Chapter

Phosphonates: Their Natural Occurrence and Physiological Role

Paweł Kafarski

Abstract

The first natural compound containing carbon-to-phosphorus bond—ciliatine was discovered 60 years ago, and for four decades, phosphonates were considered simply as a biological curiosity. Finding the importance of these compounds in biogeochemical phosphorus cycling, their role in methane production, as well as discovery of numerous phosphonates and phosphonopeptides of promising antibacterial and antifungal activities has stimulated the development of studies on this class of compounds, especially on their metabolism and biochemistry. These studies are driven by the use of $^{31}$P NMR and by a clever combination of genomics and innovative chemistry by using the method of selective labeling of metabolites. These studies revealed unusual and interesting chemistry of these compounds.

Keywords: C—P bond, phosphonates, ciliatine, phosphonopeptides, mimetics, antibiotics, $^{31}$P NMR, genome mining

1. Introduction

Phosphonates are organophosphorus compounds characterized by a stable carbon-to-phosphorus (C—P) bond, which usually resists biochemical, thermal, and photochemical decomposition. The first phosphonate (compound 1, Figure 1), being an analog of β-alanine and taurine, was isolated in 1959 from ciliated protozoa in the rumen of sheep [1]. That was the cause why its discoverers—M. Horiguchi

![Figure 1. Ciliatine (2-aminoethylphosphonic acids) and its derivatives found in lipids, glycans, glycoproteins, and bile acids.](image)
and M. Kandatsu, named it ciliatine. This amino acid was then considered as a possible marker of the content of protozoa in sheep rumen, which appeared further to be misleading. For many years, natural compounds containing the C—P bond had been considered as curiosity being only scarcely studied. This is not the case in science currently because of their involvement in the global phosphorus cycle and in oceanic methane production. Some aspects of their occurrence, environmental role, biochemistry, and biological functions have been reviewed [2–5]. This chapter will concentrate on discussion of chemical diversity of the naturally occurring phosphonates and on the indication of open problems, which have not yet been solved.

2. Occurrence of carbon-to-phosphorus bond

The discovery of ciliatine stimulated intensive studies on the distribution of phosphonates in nature. Despite the fact that early studies were hampered by the lack of simple and sensitive methods for the identification of the presence of carbon-to-phosphorus bond in natural samples, it was found to exist in protozoa, bacteria, coelenterates, and mollusks [6–11]. Presumably, the unbreakable record is held by the snail Helisoma sp. freshly laid eggs, which contain over 95% of total phosphorus in phosphonate form [12]. Upon embryonic development, phosphonate is converted into phosphoric acid and subsequently incorporated into cellular constituents. It is believed that the physiological role of incorporation of phosphonates into the lipid fraction might function as a means to protect the eggs against predators, because they are presumably not able to disrupt and digest such membranes.

The advent of $^{31}$P NMR for the analysis of tissue extracts, body fluids, and later—whole cells provided an effective tool for tracking the forms of phosphorus and its interchanges during organism development and growth. Quite paradoxically, the availability of $^{31}$P NMR was accompanied with a significant decrease in the number of papers dealing with distribution of phosphonates in various species. Applications of this simple technique enabled the determination of the presence of C—P bond in bacteria and bacterial communities [13, 14], cyanobacteria [15], sponges [16], higher fungi [17, 18], or even human specimens [19]. However, these studies did not explain if phosphonates are synthesized de novo or are introduced to these organisms by cohabiting organisms or diet. On the other hand, phosphonate xenobiotics are quite massively released into environment [20], and various organisms might use them, or products of their decomposition, as building blocks of more complex structures.

Next, gene-based methods for assessing the abundance and identity of biological phosphonate producers were applied. This approach based on knowledge regarding C—P compound biosynthesis. Thus, with a single exception [21], all the known phosphonates are derived from phosphoenolpyruvate by isomerization to phosphonopyruvate in a reaction catalyzed by the phosphoenolpyruvate mutase, followed by its fast utilization because the reaction of formation of the C—P bond is thermodynamically unfavorable (see Figure 3). Most common, decarboxylation of phosphonopyruvate by phosphonopyruvate decarboxylase to produce phosphonoacetaldehyde is the next, irreversible step [3, 22–24]. Mining in genome databases for genes related to these two enzymes, as well as their homologs, enabled to determine that 10–15% of bacterial species are able to produce phosphonates [23–25].

Discovery that phosphonates form around 10% of dissolved and particulate phosphorus in the oceans [15, 25, 26] brought the increasing recognition of the importance of these compounds in biogeochemical phosphorus cycling and an awareness of the interdependence between the global phosphorus cycle and those of the other biologically significant elements [27, 28]. It is important because
phosphorus availability has been shown to be a key determinant of marine phytoplankton productivity [15]. Phosphonates are mostly concentrated in dissolved organic phosphorus (DOP), an integral and dynamic part of the marine organic matter pool. The composition of the DOP pool is complex and largely unknown, but phosphonates account for one third of its high molecular weight fraction. Thus, they seem to be an important resource of this element for aquatic organisms; however, the understanding of their utilization by eukaryotic phytoplankton is severely limited [29, 30]. They most likely occur in a form of polysaccharides esterified with methylphosphonate (compound 2, Figure 1) and 2-hydroxyethylphosphonate (compound 3, Figure 1). These compounds have been mainly found in *Nitrosopumilus maritimus*, one of the most abundant organisms on the planet and a resident of the oxygen-rich regions of the open oceans [31, 32].

Up to 4% of the methane on Earth comes from the oxygen-rich waters through the cleavage of the highly unreactive carbon-to-phosphorus bond in methyl phosphonate [32]. The production of methylphosphonic acid (MPn) by cyanobacteria or marine archaea related to *N. maritimus* and its subsequent decomposition by phosphate-starved bacterioplankton may partially explain the production of methane in oceanic and lake surfaces [33–35]. The concentration of methane in the upper ocean being above equilibrium with the atmosphere is known as the oceanic methane paradox [36, 38].

Some researchers believe that phosphonates are a form of relic of evolution. Being of slightly lower formal oxidation state, they might predominate in prebiotic reductive conditions [37]. This assumption, although debatable, finds some support by finding several phosphonic acids in Murchison meteorite [39].

