carbons.[19] Therefore, in the creation of the monocyclic β-lactam ring of proclavaminate, the N-C-4′ bond is formed with net retention of configuration, in keeping with either 0 or an even number of inversions at the carbon that becomes C-4′. In contrast, monocyclic β-lactam formation in nocardicin A (**6**, Scheme I) from L-serine (**5**) occurs with inversion of configuration.[5] Although no intermediates between glycerate and proclavaminate have been unequivocally established,[20] the results of the present experiments suggest several possible mechanisms for β-lactam formation more complex than the simple S_N2 process apparent in nocardicin biosynthesis[5] and distinct from the oxidative cyclization involved in isopenicillin N formation.[6]

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Registry No. 1a, 124603-44-7; **1b**, 124603-45-8; **2**, 112296-12-5; **4**, 58001-44-8; **7**, 85270-00-4; **8**, 124603-41-4; **9**, 124649-51-0; **10**, 100188-51-0; **11**, 124649-49-6; **12**, 124603-40-3; **13**, 124649-50-9; **14**, 124603-42-5; **15**, 124603-43-6; CS, 111693-82-4; benzyl bromoacetate, 5437-45-6.

(19) Townsend, C. A.; Ho, M.-f. *J. Am. Chem. Soc.* **1985**, *107*, 1066-1068.

(20) On the basis of whole-cell incorporation experiments, 3-hydroxypropionate has been claimed to be a specific precursor of the β-lactam segment of clavulanic acid (Gutman, A. L.; Ribon, V.; Boltanski, A. *J. Chem. Soc., Chem. Commun.* **1985**, 1627-1629). Despite very considerable effort using three fermentation media, we have been unable to duplicate the findings of this report. Low, nonspecific incorporations of radiolabel into clavulanic acid were obtained comparable to cysteine or β-alanine (Mao, S.-s.; Townsend, C. A., unpublished).

Structural Effects on the Yields of Singlet Molecular Oxygen ($^1\Delta_gO_2$) from Alkylperoxyl Radical Recombination

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Howard and Ingold,[1] following a suggestion of Russell, first reported singlet molecular oxygen from peroxyl terminations in 1968 (eq 1):

$$2R_1R_2CHOO^\bullet \rightarrow R_1R_2CO + R_1R_2CHOH + {}^3O_2 + {}^1O_2 \quad (1)$$

This reaction is important because it is a common termination step of the ubiquitous autoxidation process including many biological systems.[2] The reactions of the singlet oxygen formed must be taken into account in any comprehensive modeling scheme of these oxidation processes. Finally, we wondered whether reaction 1, possibly carried out under specialized conditions (e.g., combustion), might furnish singlet oxygen in synthetically useful yields.

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(2) (a) Kanofsky, J. R. *J. Biol. Chem.* **1984**, *259*, 5596-5600. (b) Kanofsky, J. R. *J. Org. Chem.* **1986**, *51*, 3386-3388 and references therein.

Table I. Yields of $^1O_2$ from Radical-Initiated Autoxidation of Hydrocarbons[a]

substrate, MBHT[b]	T, °C	% $^1O_2$	substrate, MBHT[b]	T, °C	% $^1O_2$
PhMe, 0.050	79.8	6.0 ± 0.4	n-$C_{12}H_{26}$[d]	79.8	3.9 ± 0.3
PhEt	69.9	11.5 ± 1.2	n-Bu_2O	79.6	9.1 ± 0.6
PhEt, 0.020	79.6	14.0 ± 1.1	c-$C_5H_{10}CO$, 0.042	79.5	4.6 ± 0.5
$PhCMe_3$	79.8	3.4 ± 0.3	Me_2NCHO	77.2	0.0 ± 0.4
Ph_2CH_2	69.4	11.3 ± 0.6	$MeN(COC_3H_6)$[e]	79.5	0.0 ± 0.4
0.6 M fluorene	79.5	6.1 ± 0.3	$PhCH_2CN$, 0.050	80.0	0.0 ± 0.4
Ph_2CH_2, 0.020	79.2	11.6 ± 0.7	Me_3COOH[f]	79.8	0.0 ± 0.3
1-Me-naphthalene, 0.051	77.2	4.7 ± 0.3	TOOH[g]		7.8 ± 0.5
c-C_8H_{16},[c] 0.050	77.8	6.7 ± 0.4	CH_3CN[h]	65.2	0.5 ± 0.1
c-C_8H_{14}	78.5	6.4 ± 1.3			

[a] Solutions (5.0 mL) in Au-coated, water-jacketed cell; IR detected with North Coast Model E0817 instrument with phase-sensitive detection at 100 Hz. Average of three measurements relative to areas under decay curves of N02 under the same conditions. [b] Initial concentration of di-*tert*-butyl hyponitrite. Cage effect (f = escaped radical pairs) assumed 0.89, the value in $PhCMe_3$,[3] unless noted otherwise. [c] f = 0.85 determined experimentally from induction period as described.[3] [d] f = 0.84 estimated from relations derived by Kiefer and Traylor (Kiefer, H.; Traylor, T. G. *J. Am. Chem. Soc.* **1967**, *89*, 6667-6671) and published viscosity data (Stephen, K.; Incase, K. *Viscosity of Dense Fluids*; Plenum Press: New York, 1979). [e] *N*-Methylpyrrolidone. [f] Initially 0.25 M in $PhCMe_3$ solution. [g] Initially 0.10 M in $PhCMe_3$ solution. [h] Initiated with benzyl hyponitrite (0.060 M) with f = 0.65.[17]

