Kinetic Isotope Effects in the Reactions of Aryl-^{18}O-2,4-dinitrophenyl Dibenzyl Phosphate and Aryl-^{18}O-2,4-dinitrophenyl Phosphate. Evidence for Monomeric Metaphosphate

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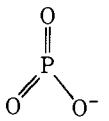
Abstract: The ^{18}O kinetic isotope effect for the hydrolysis of aryl-^{18}O-2,4-dinitrophenyl phosphate is 2.04% and is interpreted in terms of substantial P-O bond breaking to the 2,4-dinitrophenoxide leaving group in the transition state to generate monomeric metaphosphate ion. The kinetic isotope effect in the buffer-catalyzed hydrolysis of aryl-^{18}O-2,4-dinitrophenyl dibenzyl phosphate is 0.70% and support the S_N2-type mechanism for the nucleophilic reaction. Study of the pyridine, dioxane, and 2,6-lutidine catalyzed hydrolysis of 2,4-dinitrophenyl phosphate dianion indicates that the degree to which the monomeric metaphosphate ion exists as a free species determines whether S_N1 or S_N2 type reactivity is observed.

The role of the monomeric metaphosphate ion intermediate, **1**, in the unimolecular decomposition of phosphate



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monoesters has been the subject of much investigation. Numerous studies on such esters as methyl phosphate,[2] acetyl phosphate,[3] and aryl phosphates[4] have attempted to use pH-rate profile data, entropy of activation comparisons, solvent effects, etc., to confirm the metaphosphate hypothesis. In addition, actual trapping of the monomeric metaphosphate species in the gas phase[5] or in nonaqueous solution[6] has been recently demonstrated. While a substantial body of data thus now exists[7,8] which appears consistent only with the metaphosphate mechanism, no one piece of evidence is conclusive in itself, and support for this mechanism must rest upon the accumulation of a sufficient number of reasonable arguments. In a previous communication[9] we reported preliminary ester oxygen ^{18}O isotope effect data on the hydrolysis of the dianion of a monoaryl phosphate ester ($ArO^*PO_3^{2-}$) which supported the metaphosphate mechanism. In the present study and the accompanying paper[10] we wish to further support our previous conclusion and present new data on reactions of a phosphate triester which allows a clear distinction between unimolecular and biomolecular mechanisms for reaction at phosphorus.

Experimental Section

Materials. Buffers and phosphate ester syntheses are described in the accompanying paper[10] and previous communication.[9]

Kinetics. Kinetics were determined on a Zeiss PMQ-II or a Cary 16K UV-visible spectrophotometer. Reactions were run in simultaneous sets of four (two ^{16}O and two ^{18}O samples) and followed by the appearance of 2,4-dinitrophenoxide for at least 2 half-lives and the infinity absorbance values taken after 10 or more half-lives. Excellent first-order rate constant (errors less than ±0.5%) were determined both by hand plotting of the absorbance data and by computer calculation with an infinity varying, least-squares program. All errors are standard deviations. In addition in later runs a direct digitalization of the Cary 16K/Varian G2500 log recorder spectrophotometer output was used and a punch tape record directly computer analyzed. Similar, though less accurate rate constants were obtained by this approach. Since the ^{18}O isotope effects were determined by a direct kinetic approach,[11] comparing the directly measured rate of hydrolysis of the aryl-^{18}O ester to that of the aryl-^{16}O esters, great care must be taken to obtain precise and accurate rate constants.

Results

Representative rate constants are presented in Table I for the hydrolysis of aryl-^{16}O- and -^{18}O-2,4-dinitrophenyl phosphate (>94% aryl ^{18}O). Since no discernible trend in the isotope effect in the temperature range 39-55 °C and the pH range 4.4-8.0 in the phosphate buffer solution was observed, all 32 runs were averaged together to give an isotope effect of 1.0204 ± 0.0044. Since this value is more than four standard deviations from unity (i.e., no isotope effect) we can state with greater than 99% confidence[12] that at least some isotope effect is experimentally verifiable. It should be noted that an isotope effect less than 1.000 was never observed in any of the 32 kinetic runs made.

