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REVIEW

The formation and transformation of phosphorus–carbon bonds in living organisms

John S Thayer
Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221, USA

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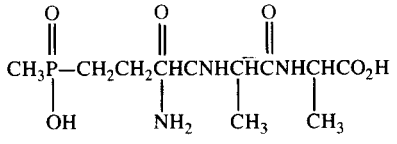
INTRODUCTION

Compounds containing phosphorus–carbon bonds were first discovered inside living organisms in 1959, when Horiguchi and Kandatsu reported the isolation of 2-aminoethylphosphonic acid (**I**) from sea anemones.[1] within the next few years, enough additional reports had been published to enable the writing of two reviews.[2,3] Two monographs, on one organometallic compounds in biological systems,[4] and the other on organometallic compounds in the environment,[5] discussed these compounds briefly. Nevertheless, despite a substantial literature, few individuals not actually working in this area appear to be aware that biogenic formation of phosphorus–carbon bonds can occur. This reveiw will endeavor to improve that situation by discussing recent work on biogenic organophosphorus compounds, with particular emphasis on mechanisms by which phosphorus–carbon bonds may be formed or broken and their relationship to corresponding mechanisms in the rapidly developing area of bio-organometalloidal chemistry.

$NH_2CH_2CH_2PO_3H_2$

I

Two compounds account for the great majority of biogenic organophosphorus species reported: the previously mentioned species **I** (occasionally referred to as 'ciliatine'), and a tripeptide (**II**, L-alanyl-L-alanylphosphinothricin, commonly named 'bialaphos'). These two compounds will receive separate discussion. Other biogenic compounds will be mentioned as a group.



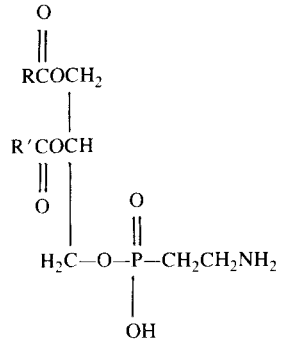
II

CILIATINE

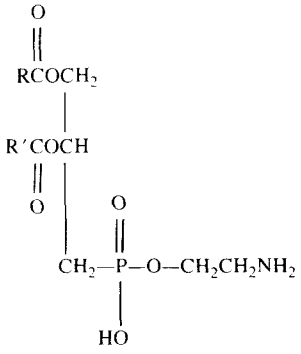
The distribuiton of this compound among organisms has been reviewed elsewhere.[6] Whilst it has occasionally been found as a free molecule, **I** usually occurs bound to larger molecules. Such derivatives usually have the prefix 'phosphono-' in their name; this is used as a counterpart to 'phospho-' in such terms as phosphonolipids, phosphonoproteins, phosphonoglycans, etc. Phosphonolipids are by far the most frequently reported examples of such materials and may represent the most common type of organophosphorus compounds found in nature.[6,7]

Baer and Stanacev[8] originally coined the term 'phosphonolipid' to describe two types of compounds analogous to phospholipids: molecules having a nitrogen-bearing portion bonded by carbon to the phosphorus atom (structure **III**), or molecules having a polydiester portion bonded to phosphorus through carbon (structure **IV**). To date, only compounds having structure **III** have actually been found in organisms.

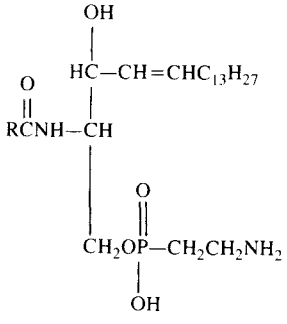
All naturally occurring phosphonolipids reported to date are esters of **I** or its *N*-mono-, di- or tri-methyl derivatives; a typical phosphonolipid structure is represented by **V**. Phosphinolipids, containing a carbon–phosphorus–carbon (C–P–C) linkage, have been prepared in the laboratory,[8] but have not yet been found in nature. Phosphonolipids usually contain only one ciliatine per molecule, but a few contain two[9] or even three.[10]



