e.g., **1** → **6** or **7**) unless the intermediate is efficiently captured by protonation.



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(10) Measured by integrated –CHO proton or –CDO deuteron resonances relative to *p*-dichlorobenzene or benzene-d_6 internal standards, respectively. The products from **3**-d_6 are **4**-d_6 and **5**-d_6 formed in 76 and 24% yields, respectively.
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Chemistry of Superoxide Ion. 4. Singlet Oxygen Is Not a Major Product of Dismutation[1]

Sir:

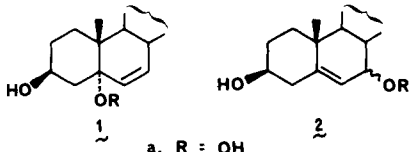
Since McCord and Fridovich discovered the enzyme, superoxide dismutase, which catalyzes the dismutation of superoxide radical anion ($O_2^{-}\cdot$) to give O_2 and H_2O_2[2] (reaction 1), the mechanism of the biological toxicity of $O_2^{-}\cdot$ has been a subject of great interest. There have been many mechanisms suggested for this toxicity: one is that singlet oxygen, known to react with many biological molecules, may be produced during the uncatalyzed dismutation of $O_2^{-}\cdot$ in water. While several reports have produced suggestive evidence for this reaction,[3] several others have produced moderately convincing negative evidence,[4] and others have produced inconclusive results.[1a,5]

$$2O_2^{-}\cdot + 2H^+ \rightarrow H_2O_2 + O_2(^1\Delta_g \text{ or } ^3\Sigma_g?) \qquad (1)$$

One problem with all of these studies is that the amount of $^1O_2$ produced (or the upper limit for its production) has not been determined quantitatively. Another problem is that the quenching by $O_2^{-}\cdot$ of any $^1O_2$ produced (reaction 2), which has been shown to have a rate constant of $\sim 10^9$ M^{-1} s^{-1} in dipolar aprotic solvents,[1a] could obscure the formation of $^1O_2$ in chemical model systems. We have designed a novel and generally useful technique for the specific and quantitative detection of singlet oxygen in aqueous systems. This technique avoids both difficulties mentioned above, and we report that reaction 1 produces *at most a few tenths of a percent* of $^1O_2$ under the most favorable conditions that we have studied.

$$O_2^{-}\cdot + {}^1O_2 \rightarrow {}^3O_2 + O_2^{-}\cdot + 22 \text{ kcal} \qquad (2)$$

Cholesterol gives a characteristic product with singlet oxygen, the 5α-hydroperoxide (**1a**), which is distinct from the products of radical autoxidation, which include the 7α- and 7β-hydroperoxides (**2a**).[6] Since cholesterol is virtually insoluble



in water, we used [4-^{14}C]cholesterol supported on polystyrene latex microbeads (Ⓟ-chol) in buffer.[7]

To ~28 mL of the stirred disperson, $(CH_3)_4N^+O_2^{-}\cdot$ (0.1 M)[8] in 25 mL of dry Me_2SO was added at 1.7 mL/h through 1-mm Teflon tubing with a syringe pump. Following reaction, the organic products were extracted with CH_2Cl_2 and hydroperoxides were reduced with $(C_6H_5)_3P$. TLC of the mixture was carried out with added known products,[6] and the bands corresponding to cholesterol, the $^1O_2$ product [5α-diol (**1b**)], and the 7α- and β-diols (**2b**) were scraped from the plate, extracted, and counted.[9]

Table I. Yields of $^1O_2$ (Percent) Based on $O_2^{-}\cdot$ Added at Various pHs, Corrected for Trapping Efficiency[a]

pH	yield of $^1O_2$, % × 10^4	pH	yield of $^1O_2$, % × 10^4
4	2.7	7	7.1
6	6.0	8	2.8
		10	16

[a] See ref 8c.

To quantitate the amount of singlet oxygen formed, the following method was used. To a suspension of Ⓟ-chol, prepared as above, histidine (5×10^{-4} M) and methylene blue (2×10^{-6} M) were added. The amount of 5α product produced after irradiation for 10 min was determined as above. Then the amount of histidine which had reacted in the same experiment was determined in the aqueous layer after the extraction of the cholesterol.[10] From the amount of histidine reacted, corrected for its trapping efficiency for $^1O_2$ at the concentration used,[11] the amount of $^1O_2$ generated photochemically in this calibration experiment could be determined, and thus the trapping efficiency (moles of 5α product/moles of $^1O_2$ produced) of the Ⓟ-chol system was found to be 2.5×10^{-5}. Although this efficiency is low, because of the sensitivity of the ^{14}C radioassay, it is sufficient.[12]

As a final control, two 25-mL solutions containing Ⓟ-chol and rose bengal were photooxidized under the same conditions. To one of them, $(CH_3)_4N^+O_2^{-}\cdot/Me_2SO$ (0.1 M) was added at 8.8 mL/h (six times the rate used in the dismutation experiment); the $O_2^{-}\cdot$ addition caused a decrease of 7 ± 6% in the amount of 5α product formed, so that $O_2^{-}\cdot$ quenching of $^1O_2$ is not significant under the conditions.[13]

The results of the experiments are shown in Table I.[8c] The fraction of oxygen appearing as $^1O_2$ is listed as a function of pH. The amounts found in the range pH 4–8 are probably not significantly >0. The amount found at pH 10, although small, may be significant, but further work will be necessary to establish this. *Thus, we conclude that $^1O_2$ is produced in amounts of no more than 0.2% under the quantitative conditions that we have studied, which include correction for trapping efficiency and $O_2^{-}\cdot$ quenching.* Thus, $^1O_2$ appears to be an *unlikely* candidate for the biological toxicant, at least under these conditions.

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