in the presence of 7-azatryptophan, this *E. coli* strain showed roughly 20% of the β-galactosidase activity of bacteria grown in tryptophan (5.3 A_{420}/mg of protein vs 28.2 A_{420}/mg of protein[24,25]).

These data point to the importance, the versatility, and the usefulness of 7-azatryptophan as a probe of protein structure and dynamics.

Direct Measurement of Transition-State Bond Cleavage in Hydrolysis of Phosphate Esters of *p*-Nitrophenol

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We have found a simple technique to measure the degree of bond breaking in the transition state which should be applicable to any reaction where *p*-nitrophenol is the leaving group. The breaking of a bond to *p*-nitrophenol in a displacement reaction involves the development of delocalized negative charge in the phenol corresponding to the degree of bond breaking. The delocalization of this negative charge occurs through the contribution of a quinonoid resonance form in which the bond order between nitrogen and oxygen decreases and the bond order between nitrogen and carbon increases. Since nitrogen–oxygen bonds are more stiffening than nitrogen–carbon ones, ^{15}N will enrich in the uncharged phenol, and a normal isotope effect will be observed on formation of the phenolate anion. The size of this isotope effect in a displacement reaction affords a measure of the magnitude of charge developed on the *p*-nitrophenol leaving group in the transition state.

Though small, these isotope effects are well within the measurable range when one uses the competitive method and an isotope ratio mass spectrometer to measure isotopic discrimination. Since the spectrometer measures nitrogen as N_2, the nitrogen in the *p*-nitrophenol must be converted to N_2 via reduction, Kjeldahl digestion, and oxidation.[1] The natural abundance of ^{15}N in the substrate serves as the label, so no synthesis of labeled substrate is necessary.[2]

We have determined the equilibrium ^{15}N isotope effect for deprotonation of *p*-nitrophenol by partitioning half-ionized material between water and methylene chloride. A prior experiment with fully protonated *p*-nitrophenol determined the equilibrium constant for partitioning $K_{organic/aqueous}$ to be 6.2, and the equilibrium isotope effect on partitioning $^{15}K_{part}$ as 1.0005 ± 0.0001.[3] With half-ionized nitrophenol, all of the deprotonated compound stays in the aqueous layer and the protonated phenol partitions between the two layers. The equilibrium isotope effect on deprotonation was found to be 1.0023 ± 0.0001.[4]

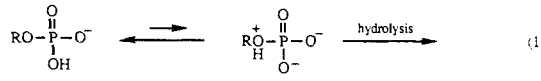
Table I. Isotope Effects on Phosphate Ester Hydrolysis Reactions and on Deprotonation of Nitrophenol

	reactn conditns[a]	$^{14}k/^{15}k$
monoester[b]	pH 4, 100 °C, 1 h	1.0004 ± 0.0002
monoester[b]	pH 10, 100 °C, 8.5 h	1.0028 ± 0.0002
diester[c]	pH 0.5, 100 °C, 14 h	1.0009 ± 0.0002
diester[c]	pH 13, 100 °C, 2.5 h	1.0016 ± 0.0002
triester[d]	pH 12, 22 °C, 15 min	1.0007 ± 0.0001
deprotonation		1.0023 ± 0.0001[e]

[a] Times given are the half-lives for hydrolysis. [b] *p*-Nitrophenyl phosphate. [c] 3,3-Dimethylbutyl *p*-nitrophenyl phosphate. [d] Diethyl *p*-nitrophenyl phosphate. [e] Equilibrium isotope effect.

The utility of this new method for probing transition-state structures is demonstrated in a study of phosphate ester hydrolysis reactions. We have measured the ^{15}N isotope effect in the hydrolysis reactions of a phosphate monoester, a diester, and a triester with *p*-nitrophenol as the leaving group, under a variety of conditions. Our results appear in Table I.

The generally accepted mechanism[5,6] for the hydrolysis of monoanions of phosphomonoesters involves a preequilibrium proton transfer to the bridge oxygen atom followed by P–O bond cleavage or, for good leaving groups, rate-limiting proton transfer followed by P–O bond cleavage (eq 1).