### 3. Ciliatine (AEP, 2-aminoethylphosphonic acid)

Ciliatine (compound 1) is the most ubiquitous phosphonate present in lower organisms and occurs in remarkably high amounts. It is either presented in a free, unbound form being a common intermediate in numerous phosphonate biosynthetic pathways or incorporated into lipids and glycans. It is not surprising if considering that ciliatine is a formal analog of common component of lipids—phosphoethanolamine (compound 4). Most of the studies on natural occurrence of ciliatine and its lipids had been published in 1960–1990 and are comprehensively reviewed [2–5]. Only single paper was published after this period. As shown in Figure 1, its methylated forms, namely *N*-methyl, *N*,*N*-dimethyl-, and *N*,*N*,*N*-trimethylciliatine (compounds 5, 6, and 7), were also found in lipid fractions of some organisms albeit in significantly smaller quantities. Compound 7 is an analog of the most common component of lipids—phosphocholine (compound 8). The presence of an unusual aminophosphonate—(R)-2-amino-1-hydroxyethylphosphonic acid (compound 9) and its acetyl derivative has been determined in lipid fractions of *Bacteriovorax stolpii* [40, 41]. Its configuration was elegantly determined by a combination of chemical synthesis and biochemical studies [42].

Lipids containing aminophosphonates are called phosphonolipids. There are two classes of these compounds—glycerophosphonolipids and sphingophosphonolipids (representative structures are shown in Figure 2). They have been isolated from numerous organisms including humans, mammals (sheep, goats, and rats), egg yolk, fish, insects, sea anemones, sponges, numerous species of freshwater and marine mollusks, seeds of plants, protozoa, and bacteria [3, 43–46]. Usually they are a small fraction of the total lipids present, and their isolation and exact identification/characterization are difficult and cumbersome.
The physiologic function of phosphonolipids is still unknown, and the suggested protecting role against predators resulting from their stability toward hydrolysis by lipases and phosphatases has not been proved so far. Moreover, the distribution and abundance of phosphonolipids among organisms vary with species, tissue, or cellular location. For example, vertebrates have sphingophosphonolipids as components of nervous tissue sphingomyelin, while invertebrates frequently contain high levels of these lipids as outer membrane components.

Whereas phosphate is a common modification of polysaccharides, there are only a few examples of polysaccharides containing phosphonate moieties. Their characterization/identification was made possible as well as substantially accelerated by the development of glycomics [47]. Ciliatine and compound 9 have been found to be bound to the sugar moieties of variable glycans (see Figure 2 for schematic structures). Their occurrence was documented in fractions of glycosphingolipids (lipids) of many lower marine phyla [2, 12, 48], bacterial exopolysaccharides (secreted polysaccharides into the environment), and outer membrane components.
and glycoproteins deriving from marine snails, common jellyfish and locusts [51-54]. Genome scanning led to the identification of methylphosphonic acid (compound 2) in the exopolysaccharide of the marine archeon *N. maritimus*. Its function is not known, but it is ultimately a major source of methane production by the oceans [32].

Similarly as in the case of phosphonolipids, the physiological role of phosphoglycans is not known and thus awaits determination. This might be important in the context that the glycans are essential molecules being well known to enable adaptive response to environmental changes [55]. The speculative roles of phosphoglycans include cell–cell signaling or their action as phosphorus reservoirs in the environments of low phosphate concentration. The second assumption might be supported by the conservation of phosphonolipids at the expense of phosphodiester in starved conditions by the oyster *Crassostrea virginica* [56]. Other possibility is demonstrated by the fact that *Bacteroides fragilis*, a part of the normal microbiota of the human colon, produces a capsular polysaccharide complex containing ciliatine, which is directly involved in abscess formation in animal models when bacteria are displaced into the bloodstream [57].

It is also important to mention that the phosphonic analog of taurocholic acid was found in the gall bladders of cows [58]; however, this finding may require additional confirmation.

### 4. Low-molecular phosphonates metabolically related to ciliatine

Biosynthesis of phosphonates starts from rearrangement of phosphoenolpyruvate (compound 10) into phosphonopyruvate (compound 11), a reaction catalyzed by phosphonoenolpyruvate mutase. In this equilibrium process, the thermodynamics favors phosphonoenolpyruvate by a factor of at least 500. Thus, phosphonopyruvate has to be rapidly converted into metabolically useful compounds, most favorably in the irreversible reactions. Consequently, it is a key substrate in the synthesis of ciliatine (compound 1), phosphonoalalnine (compound 12), 2-hydroxyethylphosphonic acid (compound 3), phosphonoacetaldehyde (compound 13), phosphonomethylmalic acid (compound 14), and 2-keto-4-hydroxy-5-phosphonopentanoic acid (compound 15). Most of the enzymes involved in the production of these compounds have been isolated and characterized and comprehensively reviewed [2, 59]. The metabolic relationships between these compounds and their precursor role in the synthesis of phosphonate antibiotics are shown in Figure 3.

Low-molecular antibiotics such as fosfomycin (compound 17) [60], fosfonochlorin (compound 18 produced by several strains of *Fusarium* and *Talaromyces flavus*) [61], nitrilaphos and hydroxynitrilaphos (compounds 19 and 20 found in cultivating media of *Streptomyces*) [62], and herbicidal phosphonothrixin (compound 21, produced by *Saccharothrix*) [63] might be also considered as low-molecular compounds related to ciliatine.

Only one of them—*fosfomycin* (also known as *Monuril, Monurol, or Monural*), produced by *Pseudomonas* and *Streptomyces*, has found limited use as therapeutic agent to cure urinary tract infections and diabetic foot [60]. It is an active site directed covalent inactivator of muramyl ligase A, the first enzyme of peptidoglycan synthesis, and causes disruption of bacterial cell wall. Unfortunately, bacteria adapted to be able to open the epoxy ring functionality of fosfomycin, thus resulting in the compound deactivation/degradation of this antibiotic and in the microorganism ability to readily develop drug resistance [64]. Quite interestingly, pathways for the biosynthesis of fosfomycin in *Streptomyces* and *Pseudomonas* are different.
(see Figure 3). This shows that synthesis of natural phosphonates does not have to be normalized; many metabolic pathways are still yet to be discovered.