As shown in Figure 1, the apparent catalysis by pyridine (noted earlier by Kirby and Varvoglis[13]) prompted a kinetic isotope effect study on the pyridine-catalyzed hydrolysis of the aryl phosphate monoester. Although the precision of the measurements was not as high as in the uncatalyzed reaction, a similar isotope effect is noted. Again, all pyridine buffer reactions were analyzed as a single set, giving an isotope effect of 1.0169 ± 0.0157.

Kinetic isotope effects in the buffer-catalyzed hydrolysis of aryl-^{18}O-2,4-dinitrophenyl dibenzyl phosphate are presented in Table II. The oxygen-18 isotope effects are 1.007, 1.009, 1.006, and 1.003 for reaction with formate, acetate, phosphate, and carbonate, respectively. (The runs at different buffer ratios and concentrations were considered as a single set). Since the variation among the different buffer-catalyzed isotope effects is smaller than the probable error for these measurements (>0.5%), all of the values were averaged together, yielding an isotope effect of 1.0070 ± 0.0038.

Discussion

Heavy-atom isotope effects have been shown to be a powerful investigative tool of the mechanistic chemist.[14,15] Large (2-6%) ^{13}C, ^{18}O, and ^{15}N isotope effects have been demonstrated and theoretically justified[16] to indicate substantial bond breaking or bond order change in the transition state. Kinetic ^{18}O isotope effects have proven to be very useful in the elucidation of addition and nucleophilic substitution mechanisms at acyl and saturated carbon centers, respectively.[15,18] We report here the first study of ^{18}O isotope effects in the reactions of phosphate esters.

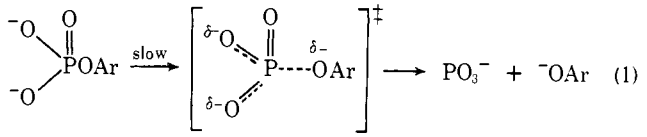
Our observation of a 2% kinetic isotope effect in the spontaneous hydrolysis of the P-O(Ar)bond[9] in 2,4-dinitrophenyl phosphate strongly indicates that substantial P-O bond breaking occurs in the transition state. This is consistent with

Table I. Selected Rate Constants for the Hydrolysis of Aryl-^{18}O- and -^{16}O-2,4-dinitrophenyl Phosphate

Conditions[a]							
Buffer concn, M	pH	Temp, °C	k_0^{16},[b] $s^{-1} \times 10^3$		k_0^{18},[b] $s^{-1} \times 10^3$		k_0^{16}/k_0^{18}[d]
			Phosphate				
0.025	7.72	51.1	0.4733	0.4695	0.4663	0.4620	1.0157
0.025	7.72	51.1	0.4737	0.4652	0.4558	0.4577	1.0244
0.010	7.70	51.0	0.4473	0.4500	0.4416	0.4400	1.0193
0.010	7.70	51.0	0.4464	0.4445	0.4374	0.4360	1.0249
0.010	7.70	51.0	0.4333	0.4301	0.4255	0.4185	1.0200
0.005	7.62	51.1	0.4799	0.4849	0.4716	0.4716	1.0230
0.010	7.70	39.0	0.08101	0.07931	0.07815	0.07830	1.0184
0.010	7.70	38.9	0.07736	0.07803	0.07658	0.07635	1.0161
0.05	8.0	55.0	0.8402		0.8160	0.8235	1.0225
0.05	4.4	51.0	0.07019		0.06869		1.0226
			Pyridine				
0.2	7.3	39.6	0.2998	0.3022	0.2986	0.2953	1.0136
0.2	7.3	39.6	0.3005		0.2997	0.2973	0.9933
0.2	7.3	39.6	0.2863	0.2823	0.2844	0.2839	1.0005
0.4	7.4	39.6	0.4205	0.4191	0.4148	0.4163	1.0102
0.4	7.4	39.6	0.4115	0.4094	0.4035	0.4070	1.0128
0.4	7.4	39.2	0.4070	0.4069	0.4061	0.3985	1.0116
0.6	7.5	39.6	0.5277	0.5286	0.5019	0.4995	1.0548
0.6	7.5	39.2	0.5325	0.5324	0.5209	0.5121	1.0309
0.6	7.5	39.6	0.5243	0.5250	0.5217	0.5238	1.0036
0.6	7.5	39.8	0.5127	0.5102	0.5039	0.4983	1.0207
0.6	7.5	39.2	0.4938	0.4870	0.4800	0.4850	1.0164
0.6	7.5	39.2	0.5105	0.5121	0.5009	0.4918	1.0301
0.8	7.5	39.6	0.6058	0.6092	0.5980	0.5915	1.0214