III



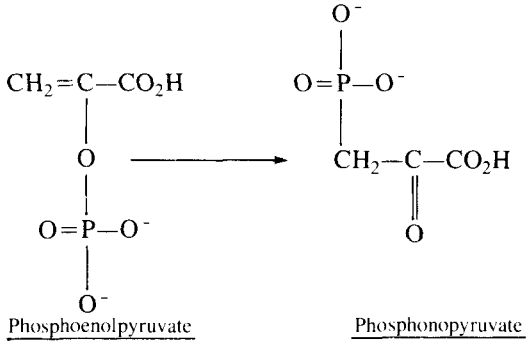
IV



V

Phosphonolipids have usually been isolated from invertebrates, such as the sea hare, *Aplysia kurodai*,[9,10] but their distribution is considerably more widespread than this might suggest,[6] and they have been found in many species of animals[6] and plants,[11] including such vertebrate sources as beef brain[12] and human sperm;[13] additional sources will doubtless be reported in the future. The proportion of phosphonolipids to all phosphorus-containing lipids varies, being about 20% in the ciliate protozoan *Tetrahymena thermophila*.[14] Biochemical roles for these compounds in organisms apparently arise from the presence of the phosphorus–carbon bond,[6,7] the existence of which will alter various physical properties (e.g. solubility in lipids) and related chemical properties (e.g. resistance to some enzymes such as hydrolases).[6,7]

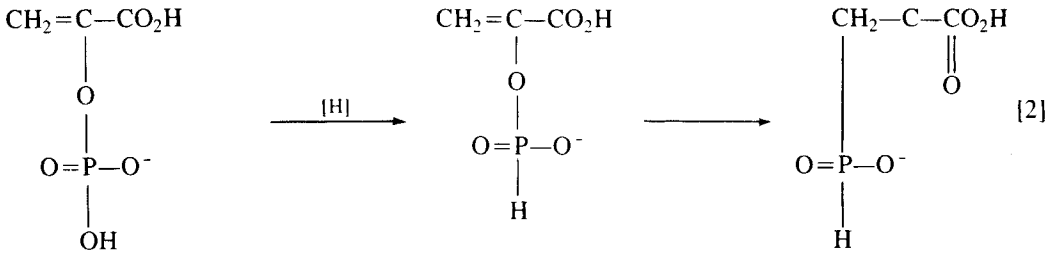
Research on the formation and rearrangements of biogenic phosphonates has been reviewed by Smith.[15] The crucial step, in which the phosphorus–carbon bond forms, is the rearrangement of phosphoenolpyruvate to phosphonopyruvate (Eqn [1]).



Phosphoenolpyruvate Phosphonopyruvate [1]

Mechanistic studies by Horiguchi and Rosenberg[16] confirmed that the carbon–phosphorus (C–P) linkage of **I** originated from the C-3 carbon and the phosphorus atom of phosphoenolypyruvate, and that 3-phosphonopyruvate formed when extracts from cells of *Tetrahymena pyriformis* were treated with phosphoenolpyruvate.[17] The various *N*-methyl derivatives of **I** apparently form after **I** itself has been synthesized. Cells of *T. thermophila* absorbed *N,N*-dimethylaminoethylphosphonate at the expense of phosphatidylethanolamine and phosphatidylcholine, but not at the expense of **I**-containing phosphonolipids;[10] these dimethylamino derivatives were not subsequently methylated to *N,N,N*-trimethylaminoethylphosphonate.

Other derivatives of **I** are less common and less well characterized. Ciliatine can bond to proteins or oligosaccharides to give phosphonoproteins or phosphonoglycans respectively.[6] These derivatives apparently form by reaction of previously formed **I**. As research in this area continues to develop and expand, more such compounds will undoubtedly be reported.

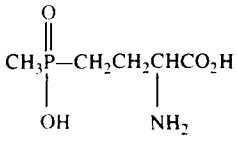


[2]

