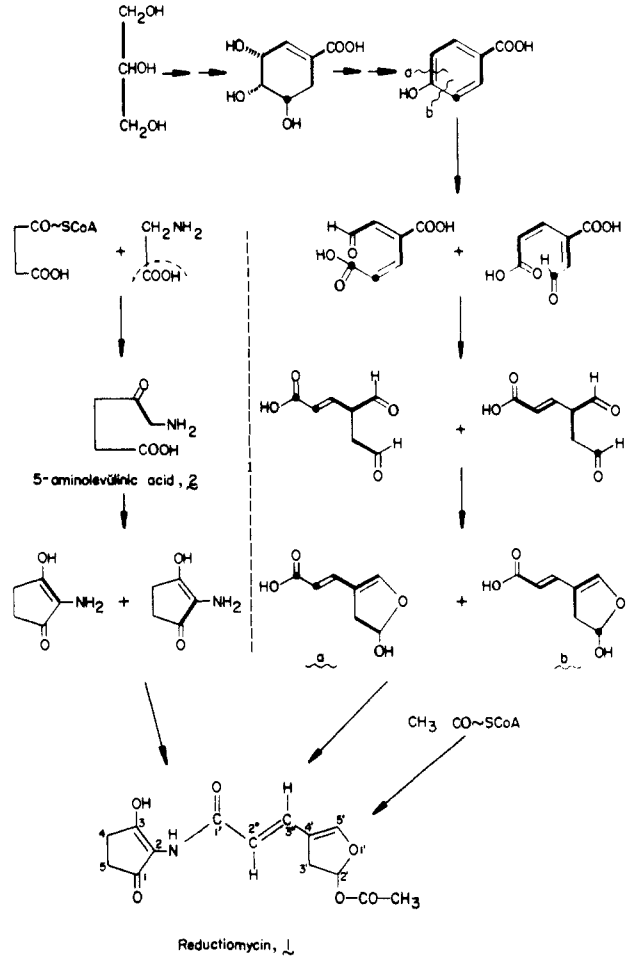
Table I

^{13}C chem shift, ppm	assignment	^{13}C enrichments, %; J_{cc}, Hz, in **1** derived from [U^{13}-C$_3$]glycerol	[1,2-^{13}C$_2$]acetate	[4,5-^{13}C$_2$]-**2**[a]	4-hydroxy[7-^{13}C]-benzoic acid
20.92	CH_3CO	4.35; 60	11.5; 60		
25.52	4	4.35; 45	15.6; 45		
32.14	5	4.35; 40	15.6; 40		
34.19	3′	6.7; 41	3.1		
98.45	2′	4.7; 41	3.1		
114.68	4′	6.3; 78, 64, 41	2.2		
115.09	2	3.7	3.2	3.7; 62.6, 80	
115.35	2″	3.5; 68, 73.5	1.8		
135.48	3″	5.9; 64, 73.5, 11	2.1		
150.52	5′	6.7; 78, 11	2.1		64
165.71	1″	4.2; 68	2.4		
169.53	CH_3CO	3.8; 60	11.5; 60		
173.90	3	4.7; 45	12.5; 45	1.8; 80	
197.56	1	3.95; 40	11.5; 40	1.8; 62.6	

[a] Signals for which no figures are given showed no significant enrichment.

Scheme I



These results point to the shikimate pathway as the source of the dihydrofuran moiety of **1**. [U-^{13}C$_3$]Glycerol will label shikimate as shown in Scheme I. Coupling pattern **b** implies ring cleavage between C-4 and C-5 of shikimate or a metabolite thereof. The additional presence of coupling pattern **a** reflects cleavage between C-3 and C-4, indicating that the substrate must be a symmetrical product containing all seven carbon atoms of shikimate. 4-Hydroxybenzoate was considered a plausible candidate for this ring cleavage. This notion was tested by synthesizing 4-hydroxy[7-^{13}C]benzoate[13] and feeding it (400 mg, 99% ^{13}C) to *S. xanthochromogenus*. A 64% enrichment solely in C-5′ of the resulting **1** (85 mg) confirmed that indeed 4-hydroxybenzoate or a closely related product, e.g., the corresponding aldehyde, must be the substrate for the ring cleavage reaction leading to the formation of the dihydrofuran moiety of **1**. The conclusions are summarized in Scheme I in terms of a likely pathway to reductiomycin. These results add another example to the list of natural products that arise by cleavage of an aromatic ring, like the betacyanins[14] or the anthramycin family of antibiotics.[15]

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Registry No. 1, 68748-55-0; **2**, 106-60-5; $HOCH_2CH(OH)CH_2OH$, 56-81-5; HOAc, 64-19-7; *p*-$HOC_6H_4CO_2H$, 99-96-7.

