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Phosphoiodyns A and B, Unique Phosphorus-Containing Iodinated Polyacetylenes from a Korean Sponge *Placospongia* sp.

Hiyoung Kim,[†,#] Jungwook Chin,[†,‡,#] Hyukjae Choi,[∥] Kyungryul Baek,[†] Tae-Gu Lee,[†] Seong Eon Park,[†] Weihong Wang,[†] Dongyup Hahn,[†] Inho Yang,[†] Jihye Lee,[†] Bora Mun,[†] Merrick Ekins,[⊥] Sang-Jip Nam,[*,§] and Heonjoong Kang[*,†,‡]

Center for Marine Natural Products and Drug Discovery, School of Earth and Environmental Sciences, Seoul National University, NS-80, Seoul, 151-747, Korea, Research Institute of Oceanography, Seoul National University, NS-80, Seoul, 151-747, Korea, College of Pharmacy and Research Institute of Life and Pharmaceutical Sciences, Sunchon National University, Suncheon, 540-950, Korea, College of Pharmacy, Yeungnam University, 214-1 Dae-dong, Gyeongsan, 712-749, Korea, and Queensland Museum, P.O. Box 3300, South Brisbane, Queensland, 4101, Australia

sjnam@sunchon.ac.kr; hjkang@snu.ac.kr

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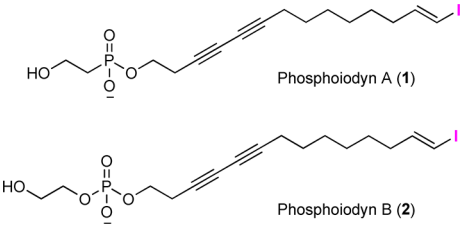
ABSTRACT



Two unprecedented phosphorus-containing iodinated polyacetylenes, phosphoiodyns A and B (1−2), were isolated from a Korean marine sponge *Placospongia* sp. Their structures were elucidated by spectroscopic data analysis. Phosphoiodyn A exhibited potent agonistic activity on human peroxisome proliferator-activated receptor delta (hPPARδ) with an EC_{50} of 23.7 nM.

Polyacetylenic natural products characterized by carbon−carbon triple bonds or alkynyl functional groups are widely distributed in plants, bacteria, vertebrates, marine algae, and marine invertebrates.[1] A number of polyacetylenes have been isolated from marine sponges, and among them the genus *Callyspongia* is a particularly rich source of polyacetylenes. The approximately 25 polyacetylenes from *Callyspongia* spp. account for over half of all discovered sponge polyacetylenes.[2] Polyacetylenes vary by a number of structural features, including the number of triple bonds, chain lengths, and the substituted functional groups.[3] They exhibit diverse bioactivities such as cytotoxic, antiviral, antifouling, RNA-cleaving, and enzyme−inhibitory activities.[2,3]

PPARδ is a ligand-activated transcription factor which regulates lipid and glucose metabolism[4] through activating

[†] SEES, Seoul National University.

[‡] Research Institute of Oceanography, Seoul National University.

[§] Sunchon National University.

[∥] Yeungnam University.

[⊥] Queensland Museum.

[#] These authors contributed equally.

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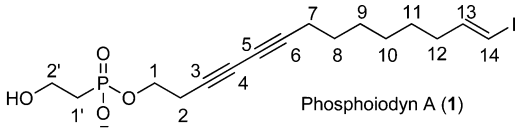
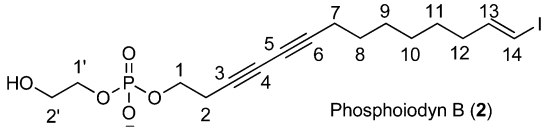
oxidative genes and energy uncoupling, and increasing mitochondrial biogenesis along with the improvement of insulin sensitivity in mouse models.[5] PPARδ has been also implicated as a key regulator in lipid homeostasis and inflammation, the two key determinants in atherosclerosis.[6] Overall, PPARδ is a novel drug target for metabolic disorders, and selective PPARδ agonists could become novel drugs to treat diseases related to metabolic disorders such as obesity and type II diabetes.[7]





As part of our continuing search for new PPARδ selective agonists from marine natural products, bioactivity-guided fractionation followed by HPLC chromatography gave two unprecedented phosphorus-containing iodinated polyacetylenes, phosphoiodyn A and B (**1**–**2**), from a Korean marine sponge *Placospongia* sp. Herein, we describe the isolation and structural elucidation of phosphoiodyns as well as biological activity of phosphoiodyns.

