to allow condensation of all the material, the mixture was analyzed by GC.

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Supplementary Material Available: Kinetic analysis of the cross-dimerizations (1 page). Ordering information is given on any current masthead page.

Hydrocarbon and Phosphate Triester Formation during Homolytic Hydrolysis of Organophosphonium Ions: An Alternate Model for Organophosphonate Biodegradation

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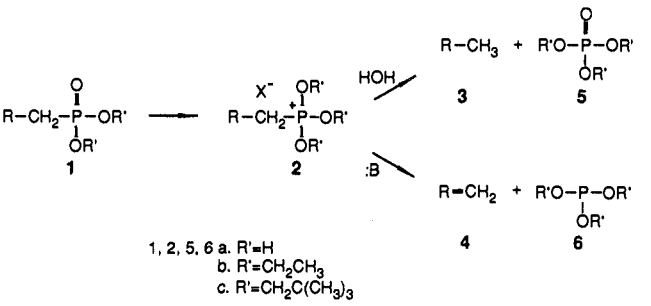
Abstract: Treatment of organotrineopentoxyphosphonium trifluoromethanesulfonates with base and organic peroxides results in carbon to phosphorus (C-P) bond cleavage. The products of the homolytic hydrolysis are hydrocarbons and trineopentyl phosphate. Reaction of the organophosphonium ions with only base leads to oxygen to phosphorus (O-P) bond cleavage with a complete absence of C-P bond cleavage. The likely intermediacy of a pentacovalent phosphonyl radical during the homolytic hydrolysis provides the basis for an alternate mechanistic formulation for the C-P bond cleavage observed during organophosphonate biodegradation. This formulation is unique in its prediction of inorganic phosphate as the immediate phosphorus-containing product of microbe-mediated C-P bond cleavage.

Microbial degradation of organophosphonates **1a** (Scheme I) involving cleavage of carbon to phosphorus (C-P) bonds provides a unique challenge to chemical modeling due to the apparent exploitation of chemistry that lacks direct literature precedent. All inorganic phosphate **5a** necessary for survival is derived by the microbe from the organophosphonate **1a** phosphorus. The remainder of the organophosphonate is not utilized by the microbe as in the case of alkylphosphonate **1a** biodegradation, where (Scheme I) alkanes **3** and small amounts of alkenes **4** are generated. Although various chemical mechanisms[1] have been formulated that can account for alkane and alkene formation during organophosphonate biodegradation, one type of mechanistic formulation has been overlooked prior to this report.

Hydrolysis of phosphonium ions[2] and phosphorus ylides[3] has long been precedented to result in C-P bond cleavage with, in certain cases, generation of alkanes and alkenes. Likewise, hydrolysis of organophosphonate C-P bonds catalyzed by microbes might involve initial activation of the organophosphonate as an organophosphonium ion **2a** (Scheme I). Alkanes would then be the dominant product of alkylphosphonate biodegradation. The trace levels of alkene formation might arise from a small amount of competing β-eliminative fragmentation of the organophosphonium ion.

Scheme I



Unfortunately, the relevance of organophosphonium ion and phosphorus ylide hydrolysis to microbial cleavage of organophosphonate C-P bonds is rather indirect. Hydrolyses of organophosphonium ions[4] structurally similar to those that could be generated during microbial degradation of organophosphonates have not been examined. This investigation thus began with the preparation and characterization of biologically relevant models in the form of organotrialkoxyphosphonium ions (**2b**, **2c**). Hydrolysis of these organophosphonium ions under appropriate conditions has been discovered to result in facile C-P bond cleavage.

Results and Discussion

Preparation of the Organophosphonium Models. Organophosphonium ions that most closely resemble the putative reactive intermediate formed during microbial degradation of organophosphonates are found as reactive intermediates during Arbuzov

(1) (a) Cordeiro, M. L.; Pompliano, D. L.; Frost, J. W. *J. Am. Chem. Soc.* **1986**, *108*, 332. (b) Shames, S. L.; Wackett, L. P.; LaBarge, M. S.; Kuczkowski, R. L.; Walsh, C. T. *Bioorg. Chem.* **1987**, *15*, 366. (c) Frost, J. W.; Loo, S.; Cordeiro, M. L.; Li, D. *J. Am. Chem. Soc.* **1987**, *109*, 2166. (d) Avila, L. Z.; Frost, J. W. *J. Am. Chem. Soc.* **1988**, *110*, 7904. (e) Avila, L. Z.; Frost, J. W. *J. Am. Chem. Soc.* **1989**, *111*, 8969.