[a] Ionic strength maintained at 0.05 M with KCl in phosphate buffers and 1.0 M in pyridine buffers. [b] ^{16}O and ^{18}O samples were run concurrently in sets of three or four. Results from nonlinear, weighted least-squares, infinity varying, computer calculation. [c] Temperature precision during a given run is ±0.05 °C. Accuracy of the temperature from one run to another is ±0.20 °C. [d] Reported isotope effects for the phosphate buffer reactions are calculated from either the infinity varying, least-squares program, a fixed, experimental infinity value, least-squares program, or the average of the two methods. The criterion was which calculation yielded the smallest sum of the squares of the residuals in the data points and also produced no curvature in the linearized plots.

the earlier work of Kirby and Varvoglis[4b] and Bunton et al.,[19] who have concluded that the reaction proceeds via a monomeric metaphosphate ion intermediate, involving unimolecular breakdown of the aryl ester dianion (eq 1).



(1)

The extent of P-O bond breaking in the transition state may be approximated by calculating the frequency of the P-O(Ar) stretch in the transition state, $\nu^{\ddagger}$, consistent with the observed isotope effect:[14-15]

$$k_{16}/k_{18} = e^{[hc(\Delta\nu^r - \Delta\nu^{\ddagger})/2kT]} \quad (2)$$

and assuming $\nu_{16}/\nu_{18} = 1.017$ for both the reactant (r) and transition state (‡) frequencies.[15] The aryl P-O stretching frequency in the reactant (~1200 cm^{-1})[20] is reduced to ~650 ± 100 cm^{-1} in the transition state according to this calculation and is certainly consistent with the above monomeric metaphosphate mechanism, which requires substantial P-O bond breaking in the transition state (i.e., reduced P-O stretching frequency).[21]

Nucleophilic Catalysis in the Hydrolysis of 2,4-DNPP. Although the 2,4-DNPP dianion apparently hydrolyzes via an $S_N1(P)$ type mechanism, other reactions of the monoester dianion suggest $S_N2(P)$ type of reactivity. The second-order reaction with pyridine (Figure 1) and other nucleophiles[13] has been previously noted by Kirby and Varvoglis. The large negative entropy of activation, $\Delta S^{\ddagger} = -23.9$ eu, and a small

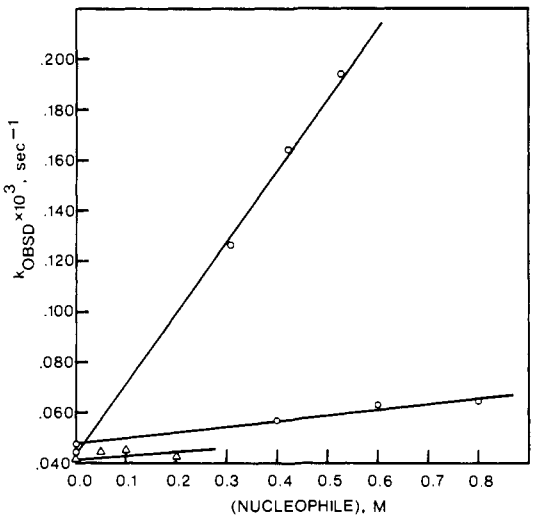


Figure 1. Plot of the pyridine (○), 2,6-lutidine (△), and 1,4-dioxane (○) catalyzed hydrolysis of 2,4-dinitrophenyl phosphate, $T = 30$ °C, $I = 1.0$ M, pH 7.1. Pyridine data has been corrected for the self-association of pyridine (ref 23).