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(15) Hurley, L. H. *Acc. Chem. Res.* **1980**, *13*, 263.

Degradation and Detoxification of Organophosphonates: Cleavage of the Carbon to Phosphorus Bond

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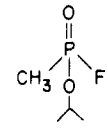
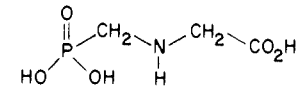
Ranging from the acetylcholinesterase inactivator sarin (**1**), to the herbicide glyphosate (**2**), organophosphonates rank among



1



2

the most biocidal of all organic molecules. Organophosphonates are characterized by a carbon atom covalently bonded to phosphorus. This bond is inert to vigorous acid and base hydrolytic conditions as well as to the action of phosphatase. Nonetheless, *Escherichia coli*[1,2] can cleave carbon to phosphorus bonds. This

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Table I

Organophosphonate	Products[a]	Mole Ratio of Products[b] (saturated : unsaturated)	
		E. coli	$Pb(OAc)_4$
$CH_3P(O)(OH)OH$ **3**	CH_4	–	–
$CH_3CH_2P(O)(OH)OH$ **4**	— (**9**), ═ (**10**)	30:1	8:1
$CH_3CH_2CH_2P(O)(OH)OH$ **5**	∧ (**11**), ⩘ (**12**)	60:1	5:1
$CH_3CH_2CH_2CH_2P(O)(OH)OH$ **6**	∧∧ (**13**), ⩘∧ (**14**)	600:1	6:1
$CH_3CH_2CH_2CH_2CH_2P(O)(OH)OH$ **7**	∧∧∧ (**15**), ⩘∧∧ (**16**)	90:1	7:1
$CH_3CH_2CH_2CH_2CH_2CH_2P(O)(OH)OH$ **8**	∧∧∧∧ (**17**), ⩘∧∧∧ (**18**)	2000:1	5:1

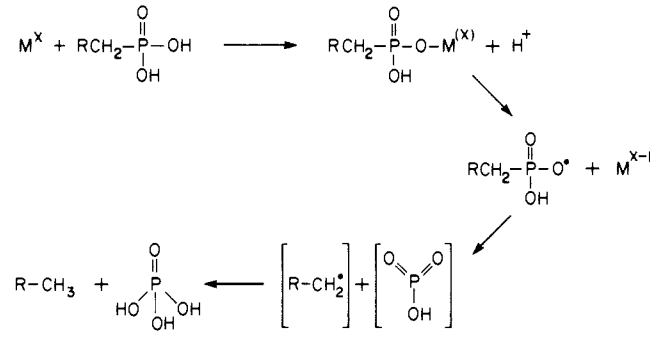
[a] Products were initially identified by coinjection with authentic samples on 0.19% picric acid on Graphpac GC, 80% Porapak N + 20% Porapak Q, Carbosphere, or Alumina F1. Further characterization followed GC–MS of the gaseous products and comparison with fragmentation patterns of authentic samples. [b] Ratios determined relative to internal standards by using an HP 3392 A integrator.

degradation raises questions concerning the chemical mechanism involved in the cleavage reaction and suggests a possible means for detoxification of organophosphonates.

Remarkably, the predominant degradation product of *E. coli*[6,7] growth on methylphosphonic acid **3** (Table I)[8] is methane gas. This degradation proved to be a general phenomenon. Ethane, propane, butane, pentane, and hexane were generated when *E. coli* were grown on ethyl-, propyl-, butyl-, pentyl-, and hexylphosphonic acids, respectively (Table I). Closer examination of the headspace over *E. coli* growths on the respective alkylphosphonic acids revealed the presence of ethene, 1-propene, 1-butene, 1-pentene, and 1-hexene as minor products.