(1)

If this mechanism is correct, there will be no development of negative charge on the leaving group, and the small isotope effect may reflect the difference between an O–phosphoryl versus an O–H bond to the nitrophenol. However, MNDO, AM1, and PM3 calculations performed in our laboratory all indicate that a monoester protonated at the bridge position has no stability and therefore cannot be an intermediate. The computational and isotope effect results are consistent with a mechanism where proton transfer is driven by and lags slightly behind P–O bond fission in a concerted, asynchronous process.

The isotope effect on deprotonation might be expected to represent the upper limit for these isotope effects, since it formally represents completely breaking the bond to the phenolic oxygen. This is not the case experimentally (Table I) as the isotope effect for hydrolysis of *p*-nitrophenyl phosphate at pH 10 is larger than the deprotonation effect. There are three reasons why the equilibrium isotope effect on deprotonation will not represent the maximum observable effect. The first is the aforementioned difference between an O–P versus an O–H bond. Second, the observed equilibrium deprotonation effect in water will be diminished by hydrogen bonding of solvent to the phenolic oxygen, which will lessen the contribution of the quinonoid resonance form. In the transition state of a displacement reaction, the phenolic oxygen will not be similarly solvated. Third, and probably more important, the monoester dianion is thought[6] to hydrolyze by a dissociative mechanism with almost no bond order to the *p*-nitrophenol leaving group. In such a transition state, the negative charge on the PO_3 unit will be in close proximity to the phenolate oxygen, and charge repulsion will increase the contribution of the quinonoid resonance form and hence the ^{15}N isotope effect. The isotope effect observed for this reaction is thus in agreement with the accepted mechanism.

Phosphodiesters undergo both acid- and base-catalyzed hydrolysis. The pH profile[7] in the acid region indicates that the

(1) The general method used in this lab for measuring ^{15}N isotope effects has been described: Hermes, J. D.; Weiss, P. M.; Cleland, W. W. *Biochemistry* **1985**, *24*, 2959. A detailed description of the techniques used in the production and isolation of N_2 from nitrophenol can be found in the following: Weiss, P. M. Heavy Atom Isotope Effects Using the Isotope Ratio Mass Spectrometer. In *Enzyme Mechanisms From Isotope Effects*; Cook, P. F., Ed.; CRC Press, Inc.: Boca Raton, FL; Chapter 11, in press.

(2) Reactions are allowed to run to about 50% completion and assayed to determine the exact fraction of reaction. The product is separated from the residual substrate, the nitrogen from each separately converted to N_2, and the isotopic composition determined by the isotope ratio mass spectrometer. The isotopic composition of the starting material is separately determined. Isotope effects are calculated from the isotopic composition of the starting material and residual substrate, and independently from those of the starting material and product. The equations used for isotope effect calculations can be found in the following: O'Leary, M. H. *Methods Enzymol.* **1980**, *64*, 83.

(3) $^{15}K_{part}$ was calculated as $R_{aqueous}/R_{organic}$. $R_{aqueous}$ and $R_{organic}$ refer to the isotopic ratios of *p*-nitrophenol in the aqueous and organic layers, respectively. The isotopic ratios are the ratios of the ^{15}N-to-^{14}N ratio in the sample to that of a standard sample of N_2 gas, as measured by the isotope ratio mass spectrometer.

(4) Calculated as $R_{protonated}/R_{deprotonated}$ where these values refer to the isotopic ratios of the protonated and deprotonated nitrophenol in the aqueous layer. $R_{protonated}$ was found by multiplying the isotopic ratio of nitrophenol in the organic layer by 1.0005. $R_{deprotonated}$ was calculated from $R_{protonated}$, the observed combined isotopic ratio of both species in the organic layer (R_{obsd}), and the fraction of protonated nitrophenol in the aqueous layer (f) using the equation $R_{deprotonated} = R_{obsd} - (f \times R_{protonated})/(1 - f)$.

(5) Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 414.

(6) Benkovic, S. J.; Schray, K. J. In *Transition States of Biochemical Processes*; Gandour, R. D., Schowen, R. L., Eds.; Plenum Press: New York, 1978; Chapter 13. Westheimer, F. N. *Chem. Rev.* **1981**, *81*, 313.

(7) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 510.

