Oxidatively Initiated Phosphorylation Reactions of Phosphine

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Introduction of phosphine into alcoholic solutions of *m*-chloroperoxybenzoic acid results in mixtures of phosphinic and phosphonic acids, monoalkyl dihydrogen phosphinates, monoalkyl hydrogen phosphonates, dialkyl hydrogen phosphonates, and trialkyl phosphates, as analyzed by ^{31}P NMR spectroscopy and GC/MS. The requirement for peracid indicates the intermediacy of oxidatively generated reactive phosphorylating species. Steric factors appear to be important since the extent of phosphorylation varies inversely with the steric bulk of the alcohol used, i.e., ester formation with methanol > ethanol > *n*-propanol ≫ 2-propanol >>> *tert*-butyl alcohol. Imidazole is also N-phosphorylated under comparable conditions. Phosphine administered to rats, orally as a suspension of zinc phosphide in corn oil, is excreted in the urine as phosphinic and phosphonic acids, thereby suggesting that an analogous oxidation sequence may occur in vivo. Thus, the toxicological properties of phosphine may reflect a combination of its reactivity as a nucleophile per se and the electrophilic character of the intermediates arising on its oxidation which could lead to derivatization of critical bionucleophiles.

INTRODUCTION

Phosphine is used as a fumigant for the control of insects and, in the form of zinc phosphide, as a bait for rodents. It is normally generated by hydrolysis of aluminum, magnesium, or zinc phosphide (Fluck, 1973). Phosphine is also important in the manufacture of pharmaceuticals, oil additives, and flame retardants and as a dopant in the electronics industry (WHO, 1988).

Phosphine is both a potent nucleophile and a powerful reducing agent. It interacts with various hemoproteins including cytochrome oxidase, catalase, and peroxidase and leads to their slow deoxygenation since phosphine generally has a greater affinity for oxygen. Relative to its redox behavior, the accumulation of oxygen-derived free radicals may be a factor in its acute toxicity since these eventually break down cell integrity by disrupting enzymes associated with oxygen defense (WHO, 1988; Price, 1985; Nakakita, 1987; Bolter and Chefurka, 1990; Chaudhry and Price, 1990). Phosphine also causes chromosomal aberrations in plants (Bakheit et al., 1985; Younis et al., 1989), rodents (Duma et al., 1977), and humans (Garry et al., 1989) and sterility or mutagenic effects in insects (Al-Hakkak, 1988; Al-Hakkak et al., 1985).

The destruction of cell integrity by phosphine may involve, in addition to the parent compound, various activated species generated during its oxidation (Chaudhry and Price, 1990). Oxidative bioactivation may also be a factor in the chromosomal damage and mutagenesis, since these lesions are often associated with compounds that are inherently reactive as electrophiles, or which become nucleophilic following their metabolic activation, and lead to relatively stable derivatives of biological nucleophiles (Sipes and Gandolfi, 1986). In this context, mammals and insects oxidize phosphine to various oxy acids of phosphorus (WHO, 1988; Robinson and Bond, 1970; Price, 1981). The initial oxidation product is presumably phosphine oxide which, although too reactive to isolate, has been demonstrated by low-temperature infrared techniques (Streitwieser et al., 1987; Withnall and Andrews, 1987). Sequential oxidation products observed in aqueous solution are phosphinic, phosphonic, and phosphoric acids (Scheme I). In the former two, the phosphorus center is electrophilic and should be reactive toward nucleophiles.

Scheme I

$$PH_3 \rightarrow P(O)H_3 \rightarrow H_2P(O)OH \rightarrow HP(O)(OH)_2 \rightarrow HOP(O)(OH)_2$$

Several aspects of the oxidative bioactivation that many phosphorus-containing pesticides and toxicants undergo have been successfully modeled using chemical oxidants such as *m*-chloroperoxybenzoic acid (MCPBA) or magnesium monoperoxyphthalate (MMPP) in a variety of protic, aprotic, hydroxylic, and nonhydroxylic solvents. Reactive electrophiles generated in these peracid oxidations can be conveniently trapped with methanol or other alcohols as the corresponding alkyl phosphates (Segall and Casida, 1983; Bielawski and Casida, 1988), and ^{31}P NMR spectroscopy allows direct and continuous monitoring of the reaction mixtures to detect short-lived intermediates.

