neighboring n,π^* are not far apart in a planar configuration as shown by our calculations (Tables I and III).

Further support for the lowest triplet energy level of 12-MBAQ and 7,12-DMBAQ being n,π^* may be derived from the experimental S_1–T_1 splitting energy values. The magnitude of splitting is low for the latter quinones ($\sim$1000 cm^{-1}), which is of the order of splitting for $S_{(n,\pi^*)}$–$T_{(n,\pi^*)}$ in quinones. For the other quinones, this splitting is 2–3-fold greater. Also, the substantial red-shift in the visible absorption bands (Figure 1) of 12-MBAQ and 7,12-DMBAQ may be due to the consequences of the distortion discussed earlier. Finally, it is easy to explain the behavior of the quinone 7-MBAQ being very similar to the unsubstituted quinone. Here the methyl substitution is far away from the bay region and very probably has no consequence on the planarity of the ring structure.

Registry No. 1a, 74877-25-1; **1b**, 111238-10-9; **1c**, 71989-02-1; **1d**, 70092-13-6.

Chemistry of Singlet Oxygen. 51. Zwitterionic Intermediates from 2,4-Hexadienes

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Abstract: The three isomeric 2,4-hexadienes give nearly identical product distributions with singlet oxygen. Singlet oxygen causes rapid interconversion of the isomers, and methoxy hydroperoxides are formed from all three dienes in methanol (*E,Z* and *Z,Z* ~25%, *E,E* <10%). These observations are explained by intermediate zwitterions that revert to isomerized dienes in competition with collapse to products or capture by methanol.

Singlet oxygen undergoes Diels–Alder [2 + 4] addition to dienes to give endoperoxides, "ene" reaction with olefins with abstractable allylic hydrogens to give allylic hydroperoxides, and [2 + 2] addition to give dioxetanes.[1] Although details of the reactions are still unclear and numerous mechanisms have been proposed, the Diels–Alder process is widely believed to be a simple concerted reaction. However, methanol adducts formed in the reaction of singlet oxygen with structurally restricted dienes such as 2,4-dimethyl-2,4-hexadiene[2] and indenes[3] provide evidence for nonconcerted pathways involving polar or biradical intermediates. We now report evidence for such intermediates in the reaction of singlet oxygen even with simple dienes.

Results and Discussion

The (*E,E*)-, (*E,Z*)-, and (*Z,Z*)-2,4-hexadienes were photooxidized at −78 °C in CD_2Cl_2 with tetraphenylporphine as sensitizer. The *E,E* isomer gives the expected *cis*-endoperoxide (A) as the major product (Scheme I). However, the *E,Z* isomer also yields the *cis*-endoperoxide as the major product as Gollnick reported.[4] The product distribution from the *Z,Z* isomer is nearly identical with that of the *E,Z* isomer. All three dienes also give a complex mixture of hydroperoxides, acetaldehyde, and *cis*- and *trans*-2-butenal (Table I).

The aldehyde products apparently come from cleavage of dioxetanes. However, attempts to observe dioxetanes or other reactive intermediates by NMR were unsuccessful at −80 °C, even using filtered light (>450 nm), which left the product distribution unchanged. Pure *cis*-endoperoxide (A) was isolated and characterized by several spectroscopic techniques, including DEPT and 2-D NMR experiments (Figure 1). The *trans*-endoperoxide was characterized as a mixture with the cis (Figure 2). The IR and mass spectral data are in accord with those reported by Gollnick[4] and Matsumoto.[5] The *cis*-endoperoxide exhibits temperature-dependent proton and carbon NMR spectra, indicating slow half-chair–half-chair interconversion,[6] coalescing at −50 °C.

The doubly allylic hydroperoxides decompose at room temperature or upon attempted chromatography and were therefore characterized by NMR in product mixtures. The ^{1}H NMR spectra have peaks whose chemical shifts and relative areas are consistent with the assigned structures (broad proton singlets at 8–9 ppm that disappear when methanol is added, complicated multiplets in the vinyl region, and doublets around 2.0 ppm); there are also corresponding peaks in the ^{13}C spectra.

