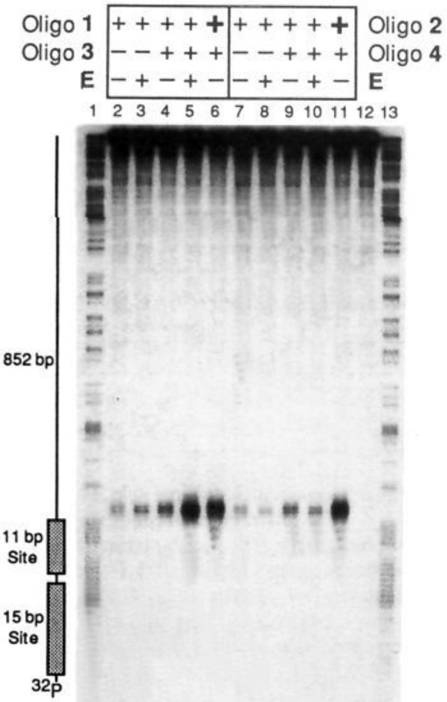

Figure 2. Autoradiogram of a denaturing polyacrylamide electrophoresis gel of reaction products from affinity cleavage reactions with the oligonucleotides described below and a ^{32}P-labeled restriction fragment from pMD5556 containing the target site shown in Figure 1. The radiolabeled restriction fragment was prepared by digestion of pMD5556 with *Eco*RI followed by incubation with [α-^{32}P]dATP and [α-^{32}P]TTP in the presence of Sequenase. Digestion with *Xmn*I and subsequent gel purification of the 852-bp fragment yielded the DNA used in the experiments below. Affinity cleavage reactions were performed by preincubating the desired oligonucleotides together with Tris–acetate, pH 7.0 (25 mM), NaCl (10 mM), spermine (1 mM), $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (2 μM), sonicated calf thymus DNA (100 μM in bp), MeOH (10% v/v), 30000 cpm ^{32}P-labeled DNA, and 50 μM echinomycin (E), where noted below, in a volume of 40 μL at 37 °C for 1 h. The cleavage reactions were initiated by addition of dithiothreitol (4 mM, final concentration) and allowed to react for 6 h at 37 °C, at which time they were terminated by NaOAc/EtOH precipitation. The samples were washed with 70% EtOH, dissolved in H_2O, dried in vacuo, dissolved in formamide–TBE loading buffer, heated to 95 °C for 3 min, and subjected to electrophoretic separation on an 8% denaturing polyacrylamide gel (19:1, monomer:bis). Except where noted below, oligonucleotides **1** and **2** are used at 100 nM, oligonucleotides **3** and **4** at 1 μM, and E at 50 μM. Lanes 1 and 13, adenine-specific sequencing reaction;[13] lane 2, oligonucleotide **1**; lane 3, **1** with E; lane 4, **1** and **3**; lane 5, **1** and **3** with E; lane 6, **1** (1 μM) and **3**; lane 7, **2**; lane 8, **2** with E; lane 9, **2** and **4**; lane 10, **2** and **4** with E; lane 11, **2** (1 μM) and **4**; lane 12, no oligonucleotide.

and cleaves as well as **1** under similar conditions in spite of its insensitivity to E (lane 6).

To determine the magnitude of ligand-mediated control, the quantitative affinity cleavage titration[11] was used to obtain binding isotherms and equilibrium binding constants for **1** alone, **1** in the presence of 1.0 μM **3**, and **1** in the presence of 1.0 μM **3** and 50 μM E (Figure 3). These experiments yielded values of $(3.0 \pm 0.4) \times 10^5$, $(1.3 \pm 0.2) \times 10^6$, and $(4.1 \pm 1.4) \times 10^6$ M^{-1}, respectively. This analysis demonstrates that the 5-bp dimerization domain, created by the addition of **3**, increases the affinity of **1** for its site 4.3-fold. Addition of echinomycin to the dimeric **1**,**3** system further enhances the binding of **1** by a factor of 3.2. We conclude that ligand-mediated control of dimerization in cooperatively binding oligonucleotides to single sites on double helical DNA is attainable, and we envision the design of further ligand-promoted intermolecular nucleic acid assemblies.