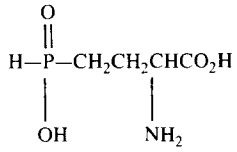
BIALAPHOS

This compound was first isolated by German scientists[18] from a culture of *Streptomyces viridochromogenes*. Their characterization included the isolation of a new amino-acid, phosphinothricin, **VI**. They reported that this compound was an antibiotic and inhibited glutamine synthetase.[18] Japanese scientists subsequently determined the biosynthetic pathway for this compound[19–25] and isolated intermediates containing a hydrogen–phosphorus–carbon (H–P–C) grouping.[20] Using isotopic labelling, these workers reported that: (1) the methyl group on the phosphorus atom of **VI** (and, by extension, of **II**), came from methionine; (2) the end aminoacetate group came from acetate ion (from glucose via acetylcoenzyme A); and (3) the two-carbon chain joining the methylphosphinyl and aminoacetyl groups also came from glucose through phosphoenolpyruvate.[19] In a separate experiment, they observed that phosphonopyruvic acid was not incorporated into **II**; however, compound **VII**, containing a phosphorus–hydrogen (P–H) linkage, readily converted to **II** and was detected in the growth medium of *Streptomyces hygroscopicus*.[20] The researchers therefore postulated the following sequence, involving reduction of phosphoenolpyruvate to phosphinoenolpyruvate, followed by migration and subsequent metabolism (Eqn [2]).[21]

Phosphite ion did not stimulate production of **II**, and was therefore considered unlikely to be a precursor. When carbon-labelled **II** was administered orally (1.85 mg kg^{-1}) to mice, 89.2% of the label emerged in feces and 7.9% in urine after 4 h.[26] The major metabolite was **VI**, but other unidentified metabolites were also detected.[26] The genes responsible for bialaphos biosynthesis by *S. hygroscopicus* have been isolated and characterized.[27–30] A mutant strain of *S. viridochromogenes* that showed resistance to **VI** had the gene conferring that resistance cloned to other bacterial species.[31]



VI



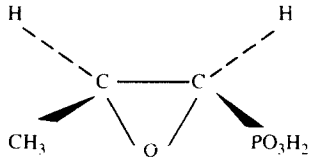
VII

While **II** can act as an antibiotic, its major use to date has been as a nonselective herbicide.[32] Plants treated with **II** accumulate ammonia to toxic levels,[33,34] due to the inhibition of glutamine synthetase.[32–34] The phosphinothricin portion is the phytotoxic part of **II**, and phosphinothricin itself is currently being prepared as a herbicide with the name 'glufosinate'. Both **II** and **VI** are rapidly degraded by soil micro-organisms.[35]

OTHER BIOGENIC ORGANOPHOSPHORUS COMPOUNDS

All such compounds reported so far are alkylphosphonic acids. The most intensively studied is (−)-(1*R*,2*S*)-1,2-epoxypropylphosphonic acid (**III**) (commonly called 'phosphonomycin' or 'fosfomycin'). Originally isolated from cultures of *Streptomyces* species,[36] this compound has been extensively used in clinical and toxicological studies for its antibiotic properties. Recently, it has also shown ability to decrease toxic side effects of some antitumor drugs.[37,38]

Various other substituted propylphosphonic acid derivatives have also been found in *Streptomyces* cultures.[6] The compound 1-amino-2-phosphonopropionic acid has been reported in the protozoan *T. pyriformis* and in various species of coral.[6,39] Methylphosphonic acid has been reported in natural waters;[40,41] whilst this was attributed to anthropogenic sources, the possibility of biogenic origin could not be ruled out.



VIII

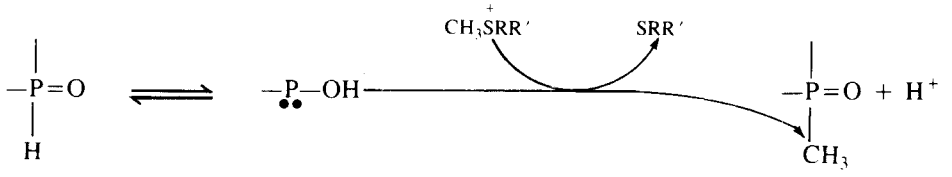
BIOGENIC FORMATION OF PHOSPHORUS–CARBON BONDS

The conversion of phosphoenolpyruvate to phosphonopyruvate (Eqn [1]) is the crucial step in the biogenesis of phosphorus–carbon bonds. Few specific details have been reported, making any discussion necessarily speculative. Takada and Horiguchi[17] investigated this rearrangement, using cell-free extracts, and isolated the product as the diphenylhydrazone derivative; conversion was low, even after 2 h at 30°C. Recently a magnesium-activated enzyme, named phosphomutase, has been isolated from cells of *T. pyriformis*,[42,42a] which catalyzed this rearrangement. The authors reported that, at equilibrium, the ratio of phosphoenolpyruvate to phosphonopyruvate was at least 500.