Scheme I

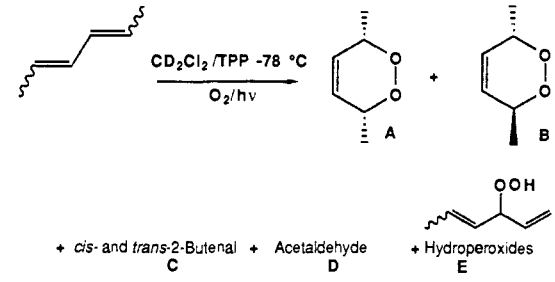

Table I. Products of Photooxidation of 2,4-Hexadienes

	product, %				
isomer	A	B	C	D	E
E,E	74	7	3	4	11
E,Z	46	12	12	8	23
Z,Z	42	10	12	11	25

(1) Review: A. A. Frimer In *Singlet Oxygen*; A. A. Frimer, Ed.; CRC: Boca Raton, FL, 1985; Vol II.
(2) (a) Manring, L. E.; Kanner, R. C.; Foote, C. S. *J. Am. Chem. Soc.* **1983**, *105*, 4707, 4710. (b) Gollnick, K.; Griesbeck, A. *Tetrahedron* **1984**, *40*, 3235.
(3) (a) Fenical, W.; Kearns, D. R.; Radlick, P. *J. Am. Chem. Soc.* **1969**, *91*, 2655, 3396, 7771. (b) Hatsui, T.; Takeshita, H. *Bull. Soc. Chem. Jpn.* **1980**, *53*, 2655.
(4) Gollnick, K.; Griesbeck, A. *Tetrahedron Lett.* **1983**, *24*, 3303.
(5) Matsumoto, M.; Dobashi, S.; Kuroda, K.; Kondo, K. *Tetrahedron* **1985**, *41*, 2147.
(6) $\Delta G^{\ddagger}$ ~10.5 kcal/mol. Similar behavior has been reported for an alkoxy-substituted endoperoxide: Clennan, E. J.; Nagraba, K. *J. Org. Chem.* **1987**, *52*, 294.
(7) Ogilby, P. R.; Foote, C. S. *J. Am. Chem. Soc.* **1983**, *105*, 3423.

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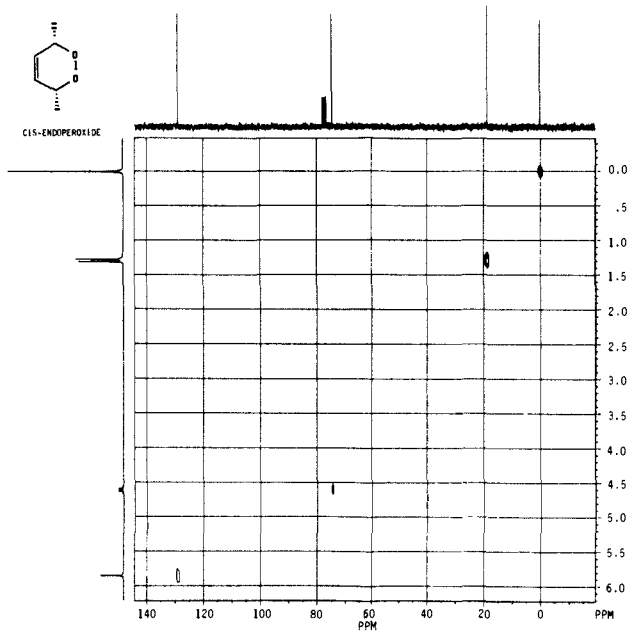


Figure 1. 2D NMR ^1H–^{13}C heteronuclear correlation of the *cis*-endoperoxide in $CDCl_3$.