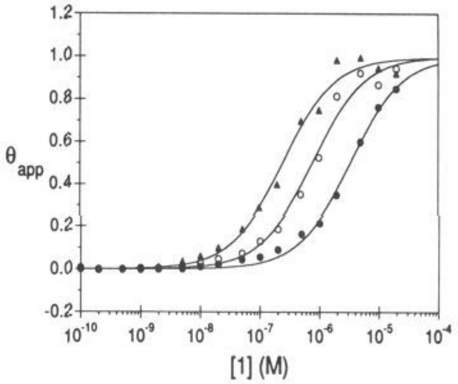

Figure 3. Binding isotherms obtained for oligonucleotide **1** alone, in the presence of 1.0 μM **2**, or in the presence of 1.0 μM **2** and 50 μM E, using the quantitative affinity cleavage titration method.[11] These experiments were performed as described in Figure 2 with the modifications described below. In each experiment, [**1**] was varied from 100 pM to 20 μM and the [$Fe(NH_4)_2(SO_4)_2$] was varied such that the [Fe(II)]/[**1**] ratio was fixed at 2.0. Reactions were preincubated for 24 h prior to initiation with DTT. After electrophoresis, the dried gels were exposed to photostimulable storage phosphorimaging plates, the plates were visualized using a Molecular Dynamics 400S PhosphorImager, and the cleavage and background intensities for each reaction were obtained. A theoretical binding curve, $I_{\text{fit}} = I_{\text{sat}}K[\mathbf{1}]/(1 + K[\mathbf{1}])$, where I_{sat} is the apparent maximum cleavage intensity and K the equilibrium association constant, was used to fit the experimental data (I_{exp}) using I_{sat} and K as adjustable parameters.[11] At least three complete data sets were used to determine each association constant. For the data shown above, I_{exp} was divided by I_{sat} to obtain θ_{app} (fractional saturation). Data for **1** alone is shown by ●, **1** and **3** by ○, and **1**, **3**, and echinomycin by ▲; each data point represents the average of three or four individual measurements.

Acknowledgment. This work was supported by the National Institutes of Health (GM-35724) and a Damon Runyon–Walter Winchell Cancer Research Fund postdoctoral fellowship for M.D.D.

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Quantum Mechanical Tunneling in the Ene Reaction of Triazolinedione with Sterically Hindered Alkenes

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The reaction of *N*-phenyl-1,2,4-triazoline-3,5-dione (PTAD) with alkenes recently has received considerable mechanistic attention.[1] For example, recent stereoisotopic studies and direct spectroscopic observations[2] of this reaction have established the formation of an aziridinium imide intermediate.[1a,d,e,g]

In the present communication we present results that show hydrogen tunneling in the ene reaction of PTAD with tetramethylethylene-d_6 (TME-d_6, **1**) and 2,2,7,7-tetramethyl-4-*cis*-

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[2] (a) Squillacote, M.; Mooney, M.; De Felippis, J. *J. Am. Chem. Soc.* **1990**, *112*, 5365–66. (b) Nelsen, S. F.; Kapp, D. L. *J. Am. Chem. Soc.* **1985**, *107*, 5548–9.

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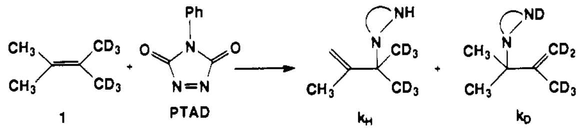
Table I. Temperature Dependence of k_H/k_D for the Ene Reaction of PTAD with TME-d_6 (**1**)

entry	solvent	temp, °C	k_H/k_D[a]
1	CCl_4	25	5.5
2		0	6.2
3		-20	9.4
4	$CHCl_3$	25	5.2
5		0	6.2
6		-20	8.8
7		-45	12.3
8		-50	12.8
9	CH_3CN	25	5.7
10		0	6.3
11		-20	8.7
12	CH_2Cl_2	25	5.8
13		0	6.3
14		-20	8.3
15		-40	10.0
16		-60	13.2
17	CH_3OH	25	5.6
18	CF_3CH_2OH	25	5.7
19		0	8.3
20	$CFCl_3$	-100	1.45[b]

[a] Determined by integration of the allylic methyl and geminal methyl hydrogens between 1.5 and 2.5 ppm on a Brucker 500-MHz spectrometer. The error was ±5%. [b] Reaction of TME-d_6 with singlet oxygen.

octene-*3,3*-d_2 (**2**). Although hydrogen tunneling has been reported for a large number of elimination and other reactions, to our knowledge this is the first case of tunneling observed for ene reactions.