Phosphoiodyn A (**1**)[8] was obtained as an amorphous solid. The molecular formula of **1** was deduced as $C_{16}H_{24}O_4PI$ based on the pseudomolecular ion peak at m/z 438.0459 $[M+H]^+$ in the HRFABMS and ^{13}C NMR data. The ^1H NMR spectrum of **1** (Table 1) displayed two methines and ten methylenes including two oxygenated methylenes. The ^{13}C NMR spectrum in combination with HSQC and HMBC spectra indicated four fully substituted carbons at δ_C 73.1, 66.1, 64.8, and 77.1 which allowed the assignment of a diyne unit. The observation of the coupling constants for C-2′ (J_{C-P} = 5.1 Hz, J_{H-P} = 13.2 Hz), C-1′ (J_{C-P} = 133.4 Hz, J_{H-P} = 17.4 Hz), C-1 (J_{C-P} = 5.1 Hz), and C-2 (J_{C-P} = 6.4 Hz) in the ^{13}C and ^1H NMR spectra are distinctive of the presence of phosphorus in the molecule.[9] The positive reaction of a modified Hanes–Isherwood reagent in thin-layer chromatography also supported the presence of a phosphorus atom in **1**.[10]

The interpretation of the COSY correlations allowed the three fragments to be assigned, as shown in Figure 1(a–c). The long-range HMBC correlations from H-7 to C-4, C-5, and C-6 and H-2 to C-3, C-4, and C-5 suggested that the two fragments (a and b) are connected through a diyne unit. The large coupling constant (J = 133.4 Hz) for C-1′ in ^{13}C NMR provided the C-1′–P attachment.[11] Lastly, the molecular formula of **1** and comparison of NMR shifts for C-13 (δ_H 6.52 and δ_C 146.5) and C-14 (δ_H 6.10 and δ_C 73.6) and the previously reported synthetic compound, (*E*)-1-iodo-1-octene,[12] permitted the attachment of an iodine atom at C-14. Thus, the complete structure of **1** was completely assigned as (*E*)-14-iodotetradeca-13-en-3,5-diyn-1-yl (2-hydroxyethyl)phosphonate.

Figure 1. Substructures of a, b, and c determined by COSY and key HMBC correlations of phosphoiodyn A (**1**).

Phosphoiodyn B (**2**)[13] was obtained as an amorphous solid. The molecular formula of **2** was established as $C_{16}H_{24}O_5PI$ from the HRFABMS m/z 454.0409 $[M+H]^+$ which had a higher mass than **1** by 16 Da, indicating an additional oxygen atom.

Table 1. 1D and 2D NMR Data for Phosphoiodyn A (**1**)[a]

no.	δ_H (J in Hz)	δ_C, mult.[b] (J_{C-P} in Hz)	HMBC
2′	3.14 (dt, 13.2, 7.5)	35.5, CH_2 (5.1)	1′
1′	1.89 (dt, 17.4, 7.5)	24.5, CH_2 (133.4)	2′
1	3.94 (dt, 7.0, 6.8)	61.9, CH_2 (5.1)	2, 3
2	2.60 (t, 6.6)	21.2, CH_2 (6.4)	1, 3, 4, 5
3		73.1, C	
4		66.1, C	
5		64.8, C	
6		77.1, C	
7	2.25 (t, 6.9)	18.2, CH_2	4, 5, 6, 8
8	1.50 (q, 7.5)	27.9, CH_2	6, 7, 9
9	1.39 (m)	28.0, CH_2	8, 10
10	1.32 (m)	28.2, CH_2	9, 11
11	1.43 (m)	28.0, CH_2	12, 13
12	2.07 (qd, 6.7, 1.0)	35.5, CH_2	11, 13, 14
13	6.52 (dt, 14.4, 7.1)	146.5, CH	11, 12, 14
14	6.10 (d,14.4)	73.6, CH	12, 13

[a] In methanol-d_4, at 700 MHz for ^1H and 175 MHz for ^{13}C NMR. [b] The numbers of attached protons were determined from ^1H, ^{13}C, and HSQC NMR spectroscopic data.

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(8) Phosphoiodyn A (**1**): $[\alpha]_D^{25}$ −4 (*c* 0.008, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 221 (4.09), 253 (3.20) nm; IR (KBr) ν_{max}: 2928, 2854, 1195, 1070 cm^{-1}; $C_{16}H_{24}O_4PI$ by HRFABMS $[M+H]^+$ m/z 438.0459 (Δ +0.02 mmu); for ^1H and ^{13}C data, see Table 1.

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The ^{1}H and ^{13}C NMR spectroscopic data (Table 2) of **2** were almost identical to those of **1** except for the small carbon coupling constant (J = 4.8 Hz) and downfield shifted signals (δ_H 4.08 and δ_C 61.8) for C-1′. These differences indicated that **2** had a phosphate group instead of a phosphonate group in the molecule. Analysis of 2D NMR spectroscopic data allowed the structure of **2** to be assigned as (*E*)-2-hydroxyethyl (14-iodotetradeca-13-en-3,5-diyn-1-yl) phosphate.