The present study focuses, using the general approaches described above, on two aspects of the oxidative chemistry and toxicology of phosphine. First, its reactivity and fate are established in the peracid oxidation system as a model for its possible oxidative bioactivation. Second, and to test the model for biological relevance, the metabolites of phosphine, as detectable by ^{31}P NMR spectroscopy, have been determined in the urine of treated rats.

MATERIALS AND METHODS

Chemicals. The chemicals used were phosphine (~1.9% mixture in nitrogen) from Matheson, zinc phosphide (technical grade) from Alfa Products, phosphinic acid (50% aqueous solution), MCPBA (80%), and MMPP (80%) from Aldrich, and sodium hypochlorite (5.25% w/v aqueous solution) from Clorox. CAUTIONARY NOTE: Phosphine is a hazardous chemical because of its inherent toxicity. It is spontaneously flammable in air, but this problem is minimized by using it as a ~1.8% mixture in an inert gas (Fluck, 1973). In the present studies excess phosphine was destroyed by venting reaction vessels through traps containing aqueous hypochlorite.

Spectroscopy and Analysis. ^{31}P NMR spectra, with and without ^{1}H decoupling, were acquired at 121 MHz on a Bruker WM-300 spectrometer equipped with an ASPECT 3000 data system. These spectra were recorded with nuclear Overhauser effect (NOE) at ambient temperature using a 45° pulse (10 μs for 90° pulse) and an interpulse delay of 1.5 s unless otherwise stated. To obtain product ratios by integration of ^{31}P resonances, ^{1}H-decoupled ^{31}P NMR spectra were acquired without NOE;

0021-8561/91/1439-2274$02.50/0

Table I. ^{31}P NMR Chemical Shifts for Phosphorus Acids, Esters, and Amide Formed on Reaction of Phosphine and MCPBA with Alcohols or Imidazole in $CDCl_3$ for 25 min at 25 °C

phosphorus-containing products	^{31}P NMR chemical shift[a]					
	CH_3OH	C_2H_5OH	n-C_3H_7OH	i-C_3H_7OH	t-C_4H_9OH	imidazole
acids						
$H_2P(O)OH$[b]	6.37 (t, 556)[c]	5.12 (t, 554)	5.08 (t, 553)	4.36 (t, 551)	4.17 (t, 550)	0.29 (t, 502)
$HP(O)(OH)_2$[b]	1.19 (d, 667)	0.46 (d, 664)	0.65 (d, 664)	0.25 (d, 663)	0.24 (d, 662)	−0.81 (d, 577)
$HOP(O)(OH)_2$						−3.58 (s)
esters or amide (R = *O*-alkyl or imidazole)						
$H_2P(O)R$[b]	16.95 (tq, 577, 13.3)	12.84 (tt, 572, 9.7)	13.48 (tt, 572, 9.0)	8.86 (dt, 568, 9.7)	0.84 (t, 566)	
$HP(O)(R)OH$	4.96 (dq, 678, 11.3)	2.45 (dt, 676, 8.5)	2.76 (dt, 676, 8.0)	0.52 (dd, 674, 8.2)	−3.06 (d, 676)	1.92 (ddd, 610, 8.9, 8.3)
$HP(O)R_2$	8.82 (ds, 708, 12.2)	4.96 (dq, 700, 9.4)	5.38 (dq, 700, 9.0)	1.54 (dt, 689, 8.1)		
$RP(O)R_2$	−0.62 (m)	−4.16 (s, 7.9)	−3.89 (not resolved)			

[a] Referenced to trimethyl phosphate at δ 0.00. Alcohol:$CDCl_3$ (v/v) or imidazole:$CDCl_3$ 1:3 (w/v). [b] Products from reaction in wet chloroform [$CDCl_3$:$CHCl_3$ (with ethanol stabilizer) 1:1 (v/v)]: $H_2P(O)OH$ δ 10.67 (t, 570), $HP(O)(OH)_2$ δ 6.54 (d, 707), and $H_2P(O)OC_2H_5$ δ 2.20 (tt, 570, 9.0). [c] Data in parentheses are multiplicities and coupling constants in hertz of the ^{31}P NMR signals.