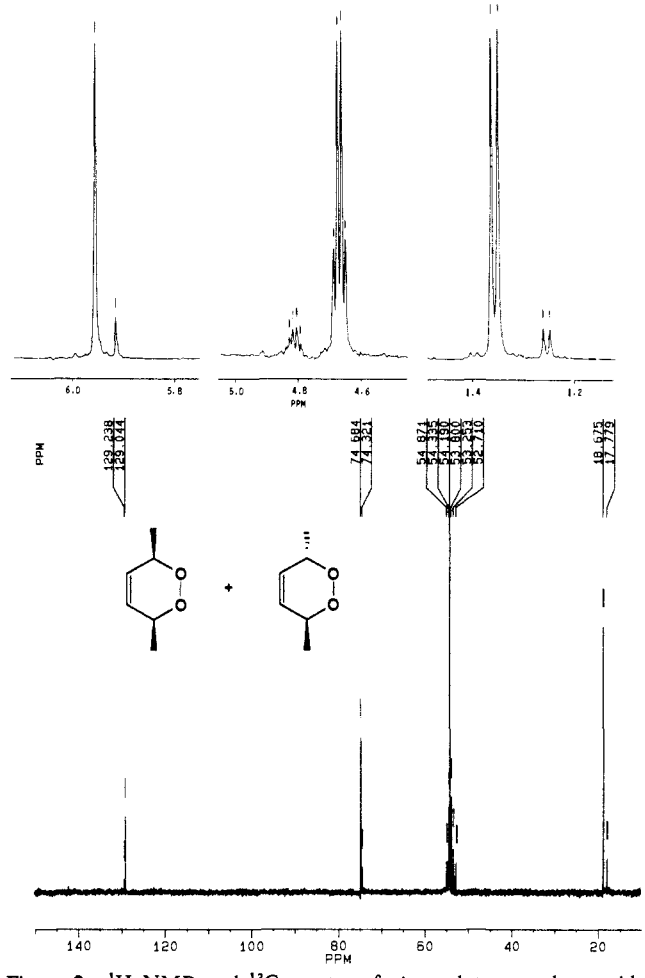


Figure 2. ^1H NMR and ^{13}C spectra of *cis*- and *trans*-endoperoxide mixture.

Rapid interconversion of the dienes during photooxidation was observed both by NMR and gas chromatography. The time course (NMR) is shown in Figure 3. Hexadienes and TPP in CD_2Cl_2 were photolyzed to completion at −78 °C. The *E,E* isomer gave a small percentage of the *E,Z* isomer, but the *Z,Z* isomer (which

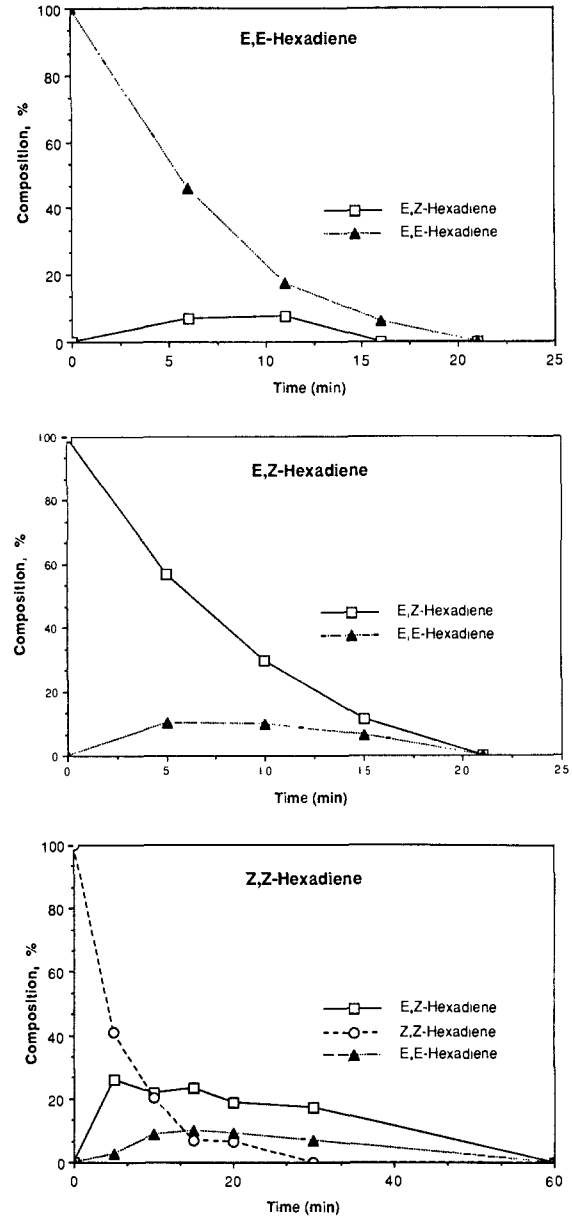


Figure 3. Time course of photooxidation and isomerization of the 2,4-hexadienes (measured by NMR). Reaction conditions are given in text.

is roughly 5 kcal higher in energy than the others[8]) was not formed from the other isomers.