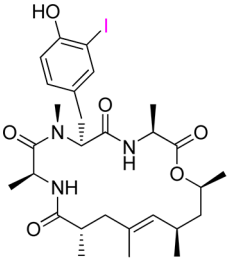
Table 2. 1D and 2D NMR Data for Phosphoiodyn B (**2**)[a]

no.	δ_H (J in Hz)	δ_C, mult.[b] (J_{C-P} in Hz)	HMBC
2′	3.12 (br. dd)	40.8, CH_2 (3.9)	1′
1′	4.08 (br. dd)	61.8, CH_2 (4.8)	2′
1	3.96 (dt, 6.9, 7.0)	63.9, CH_2 (5.1)	2, 3
2	2.62 (m, 6.8)	21.8, CH_2 (7.8)	1, 3, 4, 5
3		73.6, C	
4		66.9, C	
5		65.4, C	
6		78.1, C	
7	2.24 (t, 7.0)	19.3, CH_2	4, 5, 6, 9
8	1.51 (m, 7.6)	28.8, CH_2	6, 7, 9
9	1.38 (m)	28.4, CH_2	8, 10
10	1.30 (m)	28.4, CH_2	9, 11
11	1.41 (m)	28.6, CH_2	12, 13
12	2.07 (m)	36.2, CH_2	11, 13, 14
13	6.50 (dt, 14.5, 7.5)	146.9, CH	11, 12, 14
14	5.99 (d,14.5)	74.7, CH	12, 13

[a] In chloroform-*d* and methanol-d_4 = 5:1, at 600 MHz for ^{1}H and 150 MHz for ^{13}C NMR. [b] The numbers of attached protons were determined from ^{1}H, ^{13}C, and HSQC NMR spectroscopic data.

Both phosphoiodyns include an iodine atom. Only a few iodinated marine natural products have been reported, and most of them were isolated from marine sponges. Geodiamolide A (**3**) was reported as an antifungal agent, whereas the bioactivities of an iodinated tyrosine derivative, dakaramine (**4**), have not been determined.[14] Phosphoiodyns are the first natural products in the class of iodinated polyacetylenes. In nature, brominated polyacetylenes have been found with various bioactivities such as antimicrobial, antifungal, and HIV-1 integrase inhibitory activities.[3,15]

Intriguingly, phosphoiodyns contain phosphorus atoms, which are rarely found in natural products. In particular, phosphoiodyn A (**1**) has a C−P bond, which had not previously been observed in marine sponges. Phosphorus plays an essential role in living organisms, such as in regulation of signaling phenomena, energy transfer, and structural chemicals.[16] The first discovery of a natural product with a C−P bond, 2-aminoethane phosphonic acid (**5**), was isolated from a protozoa in the rumen of sheep in 1959.[17] The antibiotic fosfomycin (**6**) was isolated from *Streptomycetes* spp., and the C−P bond was considered as an important pharmacophore for antibiotic activity.[18,19] Structural similarities to analogous phosphate esters and carboxylic acids allow these molecules to compete with these analogs in binding to enzyme active sites. C−P compounds have a high potential for use in important biological reactions[20] because phosphate esters and carboxylic acids play ubiquitous roles in biology, such as controlling protein phosphorylation and proteolysis. Therefore, these compounds could be drug leads in modern pharmaceuticals and medicine due to their important roles in biological processes and their structural stability.[21]



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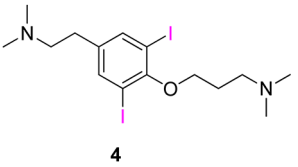

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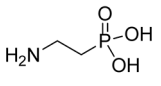

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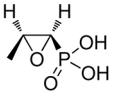

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Phosphoiodyn A (**1**) displayed highly potent hPPARδ activity (EC_{50} = 23.7 nM) in a cell-based cotransfection assay with an over 200-fold greater selectivity toward hPPARδ compared to the other subtypes, hPPARα and hPPARγ. However, phosphoiodyne B did not exhibit hPPARδ activity at a concentration of 10 μM. It appears that the C−P bond in the molecule plays an important role in hPPARδ agonism.

The C−P bond containing natural products, phosphoiodyn A (**1**), 2-aminoethane phosphonic acid (**5**), and fosfomycin (**6**), were isolated from diverse natural sources. Therefore, the true producers of phosphoiodyn A are predicted to be microorganisms associated with the sponge *Placospongia* sp. We are presently exploring the isolation of bacteria from the sponge *Placospongia* sp.

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Supporting Information Available. Experimental section, spectroscopic data for **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.