Table II. Product Distribution of Phosphorus Acids, Esters, and Amide Formed on Reaction of Phosphine and MCPBA with Alcohols or Imidazole in $CDCl_3$ for 25 min at 25 °C

phosphorus-containing products[a]	reactant and products,[b] %					
	CH_3OH	C_2H_5OH	n-C_3H_7OH	i-C_3H_7OH	t-C_4H_9OH	imidazole
acids						
$H_2P(O)OH$	13.8	20.0	22.7	32.5	48.7	63.5
$HP(O)(OH)_2$	4.3	4.7	9.7	12.3	13.5	16.2
$HOP(O)(OH)_2$	0.0	0.0	0.0	0.0	0.0	1.4
esters or amide (R = *O*-alkyl or imidazole)						
$H_2P(O)R$	4.3	6.1	6.0	13.6	24.3	0.0
$HP(O)(R)OH$	38.8	35.6	41.1	35.1	13.5	18.9
$HP(O)R_2$	29.3	27.6	17.5	6.5	0.0	0.0
$RP(O)R_2$	9.5	6.0	3.0	0.0	0.0	0.0

[a] The phosphorus-containing products do not include $HOP(O)R_2$ in any case. [b] By integration of ^{31}P NMR signals.

since it was anticipated that the various classes of phosphorus-containing products, in particular the phosphates, phosphonates, and phosphinates, would have different relaxation times (T1), the interpulse delay was set at 8 s. Positive ^{31}P chemical shifts indicate resonances downfield from external trimethyl phosphate (δ 0.00) as reference. The lock solvent was $CDCl_3$ for all MCPBA oxidations and D_2O for other oxidants indicated below.

GC/MS, following product methylation, was used to analyze the phosphine-derived acids and esters from the reaction mixtures involving ethanol and *tert*-butyl alcohol. Derivatization was achieved by treating the mixtures with excess diazomethane in ether for 60 min at 25 °C and then evaporating the solvent and redissolving the residue in $CHCl_3$. GC analyses involved a DB-5 fused silica capillary column (30 m × 0.32 mm, film thickness 0.25 μm) with 1-min splitless injection at 240 °C, helium as the carrier gas at 31 cm/s (at 200 °C), and the following temperature program: 40 °C for 1 min (for *tert*-butyl alcohol reaction) or 3 min (for ethanol reaction); 5 °C/min to 200 °C, and then hold for 1 min. MS utilized a Hewlett-Packard 5985 system with the source at 130 °C and methane at 1.0 Torr as the reagent gas for positive chemical ionization (CI).

Peracid Oxidations. Phosphine was bubbled into a $CDCl_3$ solution (3.0 mL) of MCPBA (1.4 mmol) containing either an alcohol (3.0 mL) or an imidazole (2.0 g). After 25 min at 25 °C, the reaction mixtures were analyzed by ^{31}P NMR directly and by ^{31}P NMR and GC/MS after methylation.

Two variations of the above procedure were examined. In one, wet chloroform, generated by shaking $CDCl_3$ and $CHCl_3$ (3 mL each) with deionized water (1 mL) followed by phase separation, was used directly as the solvent, as above. In the other, phosphine was bubbled through an aqueous solution (3.2 mL) of MMPP (0.70 mmol) and glucose (2.0 g) for 30 min prior to ^{31}P NMR analysis.

Other Oxidations. Sodium hypochlorite (5.25% aqueous solution) (1 mL) was added to methanol (3 mL) or to glycerol (2.4 g), and then phosphine was bubbled through the solution for times ranging from 30 min to 2.5 h. Alternatively, phosphine was bubbled for 2–5.5 h into a deionized water solution (2.2 mL) of hydrogen peroxide (1.84 mmol) with or without added horseradish peroxidase (650 units).

Metabolism. Male albino rats (Sprague Dawley, 200 g) were individually treated, orally, with a suspension of zinc phosphide (6 mg) in corn oil (200 μL) or with corn oil only. Urine was collected for 24 h and lyophilized to dryness, and then the residue was redissolved in D_2O and analyzed by ^{31}P NMR spectroscopy.

RESULTS

Products from Reaction of Peracid-Activated Phosphine with Alcohols (Tables I and II). Six phosphorus-containing products were observed when phosphine was oxidized with MCPBA in methanol, ethanol, or 1-propanol. These were identified by ^1H-coupled and ^1H-decoupled ^{31}P NMR as phosphinic and phosphonic acids and the respective monoalkyl dihydrogen phosphinates, monoalkyl hydrogen phosphonates, dialkyl hydrogen phosphonates, and trialkyl phosphates (Figure 1). The free acids have characteristic ^{31}P NMR chemical shifts, and the various esters were identified from their three-bond P–H coupling values which ranged from 7 to 14 Hz (Mavel, 1973). Resonances from products with protons bonded directly to the phosphorus center were also readily assignable from both their coupling constants ($^1J_{P-H}$ values range from 450 to 800 Hz) and their multiplicities (Mavel,