Control experiments showed that the rates and product distributions do not change with different sensitizers or when the radical-chain inhibitor 2,6-di-*tert*-butylphenol is added. The dienes do not isomerize in the absence of light, oxygen, or sensitizer, and both the rate of photooxidation and isomerization increase in going from protiated to deuteriated solvent.[9] These results are consistent with the direct involvement of singlet oxygen in the isomerization. Isomerization of (*E,Z*)-hexadiene was reported by Gollnick,[4] but direct evidence for the formation of the *E,E* isomer was not given, no products other than the endoperoxides were observed, and no isomerization of the *E,E* isomer was reported.

In methanol, the rates of both isomerization and disappearance of the *E,Z* and *Z,Z* isomers are much lower than in methylene chloride, and both the relative amount of endoperoxide and the cis/trans endoperoxide ratio decrease. Substantial amounts (~ 25% from *E,Z* and *Z,Z*, isomers, <10% from *E,E*) of new products

(8) Jensen, F.; Foote, C. S. *J. Am. Chem. Soc.* **1987**, *109*, 6376.
(9) The longer lifetime of singlet oxygen in deuteriated than protiated solvents is well documented but not completely understood. See: D. R. Kearns In *Singlet Oxygen*; Wasserman, H. H.; Murray, R. W., Eds.; Academic: New York, 1979.

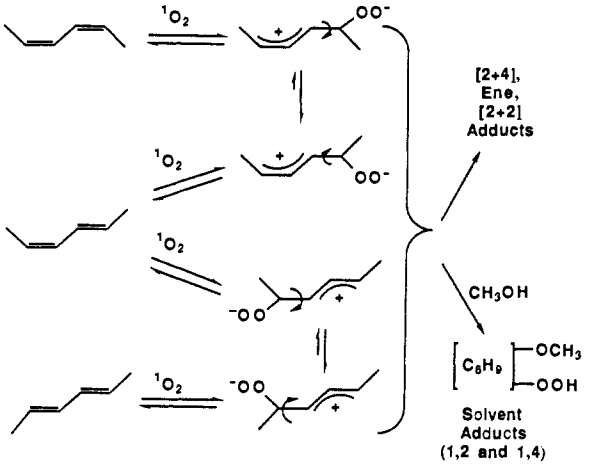
Table II. ^1H and ^{13}C NMR Chemical Shifts of the Endoperoxides and Dioxetane Cleavage Products (in ppm)

	endoperoxides				
	cis- (A)	*trans*- (B)	(*E*)-butenal	(*Z*)-butenal[a]	acetaldehyde
H(1)	1.35 (6 H, d)	1.25 (6 H, d)	9.50 (1 H, d)	10.10 (1 H, d)	9.79 (1 H, d)
H(2)	4.66 (2 H, q)	4.81 (2 H, q)	6.14 (1 H, m)	5.86 (1 H, m)	2.21 (3 H, d)
H(3)	5.96 (2 H, s)	5.92 (2 H, s)	6.90 (1 H, m)	6.74 (1 H, m)	
H(4)			2.03 (3 H, dd)	2.13 (3 H, d)	
C(1)	18.7	17.8	193.4		199.4
C(2)	74.7	74.3	134.4		30.7
C(3)	129.0	129.2	153.5		
C(4)			18.8		

[a] Low concentration prevented the measurement of the ^{13}C spectrum of (*Z*)-butenal.

are also formed. These products are also too unstable to isolate, but their NMR spectra are consistent with mixtures of isomeric methoxy hydroperoxides (several new peaks 3.5–3.7 and 9.5–10.5 ppm in the ^1H and 50–60 ppm in the ^{13}C spectra). These compounds appear to be analogous to the 1,2- and 1,4-adducts formed from 2,5-dimethyl-2,4-hexadiene in methanol.[2,3]

The formation of methanol adducts suggests a polar intermediate. Such adducts from more complex dienes have been interpreted as arising from perepoxides or zwitterions.[2,3,10] A perepoxide alone could not explain the isomerization, which requires an intermediate that can rotate around the former double bond. Zwitterionic intermediates provide the most likely explanation, both for the similarity in product distributions from the isomeric hexadienes and for the formation of solvent adducts. However, the ene products cannot be derived from the ions shown.



