ethidium–DNA complexes. At equivalent binding ratios there were no significant differences for chemical shifts between the two buffer systems. Since it is known that outside binding is eliminated for ethidium in high ionic strength buffers,[21] external interactions do not have any significant effect on the ^{31}P chemical shifts reported here. The results of this paper then indicate that the exchange-averaged ^{31}P chemical shift in DNA complexes with intercalating ligands depends strongly on the structure of the ligand. The factor which correlates most strongly with the magnitude of the chemical shift change from native DNA is the ligand unwinding angle.

The ^{31}P chemical shifts obtained for these three complexes are not sufficient for derivation of any functional dependence of ^{31}P chemical shifts on unwinding angles and such an analysis will require experiments with numerous intercalating molecules for which quantitative unwinding angles are known.

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(22) After submission of this paper, Hogan and Jardetzky (*Biochemistry* **1980**, *19*, 2079) indicated that the ^{31}P NMR signal is totally lost for a 300 base pair preparation of calf thymus DNA when complexed with ethidium. The molecular weight average of our DNA is only about 60% of that of Hogan and Jardetzky and this may account for the difference between their findings and ours. We find about a 30% loss of area in our ^{31}P signal at saturation for ethidium and this could be due to the loss of area for DNA at the upper molecular weight end of our sample. We have shown, however, that natural double-helical DNA gives measurable ^{31}P NMR signals when complexed with intercalating ligands, and this technique should prove to be quite a valuable probe of intercalation, provided DNA of sufficiently low molecular weight is used.

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Singlet Oxygen and Spin Trapping with Nitrones

Sir:

A major problem in the study of certain biological and aqueous systems is the identification and differentiation of reactive oxygen intermediates. These include the hydroxyl radical (·OH), the superoxide anion (O_2^-), and singlet oxygen ($^1O_2$). It has previously been demonstrated that nitrone spin traps can be used for the identification of both ·OH and O_2^- radicals in water,[1] and this has resulted in their use as probes in a variety of systems.[2] However, the interaction of $^1O_2$ with nitrone spin traps has been ignored. Ching and Foote[3] in 1975 demonstrated that cyclic nitrones can quench $^1O_2$ in $CDCl_3$. When the methylene hydrogens were at the C5 position (II) only physical quenching of $^1O_2$ was observed. Compound III (DMPO) is very close in structure to II and is widely used as a "spin trap".[4] This communication focuses on the reactivity of $^1O_2$ with DMPO and other nitrone spin traps in aqueous medium.

I   II   III
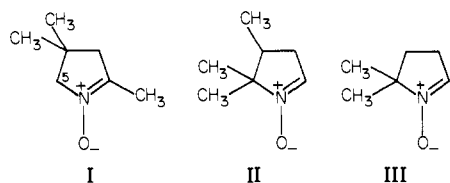
(1) Harbour, J. R.; Chow, V.; Bolton, J. R. *Can. J. Chem.* **1974**, *52*, 3549–52.

(2) For a listing of applications see: Janzen, E. G.; Wang, Y. Y. *J. Phys. Chem.* **1979**, *83*, 894–6.

(3) Ching, T.-Y.; Foote, C. S. *Tetrahedron Lett.* **1975**, *44*, 3771–4.

(4) Janzen, E. G.; Liu, J. I. *J. Magn. Reson.* **1973**, *9*, 510–2.
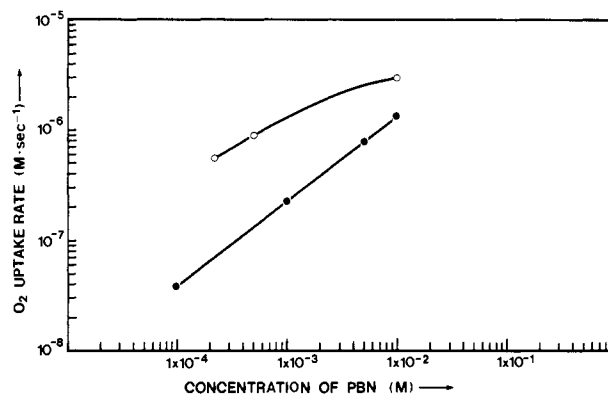
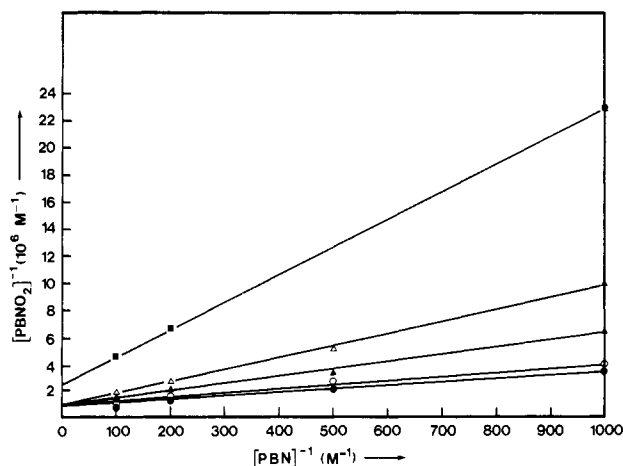**Figure 1.** Methylene blue sensitized photooxidation of PBN in H_2O (●) and D_2O (○).