(2) (a) Fenton, G. W.; Ingold, C. K. *J. Chem. Soc.* **1929**, 2342. (b) Hey, L.; Ingold, C. K. *J. Chem. Soc.* **1933**, 531. (c) Kumli, K. F.; McEwen, W. E.; VanderWerf, C. A. *J. Am. Chem. Soc.* **1959**, *81*, 3805. (d) Zanger, M.; VanderWerf, C. A.; McEwen, W. E. *J. Am. Chem. Soc.* **1959**, *81*, 3806. (e) Hoffmann, H. *Liebigs Ann. Chem.* **1960**, *634*, 1. (f) Aksnes, G.; Songstad, J. *Acta Chem. Scand.* **1962**, *16*, 1426. (g) Hudson, R. F.; Green, M. *Angew. Chem. Int. Ed. Engl.* **1963**, *2*, 11. (h) Aksnes, G.; Brudvik, L. J. *Acta Chem. Scand.* **1963**, *17*, 1616. (i) McEwen, W. E.; Kumli, K. F.; Blade-Font, A.; Zanger, M.; VanderWerf, C. A. *J. Am. Chem. Soc.* **1964**, *86*, 2378. (j) Aksnes, G.; Brudvik, L. J. *Acta Chem. Scand.* **1967**, *21*, 745. (k) Luckenbach, R. *Phosphorus* **1972**, *1*, 223. (l) Aksnes, G. *Phosphorus Sulfur Relat. Elem.* **1977**, *3*, 227.

(3) (a) Coffman, D. D.; Marvel, C. S. *J. Am. Chem. Soc.* **1929**, *51*, 3496. (b) Schöllkopf, U. *Angew. Chem.* **1959**, *71*, 260. (c) Bestmann, H. J.; Häberlein, H.; Pils, I. *Tetrahedron* **1964**, *20*, 2079. (d) Bestmann, H. J. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 583. (e) Seyferth, D.; Singh, G. *J. Am. Chem. Soc.* **1965**, *87*, 4156. (f) Isslelb, K.; Lindner, R. *Liebigs Ann. Chem.* **1966**, *699*, 40. (g) Seebach, D. *Chem. Ber* **1972**, *105*, 487. (h) Schnell, A.; Tebby, J. C. *J. Chem. Soc., Chem. Commun.* **1975**, 134. (i) Schnell, A.; Dawber, J. G.; Tebby, J. C. *J. Chem. Soc., Perkin Trans. 2* **1976**, 633. (j) Schnell, A.; Tebby, J. C. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1883.

(4) (a) Colle, K. S.; Lewis, E. S. *J. Org. Chem.* **1978**, *43*, 571. (b) Hudson, H. R.; Rees, R. G.; Weekes, J. E. *J. Chem. Soc., Chem. Commun.* **1971**, 1297. (c) Landauer, S. R.; Rydon, H. N. *J. Chem. Soc.* **1953**, 2224.

