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Chiral *O*-phosphorylated derivative of 2-hydroxy-2-phenylethylphosphonate as a valuable product of microbial biotransformation of diethyl 2-oxo-2-phenylethylphosphonate

E. Żymańczyk-Duda *, M. Brzezińska-Rodak, M. Klimek-Ochab, R. Latajka, P. Kafarski, B. Lejczak

Wrocław University of Technology, Faculty of Chemistry, Department of Bioorganic Chemistry, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

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Abstract

Biotransformation of diethyl of 2-oxo-2-phenylethylphosphonate using different fungal cells as biocatalysts yielded optically active *O*-phosphorylated derivative of 2-hydroxy-2-phenylethylphosphonate of satisfactory enantiomeric excess up to 99%.

Keywords: Biotransformation; Fungi; Phosphonates

1. Introduction

Biotransformations are biocatalytic conversions of non-natural substrates to structurally diverse products and belong to the standard means of "Green Chemistry". The use of isolated enzymes as well as whole cells of microorganisms to obtain desired compounds of various interests is a vigorously developing field of bioorganic chemistry [1,2]. Biocatalysts are usually applied when the chemical synthesis fails or when compounds of required stereochemistry have to be synthesized [3–5] or for bioremediation purposes [6]. Phosphonates are a group of compounds, whose molecules contain direct bond between phosphorus and carbon atoms. Among them there are structural analogues of carboxylic acids, which influence the activity of enzymes involved in amino acid metabolisms exerting biological effect as antibacterials, herbicides or neuromodulators [7]. Furthermore, phosphonate derivatives with two acidic groups are known as compounds of medical importance, they are potential chelating agents in bone diseases treatment [7]. Going further organophosphorus molecules with one or two stereogenic centres are valuable intermediates in asymmetric organic synthesis – they serve as chiral building blocks [8–10]. Because of that simple and effective methods to synthesize them are still prospected. This field of scientific interest is still not fully explored, although there are reports about the use of pure enzymes and whole microbial cells as biocatalysts for the synthesis of various optically active organophosphorous compounds [11–16]. We have recently described biocatalyzed synthesis of several optically pure hydroxyphosphonates via stereochemically controlled, asymmetric bioreduction using following microbial strains: *Saccharomyces cerevisiae*, *Verticillium* sp. Op14, *Cladosporium* sp. Op328, *Rhodotorula* sp. [17,18]. The characteristic feature of reported bioconversions was that no side products but the desired ones appeared. It was a consequence of on the one hand – the biocatalysis advantages and on the other – rationally designed procedures.

Here we report the very effective strategy leading to a new, non-racemic derivative of hydroxyphosphonate. Five fungal species, known from their various catalytic activities towards phosphonates were used as biocatalysts for the bioconversion of diethyl 2-oxo-2-phenylethylphosphonate into its asymmetric derivative, compound of potential biological activity – *O*-phosphorylated derivative of hydroxyphosphonate (compound **3**, Scheme 1)

2. Experimental

2.1. Substrate synthesis

Substrate was synthesized according to the literature procedure [19].

* Corresponding author.
E-mail address: ewa.zymanczyk-duda@pwr.wroc.pl (E. Żymańczyk-Duda).

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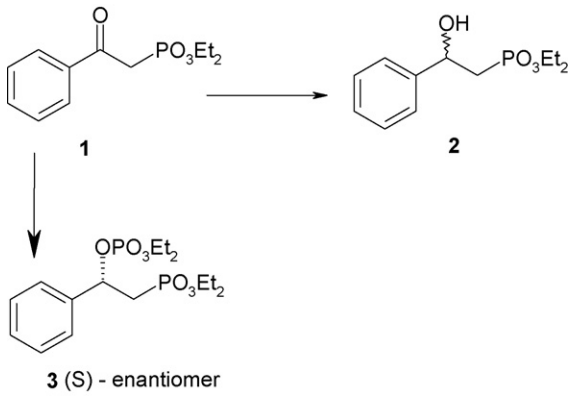


Scheme 1. Microbial bioconversion of diethyl 2-oxo-2-phenylethylphosphonate.