Zwitterionic intermediates have been proposed in the reactions of other electron-poor dienophiles such as triazolinediones[8,11] and tetracyanoethylene[12] with substituted butadienes. Methanol adducts are also formed form (*Z,Z*)- [and to a lesser extent, (*Z,E*)-] hexadiene with phenyl triazolinedione (PTAD) and were similarly rationalized.[4] However, diene isomerization was not observed with PTAD. Diazetidines appear to be secondary intermediates with PTAD and rearrange to [2 + 4] adducts. However, there is no evidence that analogous dioxetanes rearrange to endoperoxides in the hexadiene series. Diradical intermediates in the isomerization cannot be ruled out, but would not account for the solvent adducts. Calculations show that 1,4-biradicals and zwitterions are not fundamentally different, and substituents dictate the electron density at each center.[13]

Polarity, viscosity, and temperature effects as well as details of the reaction kinetics are being investigated.

Experimental Section

Materials. (*Z,Z*)-2,4-Hexadiene was from Wiley Organics; (*E,Z*)- and (*E,E*)-2,4-hexadiene were from Aldrich Chemical Co. All had isomeric purities of >98%. The isomeric purity was verified by gas chromatography on a Hewlett-Packard 5880A GC equipped with a 30-m DB-17 capillary column. With helium as the carrier gas, a head pressure of 0.5 psi and an oven temperature of 40 °C, the dienes were easily separated. NMR solvents methanol-d_4 and methylene-d_2 chloride were from Cambridge Isotope Laboratories. Tetraphenylporphine (TPP), mesoporphyrin IX dimethyl ester (MP IX), and rose bengal (RB), all from Aldrich, were used as sensitizers. Typical sensitizer solutions contained 1 mg of TPP/5 mL of methylene chloride, 2 mg of MPIX/50 mL of Freon 11, or 1.5 mg of RB/5 mL of methanol.

Reaction Conditions. Low-temperature photooxidations were performed by use of a vacuum-jacketed pipeline with temperature control with an accuracy of ±2.0 °C.[7] The sample, containing 25 μL of diene, 1.0 mL of sensitizer solution and 0.01% TMS, was placed in a 5-mm NMR tube and photolyzed at −78 °C with a 300-W Varian-Eimac xenon lamp. Oxygen, dried over KOH and Drierite, was continuously bubbled through the sample during photolysis. For the diene isomerization experiments, the reaction solutions (described above) were photooxidized at −78 °C for a given time interval. At the end of each interval the sample was allowed to warm to room temperature before ^1H NMR and gas chromatography.

Isolation and Characterization. A 200-μL aliquot of (*E,E*)-2,4-hexadiene and 5 mL of the Freon 11/MP IX sensitizer solution were pipeted into a 13 × 100 mm test tube. The solution was photooxidized at −78 °C until no diene was detectable by gas chromatography. Small amounts of sensitizer solution (0.5 mL) had to be added during the reaction due to bleaching of the dye. The solution was rotary evaporated to remove solvent and other volatile components such as acetaldehyde and butenal. The remaining solution was placed under a vacuum at 0.75 mmHg until most of the residue had been passed into a trap cooled by a dry ice/acetone bath. The trapped solution was >98% *cis*-endoperoxide by gas chromatography. A clean mixture of *cis*- and *trans*-endoperoxide was similarly isolated from the photooxidation of (*E,Z*)-2,4-hexadiene. Other compounds were characterized as components of a mixture. ^1H, ^{13}C, and 2-D NMR were used to assist in the assignments. NMR spectra were recorded on Bruker AM-500 MHz and AF-200 MHz instruments. Chemical shifts are given (in ppm) relative to TMS. NMR integration was used to measure relative product percentages.

***cis*-Endoperoxide**: isolated as a clear, yellowish liquid in 98% purity: see Table II for ^1H and ^{13}C data, IR (film) 3010, 2900, 1450, 1380, 1100, 1040 cm^{-1}; MS, *m/z* 114 (M^+), 99, 82 (base). Lit.[5]: ^1H NMR, 1.22 (6 H, d), 4.46 (2 H, q), 5.77 (2 H, s) ppm; IR, 1040, 1017 cm^{-1}; MS, *m/z* 114 (M^+), 96, 82 (base), 67, 43.

***trans*-Endoperoxide**: isolated and characterized as a mixture with the *cis*-endoperoxide. See table for ^1H and ^{13}C data.

(*E*)-Butenal: lit.[14]: ^1H NMR 10.60, 5.99, 6.85, 1.93 ppm. The peak assignments do not agree with ours. Results obtained from an authenic sample were identical with those we obtained from the reaction mixtures (See Table II).