Bialaphos has two phosphorus–carbon linkages which form separately. One bond, as previously mentioned, apparently involves the rearrangement shown in Eqn [2] which is analogous to the rearrangement of phosphoenolpyruvate but with the important difference that a phosphorus–hydrogen linkage forms first.[21] Compounds containing this linkage were abundant enough and stable enough to be detected and isolated; clearly there must exist a mechanism by which they form. The phosphorus–methyl bond may form by a mechanism previously proposed by Thayer:[4a] the hydrogen moves from phosphorus to oxygen (a rearrangement will known in organophosphorus chemistry),[43] and the resulting phosphorus(III) intermediate is methylated by *S*-adenosylmethionine [$CH_3\overset{+}{S}RR$] by the Challenger mechanism (Eqn [3]).

The methyl group in **VI** was reported to be supplied by methionine,[19] probably via *S*-adenosylmethionine.

The presence of biologically stable molecules containing phosphorus–hydrogen linkages is noteworthy and deserves discussion. Table 1 lists various reduction potentials listed in (or calculated from) Bard.[44] Their values indicate that phosphate is reduced much less readily than sulfate and arsenate, both of which are known to undergo reduction to the respective hydrogen derivatives. Hydrogen sulfide (H_2S) has long been known to be an important part of the environmental cycle for sulfur,[45] and arsine (AsH_3) can be readily released from soils.[46] Whilst stibine (SbH_3) has not yet been directly detected in nature, it has the capability of being formed biogenically, and may be a component of volatile antimony compounds released from soils under anaerobic conditions.[47] Recently phosphine (PH_3) was detected in gases released from a sewage treatment plant, and it can be generated by anaerobic bacteria.[48] Phosphine has been proposed as being formed in the corrosion of iron by anaerobic bacteria as the precursor to the observed product iron phosphide.[49] A reduced phosphorus species was reported among the corrosion products of steel by *Desulfovibrio desulfuricans*.[50] The reduction potentials also indicate that the crucial step for phosphorus–hydrogen bond formation will be the reduction of phosphorus(V) to phosphorus(III). The papers already cited in this article indicate that such reduction does occur in micro-organisms, but the mechanism remains to be worked out.



[3]

Table 1 Standard reduction potentials for some elements of Groups VA and VIA[a]

Element, E	E(V)/E(III)	E(V)/EH$_3$
P	−0.260	−0.265
As	+0.662	+0.032*
Sb	+0.363	+0.066*
S	+0.17	+0.304
Se	+1.09	+0.558
Te	+0.813	+0.659

[a] Potentials (in volts) taken from Ref. 44. Those values marked with * are calculated from listed potentials.

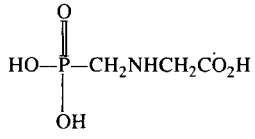
Several papers have been published on the formation of alkylphosphonates by snails.[51–54] Snails of genus *Helisoma* readily take up[33] phosphorus-labelled orthophosphate and convert it to aklylphosphonic acids, primarily ciliatine.[51,54] The phosphorus in newly-laid snail eggs exist overwhelmingly (98%) as alkylphosphonic acids, and most of that as ciliatine.[54] This is also true for the schistosomal vector snail *Biomphalaria glabrata.*[52,53] The proportion of phosphorus present as phosphonic acids decreases as the embryos develop, suggesting that these compounds might serve as a conveniently stored source of phosphorus. When snails are infected by schistosomes, the proportion of phosphorus present as aklylphosphonic acids decreases noticeably.[52]