The separate class is aminophosphonate antibacterial antibiotics possessing an amino group in the gamma position in relation to the phosphonic functional group, namely fosmidomycin (compound 22), and its derivatives denoted as FR900098 (compound 23), FR-33289 (compound 24), and FR32863 (compound 25), originally isolated from culture broths of Streptomyces as well as cyclic SF2312 (compound 26) isolated from Micromonaspora sp. [65–67]. Their structures are shown in Figure 4.

Fosmidomycin and its homologs are potent inhibitors of 1-deoxy-d-xylulose-5-phosphate reductoisomerase, an essential enzyme of the non-mevalonate pathway of isoprenoid biosynthesis being active against a broad range of enterobacteria,
but not against Gram-positive organisms or anaerobes. More importantly, they are blocking the development of isoprenoids in the parasite apicoplast, and thus, structurally modified fosmidomycin derivatives are considered as promising antimalarial agents (for representative structure, see Figure 4) [68].

*Aphanizomenon flos-aquae* is a cyanobacterium that grows in eutrophic Balgavies Loch in Scotland. From its water blooms, a novel biosurfactant of lipidic character—2-acyloxyethylphosphonate (compound 27) was isolated; however, its ecological function remains to be evaluated [69].

Two unusual placotylene A esters [69] of ciliatine (phosphoiodyn A, compound 28) and its phosphate congener—phosphoethanolamine (phosphoiodyn B) were isolated from a Korean marine sponge *Placospongia* sp. [70]. Phosphoiodyn A was found to exhibit a potent agonistic activity on human peroxisome proliferator-activated receptor delta (hPPARδ), which is thought to function as an integrator of transcriptional repression and nuclear receptor signaling [16, 71]. This compound, as well as its analogs, demonstrates significant neuroprotective activity in an *in vitro* cellular model indicating that such phosphonates may be an effective novel scaffold for the design of therapeutics for the treatment of neurodegenerative disorders [71].

5. Phosphonopeptide antibiotics

Half of the century after the discovery of ciliatine witnessed a slow progress in the isolation and identification of natural compounds containing the C—P bond with most of them being antibacterials. The majority of these compounds appeared to be peptides containing C-terminal phosphonic acids and mostly differ by their N-terminal peptide structure. They have drawn attention not only because of their bioactivity but also because of unusual and interesting chemistry associated with the biosynthesis and biodegradation of these molecules. Structures of antibiotic phosphonopeptides are shown in Figure 5.

Bialaphos (compound 29, [72]) was isolated from as the first such an antibiotic from the culture filtrates of *Streptomyces viridochromogenes* and *Streptomyces hygroscopicus* [72–74]. Further studies indicated that its antibacterial activity is a result of active transport of the peptide across bacterial membrane followed by hydrolysis of the peptide and release of terminal phosphonate—phosphinothricin, which inhibits glutamine synthetase. This enzyme converts glutamic acid and ammonia into glutamine; this reaction is an important step of the nitrogen metabolism in bacteria and plants [75]. That activity of phosphinothricin resulted in its introduction to agriculture as a popular herbicide, and it is sold as ammonium salt under the name glufosinate. Its application causes accumulation of ammonia in plants and consequently plant death [76]. It is worth to notice that bialaphos also exerts herbicidal activity and was applied in Japan [77]. Its activity relies on hydrolysis of bialaphos in plant tissues and release of herbicidal phosphinothricin. Further studies on bialaphos resulted in isolation of tetrapeptide trilaphos (compound 30) [78] and phosalacine (compound 31) [79] both of the same mechanism of action. Finally, studies on biosynthesis of this compound resulted in the identification of its desmethyl analog 32, which is an intermediate in bialaphos metabolism.

The antibacterial activity of bialaphos is typical for all the phosphonopeptides. Peptide parts of these antibiotics usually function as a targeting unit. Thus, the peptides are efficiently transported through bacterial (or fungal) membranes and after hydrolysis release phosphonic acid, which exerts its toxic action by inhibiting parasite vital enzymes—in this case glutamine synthetase. This mechanism of action is shown schematically in Figure 6.
The following years brought the discovery of a family of antibiotics called rhizocticins (compounds 33–36) \[80, 81\], plumbemycins (compounds 37 and 38) \[81–83\], and phosacetamycin (compound 39) \[84\], first isolated as secondary metabolites of *Bacillus subtilis* on the basis of their antifungal activity and were
Phosphonates: Their Natural Occurrence and Physiological Role
DOI: http://dx.doi.org/10.5772/intechopen.87155

later found as products of *Streptomyces plumbeus*. They form a library of di- and tripeptides containing C-terminal (Z)-L-2-amino-5-phosphono-3-pentenoic acid, a mimetic of phosphonothreonine, which is the substrate for threonine synthetase. Thus, after the release from the peptide aminophosphonate acts as a potent inhibitor of this enzyme [85].

Dehydrophos (compound 40) was first isolated from the broth of *Streptomyces luridus* as a broad-spectrum antibiotic affective in chicken model of *Salmonella* infection [86]. The history of determination of its structure is rather long and led to three propositions of which the last one appeared to be reasonable and compelling. It is a dehydrophosphonopeptide, which, after the cleavage of the peptide bond, provides an analog of dehydroalanine, which is then converted into methyl acetylphosphonate (compound 41, an analog of pyruvic acid), which is strongly antibacterial by acting most likely as antimetabolite of pyruvate (Figure 6) [87]. Thus, it was considered as a lead compound for the design of novel antibacterial agents [88]. The non-typical and innovative is the application of its biosynthetic enzymes for obtaining new antibacterial phosphonopeptides [89]. Recently, the role of nonribosomal peptidyl transferase DhpH in the formation of peptide bond in dehydrophos was studied in detail using phosphonic analog of alanine and various amino acid-tRNAs as substrates [90].

Phosphonopeptides have very limited utility in human medicine because they are readily hydrolyzed in body fluids and released aminophosphonic acids that are not able to cross bacterial or fungal cell barriers and to exert antibiotic action. Additionally, they are being readily excreted through urine.

Published in 2015 work of Metcalf and van der Donk brought a significant breakthrough in studies on naturally occurring phosphonate antibiotics. By a clever combination of the mining of the genome of 10,000 of actinomycetes and selective labeling of phosphonate metabolites, they rediscovered a large number of old phosphonates and discovered 19 new compounds [24]. This opened a genetic approach in natural phosphonate chemistry and biochemistry, especially enabling the identification of metabolic pathways leading to this class of compounds. An important and instructive example here is an activation of gene cluster from *Streptomyces* sp. NRRL F-525 and its reengineering in *Streptomyces lividans*, which resulted in the isolation of O-phosphonoacetic acid serine (compound 42) [91].