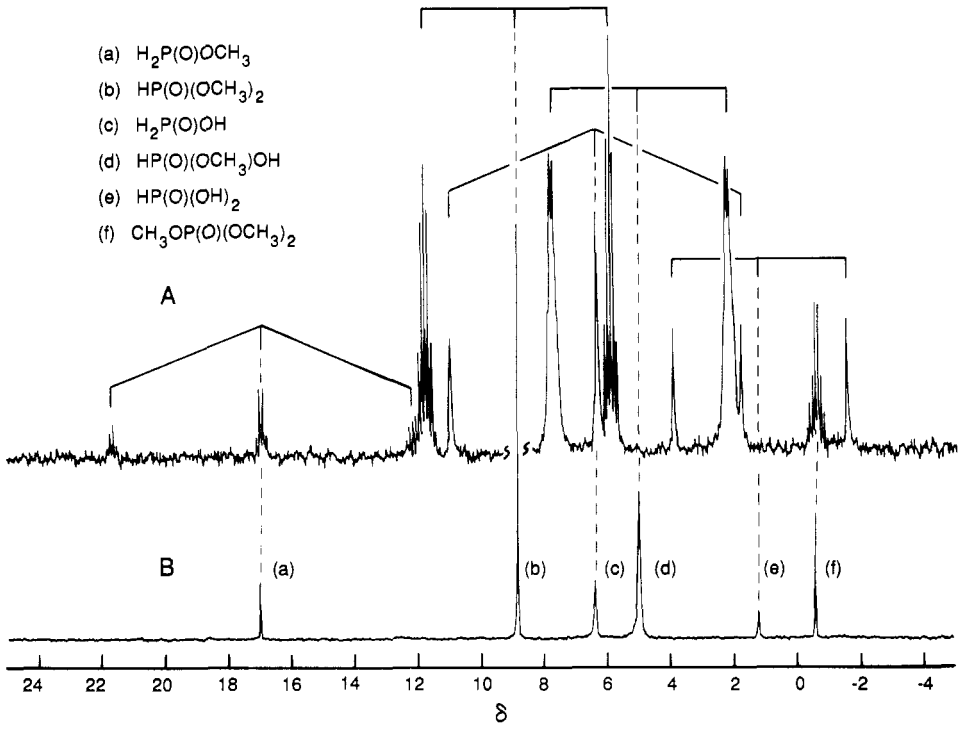

Figure 1. ^1H-coupled (A) and ^1H-decoupled (B) ^{31}P NMR spectra of the reaction mixture from phosphine, methanol, and peracid in $CDCl_3$.

Table III. GC/CI-MS Identification of Alkoxy Phosphorus Compounds Formed on Reaction of Phosphine and MCPBA with Ethanol or *tert*-Butyl Alcohol followed by Methylation[a]

products from methylation (R = C_2H_5 or *t*-C_4H_9)	alcohol and Rt,[b] min		alcohol and $[M + 1]^+$	
	C_2H_5OH	*t*-C_4H_9OH	C_2H_5OH	*t*-C_4H_9OH
$H_2P(O)OR$		7.1		123
$HP(O)(OMe)_2$	5.0	4.3	111	111
HP(O)(OR)OMe	7.2	8.4	125	153
$HP(O)(OR)_2$	9.6		139	
$MeOP(O)(OR)_2$	13.2		169	
$ROP(O)(OR)_2$	15.1		183	

[a] Reactions in $CDCl_3$ for 25 min at 25 °C followed by methylation. [b] Additional products at larger Rts are methyl 3-chlorobenzoate and 3-chlorobenzoic acid, detected for reactions in ethanol or *tert*-butyl alcohol, and ethyl 3-chlorobenzoate for reactions in ethanol. A different GC program was used for the products from the reactions in ethanol and *tert*-butyl alcohol.

1973). The extent of ester formation decreased slightly as the chain length of the alcohol increased, i.e., CH_3OH > C_2H_5OH > *n*-C_3H_7OH. For product mixtures from reactions in ethanol or *tert*-butyl alcohol structural assignments were substantiated, after methylation of the product mixture with diazomethane, by GC/MS (Table III) and ^{31}P NMR analyses.