Figure 2. Azide quenching of the methylene blue sensitized photooxidation of PBN in H_2O. [N_3^-]: (●) 0.0 M; (○) 1 × 10^{-4} M; (▲) 5 × 10^{-4} M; (△) 1 × 10^{-3} M; (■) 4 × 10^{-3} M. $PBNO_2$ is the unknown reaction product between PBN and $^1O_2$.

We have monitored the reactivity of $^1O_2$ with nitrones by measuring oxygen uptake from solution, using an oxygen electrode[5] and illuminating a 1 × 10^{-4} M aqueous solution of the $^1O_2$ sensitizer methylene blue. Figure 1 shows the rate of oxygen uptake as a function of the concentration of the spin trap α-phenyl N-tert-butylnitrone[6] (PBN). Since the lifetime of $^1O_2$ increases from 2 μs in H_2O to 20 μs in D_2O,[7] there should exist a region in the PBN concentration where this lifetime difference is manifest in relative rates of O_2 uptake for D_2O vs. H_2O (if $^1O_2$ is involved). Figure 1 shows that indeed the rate is increased when H_2O is replaced with D_2O and that a value of (rate D_2O)/(rate H_2O) of 10 is approached. This is consistent with the participation of $^1O_2$ in a chemical reaction wiith PBN.

Another method of demonstrating the participation of $^1O_2$ in a reaction is to determine whether or not the rate of the reaction is reduced upon the addition of a physical quencher of $^1O_2$. Sodium azide (N_3^-) was selected for these experiments and, when introduced to this system, resulted in a reduction in O_2 uptake. Foote[8] has shown that a constant intercept in a plot of $[AO_2]^{-1}$ vs. $[A]^{-1}$ (where A in this case is PBN) at various N_3^- concentrations can be interpreted as proof that no triplet quenching by the N_3^- occurs. In our experiments (Figure 2) this was true up to an [N_3^-] of 1 × 10^{-3}M. However, at an N_3^- concentration of 4 × 10^{-3} M a slightly higher intercept was observed. Nevertheless, using results obtained for the constant-intercept concentration range allowed the calculation of the rate constants for both azide

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(6) Janzen, E. G. *Acc. Chem. Res.* **1971**, *4*, 31–40.

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Table I. Results for Nitrone Spin Traps

spin trap	rate constants, $M^{-1}\ s^{-1}$	oxygen evolution ratio[11]	spin adduct formation
PBN	1.4×10^8	0.33	none
4-PyBN	1.4×10^8	0.30	none
4-PyOBN	1.2×10^8	0.32	$<10^{-7}$ M $a^N = 15.0$ G; $a_\beta^H = 1.7$ G
4-MePyBN	8.0×10^7	0.30	$<5 \times 10^{-7}$ $a^N = 14.8$ G; $a_\beta^H = 1.5$ G
2-SSPB	9.4×10^7	0.30	none
DMPO	1.8×10^7		$<10^{-7}$ M $a^N = 14.9$ G; $a_\beta^H = 14.9$ G

quenching, K_Q, and for the reaction of $^1O_2$ with PBN, K_A.[8] K_Q was determined to be $1.5 \times 10^9\ M^{-1}\ s^{-1}$, which can be compared to the literature value of $2 \times 10^9\ M^{-1}\ s^{-1}$.[9] K_A was found to be $1.4 \times 10^8\ M^{-1}\ s^{-1}$. Hence these quenching experiments confirm that PBN does undergo a chemical reaction with $^1O_2$ in water. No comparatively significant reaction occurred between methylene blue and PBN in the absence of oxygen or between methylene blue and N_3^- in the presence of oxygen upon illumination.