2.2. Microorganisms

Beauveria bassiana 271B (Institute of Agriculture and Forest Environmental Research, PAN, Poland). *Rhodotorula rubra* 70403, *Rhodotorula glutinis* 10134, *Geotrichum candidum* 6593 (DSMZ Germany). *Penicillium oxalicum* was a generous gift from Professor A.M. Picco (Department of Land Ecology, University of Pavia, Italy). *Cladosporium* sp. Op328 was a generous gift from Professor P. Kafarski (University of Opole, Poland).

2.3. Biotransformation – general procedure

Fungi were cultivated until the mid-log growth phase in particular cultivation medium, following the procedures described previously in literature [17]. After that the biomass was centrifuged (4500 rpm, 10 min) and the fresh cells were suspended in water (30 ml) with or without the addition of ethyl alcohol (50 μl), followed by the addition of 50 μl of the substrate. Biotransformation was carried out for 72 h at room temperature with shaking at 250 rpm. The product was isolated (after centrifugation of biocatalyst's cells, 4500 rpm, 10 min) followed by the extraction of supernatant twice with 20 ml of ethyl acetate. Then organic layer was dried over anhydrous magnesium sulphate and the volatile constituents of the reaction mixture were removed by evaporation under reduced pressure. Final product (or the mixture of products) was in the form of dense oil.

2.4. Optical rotation measurement, enantiomeric excess determination and Rf value

Optical rotation of pure product **3** (Scheme 1) is $[\alpha]_{578} = -4^{\circ}$ (room temperature; $c = 2$ in dichloromethane). Enantiomeric excess was determined by means of NMR; ^{31}P spectra were recorded with the addition of quinine as a chiral, shift reagent. Rf value is 4 (TLC, ethyl acetate).

2.5. Structural assignments

NMR spectra were recorded on Bruker Avance DRX300 instrument operating at 300.13 MHz (^{1}H), 121.499 MHz (^{31}P) and 75.46 MHz (^{13}C) and on Bruker AMX600 (2D-experiments). Measurements were made in $CDCl_3$ (99.5 at.% D) at temperature 300 K. Proton decoupling was achieved by power gated decoupling using the Waltz 16 sequence.

^{1}H NMR ($CDCl_3$): 1.28 (m, 4*CH_3, 12H), 2.8 (m, CH_2, 2H), 4.12 (m, 4*CH_2, 8H), 4.61 (m, CH, 1H), 7.20 (m, aromatic ring + $CDCl_3$, 6H); ^{31}P NMR ($CDCl_3$): 0.69 (d, 1P, $^{4}J_{PP} = 22.07$ Hz), 21.44 (d, 1P, $^{4}J_{PP} = 22.07$ Hz); ^{13}C NMR ($CDCl_3$): 16.16 (CH_3), 16.4 (CH_3), 31.62 (d, CH_2, $^{2}J_{PC} = 100.64$ Hz), 62.93 (d, CH_2, $^{2}J_{POC} = 5.9$ Hz), 64.11 (d, CH_2, $^{2}J_{POC} = 5.9$ Hz) 72.78 (dd, CH, $^{2}J_{PCC} = 162.9$H z, $^{2}J_{POC} = 7.2$Hz), 126.14, 128.46, 128.50, 140.96 (aromatic ring)

3. Results and discussion

The aim of the studies was microbial biotransformation of diethyl 2-oxo-2-phenylethylphosphonate (Scheme 1, compound **1**) into chiral compound **3** – *O*-phosphorylated diethyl hydroxyphosphonate. Following microorganisms were used, namely *Geotrichum candidum*, *Rhodotorula* sp., *Penicillium oxalicum*, *Cladosporium* sp. and *Beauveria bassiana*. The resulting compound **3** represents a structure, in which hydroxy group of phosphonate **2** is phosphorylated (Scheme 1).