(*Z*)-Butenal. See Table II for ^1H and ^{13}C data. Lit.[14]: ^1H NMR 10.04, 5.81, 6.64, 2.10 ppm; are inconsistent with our assignments (see above). Decoupling experiments unambiguously support our assignments.

Acetaldehyde. GC and NMR with authenic samples were identical with those obtained for the reaction mixtures (see Table II).

(10) (a) Zhang, J.; Foote, C. S. *Tetrahedron Lett.* **1986**, *27*, 6153. (b) Burns, P. A.; Foote, C. S. *J. Am. Chem. Soc.* **1974**, *96*, 4339. (c) Hasty, N. M.; Kearns, D. R. *J. Am. Chem. Soc.* **1973**, *95*, 3380.

(11) (a) Clennan, E. L.; Earlywine, A. D. *J. Am. Chem. Soc.* **1987**, *109*, 7104. (b) Cheng, C. C.; Seymour, C. A.; Petti, M. A.; Greene, F. D.; Blount, J. F. *J. Org. Chem.* **1984**, *49*, 2910. (c) Seymour, C. A.; Greene, F. D. *J. am. Chem. Soc.* 1980, *102*, 6384. (d) Orfanopoulos, M.; Foote, C. S.f Smonou, I. *Tetrahedron Lett.* **1987**, *28*, 15. (e) Nelsen, S. F.; Kapp, D. L. *J. Am. Chem. Soc.* **1985**, *107*, 5548.

(12) (a) Kataoka, F.; Shimizu, N.; Nishida, S. *J. Am. Chem. Soc.* **1980**, *102*, 711. (b) Kataoka, F.; Nishida, S.; Tsuji, T.; Marakami, M. *J. Am. Chem. Soc.* **1981**, *103*, 6878. (c) Fatiadi, A. J. *Synthesis* **1987**, 749.

(13) Salem, L.; Rowland, C. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 92.

(14) McGreer, D. E.; Page, D. B. *Can. J. Chem.* **1969**, *47*, 866.

Hydroperoxides. Although the hydroperoxides have been isolated as a mixture of isomers, complete characterization was not possible because of decomposition. Strong evidence in support of their structure comes from NMR analysis: ^{1}H NMR, 8.15, 8.10, and 7.92 ppm (br s) consistent with hydroperoxidic protons; 6.76, 6.30, 5.82, 5.50, and 5.32 ppm (presumably vinylic protons with very complex splitting patterns); 4.80 and 4.54 ppm (allylic protons α to oxygen); 1.73 and 1.26 ppm (alkyl protons, multiplicities could not be established because of overlapping peaks). ^{13}C NMR, peaks are also consistent with the assigned structure; 138–126 ppm (substituted olefinic carbons), 119.7 and 119.4 ppm (terminal alkene carbons); 87–80 ppm (oxygen-substituted allylic carbons); 30–18 ppm (alkyl carbons). Appropriate vinyl–vinyl, vinyl–allyl, and vinyl–alkyl couplings appear in the 2-D COSY (^{1}H–^{1}H) NMR at −20 °C.

Acknowledgment. Supported in part by NSF Grant No. CHE86-11873.

Registry No. A, 88078-74-4; B, 88078-75-5; *cis*-C, 15798-64-8; *trans*-C, 123-73-9; D, 75-07-0; E, 116053-72-6; (*E*,*E*)-2,4-hexadiene, 5194-51-4; (*E*,*Z*)-2,4-hexadiene, 5194-50-3; (*Z*,*Z*)-2,4-hexadiene, 6108-61-8.

Photolabile 1-(2-Nitrophenyl)ethyl Phosphate Esters of Adenine Nucleotide Analogues. Synthesis and Mechanism of Photolysis

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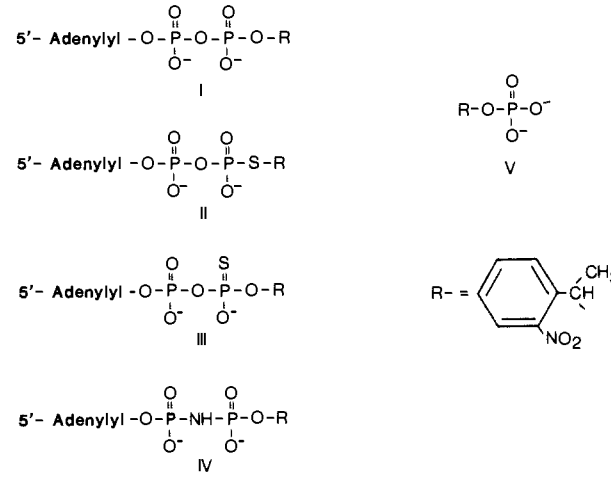
Contribution from the National Institute for Medical Research, Mill Hill, London NW7 1AA, United Kingdom. *Received March 30, 1987*