One of the examples of rediscovered compounds is fosfazinomycins A and B (compounds 43 and 44), identified 30 years after their original isolation from *Streptomyces lavendofoliæ* and *Streptomyces unzenensis* [92, 93]. They are a very specific since they contain an exotic structural feature, which is the hydrazide linkage between the carboxylic acid of peptidyl arginine and the phosphonic acid. Fosfazinomycin was also found further in one of 210 substances present in 42 actinomycetes associated with the Baltic sponge *Halichondria panacea* [94].

The genetic approach also enabled the isolation and characterization of novel of *Streptomyces* peptidomimetics such as argolaphos A and B (compounds 45 and 46) and valinophos (compound 47) [24]. Similar approach was used for the isolation of phosphonocystoximate and its hydroxylated derivative (compounds 48 and 49) [24]. Detailed NMR studies on their biosynthesis, which starts from ciliatine and its analog—compound 9, enabled to confirm the presence of intermediates such as mixtures of the (E)- and (Z)-isomers of corresponding oximes (compounds 50 and 51), substrates for the synthesis of phosphonocystoximate and its hydroxylated derivative [95]. They are formed by the action of specific flavin-dependent, oxime-forming N-oxidases. These oxidases are also able to convert the oximes 50 and 51 into corresponding nitroethylphosphonates (compounds 52 and 53) [96]. Structures of these intermediates and side products are depicted in Figure 7.
A separate group of phosphonic peptidomimetics is compounds denoted as K-26, K4, and I5B2 (compounds 54, 55, and 56, respectively) [21, 97–99], a small family of bacterial secondary metabolites, tripeptides terminated by an unusual phosphonate analog of tyrosine (see Figure 6). They are produced by three different actinomycetales and act as potent inhibitors of human angiotensin-I converting enzyme selectively targeting the eukaryotic family of the enzyme [100, 101]. These compounds derived from L-tyrosine, which suggests the existence of novel and not discovered yet reaction of carbon-to-phosphorus bond formation [21, 102].

6. Conclusions

Natural phosphonates might be considered as simple analogs of phosphate esters and/or carboxylic acids. The inherent stability of the C—P bond causes that they often display promising activities as enzyme inhibitors and therefore might be considered as drugs or agrochemicals. Moreover, the wide use of xenobiotics containing carbon-to-phosphorus bond has led to the spread of these compounds in the environment, which may result in their incorporation into variable metabolic pathways. All of this stimulate interest in these, still somewhat exotic, compounds.

The development of $^{31}$P NMR and genomics supplemented by biochemical studies resulted in the development of new detection technologies, which enormously speed out the discovery of novel naturally occurring phosphonates, identification of their metabolic pathways (both biosynthesis and degradation), and their use as lead compounds for the design of new promising medicines. With the exception of the identification of antibacterial and antifungal antibiotics, these studies are not accompanied, however, with the determination of physiologic importance of these compounds.

Acknowledgements

This work was supported by statuary grants of Wroclaw University of Science and Technology and National Science Centre, Poland (grant 2016/21/B/ST5/00115).
Conflict of interest

I declare that there is no conflict of interest that might have any bearing on research reported in this work.

Author details

Paweł Kafarski
Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland

*Address all correspondence to: pawel.kafarski@pwr.edu.pl
References

[1] Horiguchi M, Kandatsu M. Isolation of 2-aminoethane phosphonic acid from rumen protozoa. Nature. 1959;184:901-902. DOI: 10.1038/184901b0

[2] Horsman GP, Zechel DL. Phosphonate biochemistry. Chemical Reviews. 2017;117:5704-5783. DOI: 10.1021/acs.chemrev.6b00536

[3] Ju K-S, Doroghazi JR, Metcalf WW. Genomics-enabled discovery of phosphonate natural products and their biosynthetic pathways. Journal of Industrial Microbiology & Biotechnology. 2014;42:345-356. DOI: 10.1007/s10295-013-1375-2

[4] Mastalerz P, Kafarski P. Naturally occurring aminophosphonic and aminophosphinic acids. In: Kukhar VP, Hudson HR, editors. Aminophosphonic and Aminophosphinic Acids. Chichester: Wiley; 2000. pp. 1-31

[5] Petkowski JJ, Bains W, Seager S. Natural products containing “rare” organophosphorus functional groups. Molecules. 2019;24:866. DOI: 10.3390/molecules24050866

[6] Kitteredge JS, Roberts E. A carbon-phosphorus bond in nature. Science. 1969;164:37-42. DOI: 10.1126/science.164.3875.37

[7] Horiguchi M. Occurrence, identification and properties of phosphonic and phosphinic acids. In: Hori T, Horiguchi M, Hayashi M, editors. Biochemistry of Natural C-P Compounds. Shiga: Japanese Association for Research on the Biochemistry of C-P Compounds; 1984. pp. 24-52

[8] Tamari M, Kametaka M. Isolation and identification of ciliatine (2-aminoethylphosphonic acid) from phospholipids of the oyster, Crassostrea gigas. Agricultural and Biological Chemistry. 1972;36:1147-1152. DOI: 10.1271/bbb1961.36.1147

[9] Kittredge JS, Roberts E, Simonsen DG. The occurrence of free 2-aminoethylphosphonic acid in the aea anemone, Anthopleura elegantissima. Biochemistry. 1962;1:624-628. DOI: 10.1021/bi00910a013

[10] Liang CR, Rosenberg H. On the distribution and biosynthesis of 2-aminoethylphosphonate in two terrestrial molluscs. Comparative Biochemistry and Physiology. 1968;25:673-681. DOI: 10.1016/0010-406X(68)90377-0

[11] Kariotoglou DM, Mastronicolis SK. Sphingophosphonolipid molecular species from edible mollusks and a jellyfish. Comparative Biochemistry and Physiology. B. 2003;136:27-44. DOI: 10.1016/S1096-4959(03)00168-4

[12] Miceli MV, Henderson TO, Myers TC. Alkylphosphonic acid distribution in the planorbid snail Helisoma sp. Comparative Biochemistry and Physiology. B. 1987;2:603-611. DOI: 10.1016/0305-0491(87)90351-8