In Table I we present yields of singlet oxygen from reaction 1, in which the peroxyl radicals were generated continuously from different oxygen-saturated solvents by free-radical initiation with hyponitrite esters[3] (eqs 2 and 3) or directly from the hydroperoxide and initiators.

$$RONNOR \rightarrow 2RO^\bullet + N_2 \quad (2)$$

$$RO^\bullet + R_1R_2CH_2 \rightarrow R_1R_2CH^\bullet \xrightarrow{O_2} R_1R_2CHOO^\bullet \quad (3)$$

The yields were determined from the areas under the curves of chemiluminescence emission at 1.27 μm vs time,[4] relative to similar areas from the thermal decomposition of 1,4-dihydro-1,4-dimethylnaphthalene 1,4-endoperoxide (NO2).[5]

For simple hydrocarbon substrates that are expected to terminate only by reaction 1, the yields of singlet oxygen are remarkably uniform. They range from 3.4 to 6.0% for primary and from 3.9 to 14.0% for secondary alkylperoxyl terminations. The values are in the same range as found by Kanofsky by oxidation in aqueous media[2] of several hydroperoxides with α-hydrogens. With additional functional groups present in the substrate, the yields either remain about the same (n-Bu_2O, cyclohexanone) or decrease (amides, nitriles).

We find a small but measurable quantity of $^1O_2$ from autoxidation of acetonitrile. This result is pertinent to some very interesting experiments reported recently by Sugimoto, Kanofsky, and Sawyer,[6] who detected 1.27-μm emission from the electrolytic reduction on Pt of O_2-saturated acetonitrile (but not from *N*,*N*-dimethylformamide, cf. Table I). The authors ascribed the result to singlet oxygen from termination of $HOO^\bullet$ radicals bound to the electrode surface:

$$H^+ \xrightarrow{Pt/e^-} (Pt)H^\bullet \xrightarrow{O_2} (Pt)HOO^\bullet \rightarrow 0.5H_2O_2 + 0.5{}^1O_2 \quad (4)$$

(3) Lee, S. H.; Niu, Q.; Sheng, X. C.; Mendenhall, G. D. *Photochem. Photobiol.* **1989**, *50*, 249-256.

(4) Plots of starting initiator concentration (≤.08 M) vs initial detector response, or area under the decay curve of the luminescence, were linear for oxygen-saturated PhEt at 69 and at 78 °C.

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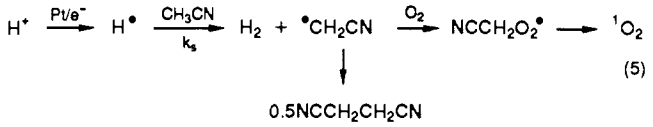
One may also consider the formation of solvent-derived radicals from a related sequence:



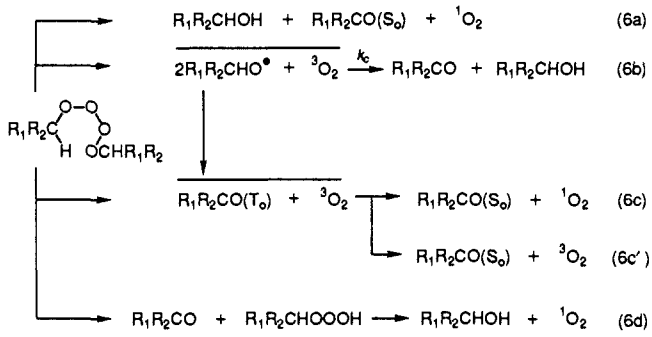
(5)

The partition of H• between solvent and oxygen will be given by

$$R = r(^{\bullet}CH_2CN)/r(^{\bullet}OOH) = k_s[H^{\bullet}][MeCN]/k_0[H^{\bullet}][O_2]$$

Experimental values of k_s,[7] k_0,[8] and oxygen solubility[9] are known, from which we estimate values of $R = 0.01$–0.2. Electrolysis of nitrogen-purged acetonitrile under the conditions described[6,10] gave a significant yield (9.0 ± 0.9%) of succinonitrile.[11] The yield fell to 0.5 ± 0.2% when the experiment was repeated while a continuous stream of oxygen was passed through the liquid. Experiments with oxygen-free solvent with anode and cathode compartments separated by a glass frit revealed succinonitrile in both compartments, consistent with the similar anodic oxidation of acetonitrile, described by Schmidt and Noack.[12] Since isotopic scrambling[13] of $^{36}O_2$–$^{32}O_2$ mixtures in autoxidizing media is well-established, we conclude that the mass spectrometric and other evidence presented[6] for reaction 4 must be tempered by a contribution from the concurrent reaction 5.