As seen from Table I, the intramolecular (product) primary isotope effect of the reaction of PTAD with TME-d_6 (**1**) depends on the reaction temperature. For example, at room temperature the k_H/k_D isotope effect measured in carbon tetrachloride, chloroform, acetonitrile, dichloromethane, methanol, and trifluoroethanol is about 5.5 (entries 1, 4, 9, 12, 17, and 18). However, at lower temperatures (entries 3, 7, 11, 15, and 19), the ratio of k_H/k_D is abnormally large, and in some cases (entries 8 and 16), its value (k_H/k_D = 12.8 and 13.2) is more than double its value at room temperature.



To a first approximation, the maximum isotope effect corresponding to the loss of the zero-point energy difference for the C–H and C–D stretches in the transition state is calculated to be about 7.4 at room temperature. In our system the measured k_H/k_D values are substantially larger than 7 at low temperatures, and they decrease sharply with an increase in temperature. They are indicative of extensive hydrogen tunneling in the reaction.

Since the ratio of the Arrhenius preexponential factors A^H/A^D and the activation energy difference ΔE_A ($E_D - E_H$) are excellent indicators of tunneling, they were calculated from the intercept and the slope of the straight line obtained by the logarithmic plot of k_H/k_D as a function of T^{-1} (K^{-1}). The ratio of the preexponential factor A^H/A^D was found to be below unity and in some cases below 0.3 (Table II). In the absence of tunneling, transition-state theory predicts that A^H/A^D would be unity or close to it. Lower A^H/A^D ratios constitute the most reliable experimental evidence cited for hydrogen tunneling.[3b] Additional evidence of hydrogen tunneling in this reaction are the large activation energy differences included in Table II. In normal cases, the expected $E_A{}^D - E_A{}^H$ difference, arising from the loss in the TS of the zero-point energy for the stretching vibrations of the C–H and C–D bonds, is usually about 1.14 kcal/mol. In our system the calculated energy differences $E_A{}^D - E_A{}^H$ are considerably larger than 1.14 kcal/mol. Again such large differences have been interpreted as evidence for proton tuneling.[3b,c]

Table II. Arrhenius Preexponential A^H/A^D Ratios and Calculated Activation Energy Differences $E_A{}^H - E_A{}^D$ of the Ene Reaction of PTAD with **1**[a] and **2**[b]

entry	solvent	A^H/A^D	$E_A{}^D - E_A{}^H$ (kcal/mol)
1	CCl_4	0.30 ± 0.06	1.80 ± 0.35
2	$CHCl_3$	0.20 ± 0.03	1.70 ± 0.30
3	CH_2Cl_2	0.60 ± 0.10	1.30 ± 0.20
4	CH_3CN	0.20 ± 0.04	1.40 ± 0.30
5	CH_2Cl_2	0.20 ± 0.04	1.60 ± 0.30

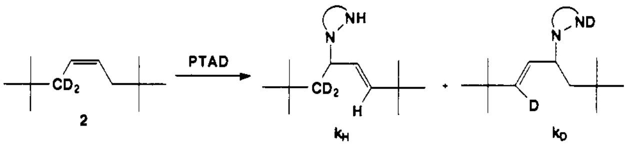
[a] Entries 1–4. [b] Entry 5.

Table III. Temperature Dependence of k_H/k_D for the Ene Reaction of PTAD with 2,2,7,7-Tetramethyl-4-*cis*-octene-*3,3*-d_2 (**2**)

solvent	temp, °C	k_H/k_D
CH_2Cl_2	25	3.2 ± 0.2
CH_2Cl_2	0	4.7 ± 0.2
CH_2Cl_2	-20	5.2 ± 0.3

Although PTAD mimics singlet oxygen chemistry,[1a,c] similar experiments with singlet oxygen have not showed any tunneling. The primary isotope effects measured earlier in the ene reaction of $^1O_2$ with TME-d_6 were temperature-independent and much smaller (k_H/k_D = 1.4)[5] than the present measured k_H/k_D. Even at −100 °C k_H/k_D = 1.45 (entry 20, Table I), a value which is similar to the one found at 0 °C (k_H/k_D = 1.4).