[13] Jayasimhulu K, Hunt SM, Kaneshiro ES. Detection and identification of Bacteriovorax stolpii UKi2 sphingophospholipid molecular species. Journal of the American Society for Mass Spectrometry. 2007;18:394-403. DOI: 10.1016/j.jasms.2006.10.014

[14] Turner BL, Baxter R, Mathieu N, Sjögersten S, Whitton B. Phosphorus compounds in subartic Fennoscandian soils at the mountain birch (Betula pubescens) - Tundra ecotone. Soil Biology and Biochemistry. 2004;36:815-823. DOI: 10.1016/j.soilbio.2004.01.011

[15] Dyhrman ST, Benitez-Nelson CR, Orchard ED, Haley ST, Pellechia PJ. A microbial source of phosphonates
in oligotrophic marine systems. Nature Geoscience. 2009;2:699. DOI: 10.1038/ngeo639

[16] Kim H, Chin J, Choi H, Baek K, Lee TG, Park SE, et al. Phosphoiodyns A and B, unique phosphorus-containing iodinated polyacetylenes from a Korean sponge Placospangia sp. Organic Letters. 2013;15:100-103. DOI: 10.1021/ol3031318

[17] Koukol O, Novák F, Hrabal R. Composition of the organic phosphorus fraction in basidiocarps of saprotrophic and mycorrhizal fungi. Soil Biology and Biochemistry. 2008;40:2464-2467. DOI: 10.1016/j.soilbio.2008.04.021

[18] Maciejczyk E, Wieczorek D, Zwyrzykowska A, Halama M, Jasicka-Misiak I, Kafarski P. Phosphorus profile of basidomycetes. Phosphorus, Sulfur and Silicon and the Related Elements. 2015;190:763-768. DOI: 10.1080/10426507.2014.992269

[19] Glonek T, Henderson TO, Hilderbrand RL, Myers TC. Biological phosphonates: Determination by phosphorus-31 nuclear magnetic resonance. Science. 1970;169:172-174. DOI: 10.1126/science.169.3941.192

[20] Studnik H, Liebsch S, Forlani G, Wieczorek D, Kafarski P, Lipok J. Aminopolyphosphonates—Chemical features and practical uses, environmental durability and biodegradation. New Biotechnology. 2015;32:1-6. DOI: 10.1016/j.nbt.2014.06.007

[21] Ntai I, Manier ML, Hachey DL, Bachmann BO. Biosynthetic origins of C-P bond containing tripeptide K-26. Organic Letters. 2005;7:2763-2765. DOI: 10.1021/ol051091d

[22] Peck SC, van der Donk WA. Phosphonate biosynthesis and catabolism: A treasure trove of unusual enzymology. Current Opinion in Chemical Biology. 2013;19:580-588. DOI: 10.1016/j.cbpa.2013.06.018

[23] Villarreal-Chiu JF, Quinn JP, McGrath JW. The genes and enzymes of phosphonate metabolism by bacteria, and their distribution in the marine environment. Frontiers in Microbiology. 2012;3:art.19. DOI: 10.3389/fmicb.2012.00019

[24] Yu JX, Doroghazi JR, Janga SC, Zhang JK, Circeo B, Grifiijn BM, et al. Diversity and abundance of phosphonate biosynthetic genes in nature. Proceedings of the National Academy of Sciences of the United States of America. 2013;110:20759-20764. DOI: 10.1073/pnas.1315107110

[25] Scott KM, Sievert SM, Abril FN, Ball LA, Barrett CJ, Blake RA, et al. The genome of deep-see vent chemolithoautitroph Thiimicrospira crunogena XCL-2. PLoS Biology. 2006;4:e383. DOI: 10.1371/journal.pbio.0040383

[26] Whitney LP, Lomas LW. Phosphonate utilization by eukaryotic phytoplankton. Limnology and Oceanography Letters. 2019;4:18-24. DOI: 10.1002/lol2.10100

[27] Chin JP, McGrath JW, Quinn JP. Microbial transformations in phosphonate biosynthesis and catabolism, and their importance in nutrient cycling. Current Opinion in Chemical Biology. 2016;31:50-57. DOI: 10.1016/j.cbpa.2016.01.010

[28] Lüke C, Speeth DR, Kox MAR, Villanueva M, Jetten MSM. Metagenomic analysis of nitrogen and methane cycling in the Arabian Sea oxygen minimum zone. PeerJ. 2016;4:e1924. DOI: 10.7717/peerj.1924

[29] Born DA, Ulrich EC, Ju K-S, Peck S, van der Donk WA, Drennan CL. Structural basis for
methylphosphonate biosynthesis. Science. 2017;358:1336-1339. DOI: 10.1126/science.aao3435

[30] Nowack B. Environmental chemistry of phosphonates. Water Resources. 2003;37:2533-2546. DOI: 10.1016/S0043-1354(03)00079-4

[31] Benitez-Nelson CR, O’Neill L, Kolowith LC, Pellecia P, Thunell R. Phosphonates and particulate organic phosphorus cycling in an anoxic marine basin. Limnology and Oceanography. 2004;49:1593-1604. DOI: 10.4319/lo.2004.49.5.1593

[32] Metcalf WW, Griffin BM, Cicchillo RM, Gao J, Chandra Janga S, Cooke HA, et al. Synthesis of methylphosphonic acid by marine microbes: A source for methane in the aerobic ocean. Science. 2012;337:1104-1107. DOI: 10.1126/science.1219875

[33] del Valle DA, Karl DM. Aerobic production of methane from dissolved water-column methylphosphonate and sinking particles in the North Pacific subtropical gyre. Aquatic Microbial Ecology. 2014;73:93-105. DOI: 10.3354/ame01714

[34] Bižić-Ionescu M, Klintzsch T, Ionescu D, Hindiyeh MY, Günthel M, Muro-Pastor AM, et al. Widespread methane formation by Cyanobacteria in aquatic and terrestrial ecosystems. BioRxiv. DOI: 10.1101/398958

[35] Beversdorf LJ, White AE, Björkman KM, Letelier RM, Karl DM. Phosphonate metabolism of Trichodesmium IMS101 and the production of greenhouse gases. Limnology and Oceanography. 2010;55:1768-1778. DOI: 10.4319/lo.2010.55.4.1768