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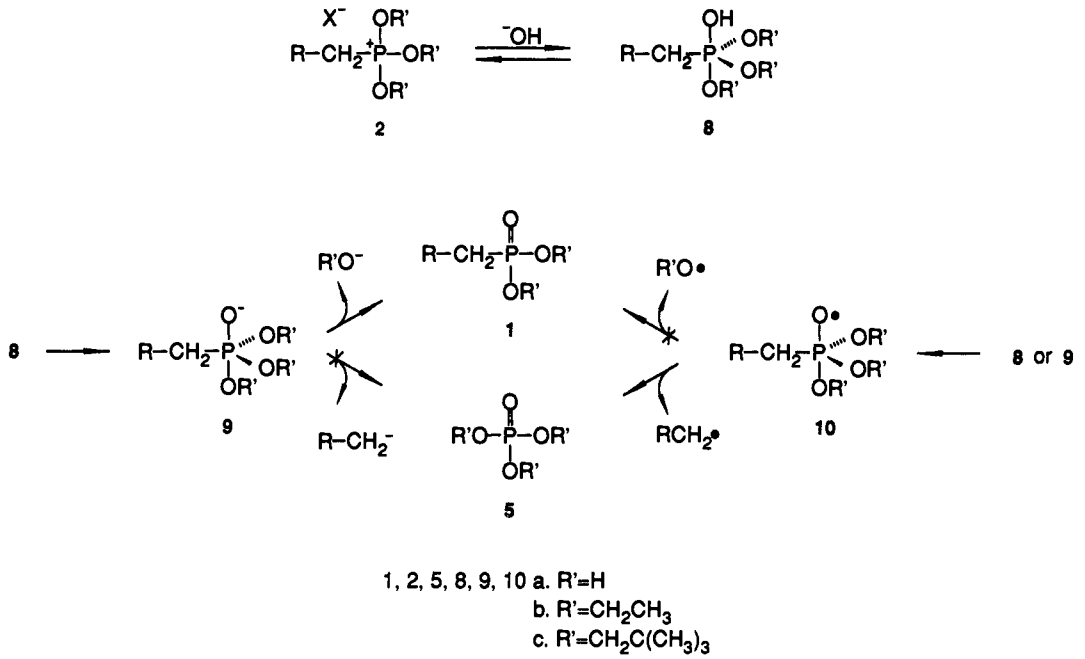
Scheme II



1, 2, 5, 8, 9, 10 a. R'=H
b. R'=CH_2CH_3
c. R'=$CH_2C(CH_3)_3$

condensations.[5] Reaction (Figure 1) of triethyl phosphite (**7b**) with ethyl iodide leads to unstable, intermediate ethyltriethoxyphosphonium iodide (**2b**, R = CH_3, X = I).[5] The nucleophilic iodide subsequently attacks a carbon attached to one of ethyltriethoxyphosphonium ion's (**2b**, R = CH_3, X = I) oxygens, leading to the formation of diethyl ethylphosphonate (**1b**, R = CH_3) and ethyl iodide. Although ethyltriethoxyphosphonium iodide (**2b**, R = CH_3, X = I) fragments rapidly, the lifetime of this type of intermediate can be lengthened by replacing ethyl iodide with ethyl trifluoromethanesulfonate as the alkylating reagent. The resulting ethyltriethoxyphosphonium trifluoromethanesulfonate (**2b**, R = CH_3, X = CF_3SO_3) has a sufficiently long lifetime in chloroform solution to be detectable by ^{31}P NMR (Figure 1a).

An alternate method[4a] (Figure 1b) for preparing ethyltriethoxyphosphonium trifluoromethanesulfonate (**2b**, R = CH_3, X = CF_3SO_3) is to react diethyl ethylphosphonate (**1b**, R = CH_3) with highly activated alkylating agents such as ethyl trifluoromethanesulfonate. At the degrading enzyme's active site, a proton could be delivered to the oxygen doubly bonded to the organophosphonate phosphorus **1a** (Scheme I), thereby generating an organophosphonium ion **2a**. Mimicking enzyme active site chemistry is difficult, but the reactivity of the oxygen in question with electrophiles can at least be approximated by examining its reactivity with the electrophilic methylene carbon of ethyl trifluoromethanesulfonate. Reaction of diethyl ethylphosphonate (**1b**, R = CH_3) with ethyl trifluoromethanesulfonate followed by dilution in chloroform affords a ^{31}P NMR (Figure 1b) indicating unreacted organophosphonate and the formation of a new chemical species. The associated resonance is identical with the ^{31}P resonance of a species derived from reaction of triethyl phosphate (**5b**) with ethyl trifluoromethanesulfonate. The ethyltriethoxyphosphonium trifluoromethanesulfonate (**2b**, R = CH_3, X = CF_3SO_3) formed by both reactions (Scheme II) illustrates that organophosphonates can be directly converted into organophosphonium ions via electrophilic attack at the oxygen doubly bonded to the phosphorus of the organophosphonate.