The product distribution from reaction 1 has been suggested to arise from spin conservation[14] in the fragmentation of a tetraoxide intermediate.[15] Some detailed pathways are as follows:



(6a)

(6b)

(6c)

(6c')

(6d)

The $^1O_2(^1\Delta_g)$ from diphenylmethane (11.3 ± 0.6%) may reasonably arise from paths 6a, 6c, and 6d as shown. Our computer modeling of the stable products indicate that reaction 6b is not significant. The yield of $^1O_2(^1\Delta_g)$ from quenching of triplet benzophenone by oxygen[16] has been measured as 29–35% and is too high to allow any combination of paths 6a and 6c in our system. The yield of triplet benzophenone from dismutation of alkoxyl radicals[17] (vertical arrow between 6b and 6c above) is only 0.15%, too low to be a significant source of singlet oxygen.

Previously we suggested[3] that cleavage of R_2O_4 into a carbonyl product and ROOOH might be a source of singlet oxygen when the hydrotrioxide decomposed (6d). This hypothesis appears to be ruled out by an experiment in which Ce^{4+} was injected into methanolic 1-tetralyl hydroperoxide (TOOH) at 25 °C and then at −78 °C.[18] The prompt IR emission resulting from the self-reaction of TOO• in these experiments was comparable at both temperatures, whereas the known *tert*-alkyl hydrotrioxides are stable[19] at −78 °C.

Perhaps 6a gives largely $^1O_2(^1\Sigma_g)$,[14,20] which partitions between $^1O_2(^1\Delta_g)$ and $^3O_2$, a known process in the condensed phase.[21] This explanation nicely accounts for the relative independence of $^1O_2(^1\Delta_g)$ yields on alkyl structure. In our system, any 760-nm emission, which could be ascribed to the $^1\Sigma_g \rightarrow {}^3\Sigma_g$ transition of molecular oxygen, must have a quantum yield below the detectable limit of about 10^{-10}.

The relative uniformity of the $^1O_2$ yields in reaction 1 may offer advantages in the study of hydrocarbon autoxidation.

Acknowledgment. This work was supported by 3M Co. and by Himont USA, Inc. We thank Dr. J. R. Kanofsky for helpful discussions of IR detection equipment.

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(9) In acetonitrile, 8.1×10^{-3} M: Achord, J. M.; Hussey, C. L. *Anal. Chem.* **1980**, *52*, 601–602. We were originally influenced by a lower value: Bruckl, N.; Kim, J. I. *Z. Phys. Chem.* **1981**, *126*, 133–150. The concentration of oxygen of course may be depleted near the electrode surface.

(10) Conditions: 0.1 M 70% $HClO_4$ in HPLC grade acetonitrile, Pt wire anode, Pt mesh cathode, 3.0 V, 4 mA, 27 h.

(11) GC analysis was carried out after neutralization with KOH, with a 25-m OV-17 capillary column programmed for 7 m at 40 °C, then 10 deg/min to 200 °C (HP 5830A gas chromatograph).

(12) Schmidt, H.; Noack, J. *Angew. Chem.* **1957**, *69*, 638. A referee suggested that HO• rather than the perchlorate radical was the active species.

(13) Bartlett, P. D.; Traylor, T. G. *J. Am. Chem. Soc.* **1963**, *85*, 2407–2710.

(14) See: Kellogg, R. E. *J. Am. Chem. Soc.* **1969**, *91*, 5433–5436 and references therein.

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(18) Methanolic $Ce(NH_4)_2(NO_3)_6$ (1.0 mL of 0.150 M) injected into 4.0 mL of 0.134 M TOOH. Both solutions were precooled for the reaction at −78 °C.

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Synthesis Using Plasmid-Based Biocatalysis: Plasmid Assembly and 3-Deoxy-D-*arabino*-heptulosonate Production

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A plasmid-based approach to microbial whole cell synthesis of 3-deoxy-D-*arabino*-heptulosonates DAH and DAHP (Scheme I) has been developed by exploiting the catalytic activity of transketolase, an enzyme that occupies a long-overlooked niche in aromatic amino acid biosynthesis. DAH and DAHP have been obtained with enzymatic synthesis[1a,c] and microbial whole cell synthesis.[1a,b] The levels (1 mM) of DAH and DAHP synthesized by microbial whole cells are significantly lower than those levels (10 mM) achieved with cell-free enzymatic synthesis.[1a] By localizing the genes encoding transketolase and DAHP synthase on a single plasmid, coupled enzyme catalysis (Scheme I) utilized during multistep, immobilized enzyme synthesis[1a] is reconstructed within the confines of an intact microbe. The result is an *Escherichia coli* strain that synthesizes substantially elevated levels of DAH and DAHP.

The activity of DAHP synthase, which catalyzes the condensation of D-erythrose 4-phosphate and phosphoenolpyruvate to form DAHP (Scheme I), is known to control the flow of carbon

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