[36] Repeta DJ, Ferrón S, Sosa OA, Johnson CG, Repeta LD, Acke M, et al. Marine methane paradox explained by bacterial degradation of dissolved organic matter. Nature Geoscience. 2016;9:884-887. DOI: 10.1038/ngeo2837

[37] McGrath JW, Chin JP, Quinn JP. Organophosphonate reveals: New insights into microbial metabolism of ancient molecules. Nature Reviews. Microbiology. 2013;11:411-419. DOI: 10.1038/nrmicro3011

[38] Schwartz AW. Phosphorus in prebiotic chemistry. Philosophical Transactions of the Royal Society B. 2006;361:1743-1749. DOI: 10.1098/rstb.2006.1901

[39] Cooper GW, Onwo WM, Cronin JR. Alkyl phosphonic acids and sulfonic acids in the Murchison meteorite. Geochimica et Cosmochimica Acta. 1992;56:4109-4115. DOI: 10.1016/0016-7037(92)90023-C

[40] Jayasimhulu K, Hunt SM, Kaneshiro ES, Watanabe Y, Giner J-L. Detection and identification of Bacteriovorax stolpii UKi2 sphingophosphonolipid molecular species. Journal of the American Society for Mass Spectrometry. 2007;18:394-403. DOI: 10.1016/j.jasms.2006.10.014

[41] Watanabe Y, Nakayima N, Hoshino T, Jayasimhulu K, Brooks EE, Kaneshiro ES. A novel sphingophosphonolipid head group 1-hydroxy-2-aminoethyl phosphate in Bdellovibrio stolpii. Lipids. 2001;36:513-519. DOI: 10.1007/s11745-001-0751-3

[42] Pallitsch K, Happl B, Stieger C. Determination of the absolute configuration of (−)-hydroxynitrilaphos and related biosynthetic questions. Chemistry-A European Journal. 2017;23:15655-15665. DOI: 10.1002/chem.201702904

[43] Moschidis MC. Phosphonolipids. Progress in Lipid Research. 1984;23:223-246. DOI: 10.1016/0163-7827(84)90012-2
[44] Muralidhar P, Radhika P, Krishna N, Venkata Rao D, Bheemasankara Rao CH. Sphingolipids from marine organisms: A review. Natural Product Sciences. 2009;9:117-142

[45] Satake M, Miyamoto E. A group of glycosphingolipids found in an invertebrate: Their structures and biological significance. Proceedings of the Japan Academy, Series B. 2012;88:509-517. DOI: 10.2183/pjab.88.509

[46] Mukhamedova KS, Glushenkova AI. Natural phospholipids. Chemistry of Natural Compound. 2000;36:329-341. DOI: 10.1023/A:1002804409503

[47] Paschinger K, Wilson IB. Analysis of zwitterionic and anionic N-linked glycans from invertebrates and protists by mass spectrometry. Glycoconjugate Journal. 2016;33:273-283. DOI: 10.1007/s10719-016-9650-x

[48] Korn ED, Dearborn DG, Fales HM, Sokolowski EA. Phosphonoglycan. A major polysaccharide constituent of the amoeba plasma membrane contains 2-aminoethylphosphonic acid and 1-hydroxy-2-aminoethylphosphonic acid. The Journal of Biological Chemistry. 1973;248:2257-2259

[49] Vinogradov E, Egbosimba EE, Perry MB, Lam JS, Forsberg CW. Structural analysis of the carbohydrate components of the outer membrane of the lipopolysaccharide-lacking cellulolytic ruminal bacterium Fibrobacter succinogenes. European Journal of Biochemistry. 2001;268:3566-3576. DOI: 10.1046/j.1432-1327.2001.02264.x

[50] Baumann H, Tzianabos AO, Brisson JR, Kasper DL, Jennings HJ. Structural elucidation of two capsular polysaccharides from one strain of Bacteroides fragilis using high-resolution NMR spectroscopy. Biochemistry.

[51] Young NM, Foote SJ, Wakachuk WW. Review of phosphocholine substituents on bacterial pathogen plicans: Synthesis, structures and interactions with host proteins. Molecular Immunology. 2013;56:563-573. DOI: 10.1016/j.molimm.2013.05.237

[52] Urai M, Nakamura T, Uzawa J, Baba T, Taniguchi K, Seki H, et al. Structural analysis of O-glycans of mucin from jellyfish (Aurelia aurita) containing 2-aminoethylphosphonate. Carbohydrate Research. 2009;344:2182-2187. DOI: 10.1016/j.carres.2009.08.001

[53] Härd K, Van Doorn JM, Thomas-Oates JE, Kamerling JP, Van der Horst DJ. Structure of the Asn-linked oligosaccharides of Apolipophorin III from the insect Locusta migratoria. Carbohydrate-linked 2-aminoethylphosphonate as a constituent of a glycoprotein. Biochemistry. 1993;32:766-775. DOI: 10.1021/bi00054a005

[54] Eckmair B, Jin C, Abed-Avandi D, Paschinger K. Multistep fractionation and mass spectrometry reveal zwitterionic and anionic modifications of the N- and O-glycans of a marine snail. Molecular & Cellular Proteomics. 2016;15:573-597. DOI: 10.1074/mcp.M115.051573

[55] Lauc G, Krištić J, Zoldoš V. Glycans—The third revolution in evolution. Frontiers in Genetics. 2014;5:art.145. DOI: 10.3389/fgene.2014.00145

[56] Swift ML. Phosphono-lipid content of the oyster, Crassostrea virginica, in three physiological conditions. Lipids. 1977;12:449-451. DOI: 10.1007/BF02533632

[57] Onderdonk AB, Kasper DL, Cisneros RL, Bartlett JG. The capsular
polysaccharide of *Bacteroides fragilis* as a virulence factor: Comparison of the pathogenic potential of encapsulated and unencapsulated strains. The Journal of Infectious Diseases. 1977;136:82-89. DOI: 10.1093/infdis/136.1.82

[58] Tamari M, Ogawa M, Kametaka M. A new bile acid conjugate, ciliatocholic acid, from bovine gall bladder bile. Journal of Biochemistry. 1976;80:371-377. DOI: 10.1093/oxfordjournals.jbchem.a131286