enthalpy of activation, $\Delta H^{\ddagger} = 15.5$ kcal/mol for the second-order reaction with pyridine contrasts with the high enthalpy of activation, $\Delta H^{\ddagger} = 25.8$ kcal/mol and a small, favorable entropy of activation, $\Delta S^{\ddagger} = 5.3$ eu for the spontaneous, first-order reaction of the phosphate monoester monoanion. (Kirby and Varvoglis report quite similar activation parameters for these reactions.[13]) The lack of a solvent isotope effect on the pyridine-catalyzed reaction and the entropy of activation

Table II. Rate Constants for the Hydrolysis of Aryl-^{18}O- and -^{16}O-2,4-dinitrophenyl Dibenzyl Phosphate, 30 °C, I = 1.0

Conditions						
Buffer (Ratio[a]) concn, M	pH	$k_0{}^{16}$,[b] $s^{-1} \times 10^3$		$k_0{}^{18}$,[b] $s^{-1} \times 10^3$		$k_0{}^{16}/k_0{}^{18}$
Formate (1/0.25), 0.1	4.6	0.1529	0.1505	0.1504	0.1500	1.0100
		0.1495	0.1493	0.1493	0.1484	1.0037
1.0	4.66	0.8934	0.9107	0.8885	0.8992	1.0092
		0.9227	0.9146	0.9141	0.9142	1.0049
Acetate (1/0.25), 1.0	6.05	0.5709	0.5717	0.5621	0.5656	1.0133
		0.5749	0.5777	0.5668	0.5683	1.0155
Acetate (1/1), 0.1	5.24	0.1355	0.1347	0.1348	0.1348	1.0022
		0.1353	0.1365	0.1348	0.1352	1.0067
1.0	5.38	0.5172	0.5200	0.5153	0.5148	1.0069
		0.5063	0.5072	0.5022	0.5043	1.0070
Acetate (1/4), 1.0	4.68	0.2886	0.2868	0.2830	0.2882	1.0074
Phosphate (3/1), 1.0	7.92	0.4350	0.4396	0.4322	0.4371	1.0060
Phosphate (1/2), 1.0	6.91	0.2977	0.2965	0.2955	0.2951	1.0061
Carbonate (3/1), 1.0	10.89	1.4270	1.4375	1.4264	1.4352	1.0010
		1.4646	1.4587	1.4589	1.4497	1.0051

[a] Base-acid ratio. [b] See footnote to Table I.

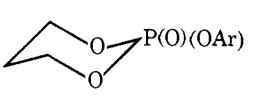
Table III. First- and Second-Order Rate Constants for the "Catalyzed" Hydrolysis of 2,4-Dinitrophenyl Phosphate, I = 1.00 M (pH 7.1)

Catalyst (temp, °C)	Concn range	k_0, $s^{-1} \times 10^3$	k_2, $M^{-1}\,s^{-1} \times 10^3$
Pyridine (20)	0.4–0.8		0.1418
(30)	0–0.8	0.04370	0.2875
(40)	0–0.8	0.1773	0.7286
(50)	0–0.8	0.6322	1.618
Lutidine (15)	0–0.2	0.006220	0.0052
(25)	0–0.2	0.02108	0.035
(30)	0–0.2	0.04162	0.042
(35)	0–0.2	0.09626	0.034
Dioxane (30)	0–0.8	0.04801	0.02229
(35)	0–0.8	0.09370	0.03988
(40)	0–0.4	0.1782	0.07875
(50)	0–0.8	0.6348	0.2618