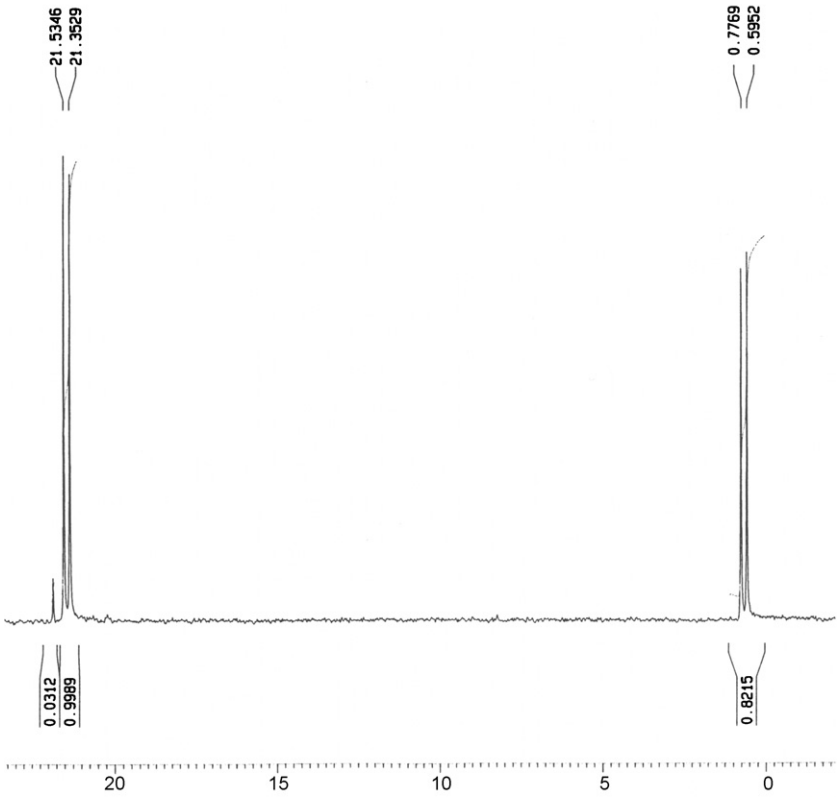
Obviously, this structural fragment has to derive from the decomposition of one substrate molecule, what was possible especially in the case of *Cladosporium* sp. (the best result obtained), because this particular strain is known from its ability to cleave the P–C bond [20]. Thus, biotransformation catalyzed by the cells of *Cladosporium* sp., carried out under very mild conditions resulted in the mixture of the products: compound **2** and **3** – both structures derived from the starting molecule. The conversion of the substrate was about 50% (the products mixture **2** and **3**) whereas the chiral *O*-phosphorylated derivative (compound **3**) of hydroxyphosphonate constituted about 98% of the final mixture (Table 1), after the reaction accomplished.

Cladosporium sp. obligatory requires some amount of ethyl alcohol as an exogenous hydrogen source for cofactor regeneration system, to maintain its catalytic activity during biotransformation proceeded under starvation conditions [17]. It is noteworthy that since biotransformation of other carbonyl derivatives of phosphonates, catalyzed by *Cladosporium* sp., always resulted in only one (*S*) – isomer of the product or in lack of the reaction [17], the absolute configuration of the compound

Table 1
The influence of biotransformation conditions[a] on the content of compound **3** in the isolated mixture of the products, determined by means of ^{31}P NMR

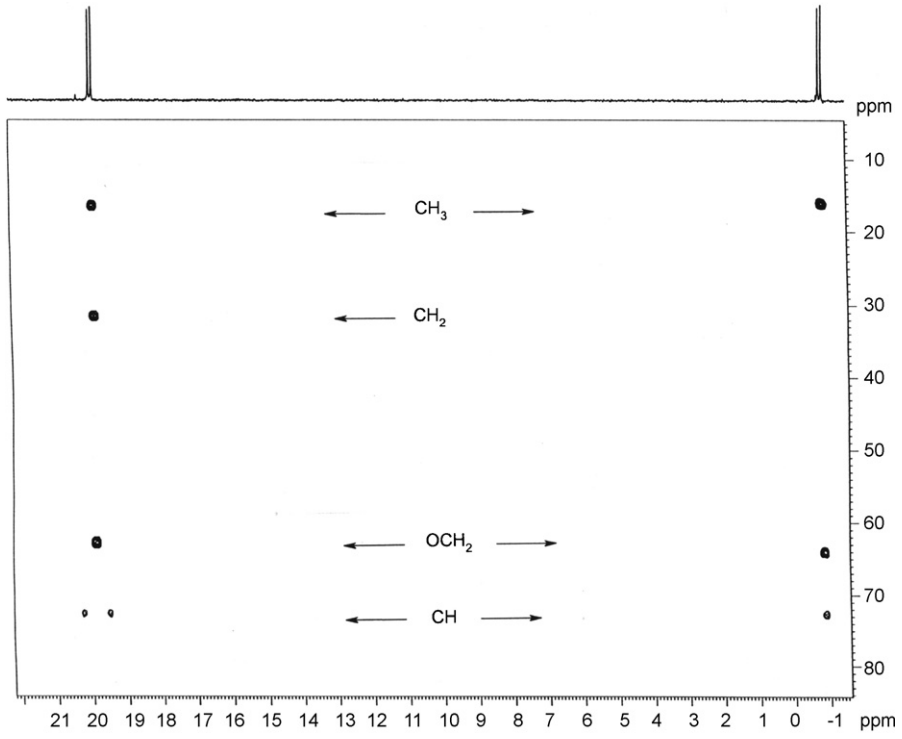
Microorganisms	% of compound **3** in the product mixture	
	No additives	Ethyl alcohol
Penicillim oxalicum	–	67
Cladosporium sp.	–	98
Beauveria bassiana	85	48
Geotrichum candidum	43	83
Rhodoturula rubra	–	62
Rhodotorula glutinis	81	15