[59] Peck SC, van der Donk W. Phosphonate biosynthesis and catabolism: A treasure trove for unusual enzymology. Current Opinion in Chemical Biology. 2013;17:580-588. DOI: 10.1016/j.chpb.2013.06.018

[60] Falagas ME, Vouloumanou K, Samonis G, Vardakas KZ. Fosfomycin. Clinical Microbiology Reviews. 2016;29:321-347. DOI: 10.1128/CMR.00068-15

[61] Takeuchi M, Nakajima M, Ogita T, Inukai K, Kodama K, Furuya K, et al. Fosfonochlorin, a new antibiotic with spheroplast forming activity. Journal of Antibiotics. 1989;42:198-205. DOI: 10.7164/antibiotics.42.198

[62] Cioni JP, Doroghazi JR, Ju JR, Yu K-S, Evans BS, Lee J, et al. Cyanohydrin phosphonate natural products from *Streptomycins regensia*. Journal of Natural Products. 2014;7:243-249. DOI: 10.1021/np400722m

[63] Takahashi E, Kimura T, Nakamura K, Arahira M, Iida M. Phosphonothrixin, a novel herbicidal antibiotic produced by *Saccharothrix* sp. ST-888. I. Taxonomy, fermentation, isolation and biological properties. Journal of Antibiotics (Tokyo). 1995;48:1124-1129. DOI: 10.7164/antibiotics.48.1124

[64] Silver LL. Fosfomycin: Mechanism and resistance. Cold Spring Harbor Perspectives in Medicine. 2017;7:a025262. DOI: 10.1101/cshperspect.a025262

[65] Iguchi E, Okuhara M, Koshaka M, Aoki H, Imanaka H. Studies on new phosphonic acid antibiotics. II. Taxonomic studies on producing organisms of the phosphonic acid and related compounds. Journal of Antibiotics. 1980;33:19-23. DOI: 10.7164/antibiotics.33.18

[66] Ohba K, Sato Y, Sasaki T, Sezaki M. Studies on a new phosphonic acid antibiotic SF2312.II isolation, physico-chemical properties and structure. Science Reports. 1986;25:18-22

[67] Okuhara M, Kuroda Y, Goto T, Okamoto M, Terano H, Kohsaka M, et al. Studies on a new phosphonic acid antibiotic III. Isolation and characterisation of FRFR-31564, FR-32863 and FR-33289. Journal of Antibiotics. 1980;33:24-28. DOI: 10.7164/antibiotics.33.24

[68] Edwards RL, Brothers RC, Wang X, Maron MI, Ziniel PD, Tsang PS, et al. MEPicides: Potent antimalarial prodrugs targeting isoprenoid biosynthesis. Scientific Reports. 2017;7:art.8400

[69] Kaya K, Morrison LF, Codd GA, Metcalf JS, Sano T, Takagi H, et al. A novel biosurfactant, 2-Acylxyethylphosphonate, isolated from Waterblooms of *Aphanizomenon flos-aquae*. Molecules. 2006;11:539-548. DOI: 10.3390/m11040539

[70] Kim H, Kim K-J, Jeon J-T, Kim S-H, Won DH, Choi H, et al. Placotylene A, an inhibitor of the receptor activator of nuclear factor-xB ligand-induced osteoclast differentiation, from a Korean sponge *Placospongia* sp. Molecules. 2014;12:2054-2065. DOI: 10.3390/m12042054

[71] Kinarivala N, Suh JH, Botros M, Webb P, Trippier PC. Pharmacophore
elucidation of phosphoiodyn A—Potent and selective peroxisome proliferator-activated receptor β/δ agonists with neuroprotective activity. Bioorganic & Medicinal Chemistry Letters. 2016;26:1889-1893. DOI: 10.1016/j. bmcl.2016.03.028

[72] Ogawa Y, Tsuruoka T, Inouye S, Niida T. Studies on a new antibiotic SF-1293. II. Chemical structure of antibiotic SF-1293. Science Reports. 1973;13:42-48

[73] Bayer E, Gugel KH, Hägele K, Hagenmaier H, Jessipow S, König WA, et al. Metabolic products of microorganisms. 98. Phosphinothricin and phosphinothricyl-alanyl-alanine. Helvetica Chimica Acta. 1972;55:224-239. DOI: 10.1002/hc.19720550126

[74] Blodgett JAV, Zhang JK, Yu X, Metcalf WW. Conserved biosynthetic pathways for phosalacine, bialaphos and newly discovered phosphonic acid natural products. The Journal of Antibiotics. 2016;69:15-25. DOI: 10.1038/ja.2015.77

[75] Wild A, Ziegler C. The effect of bialaphos on ammonium-assimilation and photosynthesis I. Effect on the enzymes of ammonium-assimilation. Zeitschrift für Naturforschung. 1984;44:97-102. DOI: 10.1515/ znc-1989-1-217

[76] Devkota P, Johnson WG. Glufosinate efficacy as influenced by carrier water pH, hardness, foliar fertilizer, and ammonium sulfate. Weed Technology. 2016;30:848-859. DOI: 10.1614/ WT-D-16-00053.1

[77] Tachibana T, Kaneko T. Development of an herbicide, bialaphos. Journal of Pest Science. 1986;11:297-304. DOI: 10.1584/ jpestics.11.297

[78] Kato H, Nagayama K, Abe H, Kobayashi R, Ishihara E. Isolation, structure and biological activity of trialaphos. Agricultural and Biological Chemistry. 1991;55:1133-1134. DOI: 10.1080/00021369.1991.10870694

[79] Omura S, Murata M, Hanaki H, Hinotozawa K, Oiwa R, Tanaka H. Phosalacine, a new herbical antibiotic containing phosphinothricin. Fermentation, isolation, biological activity and mechanism of action. The Journal of Antibiotics. 1984;37:829-835. DOI: 10.7164/antibiotics.37.829

[80] Rapp C, Jung G, Kugler M, Loeffler W. Rhizocinins—New phosphono-oligopeptides with antifungal activity. European Journal of Organic Chemistry. 1988:655-661. DOI: 10.1002/jlac.198819880707

[81] Gahungu M, Arguelles-Arias A, Fickers P, Zervosen A, Joris B, Damblon C, et al. Synthesis and biological evaluation of potential threonine synthase inhibitors: Rhizocin A and Plumbemycin A. Bioorganic & Medicinal Chemistry. 2013;21:4958-4967. DOI: 10.1016/j. bmc.2013.06.064