comparison clearly suggest an $S_N2(P)$ mechanism for the nucleophile reaction and an $S_N1(P)$ mechanism for the spontaneous reaction. However, this quite reasonable division of mechanisms does not explain several other features of the reactions of the 2,4-DNPP dianion. First, Kirby and Varvoglis report a Brønsted coefficient, $\beta \sim 0$, for the second-order reaction with various substituted pyridines.[13] Secondly, the ^{18}O kinetic isotope effect (1.7%) for the reaction of the dianion with pyridine suggests nearly the same amount of 2,4-dinitrophenoxide bond breaking in the spontaneous reaction as in the second-order reaction. (Unfortunately, the error in this value is so large as almost to preclude this assessment.) Finally, as shown in Figure 1 and Table III and earlier observed by Kirby and Varvoglis,[13] other "nonnucleophilic" molecules catalyze the hydrolysis of the monoester dianion. 2,6-Lutidine is sterically hindered from participating as a close nucleophilic partner in the reactions at tetrahedral phosphorus, yet this base accelerates the hydrolysis reaction, albeit not as effectively as pyridine (Table III). In addition other organic solvents which are not phosphorylated accelerate the hydrolysis reaction.[13] As shown in Figure 1 and Table III we confirm the "catalysis" by 1,4-dioxane, which falls in between the catalytic effectiveness of 2,6-lutidine and pyridine. Strikingly, the entropy of activation for the second-order reaction with dioxane ($\Delta S^{\ddagger} = -3.8$ eu and $\Delta H^{\ddagger} = 23.1$ kcal/mol) is similar to the entropy of activation for the spontaneous reaction.

These additional results suggest that no sharp line can be drawn between the $S_N1(P)$ and $S_N2(P)$ mechanisms for reactions of 2,4-DNPP. Apparently in all of these reactions, considerable bond breaking to the leaving group occurs in the transition state. Very little bond formation to the attacking nucleophile (such as pyridine) or surrounding solvent (including 2,6-lutidine, dioxane, or water) also occurs, resulting in formation of what may best be described as a highly reactive monomeric metaphosphate species. Westheimer and coworkers[5b] have recently shown that the methyl ester of monomeric metaphosphate is an extremely powerful electrophile, capable of phosphorylating the para position of *N*,*N*-diethylaniline. The electrophilicity is thus comparable to SO_3 and the ability of SO_3 to form adducts with sterically hindered bases and with dioxane further supports this comparison.[13] The monomeric metaphosphate intermediate must have an extremely short lifetime in the condensed phase with suitable nearby electron donors in its solvation sphere. With pyridine in this solvation sphere, the metaphosphate species lifetime must be so short that essentially one must consider the reaction a loosely coupled S_N2 displacement. With poorer nucleophiles (water, dioxane, 2,6-lutidine) the metaphosphate has a greater chance to exist as a discrete, relatively free intermediate. The dioxane (and 2,6-lutidine) likely preferentially solvates the monomeric metaphosphate. This would explain the biomolecularity of the dioxane reaction, and yet the highly favorable (unimolecular-like) entropy of activation. The dioxane and pyridine reactions must thus bridge the borderline between the S_N1 and S_N2 mechanisms.

