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Towfik H. Teherani, William J. Peer
 J. J. Lagowski, Allen J. Bard*

Department of Chemistry
 The University of Texas at Austin, Austin, Texas 78712

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Chemistry of Singlet Oxygen. 29. A Specific Three-Phase "Kautsky Test" for Singlet Oxygen¹

Sir:

In 1933, Kautsky reported experimental evidence for a "metastable, reactive state of the oxygen molecule" by observing photochemical oxidation of leucomalachite green supported on dry silica gel which was intimately mixed with a separate batch of silica gel on which was adsorbed a sensitizer (tryptaflavin).² Reaction was observed only over a rather limited range of pressures, with the maximal effect being found at 0.02 mmHg. This experiment provides strong evidence for a volatile reactive intermediate, but provides little information about its nature. Although this intermediate was probably singlet oxygen,³ neither the sensitizer nor the acceptor⁴ used have been much studied and the chemistry involved is obscure. Similar problems attend an analogous experiment carried out by Rosenberg and Shombert;⁵ indeed, this experiment was used to suggest that a *vibrationally* excited oxygen molecule was the reactive intermediate.

Bourdon and Schnuriger carried out a related experiment in which oxidation of methoxynaphthalene, rubrene, or diphenylanthracene was photosensitized by methylene blue or eosin (separated from the acceptor by stearate layers).⁶ This experiment is easier to interpret as singlet oxygen chemistry, but no product identification was reported.

Experiments in which a supported sensitizer is irradiated in a stream of O₂ and a downstream acceptor is oxidized have also been carried out, but give miniscule yields of product which often cannot readily be distinguished from that of auto-oxidation.⁷

Because of the central nature of this type of experiment to the singlet oxygen field, we wished to use a system in which both sensitizer and acceptor were well-characterized singlet oxygen reagents. We report the generation and trapping of ¹O₂ in a "three-phase" system⁸ using polymer-bound rose bengal⁹ as acceptor and a polymer-bound olefin (6-methyl-5-heptenoate, **1a**) as acceptor.¹⁰

Photooxidation of polymer-bound ester **1a** or the methyl ester **1b** in methanol containing soluble rose bengal produced a mixture of the two allylic products (**2** and **3**, analyzed after reduction to alcohols) in 1:4 ratio.¹¹ The ratio of products was the same from **1a** and **1b** (but subject to analytical difficul-

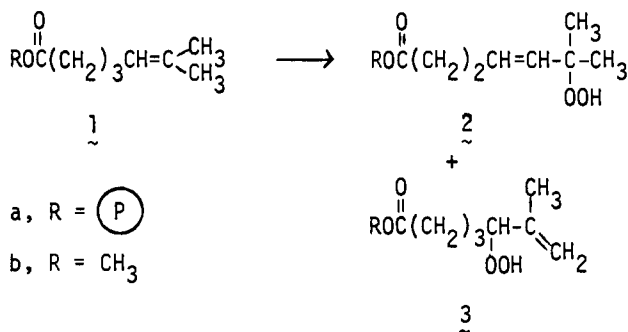


Table I. Photooxidation of P -6-Methyl-5-heptenoate (**1a**) Sensitized by P -Rose Bengal^a

conditions	irradiation time, h	yield of 2 + 3 , %	$\tau(^1\text{O}_2)$, s
CCl ₄	9	~0 ^b	7×10^{-4} ^c
air, 760 mmHg	8	~0 ^b	8.8×10^{-2} ^d
O ₂ , 25 mmHg	14	1-2	5.6×10^{-1} ^d
O ₂ , 10 mmHg	14	5-6	1.4 ^d

^a References 11 and 12. ^b <<1 % could have been easily detected. ^c Reference 13. ^d Calculated from rate constant in ref 14.

ties¹¹). No oxidation of **1a** occurred in the absence of sensitizer.

Mixtures of bound sensitizer and bound acceptor were mixed and irradiated under various conditions. The results are summarized in Table I.¹² Substantial product formation occurred when photolysis was carried out at 25 mm of O₂, and more at 10 mm; in contrast, no product formation was observed in air or in CCl₄, a solvent in which ¹O₂ has a comparatively long lifetime. The product ratio was the same as with the soluble photosensitized reaction with **1a** and **1b**.¹¹

Although the exact efficiency of the ¹O₂ trapping in this system cannot be calculated from the data, and, although the amount of ¹O₂ formed would not be easy to make reproducible because of the variability of light adsorption associated with the inhomogeneous system, it is clear that singlet oxygen does not have sufficient lifetime to diffuse efficiently from one solid phase to the other through carbon tetrachloride ($\tau(^1\text{O}_2) = 700 \mu\text{s}$) or air (8.8×10^{-2} s), but that at 25 mm of O₂ (0.56 s) or 10 mm (1.4 s) measurable trapping occurs. Since the mean radius of diffusion of singlet O₂ during its lifetime varies from $\sim 3 \times 10^{-4}$ cm in CCl₄ to ~ 3 cm at 10 mm of O₂, and is ~ 0.1 cm in air, this experiment also sets some limits for the use of the "three-phase test" for trapping short-lived species.⁸