BIOLOGICAL CLEAVAGE OF PHOSPHORUS–CARBON BONDS

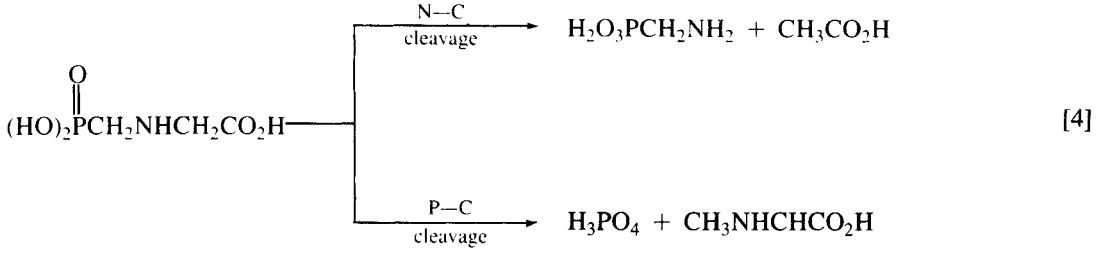
The compound *N*-phosphonomethylglycine (**IX**), commonly called 'glyphosate' has received considerable use as an herbicide. This has led to investigation into its metabolism and environmental degradation. Application of **IX** to Oregon forest ecosystems indicated that it had a half-life of 10.4–26.6 days in foliage and twice that long in soils.[55] A decomposition product, aminomethylphosphonic acid, could be detected at low concentrations in soils but decomposed rapidly.[55] This compound was also detected in the metabolism of glyphosate by a *Flavobacterium* species[56] and by *Arthrobacter atrocyaneus.*[57] A different strain of *Arthrobacter* metabolized glyphosate to *N*-methylglycine ('sarcosine') and orthophosphate.[58] These two routes involve cleavage of a nitrogen–carbon and phosphorus–carbon bond respectively (Eqn [4]).

The sarcosine is subsequently converted to glycine.[59–61] Orthophosphates, organophosphates and organophosphonates inhibited this decomposition of glyphosate.[62]

Microbiologists have investigated the ability of micro-organisms to utilize organophosphorus compounds as a source of phosphorus. Strains of both *Pseudomonas*[63] *and Escherichia coli*[64] were reported to metabolize a wide variety of alkylphosphonates. Glyphosate can serve as a phosphorus, but not a carbon, source for strains of *Flavobacterium*[56] *and Alcaligens.*[65] The organic group attached to the phosphorus is converted to a hydrocarbon and the enzyme used for this purpose has been termed a carbon–phosphorus lyase.[66,67] Trideuteromethylphosphonic acid gave trideuteromethane as a gaseous product, and cyclopropylmethylphosphonic acid gave traces of 1-butene as well as methylcyclopropane.[67]



IX



[4]

This lyase has been reported to have a specific genetic locus.[68,69]

CONCLUSIONS

Research on phosphorus–carbon bond formation or cleavage, as with most of organophosphorus chemistry, has developed independently of corresponding work on organo derivatives of other elements. As a consequence, the reported work rêmains largely unknown to most organometallic or organometalloidal chemists. Compounds containing phosphorus–carbon (P–C) bonds appear to be widespread, perhaps ubiquitous, among micro-organisms. The existence of a phosphorus–carbon lyase among various bacterial species (and doubtlessly more will be reported in the future) indicates a need for such species to protect themselves against toxic phosphonates, such as fosfomycin, that they might encounter. Phosphonolipids, largely derivatives of **I**, have been reported in both invertebrates and vertebrates.[6] On the basis of current evidence, such compounds seem to arise from symbiotic bacteria or from ingestion with food rather than from direct biosynthesis.

The most important biological route for phosphorus–carbon bond formation is the rearrangement shown in Eqn [1]. Direct methylation of phosphorus occurs in the biosynthesis of bialaphos and phosphinothricin and requires the existence, at least transiently, of a phosphorus(III) moiety. Thus, methylation of phosphorus follows the same reaction pathway as methylation of the related elements arsenic, sulfur and selenium.[4,5] The environmental occurrence of methylphosphorus compounds arising from biological processes has not been unequivocally demonstrated, and the ability of many micro-organisms to metabolize methylphosphonic acid makes any accumulation of this compound appear unlikely; however, the unexpected environmental appearance of biogenic phosphine (PH_3) and the isolation of biogenic compounds having phosphorus–hydrogen linkages makes any prediction rather risky.

Research on biogenesis and biotransformation of organophosphorus compounds has developed into an active and promising discipline, deserving more recognition than it has hitherto received. Hopefully, it will interact more completely with research on corresponding compounds of related elements, to the benefit of all concerned.

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