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protonated neutral phosphate is the reactive species. Protonation makes the diester electronically equivalent to a triester, and their similar ^{15}N isotope effects indicate a similar degree of bond breaking to the leaving group in the transition states of these two processes. The reaction in these cases is associative, with only ~25–32% bond cleavage in the transition state if one uses the value of 1.0028 as representing a fully broken bond.

The alkaline hydrolysis of phosphodiesters is an S_N2 process. The value for the ^{15}N isotope effect suggests that the P–O bond is ~57% broken in the transition state. The isotope effect data imply a less associative transition state for this reaction than for the alkaline triester or acidic diester hydrolysis.

Although the maximum isotope effect representing total bond cleavage is somewhat uncertain, comparisons within this series of reactions allow one to observe with a good degree of precision subtle differences in the transition states in the continuum of phosphoester reactions. Because the ^{15}N isotope effect is a secondary one and reaction coordinate motion does not make a contribution, it is a better measurement of P–O bond order in the transition state than the primary ^{18}O isotope effect. This method should be a useful tool for probing transition-state bond breaking in other reactions as well.

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Generation of a Stable Formaldehyde–Organoaluminum Complex and Its Synthetic Utility

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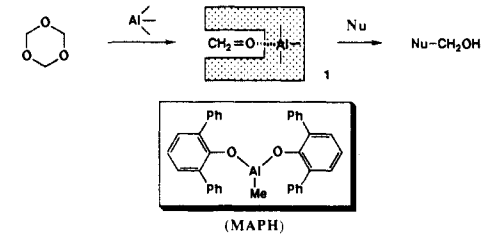
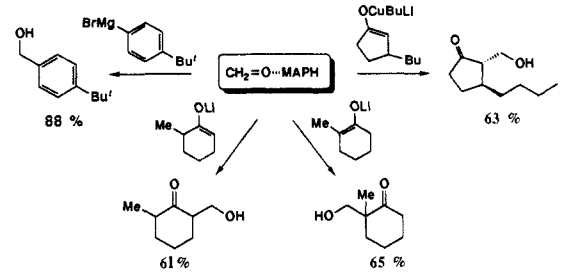
Formaldehyde (gas) is undoubtedly one of the most highly reactive C_1 electrophiles in organic synthesis, as demonstrated in a number of natural product syntheses[1] including a cytotoxic sesquiterpene, vernolepin,[2] and prostaglandins.[3] The synthetic utility of gaseous formaldehyde, in spite of its vast potential, is somewhat restricted because of its remarkably facile self-polymerization. Recent extensive efforts by Snider et al. have resulted in the development of expedient methods, i.e., the generation of formaldehyde from paraformaldehyde catalyzed by Me_2AlCl or Me_3Al and the successful trapping of in situ generated formaldehyde with various olefins.[4] In view of their Lewis acidic conditions as well as the unstable formaldehyde–aluminum complexes, however, these methods cannot be utilized for the nucleophilic addition of carbanions (organometallics, enolates, etc.) as often seen in natural product syntheses.[1-3] In this context, we have been interested for some time in the possibility that certain exceptionally bulky, oxygenophilic organoaluminum reagents might be highly effective because of their two different capabilities, the generation of formaldehyde from readily available trioxane and its stabilization as a formaldehyde–organoaluminum complex

Scheme I



(MAPH)

Scheme II



to suppress self-polymerization by the exceptionally bulky aluminum ligands (Scheme I). In this communication we report our initial results from this study.