When branched-chain alcohols were used as solvents, ester formation decreased with a concomitant increase in the formation of phosphinic and phosphonic acids. The diisopropyl and di-*tert*-butyl hydrogen phosphinates were not observed, and the major ester derivatives were the monoalkyl dihydrogen phosphinates and monoalkyl hydrogen phosphonates. Methyl dihydrogen phosphinate, formed from methylation of phosphinic acid, was detectable by ^{31}P NMR but not by GC/MS; this suggests that it decomposes in the GC injection port [see Baudler (1973)].

Products from Reaction of Peracid-Activated Phosphine in Wet Chloroform. Phosphinic acid (87%) and phosphonic acid (10%) were the major products. A minor product, ethyl dihydrogen phosphinate (3%), was also noted. The latter probably arose from the traces of ethanol present in the chloroform as a stabilizer.

Products from Reaction of Peracid, Imidazole, and Phosphine (Tables I and II). When phosphine, peracid, and imidazole were reacted in chloroform, a single imidazole–phosphorus–containing product was formed in 19% yield. On the basis of the ^{31}P chemical shift (δ 1.92) and the magnitude of the coupling constants ($^1J_{P-H}$ = 610 Hz, $^3J_{P-N-CH}$ = 8.9 Hz, and $^3J_{P-N-CH'}$ = 8.3 Hz) this was assigned as either *N*-imidazolyl hydrogen phosphonic acid or its corresponding imidazolyl 3-*N*-oxide. The other phosphorus-containing products were phosphinic and phosphonic acid.

Reactions with Other Oxidants. Although not tabulated, phosphine was oxidized to phosphinic acid by MMPP in water containing glucose and to phosphinic (major) and phosphonic (minor) acids by hydrogen peroxide alone or with horseradish peroxidase in aqueous solution. Aqueous sodium hypochlorite converted phosphine to phosphinic acid and, in the presence of added methanol, to phosphinic acid and methyl dihydrogen phosphinate. In reactions involving glycerol ^{31}P NMR analyses indicated that the products were phosphinic acid (major, δ 4.25), phosphonic acid (δ −0.45), and five phosphorus compounds derived from glycerol [δ 5.31, 3.74 (major), 2.78, −2.54, and −3.07].

Metabolism in Rats. ^{31}P NMR analyses of the urine of rats treated with zinc phosphide indicated the occurrence of phosphinic, phosphonic, and phosphoric acids. Parallel analyses of urine from control rats (treated only with corn oil) demonstrated phosphoric acid as the only phosphorus-containing compound.

DISCUSSION

The products that arise from phosphine on its reaction with peracid fall into two classes, the relative significance of which is largely solvent dependent. The first class is the series of oxy acids which represents sequential oxidations probably occurring through the intermediacy

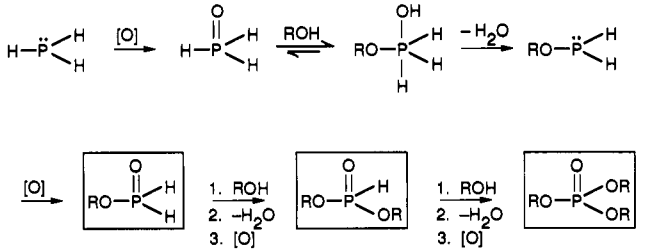

Figure 2. Proposed reactions of phosphine and MCPBA in methanol (R = CH_3). Products observed by ^{31}P NMR are shown in boxes. Phosphine is observable by ^{31}P NMR but is not seen when oxidant is present. The remaining structures are proposed intermediates. Analogous inorganic phosphorus acids (R = H) are obtained from water added to or generated in the reaction.

of the tautomeric phosphites. These tend to form in greatest amount when reactive nucleophiles are not available in the reaction medium. The second class comprises the various esters. This is of greater toxicological interest since it is indicative of reactive phosphorylating species produced during the course of oxidation and which can be trapped by added nucleophiles, including alcohols and the biologically relevant nitrogen heterocycle imidazole. It is noteworthy that esterification occurs only when the reaction cascade is initiated by the addition of oxidant, i.e., only when reactive electrophilic species are generated.