Table I lists the various nitrones which we have now studied.[10] The rate constants were obtained by measuring the relative rates under identical conditions and using the above-determined value of K_A for PBN. The spin trap DMPO gives a K_A almost 1 order of magnitude less than PBN. Thus, in selecting a nitrone for use as a "spin trap" for ESR studies in systems thought to contain $^1O_2$, DMPO would be a better choice.

It is also important to know whether the product of a $^1O_2$ and nitrone reaction leads to radical adducts, as this could lead to false conclusions in experiments whose objectives are the identification of radical intermediates. Moreover, it could lead to a diagnostic test for $^1O_2$ if a certain radical adduct is formed. Our experiments show, in fact, that extremely small quantities of spin adducts are generated with certain traps, whereas others lead to no observable adducts (Table I). In those cases where radical adducts were observed, the radical concentrations were 3 orders of magnitude less than the concentrations of either O_2 or PBN consumed. Nevertheless, the high sensitivity of ESR allows detection in these cases and misinterpretation is possible. For this reason, the splitting constants for the adducts where observed are also given in Table I. These splittings correspond to the ·OH adduct of each of the spin traps.[1,12,13] Therefore, formation of the ·OH adduct at very low concentrations could reflect $^1O_2$ generation rather than ·OH production. The ESR experiments used 1×10^{-4} M methylene blue and 1×10^{-2} M spin trap under illumination conditions similar to those for the uptake results.

The reaction product of $^1O_2$ with nitrones is unknown. However, addition of catalase to the system after illumination resulted in the evolution of O_2 and accounts for 30% of the oxygen consumed. Optical studies revealed that 7.8% PBN was consumed (monitoring decay at λ 290 nm) whereas the calculated percentage loss should have been 14%, using the measured oxygen uptake determined for the same sample. Since the product may have absorbance at this wavelength, a value of less than 14% would be expected.

This study reveals that nitrone spin traps do react chemically with $^1O_2$ with a significant rate (K_A for PBN = 1.4×10^8 M^{-1} s^{-1}) and lead to predominantly diamagnetic product(s). The technique of oxygen uptake offers a method of continuous monitoring of the chemical reactions of $^1O_2$ in aqueous medium. The

use of this same probe for pigment photochemistry in nonaqueous systems[14] suggests that this technique could be extended to virtually all solvent systems. Clearly, nitrone spin traps can be used (in conjunction with both ESR and O_2 uptake) for the detection of ·OH, O_2^- and $^1O_2$ in water. However, experimenters should be aware of the problems which can arise. In particular, if $^1O_2$ is simultaneously being produced in the systems, a rapid depletion of dissolved O_2 can occur and this may prevent a normal radical reaction which would have prevailed if the trap were absent.

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Evidence for an Electron-Transfer Mechanism in the Reduction of Ketones by Main-Group Metal Hydrides

Sir:

The reduction of ketones by hydrogen transfer from main-group metal hydrides is one of the most widely used synthetic methods in organic chemistry.[1] The mechanism of such hydrogen transfer is generally considered to be of a polar nature.[2,3] Recent reports from our laboratory and others have shown the importance of electron-transfer (ET) mechanisms in describing the alkylation of ketones by main-group organometallic compounds. In view of our results showing that magnesium alkyls react with ketones by an electron-transfer process,[4] we decided to study the reactions of ketones with main-group metal hydrides in order to determine if hydrogen is transferred in a manner similar to the alkyl groups when metal hydrides are allowed to react with ketones.

The kind of probe used to detect an electron-transfer process in the reaction of main-group metal alkyls with ketones involves cyclization[4] or isomerization of the intermediate alkyl radical (A)[5] or isomerization of a radical anion (B)[6] (eq 1). Since it is not possible to have H· as a probe in cases involving the reactions of metal hydrides with ketones, the intermediate ketyl itself has been used as the probe (eq 2).

Normally k_2 is much greater than k_1 such that detection of the intermediate ketyl would not be possible; however, if sterically hindered ketones are employed, the electron-transfer step (k_1) should not be significantly affected, yet k_2 should be greatly decreased. Of course, the hope is that k_2 can be made to be less than k_1, in which case the ketyl could be observed spectroscopically and its disappearance with time recorded. Here we present the results of our studies, which clearly demonstrate the involvement of an electron-transfer mechanism in the reduction of ketones by main-group metal hydrides.

In the present studies, we have found that deep colored solutions are formed when dimesityl ketone (DMK) and metal hydrides such as AlH_3, BH_3, MgH_2, $HMgX$ (where X = Cl or Br) are allowed to react in THF. These highly colored solutions are EPR active, suggesting them to be paramagnetic in nature. In particular, when an equimolar amount of AlH_3[7] and DMK are mixed

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