If the large steric interactions in the transition state of the reaction of PTAD with the crowded tetramethylethylene-d_6 (**1**) are responsible for the observed tunneling effect, then one would expect some hydrogen tunneling even in alkyl-disubstituted ethylenes. To test this possibility we prepared the crowded 2,2,7,7-tetramethyl-4-*cis*-octene-*3,3*-d_2 (**2**) in high stereochemical purity.[6] This substrate is ideally suited for this purpose, because it bears two large *tert*-butyl groups on each side of the double bond in a cis configuration. The k_H/k_D isotope effect of the ene reaction of PTAD with **2** in dichloromethane is given in Table III. It is evident that it depends strongly on temperature. As the temperature decreases from 25 to −20 °C, k_H/k_D increases from 3.1 to 5.2. This dependence is again a sign of quantum mechanical tunneling. It is noteworthy that the magnitude of the isotope effect at a single temperature appears to be normal, i.e., less than 7. In this case it is only its strong temperature dependence that points to tunneling. Furthermore, the calculated energy difference $E_A{}^H - E_A{}^D$ of 1.6 kcal/mol and the ratio of the Arrhenium preexponential factors A^H/A^D of 0.2 kcal/mol constitute strong evidence for proton tunneling (Table II, entry 5).



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(6) Alkene 2,2,7,7-tetramethyl-4-*cis*-octene-*3,3*-d_2 (**2**) was prepared by the following method: $LiAlD_4$ reduction of 2,2-dimethylpropanoic acid followed by bromination of the corresponding 2,2-dimethylpropanol-*1,1*-d_2 led to the 2,2-dimethyl-1-bromopropane-*1,1*-d_2. The Grignard reagent of the previous bromide was coupled with formylpiperidine[7] to yield 3,3-dimethylbutyraldehyde-*2,2*-d_2. Wittig coupling of the previous aldehyde with the corresponding ylide gave the cis configuration of olefin **2** in 90% isomeric purity. ^1H NMR: ($CDCl_3$) δ 5.52 (bt, 2 H), 1.92 (bd, *J* = 5.6 Hz, 2 H), 0.88 (s, 18 H).

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In summary, the isotope effects, the differences in the energies of activation, and the A^H/A^D ratios in the ene reaction of PTAD with alkenes **1** and **2** have been determined. These results are in agreement with substantial hydrogen tunneling in the title reaction.

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The First Direct Observation of a Phosphenite: IR, UV, and ^{31}P NMR Spectra of 2,6-Di-*tert*-butyl-4-methylphenyl Phosphenite

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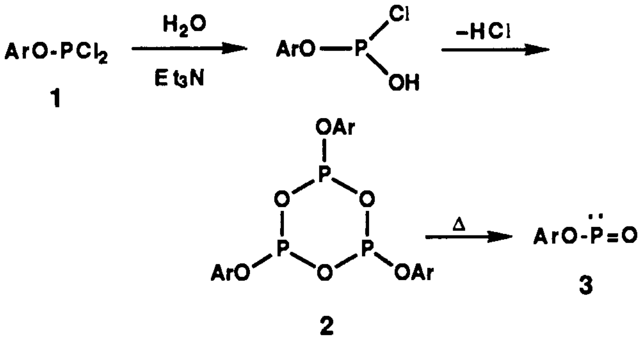
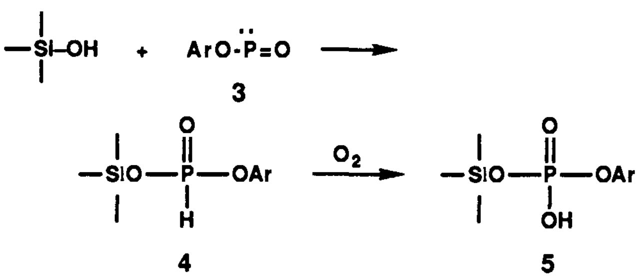
Even after the intense effort of recent years in the field of low-coordination phosphorus compounds, two-coordinate species with a p-p π-bond between phosphorus and oxygen remain a rarity;[1] except for the observation of some positive ions in mass spectra (PhO-PO,[2] Ph-PO,[3] **3**[4]), there is no record of an organic derivative bearing this feature that has been directly detected by spectroscopic techniques or that has been isolated. Evidence for the existence of such an organic compound is derived solely from trapping experiments.[1] In this communication, we report the infrared, ultraviolet, and ^{31}P NMR spectra of 2,6-di-*tert*-butyl-4-methylphenyl phosphenite (**3**), generated by the thermolysis of the cyclic trimer **2** (Ar = 2,6-di-*tert*-butyl-4-methylphenyl; prepared as reported[5] by partial hydrolysis of the sterically hindered aryl phosphorodichloridite **1**).