Phosphonic acids that readily undergo cleavage of the carbon to phosphorus bond typically have a hydroxyl group[3] or carbonyl[4] α to the phosphorus. Our initial working hypothesis involved oxidation of the alkylphosphonic acids at the carbon directly attached to phosphorus by aerobically grown *E. coli*. Degradation of the resulting hydroxyl, keto, or hydroperoxy derivatives would be a comparatively simple matter for the microorganism.[5] Exhaustive examination by this laboratory of whole cells and cell lysates of *E. coli* grown on ^{12}C and ^{13}C methylphosphonic acid failed to detect any such intermediates when analyzed by ^{31}P and ^{13}C NMR. Quantitation of intracellular phosphorus[9] and

Scheme I



headspace methane[10] revealed a 1:1 mole ratio.

One must turn to radical-based decarboxylation of alkylcarboxylic acids to find similar product mixtures.[11] The relevance of such chemistry to the observed degradation was established by reaction of lead tetraacetate with the alkylphosphonic acids which supported growth of *E. coli*. Mixtures of saturated and unsaturated hydrocarbons were obtained as products (Table I). With such chemistry as a basis, the mechanism detailed in Scheme I could be operative in *E. coli* catalyzed degradation of alkylphosphonic acids.[13] Additional evidence for the proposed mechanism follows from *E. coli* growth on (cyclopropylmethyl)phosphonic acid (**19**) (R in Scheme I = cyclopropyl). Operation of radical-based dephosphorylation in *E. coli* would lead to a cyclopropylcarbinyl radical. This reactive intermediate can undergo skeletal rearrangement[14] to give 1-butene (**14**). Identification of 1-butene in such systems is a classical approach to establish radical processes.[15] Analysis of *E. coli* growth headspace indicated cyclopropylmethane *and* 1-butene.[16]

This initial stage of examination into a biological degradation has opened hitherto unexplored aspects of organophosphorus chemistry. Radical-based dephosphorylation might also account for reported *E. coli* growth on media where (aminomethyl)phosphonic acid ($NH_2CH_2PO_3H_2$) is the exclusive source of phosphorus.[1c] This mechanistic interpretation[17] is particularly intriguing due to its potential as a vehicle for detoxification of glyphosate.

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(5) α-Hydroxyl and α-keto phosphonic acids decompose to phosphorous acid. In a second step the phosphorous acid could be oxidized to inorganic phosphate. Inorganic phosphate could be obtained directly via intermediacy of an α-hydroperoxy derivative resulting from reaction with molecular oxygen: (a) Mikolajczyk, M.; Midura, W.; Grzejszczak, S. *Tetrahedron Lett.* **1984**, 2489. (b) Mikolajczyk, M.; Grzejszczak, S.; Midura, W.; Popielarczyk, M.; Omelanczuk, J. In "Phosphorus Chemistry. Proceedings of the 1981 International Conference"; Comstock, M. J., Ed.; American Chemical Society: Washington, DC, 1981; Chapter 11.
(6) The strain used in this study was *E. coli* RB 791 (W3110 lac L8I^q), an *E. coli* K-12 variant.
(7) Growth media consisted of distilled, deionized water containing glucose (26 mM), magnesium sulfate (1.0 mM), trizma·HCl (64 mM), sodium chloride (8.0 mM), ammonium chloride (19 mM), thiamine (0.014 mM), and alkylphosphonic acid (0.2 mM). The pH of the solution was adjusted to pH 7.0.
(8) In growths of *E. coli* on methylphosphonic acid there was a 60% conversion of the starting phosphonic acid to methane gas.
(9) Inorganic phosphate was localized exclusively within the cells. No detectable concentrations were present in the growth supernatant. Intracellular phosphorus concentrations were determined by combustion analysis of harvested cells.
(10) Determined by GC relative to propane as an internal standard.
(11) (a) Kochi, J. K. *J. Am. Chem. Soc.* **1965**, *87*, 3609. (b) Kochi, J. K.; Bacha, J. D.; Bethea, T. W., III *J. Am. Chem. Soc.* **1967**, *89*, 6538. (c) Kochi, J. K.; Bacha, J. D. *J. Org. Chem.* **1968**, *7*, 2746. (d) Sheldon, R. A.; Kochi, J. K. In "Organic Reactions"; Dauben, W. B., Ed.; Wiley: New York, 1972; Chapter 4, p 279.
(12) Lead tetraacetate (0.344 g, 0.776 mmol) and the phosphonic acid (3.37 mmol) were transferred to a roundbottom fitted with a reflux condenser. All manipulations were carried out under nitrogen atmosphere. The reagents were dissolved in dimethylformamide (6.5 mL) and the solution subjected to three cycles of freeze–thaw degassing. Reaction proceeded at 81 °C under nitrogen atmosphere. The headspace of the reaction was sampled every 30 min. Reaction was complete after 90 min.
(13) For dephosphorylation with lead tetraacetate: (mmol of ethane + ethene)/(starting mmol of lead tetraacetate) = 0.02. Oxidation of valeric acid for 48 h under otherwise identical conditions used for the dephosphorylation studies: (mmol of butane + butene)/(starting mmol of lead tetraacetate) = 0.01.
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(15) Krusic, P. J.; Eagan, P. J.; Filippo, J. S., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 250.
(16) Mole ratio of methylcyclopropane/1-butene, 50:1.
(17) It is also useful to contrast this proposed mechanism with the chemistry associated with the well-characterized breakdown of another aminophosphonate, (aminoethyl)phosphonic acid ($NH_2CH_2CH_2PO_3H_2$). (a) La Nauze, J. M.; Rosenberg, H. *Biochim. Biophys. Acta* **1967**, *148*, 811. (b) La Nauze, J. M.; Rosenberg, H. *Biochim. Biophys. Acta* **1968**, *165*, 438. (c) Dumora, C.; Lacoste, A.; Cassaigne, A. *Eur. J. Biochem.* **1983**, *133*, 119. (d) La Nauze, J. M.; Rosenberg, H.; Shaw, D. C. *Biochim. Biophys. Acta* **1970**, *212*, 332. (e) La Nauze, J. M.; Coggins, J. R.; Dixon, H. B. *Biochem. J.* **1977**, *165*, 409.