[82] Park BK, Hirota A, Sakai H. Studies on new antimetabolite produced by microorganism. III. Structure of plumbemycin A and B, antagonists of L-threonine from Streptomyces plumbeus. Agricultural and Biological Chemistry. 1977;41:573579. DOI: 10.1271/ bbb1961.41.573

[83] Borisova SA, Circello BT, Zhang JK, van der Donk WW, Metcalf WW. Biosynthesis of rhizocinins, antifungal phosphonate oligopeptides produced by Bacillus subtilis ATCC6633. Chemistry & Biology. 2010;17:28-37. DOI: 10.1016/j.chembiol.2009.11.017

[84] Evans BS, Zhao C, Gao J, Evans CM, Ju K-S, Doroghazi JR, et al. Discovery of the antibiotic phosacetamycin via a new mass spectrometry-based method for phosphonic acid detection. ACS
Chemical Biology. 2013;8:908-913. DOI: 10.1021/cb400102t

[85] Kugler M, Loeffler W, Rapp C, Kern A, Jung G. Rhizoctin A, an antifungal phosphono-oligopeptide of Bacillus subtilis ATCC 6633: Biological properties. Archives of Microbiology. 1990;153:276-281. DOI: 10.1007/BF00249082

[86] Johnson RD, Kastner RM, Larsen SH, Ose EE. Antibiotic A53868 and process for production thereof. U.S. patent 4,482,488; 1988

[87] Circello BT, Miller CG, Lee J-H, van der Donk WA, Metcalf WW. The antibiotic dehydrophos is converted to a toxic pyruvate analog by peptide bond cleavage in Salmonella enterica. Antimicrobial Agents and Chemotherapy. 2011;55:3357-3362. DOI: 10.1128/AAC.01483-10

[88] Jiménez-Andreu MM, Quintana AL, Aínsa JA, Sayago FJ, Cativiela C. Synthesis and biological activity of dehydrophos derivatives. Organic & Biomolecular Chemistry. 2019;5:1097-1112. DOI: 10.1039/c8ob03079k

[89] Bougioukou DJ, Ting CP, Peck SC, Mukherjee S, van der Donk WA. Use of the dehydrophos biosynthetic enzymes to prepare antimicrobial analogs of alaphosphin. Organic & Biomolecular Chemistry. 2019;4:822-829. DOI: 10.1039/C8OB02860E

[90] Bougioukou DJ, Mukherjee S, van der Donk WA. Revisiting the biosynthesis of dehydrophos reveals a tRNA-dependent pathway. Proceedings of the National Academy of Sciences of the United States of America. 2013;110:10952-11057. DOI: 10.1073/pnas.1303568110

[91] Freestone TS, Ju K-S, Wang B, Zhao H. Discovery of a phosphonoacetic acid derived natural product by pathway refactoring. ACS Synthetic Biology. 2017;6:217-223. DOI: 10.1021/acssynbio.6b00299

[92] Gunji S, Arima K, Beppu T. Screening of antifungal antibiotics according to activities inducing morphological abnormalities. Agricultural and Biological Chemistry. 1983;41:2016-2069. DOI: 10.1080/00021369.1983.10865911

[93] Gao J, Ju K-S, Yu X, Velásques JE, Mukherjee S, Lee J, et al. Use of phosphonate methyltransferase in the identification of the fosfazinomycin biosynthetic gene cluster. Angewandte Chemie, International Edition. 2014;53:1334-1337. DOI: 10.1002/anie.201308363

[94] Schneemann I, Nagel K, Labes A, Wiese J, Imhoff JF. Comprehensivei of marine Actinobacteria associated with the sponge Halichondria panacea. Applied and Environmental Microbiology. 2010;76:3702-3714. DOI: 10.1128/AEM.00780-10

[95] Goettge MN, Cioni JP, Ju K-S, Pallitsch K, Metcalf WW. PcxL and HpxL are flavin-dependent, oxime-forming N-oxidases in phosphonocystoximic acid biosynthesis in Streptomyces. The Journal of Biological Chemistry. 2018;293:6859-6868. DOI: 10.1074/jbc.RA118.001721

[96] Pallitsch K, Kalina T, Stanković T. Synthetic phosphonic acids as potent tools to study phosphonate enzymology. Synlett. 2019;30:770-776. DOI: 10.1055/s-0037-1611460

[97] Yamato M, Koguchi T, Okachi R, Yamada K, Nakayama K, Kase H, et al. K-26, a novel inhibitor of angiotensin I converting enzyme produced cy an Actinomycete K-26. The Journal of Antibiotics. 1986;39:44-52. DOI: 10.7164/antibiotics.39.44

[98] Hirayama N, Kasai M, Hirata K. Structure and conformation of a novel...
inhibitor of angiotensin I converting enzyme—A tripeptide containing phosphonic acid. International Journal of Peptide and Protein Research. 1991;38:20-24. DOI: 10.1111/j.1399-3011.1991.tb01404.x

[99] Kido Y, Hamakado T, Anno M, Miyagawa E, Motoki Y, Wakamiya T, et al. Isolation and characterization of I5B2, a new phosphorus containing inhibitor of angiotensin I converting enzyme produced by Actinomadura sp. The Journal of Antibiotics. 1984;37:965-969. DOI: 10.7164/antibiotics.37.965

[100] Kramer GJ, Mohd A, Schwager LSU, Masuyer G, Acharya KR, Sturrock ED, et al. Interkingdom pharmacology of angiotensin-I converting enzyme inhibitor phosphonates produced by Actinomycetes. ACS Medicinal Chemistry Letters. 2014;5:346-351. DOI: 10.1021/ml4004588

[101] Masuyer G, Cozier GE, Kramer GJ, Bachmann BO, Acharya KR. Crystal structure of a peptidyl-dipeptidase K-26-DCP from Actinomyces complex with its natural inhibitor. The FEBS Journal. 2016;283:4357-4369. DOI: 10.1111/febs.13928

[102] Ntai I, Phelan VV, Bachmann BO. Phosphonopeptide K-26 biosynthetic intermediates in Astreosporangium hypotensionis. Chemical Communications. 2006:4518-4520. DOI: 10.1039/b611768f