Ethyltriethoxyphosphonium trifluoromethanesulfonate (**2b**) is stable in dry organic solvents such as chloroform and acetonitrile. Unfortunately, isolation and characterization of this phosphonium ion is complicated by its facile fragmentation to diethyl ethylphosphonate (**1b**, R = CH_3). This lack of stability precluded use of ethyltriethoxyphosphonium trifluoromethanesulfonate to model C–P bond cleavage during heterolytic and homolytic hydrolysis of organophosphonium ions. Instead, organotrineopentoxyphosphonium trifluoromethanesulfonates (**2c**, X = CF_3SO_3) were used. The attenuated nucleophilicity of trifluoromethanesulfonate

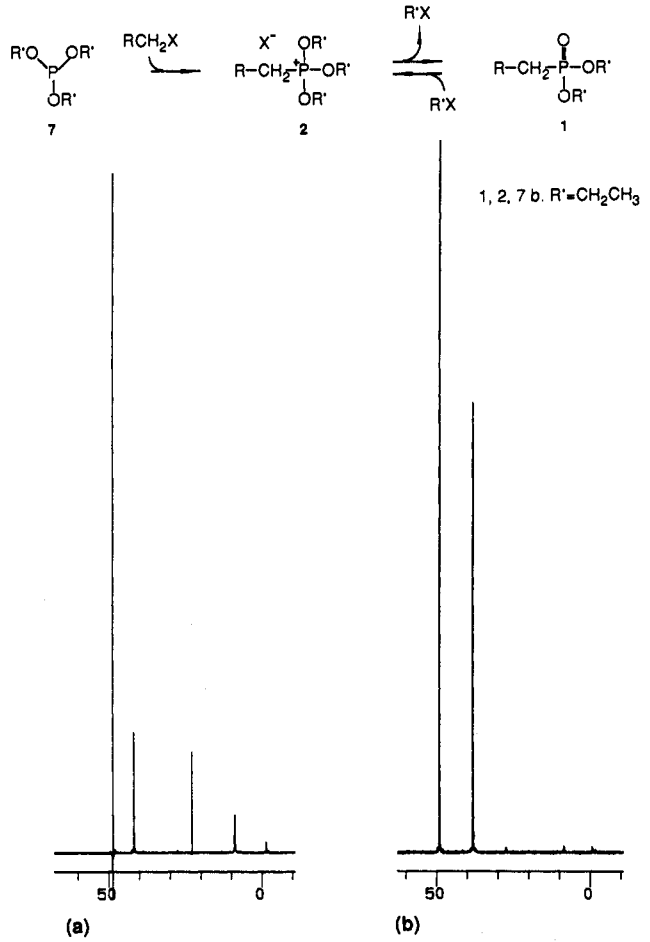
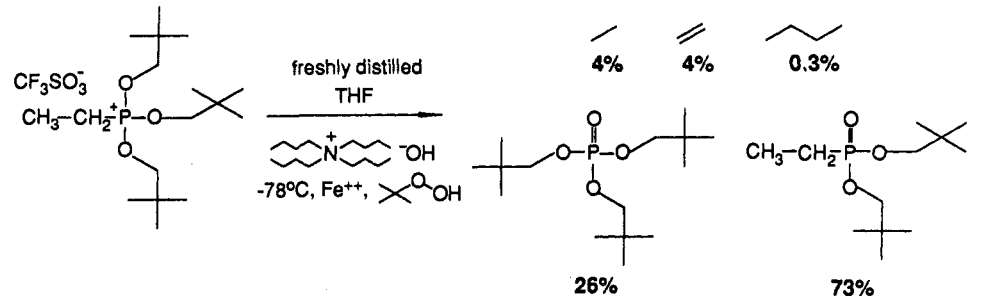


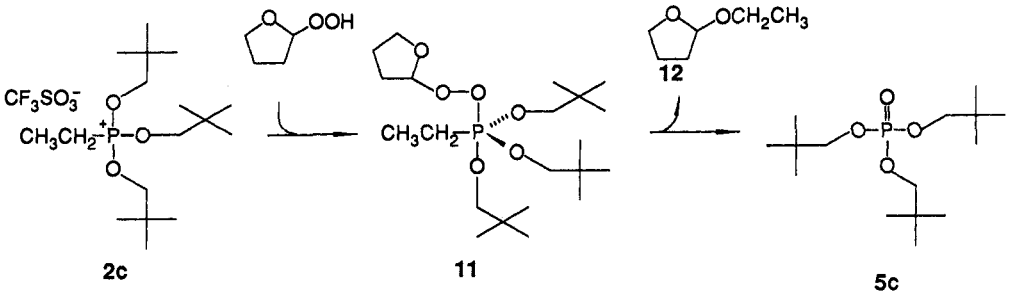
Figure 1. ^{31}P NMR ($CDCl_3$) of crude reaction mixtures containing ethyltriethoxyphosphonium trifluoromethanesulfonate (**7b**) (δ = 48) formed by reaction of ethyl trifluoromethanesulfonate with (a) triethyl phosphite (**7b**) (δ = 139) and (b) diethyl ethylphosphonate (**1b**) (δ = 39). Triethyl phosphite, but not diethyl ethylphosphonate, is completely consumed during reaction with ethyl trifluoromethanesulfonate.