We believe the polymer-bound system used here may be of general utility for trapping ¹O₂, both in gas-phase systems and in liquid media where ¹O₂ is homogeneously generated. It does not appear to be useful in heterogeneous liquid ¹O₂-generating systems such as the three-phase system because of the short diffusion radii for ¹O₂ in solution.

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- The acceptor was prepared by coupling 6-methyl-5-heptenoic acid (K. Mori, M. Matsui, *Tetrahedron*, **25**, 5023 (1969)) to Merrifield's resin (chloromethylated polystyrene, Sigma Chemicals) by heating at 60 °C for 24 h in dry purified *N,N*-dimethylformamide; 0.57 mequiv/g were bound (Br₂ titration). Infrared showed ester absorption. The bound ester **1a** could be cleaved with NaOMe to give **1b**.
- Resin-bound products were reduced (NaBH₄) and subjected to ester interchange using NaOMe-dry CH₃OH. Products were gas chromatographed, and isolated samples characterized by IR, NMR, and mass spectral analysis.

The exact ratio of alcohols in the product could not be determined because considerable dehydration (to the diene, identified by IR and mass spectroscopy) accompanied gas chromatography. Both alcohols dehydrate, as shown by rechromatography of GLC-collected pure samples. (These alcohols are high enough in molecular weight to require high injector and column temperatures for gas chromatography.) While the experiments were not designed to measure the singlet O_2 trapping efficiency of the resin ester, a rough calculation suggests that, at a concentration of 0.057 mequiv of ester/20 mL, $\sim 4/1000$ of the 1O_2 formed was trapped. If the methyl ester had been dissolved at the same concentration, $\sim 3\%$ should have been trapped; thus the resin bound ester is (very roughly) $1/10$ as efficient as the methyl ester ($\beta = 0.11$ M) at trapping 1O_2 under these conditions.

- (12) Gas-phase photolyses were run in 500-mL round-bottom flasks loaded with 100 mg of each polymer-bound species. The flask was evacuated to 10 μ and then refilled to the desired pressure using an oxygen-filled balloon. The flask was then irradiated with a 650-W Sylvania DWY tungsten-halogen lamp through a 2% sodium dichromate filter solution ~ 0.5 cm from the beads. The CCl_4 -phase run was carried out using 200 and 50 mg of polymer-bound acceptor **1a** and sensitizer, respectively, in 20 mL of CCl_4 with the same lamp and filter setup. Products were analyzed as described in ref. 11.
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- (15) Department of Chemistry, University of Pittsburgh, Pittsburgh, Pa. 15260.

S. Wolf, C. S. Foote,* J. Rebek, Jr.*¹⁵

Department of Chemistry, University of California
Los Angeles, California 90024

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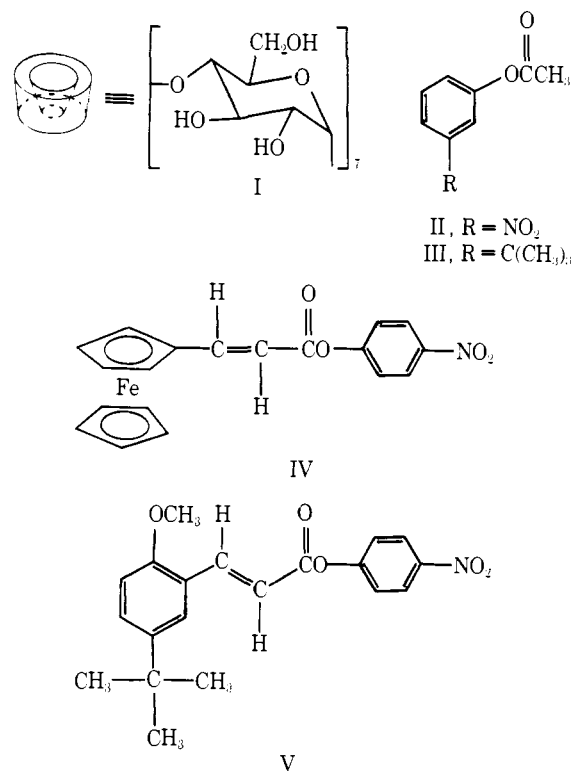
Very Fast Acylation of β -Cyclodextrin by Bound *p*-Nitrophenyl Ferrocinnamate