[a] Biocatalysis was carried out according to general procedure described in Section 2.



Scheme 2. ^{31}P NMR spectra of compound **3**.

3 suppose to be (*S*). In the case of other microorganisms examined, compound **3** (Scheme 1) was also found as a major product of biocatalysis. The use of *Beauveria bassiana* also allowed obtaining desired product (85% of the final products mixture), even without any additives (Table 1). Supplementation of the biocatalytic medium with ethanol increased the amount of compound **3** (still the chemical yield of the isolated mixture was close to 50%) for *Penicillium oxalicum*, *Geotrichum candidum*



Scheme 3. Fragment of ^{31}P, ^{13}C COSY spectra of reaction product – compound **3**.

Scheme 4. Tautomerization of the substrate.

and *Rhodotorula rubra*, whereas for *Beauveria bassiana* and *Rhodotorula glutinis* the decrease of its amount was observed. It has to be stressed that in every case the biocatalytic process was stereospecific which was indicated by measurement of optical rotation of compound **3.** The spectroscopic characterisation of the obtained product began from one-dimensional NMR experiments (^{1}H, ^{31}P and ^{13}C). The analysis of ^{31}P NMR data shows that there are two interacting phosphorus atoms (phosphonate and phosphate) in one molecule, which are coupled with 22.07 Hz value of coupling constant (Scheme 2).

In order to determine the structure of this product two-dimensional NMR measurements were done (HMBC, HMQC, ^{31}P, ^{13}C COSY and ^{31}P,^{1}H COSY). Based on these results, it was possible to assume that two phosphorus atoms are located on alkyl fragment of carbon chain, which might be derived from the phosphorylation of hydroxyl group of the compound **2** (Scheme 1). A very strong evidence of this hypothesis was found on ^{31}P, ^{13}C COSY spectra (Scheme 3).

The chemical mechanism of this synthesis seems to be puzzling; however, some explanation might be derived from the existence of *keto-enol* tautomerism of the starting substrate (Scheme 4).

As can be seen from NMR spectra (data not shown), compound **1** exists as a mixture of 90% of *enol* and 10% of *ketol* form. It is not surprising if taking into consideration the fact that the *enol* form is stabilized by the action of electron withdrawing phosphonate moiety. The decomposition of P–C bond – biodegradative way [21] – is the first step of the conversion of the substrate **1**, which provided diethyl phosphate, subsequently used for phosphorylation – esterification – of *enol* form of substrate **1** or product **2** (Scheme 1). One can speculate that there may be three enzymes of different activities involved in the described reaction: C-P liase, reductase and phosphotriesterase. At the beginning at least two different moieties of the substrate (compound **1**, Scheme 1) are simultaneously bioconverted by two enzymes. One molecule is cleaved (decomposition of C–P bond) by C-P liase, which resulted in two products: acetophenone and diethyl ester of phosphoric acid. The second molecule is reduced to corresponding hydroxy derivative by reductase. Finally, the biotransformation medium contents the mixture of optically active hydroxyphosphonate and diethyl phosphate, so the next step – hydroxy group esterification is catalyzed by phosphotriesterase. Despite the control tests, the stereochemistry and the yield of the conversion confirm the enzymatic mechanisms of the reaction.

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