(5) Bhattacharya, A. K.; Thyagarajan, G. *Chem. Rev.* **1981**, *81*, 415.

Scheme III



Scheme IV



and steric hindrance to attack at the methylene carbon of the neopentyl substituents enabled the organotrineopentoxyphosphonium trifluoromethanesulfonates (**2c**, X = CF_3SO_3) to be isolated, purified by crystallization, and characterized. These organophosphonium ions were prepared by converting the appropriate alcohol to a trifluoromethanesulfonate followed by condensation with trineopentyl phosphite.

Heterolytic and Homolytic Hydrolyses. Reaction of hydroxide with tetraalkylphosphonium ions yields hydrocarbon and trialkylphosphine oxide and is characterized by a rate expression that is second order in hydroxide concentration.[2c-f,h,j,l] A parallel mechanism for organotrineopentoxyphosphonium trifluoromethanesulfonate (**2c**, X = CF_3SO_3) hydrolysis (Scheme II) would proceed through organohydroxyphosphorane **8c** and organohydroxyphosphorane anion **9c**. To assess possible relatedness to tetraalkylphosphonium ion hydrolysis, organotrineopentoxyphosphonium trifluoromethanesulfonates (**2c**, X = CF_3SO_3) were reacted with tetrabutylammonium hydroxide in tetrahydrofuran solvent. Both at room temperature and at −78 °C, only dineopentyl organophosphonate (**1c**) formation was observed. This indicates that oxygen to phosphorus (O–P) bond cleavage (Scheme II, **9c** to **1c**) is proceeding to the complete exclusion of C–P bond cleavage (Scheme II, **9c** to **5c**) during heterolytic hydrolysis.

A difficulty with using extant organophosphonium ion or phosphorus ylide hydrolysis precedent for organophosphonate biodegradation follows from the nature of the groups attached to phosphorus. There is only the choice of C–P bond cleavage for these molecules with leaving group ability determined by carbanion stability at the carbon atom formerly attached to phosphorus.[2a,b,e] In contrast, both C–P and O–P bond cleavage can occur with organotrineopentoxyphosphonium ions (**2c**). Although the free energy change associated with phosphate formation might be expected to favor C–P bond cleavage, the stability of an alkoxide relative to an unstabilized carbanion likely introduces a kinetic factor that drives heterolytic hydrolysis toward O–P bond cleavage. This direction of bond breakage might be altered by a homolytic hydrolysis (Scheme II). Intermediacy of a pentacovalent organophosphonyl radical **10c** could conceivably direct the hydrolysis toward C–P bond cleavage with the simultaneous formation of alkyl radical and trineopentyl phosphate (**5c**) as opposed to O–P bond cleavage and the generation of alkoxy radical and dineopentyl organophosphonate (**1c**). Biological homolytic hydrolysis presumably could follow from abstraction of a hydrogen atom from organohydroxyphosphorane (**8a**) or oxidation of deprotonated organohydroxyphosphorane (**9a**).

