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(9) **3**-d_6 was prepared from 1,3-cyclopentadiene-d_6 by singlet oxygenation followed by reduction with diimide.[1d]
(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:

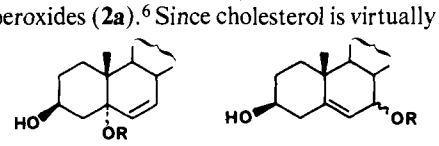
Since McCord and Fridovich discovered the enzyme, superoxide dismutase, which catalyzes the dismutation of superoxide radical anion ($O_2^{-}\cdot$) to give O_2 and H_2O_2[2] (reaction 1), the mechanism of the biological toxicity of $O_2^{-}\cdot$ has been a subject of great interest. There have been 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



1 2

a, R = OH
b, R = H

($^1O_2$ Product) (Radical Product)

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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(7) A solution (50 μL) of TLC-purified [4-^{14}C]cholesterol and 0.5 mL of diethyl ether were stirred with 0.2 mL of a 10% aqueous suspension of polystyrene latex beads (Dow Diagnostic, 0.82-μm average diameter) for 30 min; 2–3 mL of distilled H_2O was added; and the ether was removed by passing N_2 over the sample. The appropriate 0.1 M buffer (25 mL) (pH 4, acetate; 6, 7, and 8, phosphate; 10, carbonate) was then added.

(8) (a) Prepared by the method of A. D. McElroy and J. S. Hashman, *Inorg. Chem.*, **3**, 1798 (1964). *Caution:* explosions have been reported during this reaction. Extraction should be carried out in all-glass apparatus; avoid paper thimbles. (b) Material so prepared had mp 101 °C uncorr (lit.[8a] mp 97 °C) and assayed 96–98% $O_2^{-}\cdot$ by O_2 evolution. (c) A control showed that 60% of the original $O_2^{-}\cdot$ remained in the Me_2SO solution after 19 h. (Most of the losses occur during the first hour.) The figures in Table I are not corrected for these losses. The reduction of 10^{-3} M nitroblue tetrazolium in Me_2SO was used to assay $[O_2^{-}\cdot]$. Solutions were diluted 10-fold with Me_2SO and absorbance of the product (ϵ_{685} 85 000 M^{-1} cm^{-1}) was recorded.

(9) Using a scintillation counter; 10 mL of Biofluor (NEN) was added; the internal standard technique was used to correct for quenching.

(10) The aqueous layer was centrifuged and the amount of histidine reacted measured by the Pauly reagent;[14] unreacted controls were used for comparison. Controls also established that the extraction process did not remove histidine, and that buffer, histidine photooxidation products, or methylene blue did not interfere with the analysis.

(11) The fraction of $^1O_2$ trapped is $[A]/(\beta + [A])$ and was 1/8 at the 5×10^{-4} M concentration of histidine used.[16] Controls showed that the Ⓟ-chol did not trap a substantial fraction of the $^1O_2$ generated.

(12) Subsequent experiments with different preparations gave slightly lower efficiencies; however, the general conclusions are not affected.

(13) It is interesting to calculate the steady-state concentration of $[O_2^{-}\cdot]$ present. From the rate of addition of $O_2^{-}\cdot$ and the rate of decay at pH 7, calculated from Czapski's relationships[15] (6.36×10^5 M^{-1} s^{-1}), the steady-state concentration of $O_2^{-}\cdot$ is calculated to be 3.9×10^{-6} M and the fraction of $^1O_2$ quenched (from the $O_2^{-}\cdot$ quenching rate[1a]) to be ~1.6%. The steady-state concentration of $O_2^{-}\cdot$ increases with pH because of the slower dismutation of $O_2^{-}\cdot$;[15] however, even at pH 10, the amount of $^1O_2$ quenching is calculated to be no more than 20% under the conditions of the yield experiment at pH 10.

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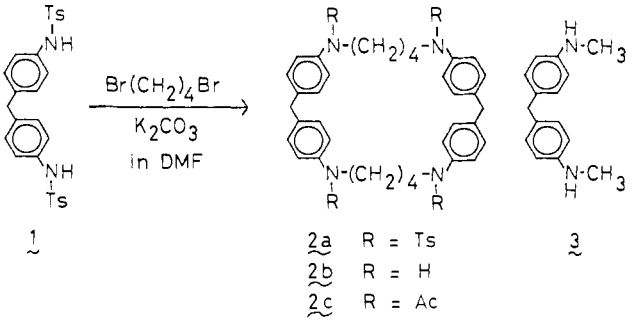
Host–Guest Complex Formation between a Water-Soluble Polyparacyclophane and a Hydrophobic Guest Molecule