The products observed from the oxidation of phosphine by peracids in the presence of hydroxylic scavengers can be rationalized by the reaction sequence shown in Figure 2. Phosphine oxide is presumably the initially formed product and a candidate phosphorylating agent; others, in order, are the alkyl dihydrogen phosphinate and dialkyl hydrogen phosphonate. The product distributions noted are interpretable in the context of a fairly complex set of competing reactions and intermediate equilibria. Consistent with the involvement of various 5-coordinate intermediates, the product distributions in the overall reaction sequence are sensitive to steric bulk. For example, the formation of monoesters [HP(O)(R)OH] decreases in the order primary alcohol ≥ secondary alcohol ≫ tertiary alcohol, and a similar pattern pertains to the di- and triester products [$HP(O)R_2$ and $RP(O)R_2$] but with the percentages of the ethyl and propyl ester derivatives decreased relative to the methyl esters. While the same steric factors presumably operate in the further esterifications, the product yields are also influenced by the loss of an alkoxy substituent competing with loss of hydroxide in the multiply substituted trigonal bipyramidal intermediates.

The N-phosphorylation of imidazole by phosphine occurs only on the addition of peracid and therefore must also involve oxidative activation. However, either competing or subsequent oxidation at the other nitrogen center of the imidazole is also plausible, and the product may be the *N*-imidazolyl hydrogen phosphonic acid or its 3-*N*-oxide.

The toxicological relevance of these chemical studies is implicit in the observation that rats exposed to phosphine excrete phosphorus oxy acids in their urine. Consistent with this result, phosphine has also been observed to be oxidized in other organisms (WHO, 1988) to phosphinic, phosphonic, and phosphoric acids. Thus, phosphine is metabolically oxidized and, ipso facto, potent phosphorylating species must be generated in situ. Although the significance and fate of such electrophiles generated in vivo remain unknown, it is interesting to speculate that if the oxidation of phosphine is carried out by a hemoprotein oxidase, then phosphine may serve as both a substrate and an inhibitor; i.e., the activated intermediates may phosphorylate nucleophiles at or near the active site of the oxidase. Mechanistic similarities have been previously noted between cytochrome P_{450} oxidases and peracids (Casida and Ruzo, 1986).

In summary, the chemical toxicology of phosphine may involve multiple features of its reactivity. First, phosphine is a good nucleophile and might interact directly with the iron atom in various hemoproteins involved in oxygen and electron transport, e.g., cytochrome *c* oxidase. Second, it is a powerful reducing agent and can enter into numerous redox processes, including those that lead to the accumulation of radical species or the production of superoxide. Third, as established in the present study, it can undergo oxidative conversion to electrophilic species which may react in vivo with biological nucleophiles conceivably including DNA or other critical sites to disrupt chromosomal functions.

ACKNOWLEDGMENT

Supported in part by National Institutes of Health Grants 5 PO1 ES00049 and 5 RO1 ES04863. We thank Shao-Yong Wu, Kathryn Moorhouse, and Judith Engel for suggestions and assistance and Mark Sanders for the GC/MS measurements.

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Received for review July 1, 1991. Accepted August 13, 1991.

Registry No. PH_3, 7803-51-2; CH_3OH, 67-56-1; C_2H_5OH, 64-17-5; *n*-C_3H_7OH, 71-23-8; *i*-C_3H_7OH, 67-63-0; *t*-C_4H_9OH, 75-65-0; $H_2P(O)OH$, 6303-21-5; $HP(O)(OH)_2$, 13598-36-2; $HOP(O)(OH)_2$, 7664-38-2; $H_2P(O)OMe$, 14684-31-2; $H_2P(O)OC_2H_5$, 14684-32-3; $H_2P(O)OC_3H_7$-*n*, 18108-08-2; $H_2P(O)OC_3H_7$-*i*, 51763-59-8; $H_2P(O)OC_4H_9$-*t*, 66447-17-4; $HP(O)(OMe)OH$, 13590-71-1; $HP(O)(OC_2H_5)OH$, 15845-66-6; $HP(O)(OC_3H_7$-*n*$)OH$, 42023-31-4; $HP(O)(OC_3H_7$-*i*$)OH$, 42800-31-7; $HP(O)(OC_4H_9$-*t*$)OH$, 16540-40-2; $HP(O)(OMe)_2$, 868-85-9; $HP(O)(OC_2H_5)_2$, 762-04-9; $HP(O)(OC_3H_7$-*n*$)_2$, 1809-21-8; $HP(O)(OC_3H_7$-*i*$)_2$, 1809-20-7; $P(O)(OMe)_3$, 512-56-1; $P(O)(OC_2H_5)_3$, 78-40-0; $P(O)(OC_3H_7$-*n*$)_3$, 513-08-6; imidazole, 288-32-4; *N*-imidazolyl hydrogen phosphonic acid 3-*N*-oxide, 137175-10-1.