Triester Hydrolysis. On the basis of a series of structure-reactivity relationships in the oxyanion-catalyzed hydrolysis of the cyclic dialkyl monoaryl phosphates, **2**, Khan and Kirby[24]



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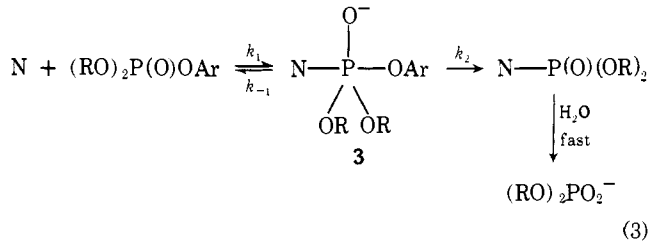
have proposed the following displacement reaction sequence (eq 3). The slopes (β) of the leaving group, linear free energy plots for attack of different nucleophiles on the aryl triesters, **2**, were −0.35 (for hydroxide and hydroperoxide attack), −0.54 (for carbonate), −0.65 (for phosphate dianion), and −0.88 (for acetate). This increasing sensitivity to leaving group pK_a with decreasing nucleophilicity of the attacking oxyanion is consistent with partial rate-limiting breakdown of the pen-



(3)

tacovalent intermediate, **3** (i.e., $k_{-1} \gtrsim k_2$), for displacement reactions with poor nucleophiles. The observation of a moderately large, negative β even for hydroxide attack on triesters suggests breakdown of the intermediate to products is always at least partially rate limiting for the triester oxyanion reactions. Our ^{18}O isotope effect data on the reactions of the acyclic triester, 2,4-dinitrophenyl dibenzyl phosphate, fit very nicely into this picture. The ^{18}O isotope effect of 0.7 ± 0.4% suggests a small (though possibly statistically insignificant) degree of P-O(aryl) bond breaking in the transition state. Analysis by eq 2 indicates that the P-O(Ar) stretching frequency of 1200 cm^{-1} in the triester is reduced to only ca. 1030 ± 100 cm^{-1} in the transition state.[25] The much smaller amount of bond breaking in the transition state for hydrolysis of the triester than in the transition state for hydrolysis of the monoester is thus consistent with the smaller Brønsted coefficients for the reaction of the triester. The Brønsted slope for the "spontaneous" hydrolysis of various dianionic benzoyl phosphates and aryl phosphates is −1.23[4] while, as noted, triester Brønsted coefficients vary between −0.35 and −0.88 for reaction with various oxyanions.[24]

Intriguingly, the triester kinetic isotope effect does appear to decrease as the nucleophilicity of the attacking oxyanion increases. This suggests that the addition step is more rate determining for the more basic nucleophiles and even less P-O(Ar) bond breaking therefore occurs in the transition state for the more basic nucleophiles. Note that this is consistent with the curved Brønsted plot of the previous paper. One must also add that a simple $S_N2(P)$ transition state with varying degrees of P-O(Ar) bond breaking would equally satisfy the available data on the reactions of triesters.[24]

Conclusions

The observed kinetic isotope effect in the hydrolysis of 2,4-dinitrophenyl phosphate may definitely rule out only those mechanisms which do not involve a rate-determining P-O bond cleavage (such as rate-determining hydration or rate-determining pseudorotation of a pentacovalent intermediate[26,28]). While alternative schemes involving either rate-limiting breakdown of a pentacovalent intermediate or direct S_N2-type displacement unfortunately are not completely eliminated, it is especially significant that a considerably smaller isotope effect is observed in the nucleophilic-catalyzed hydrolysis of 2,4-dinitrophenyl dibenzyl phosphate. Hydrolysis of the triester must necessarily proceed via either an addition-elimination or a direct $S_N2(P)$ mechanism. This indicates (but again certainly doesn't prove) that the large degree of P-O bond breaking in the hydrolysis of the monoester results from the unique unimolecular monomeric metaphosphate pathway by which the monoester reacts.

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Note Added in Proof. Dr. Jack Kirsch has informed us that the ^{18}O isotope effect on the ionization constant of phenol-labeled 2,4-dinitrophenol is 1.0210 ± 0.0003. As discussed in ref 21, this smaller than normal equilibrium isotope effect confirms our interpretation that the 1.0204 kinetic isotope effect for DNPP represents nearly *complete* P-O bond breaking in the transition state.

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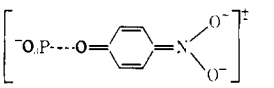

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