Reactions of organotrineopentoxyphosphonium ions (**2c**, X = CF_3SO_3) with basic hydroperoxides both in the presence and in the absence of metal ions were explored as potential routes for achieving homolytic hydrolysis. Ethyltrineopentoxyphosphonium trifluoromethanesulfonate was reacted (Scheme III) with *tert*-butyl hydroperoxide, tetrabutylammonium hydroxide, and Fe^{2+} in tetrahydrofuran at −78 °C. Ethane and trineopentyl phosphate formation are indicative of C–P bond cleavage. Ethene and butane are the expected products of disproportionation and dimerization of the ethyl radical. Homolytic hydrolysis of organotrineopentoxyphosphonium ions leading to C–P bond cleavage can also be achieved without addition of a metal ion when 2-hydroperoxytetrahydrofuran[6] is used. Because of its simplicity, these reaction conditions were used for examining the C–P bond cleavage of a number of organotrineopentoxyphosphonium ions. The products and yields derived from reaction of organotrineopentoxyphosphonium trifluoromethanesulfonates (R = CH_3, CH_3CH_2, CH_2CH, and C_6H_5) with tetrabutylammonium hydroxide and an approximately stoichiometric amount of 2-hydroperoxytetrahydrofuran in tetrahydrofuran at −78 °C are summarized in Table I. If the reaction is run at room temperature, only dineopentyl organophosphonate is formed.

Alternate Path for Organophosphonium Ion C–P Bond Cleavage. Inspection of Table I indicates an excellent reaction mass balance with trineopentyl phosphate and dineopentyl organophosphonate accounting for 93–99% of the organotrineopentoxyphosphonium ion consumed by reaction with 2-hydroperoxytetrahydrofuran. At the same time, the yields of hydrocarbons suggested that C–P bond cleavage may be leading to products other than hydrocarbons. Closer examination of the reaction of ethyltrineopentoxyphosphonium ion with 2-hydroperoxytetrahydrofuran resulted in the identification of (±)-2-ethoxytetrahydrofuran (**12**, Scheme IV), which was formed at levels comparable to the combined yields of ethane and ethene.

Mechanisms that can explain 2-ethoxytetrahydrofuran formation (Scheme IV) proceed through an organoperoxyphosphorane that is structurally reminiscent of the peroxy intermediates formed during Baeyer–Villiger oxidations. However, there is little other similarity between these two reactions. Baeyer–Villiger insertion of oxygen between C–P bonds is rare and apparently is restricted to systems having a high degree of C–P bond angle strain that is relieved upon insertion of the oxygen

(6) (a) Robertson, A. *Nature* **1948**, *162*, 153. (b) Rhein, H.; Criegee, R. *Angew. Chem.* **1950**, *62*, 120.

Scheme V

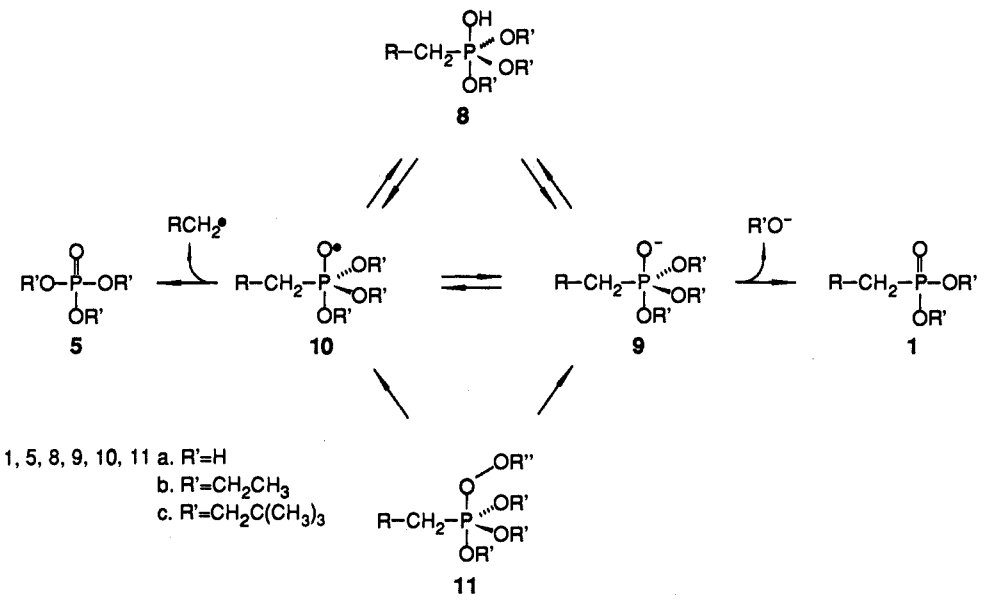
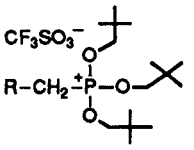
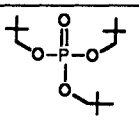
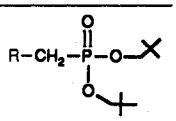