Attempted reaction of exceptionally bulky methylaluminum bis(2,6-di-*tert*-butyl-4-methylphenoxide) (MAD)[5] in CH_2Cl_2 with gaseous formaldehyde followed by addition of 1-(trimethylsiloxy)-1-cyclohexene resulted in formation of the Friedel–Crafts alkylation products, 2-*tert*-butyl-6-(hydroxymethyl)-4-methylphenol (42%) and 2,6-di-*tert*-butyl-4-(hydroxymethyl)-4-methyl-2,5-cyclohexadienone (18%). Switching aluminum reagents from MAD to methylaluminum bis(4-bromo-2,6-di-*tert*-butylphenoxide)[6] suppressed the Friedel–Crafts alkylations without any ene-product formation. In marked contrast, however, methylaluminum bis(2,6-diphenylphenoxide) (abbreviated to MAPH) is capable of forming a 1:1 coordination complex **1** with formaldehyde, as confirmed by the subsequent ene reaction with 1-(trimethylsiloxy)-1-cyclohexene, giving the desired 6-(hydroxymethyl)-1-(trimethylsiloxy)-1-cyclohexene in 61% yield after workup with saturated $NaHCO_3$.[7] We then examined the stability of the CH_2=O·MAPH complex and found that it was stable at 0 °C for 5 h and thereafter gradually decomposed at room temperature.[8] The use of simple trioxane as a formaldehyde source offers major advantages. Indeed, treatment of trioxane with MAPH (3 equiv) in CH_2Cl_2 at 0 °C for 1 h successfully yielded the CH_2=O·MAPH complex, which was confirmed by subjection to ene reactions with various olefins (Table I). Our method is obviously far superior in regioselectivity to previously known procedures.[4] The eminent chemoselectivity is also seen in the ene reaction of dihydrocarvone and geranyl acetate (entries 9 and 10). In addition, the CH_2=O·MAPH complex can be utilized as a stable source of gaseous formaldehyde for the nucleophilic addition of various carbanions, as illustrated in Scheme II. Consequently, it is no longer necessary to generate form-

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(2) (a) Grieco, P. A.; Hiroi, K. *J. Chem. Soc., Chem. Commun.* **1972**, 1317. (b) Grieco, P. A.; Hiroi, K. *Tetrahedron Lett.* **1973**, 1831; **1974**, 3467. (c) Grieco, P. A.; Noguez, J. A.; Masaki, Y. *Ibid.* **1975**, 4213. (d) Grieco, P. A.; Nishizawa, M.; Burke, S. D.; Marinovic, N. *J. Am. Chem. Soc.* **1976**, *98*, 1612.

(3) (a) Stork, G.; Isobe, M. *J. Am. Chem. Soc.* **1975**, *97*, 4745. (b) Stork, G.; Isobe, M. *Ibid.* **1975**, *97*, 6260.

(4) (a) Snider, B. B.; Rodini, D. J.; Kirk, T. C.; Cordova, R. *J. Am. Chem. Soc.* **1982**, *104*, 555. (b) Snider, B. B.; Cordova, R.; Price, R. T. *J. Org. Chem.* **1982**, *47*, 3643. (c) Snider, B. B.; Phillips, G. B. *Ibid.* **1983**, *48*, 2789. (d) Snider, B. B. In *Selectivities in Lewis Acid Promoted Reactions*; Schinzer, D., Ed.; Kluwer Academic Publishers: London, 1989; pp 147–167.

(5) (a) Maruoka, K.; Itoh, T.; Yamamoto, H. *J. Am. Chem. Soc.* **1985**, *107*, 4573. (b) Maruoka, K.; Araki, Y.; Yamamoto, H. *Ibid.* **1988**, *110*, 2650. (c) Maruoka, K.; Itoh, T.; Sakurai, M.; Nonoshita, K.; Yamamoto, H. *Ibid.* **1988**, *110*, 3588.

(6) Maruoka, K.; Nonoshita, K.; Banno, H.; Yamamoto, H. *J. Am. Chem. Soc.* **1988**, *110*, 7922; **1990**, *112*, 316.

(7) Since dimethylaluminum 2,6-diphenylphenoxide and methylaluminum bis(2-phenylphenoxide) as MAPH analogues are totally ineffective for this purpose, the origin of the remarkable effect of MAPH for stabilizing formaldehyde is worthy of comment. In a space-filling model of MAPH, two phenyl groups are parallel to each other in front of the Lewis acidic aluminum so that formaldehyde by coordination to MAPH is electronically stabilized by a sandwich structure between these two phenyl groups.

(8) These results were determined by the subsequent ene reaction of the CH_2=O·MAPH complex (3 equiv) with 1-(trimethylsiloxy)-1-cyclohexene at −78 °C for 1 h. The reaction conditions for generation of the CH_2=O·MAPH complex and the yields of the ene product follow: 61% (0 °C, 1 h); 48% (0 °C, 3 h); 52% (0 °C, 5 h); 16% (25 °C, 1 h); 2% (25 °C, 24 h).

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