Chasar et al.[4] had previously sublimed **2** (220 °C, 1-1.2 kP$_a$) and obtained a sublimate from which the dimer of the phosphenite was isolated. This suggested that the monomeric phosphenite was present in the gaseous products from the pyrolysis, possibly deriving a measure of stabilization from the space demand of the O-substituent.

To prove the intermediacy of phosphenite **3** in the gas phase, we employed a technique that we have developed for detecting alkyl metaphosphates (RO–PO$_2$) in solution;[6] we exposed silica gel to the gaseous products with the expectation that the powerfully electrophilic[1] P=O group would attack the surface hydroxy groups as it attacks alcohols in solution and create the H-phosphonate group on the surface (**4**). We employed an evacuated (0.15 mm) Kugelrohr apparatus with the first bulb serving as the reactor for decomposing the solid trimer (0.29 g), the second as the reactor containing solid silica gel (1.2 g, Aldrich 70–230 mesh), the whole assembly being rotated inside the oven (250 °C) to keep fresh silica surface exposed to **3**. After 1 h, the silica gel was removed, washed three times with 2-propanol, and the CP-MAS ^{31}P NMR spectrum then recorded (Bruker 200 MHz, CaHPO$_4$ as reference). The major signal at δ −4 is consistent with the expected H-phosphonate **4**; the ethyl ester of the same H-phosphonate has δ +4, but as is typical of phosphates, it can be expected that the silyl group will cause an upfield shift of 8–10 ppm.[7] A small signal at δ −16 is attributed to the corresponding phosphate (**5**), since exposing the silica to oxygen enhanced this signal and reduced that for **4**. A small signal at δ +7 may be due to adsorbed ArOPH(O)OH from hydrolysis. We conclude that the gas phase did indeed contain the phosphenite **3**.



Trimer **2** was then thermolyzed at 250 °C and 10^{-5} mm in such a way as to allow deposition of **3** on a KBr window at 12 K in an APD Cryogenics Displex closed-cycle helium cryostat.[9] The infrared spectrum was recorded at 12 K and then at several temperatures up to 272 K. A strong band at 1235 cm^{-1} at 12 K is attributed to stretching of P=O in phosphenite **3**; values summarized[1] for phosphenous acid and its halo derivatives in argon matrices show a range from 1252.6 to 1292.2 cm^{-1}. The signal was noticeably diminished on reaching 150–200 K and essentially disappeared by 270 K. As it disappeared, a new strong signal appeared at 820 cm^{-1} assignable to P–O–P bending[9] as would be present in either the dimeric or trimeric condensation product of phosphenite **3**. That the low temperature was not in some way responsible for the observed spectral changes was shown by recooling the sample on the KBr window to 12 K, without effect on the IR spectrum.

A film of **3** was similarly deposited on a quartz plate in the Displex apparatus at 12 K, and the ultraviolet spectrum was recorded.[8] The spectrum consisted only of two equal-intensity bands at 271 and 278 nm. These signals disappeared by the time the temperature was raised to 272 K; the spectrum then resembled that of the pure trimer **2** in possessing only end absorption at 200

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[8] The Displex is equipped with a standard DMX-15 optical spectroscopy vacuum shroud. Suprasil windows were used on the shroud for UV–vis spectroscopy, KBr windows for IR spectroscopy. The pyrolysis reaction chamber consisted of a 15 cm long × 4 mm (i.d.) Pyrex tube with a bulb at one end, attached with epoxy resin to a stainless steel plate with a hole drilled in it to allow effusion of pyrolysis volatiles from the bulb through the plate. The reaction chamber, charged with about 30–50 mg of **2**, was attached in an orientation perpendicular to the optical windows of the DMX-15 shroud, placed over the Displex cooling stage and its attached spectroscopic sample holder, and the Displex cooling assembly then evacuated to <0.01 mTorr. The sample holder was then precooled to 11–12 K, the DMX-15 shroud was rotated so that the shroud plate holding the pyrolysis chamber faced the sample holder inside, and the chamber was suddenly heated to 250 °C by placement inside a preheated Kugelrohr oven. Heating was continued for about 60 s, then the heat source was removed, and the outer shroud rotated to align the sample holder optical disk with the shroud optical windows. A visually transparent film of neat material was deposited as a matrix on the cold sample window. Spectra (UV–vis by Shimadzu UV-260, IR by Perkin Elmer 1420) were subsequently acquired at 12 K and then at 50, 100, 200, and 270 K.

[9] For the dimer, P–O–P bending has been noted at 853 cm^{-1} (Nujol).[4]

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