Acknowledgment. We are indebted to Professor J. I. Brauman for invaluable suggestions and to K. N. Smith and D. H. Li for experimental assistance. Research was partially funded by the Department of the Army (Contract DAAG29-85-K-0242).

Mechanism of Formation of the π-Allyl Palladium Chloride Complex from Methylenecyclohexane and Palladium Chloride

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The formal insertion of palladium into the allylic carbon–hydrogen bond of an olefin to provide a π-allyl complex is of interest as a prototypical transition-metal insertion and as a source of η^3 complexes for synthesis. While there is general agreement that the first step of the mechanism involves π-complexation of palladium with the olefin,[1,2] there are at least three possibilities for the subsequent insertion. These are shown in Scheme I for $PdCl_2$ and methylenecyclohexane as (a) explicit palladium insertion into the carbon–hydrogen bond to give a palladium hydride intermediate which loses hydrogen chloride,[3] (b) removal of the hydrogen as a proton by bound chloride,[4] or (c) removal of the proton by an external base.[5] The product of this step can dimerize to give the well-characterized η^3 complexes.[1] The three mechanisms are distinguished by their transition-state geometries, which under the analysis of More O'Ferrall,[6] could be reflected in the magnitude of the deuterium isotope effect for removal of the allylic hydrogen. We wish to report evidence which suggests that (a), the most popular path, is not operative with methylenecyclohexane and that transfer of the hydrogen as a proton by (b) or (c) are consistent with our analysis.

If the geometry for intramolecular hydride abstraction by palladium in pathway (a) approximates a four-membered ring with a hydrogen-transfer angle of 90–100°, model calculations predict a k_H/k_D of 1.7–2.3 at 25 °C.[6] As a test of this prediction for palladium we have found the isotope effect for the palladium-catalyzed isomerization of methylenecyclohexane to 1-methylcyclohexene, a reaction that may involve a reversible addition of a palladium hydride species in a four-center transition state,[7] to be 1.8 ± 0.5 at 60 °C.