Abstract: A general method is described for preparing photolabile 1-(2-nitrophenyl)ethyl esters of phosphate and thiophosphate compounds. The method is based on selective alkylation of weakly ionizing phosphate groups by a new alkylating agent, 1-(2-nitrophenyl)diazoethane. ATP and the widely used structural analogues of ATP, 5′-adenylyl imidodiphosphate (ATP(β,γNH)) and adenosine 5′-(3-thiotriphosphate) (ATP(γS)), were alkylated on the terminal (γ) phosphate group. ATP(γS) was alkylated on oxygen or on sulfur in approximately equal amounts. Photolysis of P^3-1-(2-nitrophenyl)ethyladenosine 5′-triphosphate, commonly called "caged ATP", was analyzed spectroscopically at pH values close to neutral in aqueous solvents by use of laser pulse photolysis. The kinetics of formation of the three products, ATP, 2-nitrosoacetophenone, and H^+, were each monitored, as well as the kinetics of formation and decay of an intermediate presumed to be an *aci*-nitro compound (apparent ϵ_{406nm} = 9.1 × 10^3 M^{-1} cm^{-1}). For caged ATP in the presence of 3 mM $MgCl_2$, the *aci*-nitro intermediate and H^+ formed first at >10^5 s^{-1} followed by the decay of the intermediate at 86 s^{-1} at pH 7.1, 21 °C, and ionic strength 0.18 M. ATP, monitored by a biochemical assay, and 2-nitrosoacetophenone, monitored by a characteristic absorption band at 740 nm, were formed simultaneously with the decay of the intermediate under all conditions tested. The rate of decay of the *aci*-nitro intermediate was therefore used as a measure of the rate of release of the nucleotide analogues from their photolabile precursors. At pH 7.1, 0.18 M ionic strength, and 21 °C the rate constants ranged from 35 to 250 s^{-1} and displayed a similar dependence on pH as caged ATP. The steady-state quantum yields of the 1-(2-nitrophenyl)ethyl phosphate esters were in the range 0.49–0.63. The deleterious effect of 2-nitrosoacetophenone on biological materials can be avoided by having thiols present. The reaction kinetics of dithiothreitol and 2-nitrosoacetophenone was described by a two-step process, the first step having a rate constant of 3.5 × 10^3 M^{-1} s^{-1} and the second 45 s^{-1} at pH 7.0, 21 °C, and ionic strength 0.18 M.

An emerging technique in biochemical research is the controlled release of substrates within intact biological systems by rapid photolysis of photolabile precursors (reviewed by Gurney and Lester[1]). One of the first photolabile precursors of an important biological substrate, ATP, was the 1-(2-nitrophenyl)ethyl P^3-ester of ATP, I, commonly termed "caged ATP", that on photolysis releases ATP at 220 s^{-1} at pH 7 and 21 °C.[2] This property of caged ATP together with the high-energy output and time resolution afforded by lasers have permitted a range of physiological experiments where diffusional delays of ATP may be overcome by the use of caged ATP. For example, the mechanism of force transduction in muscle has been studied extensively by this approach[3] where up to 77% conversion of caged ATP to form several millimolar ATP can be achieved in a single laser pulse.[4]

2-Nitrobenzyl photochemistry has provided the basis for kinetic studies of other physiological systems. Variations on the caged ATP theme include its application to cyclic nucleotides,[5] protons,[6] Ca^{2+},[7] carbachol,[8] *myo*-inositol 1,4,5-triphosphate,[9] and photosensitive Ca^{2+} channel blockers.[10] However, a major limitation to a more general application of this approach to a variety of regulatory cellular processes has been the considerable time required for synthesis and characterization of new compounds. We



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(1) Gurney, A. M.; Lester, H. A. *Physiol. Rev.* **1987**, *67*, 583–617.

0002-7863/88/1510-7170$01.50/0