Sir:

In the study of the cyclodextrins as enzyme models, particular interest has surrounded reactions in which a substrate, bound into the cavity of the cyclodextrin, reacts with one of the hydroxyl groups on the rim of the molecule.¹ For example, Bender² has studied the acylation of β -cyclodextrin (I) by bound *m*-nitrophenyl acetate (II), *m*-*tert*-butylphenyl acetate (III), and related compounds. In water solution at pH 10.6, he reports that ester III acetylates a β -cyclodextrin hydroxyl group 250 times as rapidly as it acetylates water (hydrolyzes) in the absence of cyclodextrin at the same pH. We have reported³ that such reactions are accelerated if the solvent is changed to 60% Me_2SO/H_2O , with the acylation of cyclodextrin by III being 500 times as fast as hydrolysis in this medium, and 13 000 times as fast in this medium as is hydrolysis with the same buffer in H_2O solvent.

On the basis of such data some pessimists have concluded that cyclodextrins can give selective reactions, but with only modest rate accelerations over the control. However, it seemed to us that the optimal⁴ systems had not yet been examined. Entropy factors should be more favorable for acylation processes in which the acyl group, not the leaving group, is bound into the cyclodextrin cavity. Furthermore, molecular models suggest that certain derivatives of ferrocene should be particularly well held by binding to β -cyclodextrin. We have previously shown³ that ferrocene itself is strongly bound. We now wish to report that the acylation of β -cyclodextrin by the *p*-nitrophenyl ester of ferrocinnamic acid⁵ (IV) is accelerated by over 50 000-fold compared with hydrolysis by buffer alone. The actual rate achieved is comparable with that for acylation of the enzyme chymotrypsin by *p*-nitrophenyl acetate.

The substrate IV was prepared from ferrocinnamic acid⁵ and *p*-nitrophenol with dicyclohexylcarbodiimide. Its hydrolysis in 60% of dimethyl sulfoxide/40% H_2O (v/v) at 30.0 °C with a 4 mM phosphate buffer was monitored at 410 nm (*p*-nitrophenoxide ion). With a buffer⁶ which in H_2O has pH 6.8, the pseudo-first-order hydrolysis rate constant of IV was 3.5×10^{-6} s⁻¹, while *p*-nitrophenyl acetate under the same



conditions has a rate constant of 74×10^{-6} s⁻¹, 21-fold faster. The reaction of cyclodextrin with IV (0.10 mM) in the same medium was monitored at 410 nm with β -cyclodextrin concentrations ranging from 0.20 mM to 20 mM. The data (20 points) fit an Eadie plot which demonstrates that 1:1 complex is being formed, and rigorously excludes other stoichiometries.⁷ The K_d is 7 mM, while V_{max} is 0.18 s⁻¹. V_{max} is first order in hydroxide ion and unaffected by doubling the buffer concentration. Thus, the process being observed is the acylation of cyclodextrin *anion* by bound substrate IV, as had been shown^{2,3} for substrates II and III at much higher pH's. As expected from this, the product isolated is a cyclodextrin ferrocinnamate ester (λ_{max} 474 nm) which is slowly hydrolyzed on treatment with aqueous sodium hydroxide to the salt of ferrocinnamic acid (λ_{max} 451 nm).

The acylation reaction is 51 000 times faster than hydrolysis of IV in our medium; this is two orders of magnitude larger than the best previous cyclodextrin acceleration. Furthermore, our V_{max} of 0.18 s⁻¹ for IV with pH 6.8 buffer, and thus 1.8 s⁻¹ at pH 7.8, should be compared with that of the acetylation of chymotrypsin with *p*-nitrophenyl acetate over this same pH range in water, in which V_{max} is essentially constant at 3.1 s⁻¹.⁸ Our reaction is clearly comparable in rate, even though IV is 21-fold *less* reactive than is *p*-nitrophenyl acetate in a simple hydrolysis. This is more remarkable since β -cyclodextrin lacks the principal catalytic groups of the enzyme.

The comparison with the enzyme includes the effect of a solvent change meant to mimic the interior of the protein. In fact, taking our 26-fold acceleration³ produced by this solvent in the simple hydrolysis of III and applying it here, one can argue that the full acceleration on going from an aqueous hydrolysis to a cyclodextrin acylation in 60% Me_2SO is more than 1.3 million. Regardless of the details of this comparison, it is apparent that our system shows an impressive acceleration.

Although several factors can be invoked to explain this improvement over other substrates, we believe that the geometry of IV is particularly important. Molecular models suggest that IV can go to the tetrahedral intermediate in ester exchange with full retention of the optimum binding geometry in the cyclodextrin cavity, while this is *not* the case for *m*-nitrophenyl