A model of the transition state for pathway (b), constructed by using X-ray structure data from π-olefin complexes[8] for bond lengths and angles, shows the hydrogen transfer angle to be ca. 135° in the most favorable conformation. This leads to a prediction for the isotope effect of 3.5–4.2 at 25 °C by interpolation of More O'Ferrall's model. A value of 3.0–3.5 has been reported for insertion of platinum into an alkyl carbon–hydrogen bond at

Scheme I

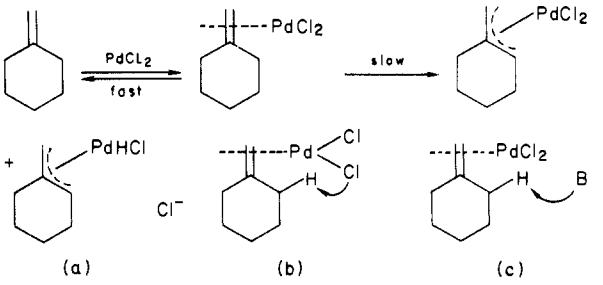


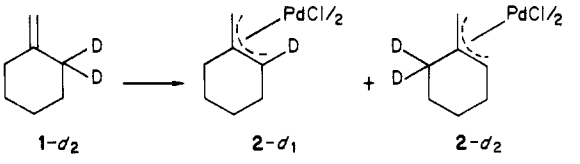
Table I. Product Isotope Effects for Conversion of **1**-d_2 to **2**-d_1 and **2**-d_2

Reaction conditions (solvent/reagents)[a]	k_H/k_D[b]
70% AcOH/$PdCl_2$ (aq), NaCl, $CuCl_2$	3.55 ± 0.1
AcOH/$PdCl_2$, NaCl, $CuCl_2$, NaOAc[c]	3.48 ± 0.1
DMF/$PdCl_2$	4.55 ± 0.1
DMF/$PdCl_2$, 21 °C	5.19 ± 0.1
DMF/$PdCl_2$, 86 °C	3.66 ± 0.1
benzene/$PdCl_2$, 2 equiv of DMF	4.32 ± 0.1

[a] Unless otherwise stated, all reactions were performed at 60 °C. [b] Analysis by GC–MS of the mixture of amines derived from **2**. [c] Conditions of Trost et al.[3c]

157 °C in a five-center transition state.[9] For pathway (c), proton abstraction by base should be linear and therefore display a maximum isotope effect of ca. 7 at 25 °C unless diminished by an unsymmetrical transition state.[10]

The measured isotope effects for the allylic palladation of **1**-d_2[11] to **2**-d_1 and **2**-d_2 by $PdCl_2$ are summarized in Table I. Reactions



in acetic acid (AcOH) with and without sodium acetate gave isotope effects of 3.5 ± 0.1 at 60 °C. This is most consistent with pathway (b), proton removal by bonded chloride with a hydrogen-transfer angle of 120–140°, a result that is also supported by stereochemical studies.[2,12] Brief mention has been made of isotope effects in similar reactions which are near these values.[12,13]

If the reaction is carried out in dimethylformamide (DMF) or in benzene containing 2 equiv of DMF, isotope effects of 4.55 and 4.32, respectively, are observed at 60 °C and values of 3.66 and 5.19 are obtaned at 21 °C and 86 °C, respectively. These values are most consistent with a nearly linear and symmetrical transition state and suggests abstraction of the proton by an external base.[14a] The DMF could act to displace the chloride ligand which then may act as an external base.

The fact that the allylic palladation and isomerization have different isotope effects rules out rate-determining formation of a π-allyl palladium hydride species as a common intermediate for these two reactions.[7] The possibility that the observed isotope effect for the formation of **2** results from slow decomposition of

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(9) Foley, P.; Whitesides, G. M. *Ibid.* **1979**, *101*, 2732.

(10) Reaction within dimers which could remove the angular constraints of (a), (b), or (c) appear geometrically difficult. Until further information is available the present test is considered to be applied to monomers.[1-5]

(11) Beak, P.; Berger, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 3848.

(12) Harvie, I. J.; McQuillin, F. J. *J. Chem. Soc. Chem. Commun.* **1978**, 747. See also: Collins, D. J.; Jackson, R.; Times, R. N. *Aust. J. Chem.* **1980**, *33*, 2663.

(13) A product isotope effect of 1.3 in the reaction of isobutene containing a single allylic deuterium atom with $PdCl_2$/NaCl/NaOAc in AcOH has been reported: Lukas, J.; Ramakers-Blom, J. E.; Hewitt, T. G.; de Boer, J. J. *J. Organomet. Chem.* **1972**, *46*, 167. Statistical correction factor allows derivation of a k_H/k_D value of 3.9.

(14) Melander, L.; Saunders, W. H. "Reaction Rates of Isotopic Molecules"; Wiley: New York, 1980; (a) p 157, (b) p 144.

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