Table I. Products Formed (% Yield) from Homolytic Hydrolysis of Organotrineopentoxyphosphonium Ions



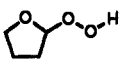
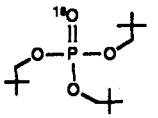
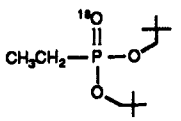
R	R—CH3 (R=CH2)		
CH_3	10 (3)	39	60
$CH_3—CH_2$	8 (3)	38	60
$CH_2=CH$	19	62	31
C_6H_5	13	38	59

atom.[7] In addition to not being associated with a strained-ring system, the collapse of organoperoxyphosphorane of Scheme IV contrasts markedly with the molecular reorganization that occurs during collapse of the organoperoxy intermediate in Baeyer–Villiger oxidations.

Organoperoxyphosphorane versus Organohydroxyphosphorane Intermediacy during C–P Bond Cleavage. Reaction conditions exploited for homolytic hydrolysis were anticipated to lead to pentacovalent organophosphonyl radical **10c** via two routes (Scheme V) that differ in the structure of the phosphorane initially formed from the organophosphonium ion **2c**. Hydroxide could first add with resulting formation of organohydroxyphosphorane (**8c**). Abstraction of a hydrogen atom from **8c** or deprotonation to **9c** followed by oxidation might afford the pentacovalent organophosphonyl radical **10c**. Alternatively, the anion of the hydroperoxide could form organoperoxyphosphorane (**11c**). Subsequent homolysis of the peroxide bond would then afford the pentacovalent organophosphonyl radical **10c**. Even during homolytic hydrolysis, O–P bond cleavage (Scheme V) was expected to compete with C–P cleavage. Phosphorane anion **9c**, a likely candidate for collapse to dineopentyl organophosphonate (**1c**), could be derived from both organohydroxyphosphorane (**8c**) and organoperoxyphosphorane (**11c**).

Differentiation (Scheme V) between the respective involvement of organohydroxyphosphorane (**8c**) or organoperoxyphosphorane (**11c**) in C–P and O–P bond cleavage was achieved by reacting ^{18}O-labeled tetrabutylammonium hydroxide and unlabeled 2-hydroperoxytetrahydrofuran with ethyltrineopentoxyphosphonium trifluoromethanesulfonate. Reaction via organohydroxyphosphorane (**8c**, R = CH_3) would lead to ^{18}O-labeled organophosphonate and organophosphate products. Organoperoxyphosphorane (**11c**, R = CH_3) intermediacy would be reflected by the lack of ^{18}O label in the phosphorus-containing products. Subsequent analysis revealed (Table II) a high degree of ^{18}O label in dineopentyl ethylphosphonate and only a small amount of ^{18}O label in trineopentyl phosphate. This labeling pattern is consistent with O–P bond cleavage proceeding almost exclusively via initial formation of organohydroxyphosphorane (**8c**, R = CH_3). Most of the C–P bond cleavage is the result of initial formation of organoperoxyphosphorane (**11c**, R = CH_3). However, some C–P bond cleavage does apparently involve initial formation of organohydroxyphosphorane (**8c**, R = CH_3) followed by conversion (Scheme II) to pentacovalent organophosphonyl radical (**10c**, R = CH_3).

Table II. ^{18}O Content of Products Formed from Reaction of Ethyltrineopentoxyphosphonium Trifluoromethanesulfonate with Tetrabutylammonium hydroxide-^{18}O and 2-Hydroperoxytetrahydrofuran

reactants/products	% ^{18}O incorp
$Bu_4N^+{}^{18}OH^-$	95
	0
	15
	91

Conclusion

Direct conversion of organophosphonates into organophosphonium ions along with formation of hydrocarbons and phosphate triester during homolytic hydrolysis of organophosphonium ions establishes a completely different mechanistic framework for viewing microbial degradation of organophosphonates. The importance of these observations is best evaluated by comparison to other mechanisms that have been proposed for the microbial cleavage of organophosphonate C–P bonds. These mechanisms and the accompanying chemical models

(7) Quin, L. D.; Kisalus, J. C.; Mesch, K. A. *J. Org. Chem.* **1983**, *48*, 4466.

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