Sir:

The design of water-soluble host compounds which have a hydrophobic cavity of definite shape and size is of great interest in relation to substrate-specific binding in aqueous solution. Of these host compounds, cycloamyloses (native and modified) have been most widely studied and thoroughly reviewed.[1] Recently another class of compounds, water-soluble paracyclophanes, in which aromatic ring(s) and methylene units are expected to compose a hydrophobic cavity, have drawn attention as artificial host compounds.[2,3]

Although several spectral studies[2a,b,3a-c] have suggested that they form inclusion complexes with hydrophobic substrates in aqueous solution, there has not been *direct evidence for "inclusion"*. We report here the first example of a crystalline complex of a water-soluble paracyclophane with a hydrophobic substrate, which was isolated from an *aqueous solution* and characterized as an *inclusion complex* by the X-ray method.

1,6,20,25-Tetraaza[6.1.6.1]paracyclophane (**2b**) was designed as a host compound and synthesized employing the known method.[4] Equimolar amounts of *N,N'*-ditosyl-4,4'-diaminodiphenylmethane[5] (**1**) and tetramethylene bromide were cyclized in DMF in the presence of potassium carbonate by high-dilution method to give **2a**,[6a] dec pt 305–306 °C, in 25% yield. Detosylation of **2a** gave **2b**,[6] mp 182.5–184 °C dec, in 67% yield after purification. The cyclic structure was confirmed on the basis of the mass spectra of **2b** and further of **2c**,[6a,c] mp 292–293 °C dec, which was obtained from **2b** in almost quantitative yield.



As **2b** was soluble in water below pH 2, the interactions of **2b** with various substrates having hydrophobic moieties were investigated in acidic aqueous solution. The fluorescence intensity of 1-anilinonaphthalene-8-sulfonate (1,8-ANS) was markedly enhanced in the presence of **2b**,[7] suggesting that 1,8-ANS was transferred into a nonpolar environment and/or subjected to a conformational change[8] by **2b**. The Benesi-Hildebrand plot[9] of the fluorescence intensity gave a straight line which indicated **2b** and 1,8-ANS formed a 1:1 complex with a dissociation constant of 1.6×10^{-4} M, comparable with other complexes from the known water-soluble paracyclophanes.[10] In the ^1H NMR spectrum the signals of 2,7-dihydroxynaphthalene moved upfield remarkably in the presence of **2b**;[11,12] this can be ascribed to a very strong shielding effect of the aromatic rings of **2b**. On the other hand the acyclic reference compound **3**[13] showed only a small effect in both the fluorescence and ^1H NMR spectra.[14] These spectral data suggest that **2b** and the substrates are in an intimate contact that does not occur without the cyclic structure of **2b**. Inclusion within the cavity of **2b** is considered to be a possible way of contact.

Furthermore **2b** formed crystalline complexes from aqueous solution with a variety of substrates having hydrophobic moieties, e.g., 1,3-dihydroxynaphthalene, 2,7-dihydroxynaphthalene, naphthalene, *p*-xylene, and durene.[15] When durene (1,2,4,5-tetramethylbenzene) was used as the substrate, the 1:1 crystalline complex which was characterized as **2b**·4HCl·durene·$4H_2O$[16] was successfully obtained, and its structure was determined by the X-ray method. Crystal data: monoclinic; space group $P2_1/n$; a = 14.552 (7), b = 22.582 (12), c = 7.238 (4) Å; β = 97.23 (4)°; V = 2359.6 Å^3; Z = 2. The crystal structure was solved by the direct method and refined by the method of block-diagonal least-squares to the final R factor of 0.065 for 3910 nonzero, independent reflections obtained by using graphite monochromated Cu Kα radiation.

As shown in Figure 1, **2b**·4HCl and durene form a *host-guest complex*[17] in which the guest molecule, durene, is *fully*

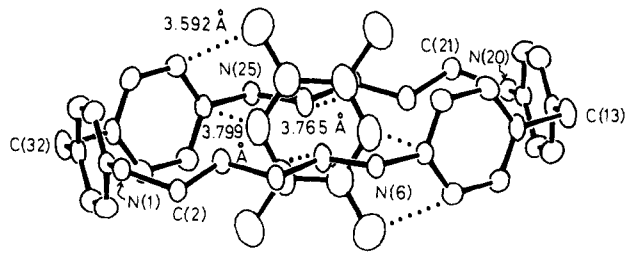


Figure 1. Perspective view of the host–guest complex of **2b**·4HCl with durene drawn by the ORTEP program.

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