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Biocatalytic oxidation of thiophosphoryl compounds: a new chemo-enzymatic approach to enantiomeric insecticidal thionophosphates and their oxons

Marian Mikołajczyk [a,*], Jerzy Łuczak [a], Piotr Kiełbasiński [a], Stefano Colonna [b]

[a] *Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Department of Heteroorganic Chemistry, Sienkiewicza 112, 90-363 Łódź, Poland*
[b] *Istituto di Chimica Organica "A. Marchesini" Universita degli Studi di Milano, Via Venezian 21, 20133 Milano, Italy*

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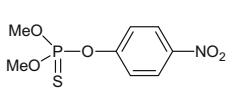
ABSTRACT

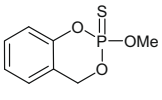
The biocatalytic oxidation of racemic *O*-*S*-dimethyl *O*-*p*-nitrophenyl phosphorodithioate **5** catalyzed by chloroperoxidase from *Caldariomyces fumago* resulted in the formation of the (−)-(*S*)-enantiomer of the corresponding oxon **4** and unoxidized substrate **5** with a (+)-(*R*)-configuration. Both compounds were obtained with very high enantiomeric excesses, 99.6% and 97%, respectively. The thionation reaction of the resulting (−)-(*S*)-oxon **4** with Lawesson's reagent gave (−)-(*S*)-phosphorodithioate **5** with full stereoselectivity, while the oxidation of unreacted substrate (+)-(*R*)-**5** with iodoxybenzene afforded oxon (+)-(*R*)-**4** with 94.9% ee.

1. Introduction

Organophosphorus compounds are widely used in agriculture as insecticides and fungicides and to some extent as herbicides.[1,2] The majority of these agrochemicals contain a thiophosphoryl group (P═S). A typical example of a commercially produced insecticide is parathion- methyl **1** and its structure is achiral. At the end of the last century, more attention was devoted to *P*-chiral organophosphorus agrochemicals for which a difference in biological activities between the enantiomeric forms has been observed. Although several such chiral organophosphorus insecticides have been obtained in enantiomeric forms, for example, salithion **2**[3] and DMPA **3**,[4] there are no commercially available single enantiomers of these compounds. This is mainly due to the difficulty in finding efficient, environmentally benign, and economic routes for a switch from racemic mixtures to single-isomer forms.[5]



Parathion-methyl **1**



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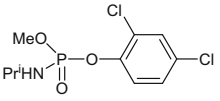
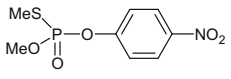
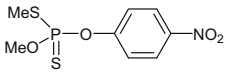


DMPA **3**

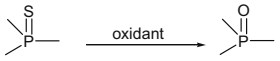


Isoparathion-methyl **4**



Parathiolothion-methyl **5**

Over the past few years, our group has been engaged in the development of enzyme-based methods for the preparative syntheses of enantiomeric organophosphorus and other heteroatom-containing compounds.[6] Therefore, we turned our attention to the possibility of using biocatalytic transformations for the synthesis of enantiomeric insecticidal thiophosphoryl structures. Raushel et al.[7] used phosphotriesterase (PTE) as a highly effective catalyst for the hydrolysis of chiral *O*-alkyl *O*-*p*-nitrophenyl phenylphosphonothionates and achieved almost complete kinetic resolution. Similarly, aminopeptidase P (AMPP) was found to stereoselectively hydrolyze racemic *O*-methyl *O*-*p*-nitrophenyl methylphosphonothionate, which also exhibited a preference for the (*S*)-enantiomer.[8]



Apart from enzymatic hydrolysis, which is the most important detoxification reaction of organophosphates, the main biodegradation pathway of thionophosphates is their oxidative desulfurization to afford the corresponding phosphoryl compounds (oxons). The latter are generally more toxic than their sulfur counterparts. The toxicity of the oxon compounds is due to the ability of these compounds to inactivate acetylcholinesterase.[9] Accordingly, the phosphorothiolates are better anticholinesterases than the phosphorothionates from which they are formed. Thus, isoparathion-methyl **4** has been found to be a better anticholinesterase agent than parathion-methyl **1**.[10] Moreover, as the thiol ester **4** is chiral at the phosphorus atom, its enantiomers showed different inhibitory action againsts various cholinesterases.[11]

* Corresponding author. Tel.: +48 42 681 58 32; fax: +48 42 684 71 26.
E-mail address: marmikol@bilbo.cbmm.lodz.pl (M. Mikołajczyk).

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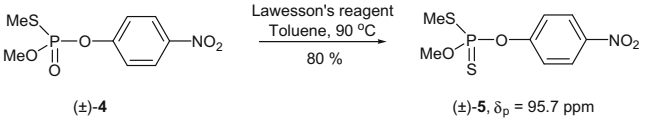
In spite of a continuous interest in the relationship between the bioactivity and absolute configuration at phosphorus, and also in many other classes of chiral phosphorus compounds, the number of synthetic approaches to chiral enantiomers of thionophosphates and the corresponding oxons are small and of limited applicability. Herein we report a novel enzymatic approach towards the efficient generation of enantiomers of these compounds through the stereoselective oxidation of racemic starting material. Our work was stimulated by the observations of Hernandes et al.[12] who reported that chloroperoxidase (CPO) from *Caldariomyces fumago* catalyzed the oxidation of a series of achiral P═S containing pesticides to their oxons. Under the conditions applied neither hydrolysis nor halogenation of substrates was observed. With this in mind, for the chloroperoxidase-mediated oxidation racemic *O*,*S*-dimethyl *O*-*p*-nitrophenyl phosphorodithioate **5** was chosen as a model insecticidal structure. The choice of dithioester **5** was also dictated by the fact that its oxidation should give oxon **4**, whose enantiomers are known and characterized. In this way the configurational relationship between enantiomeric substrates **5** and the oxidation products **4** could be established.

2. Results and discussion

2.1. Synthesis of racemic *O*,*S*-dimethyl *O*-*p*-nitrophenyl phosphorodithioate 5

The key substrate for the biocatalytic oxidation study, racemic dithiophosphate **5**, was prepared in a three-step procedure from parathion-methyl **1**, which upon treatment with trimethylamine gave the corresponding thioacid ammonium salt. The subsequent S-methylation of the latter produced isoparathion-methyl **4**. The conversion of racemic **4** into the desired dithiophosphate **5** was accomplished with Lawesson's reagent as a useful and readily available thionating agent. Thus, heating thioloester **4** and Lawesson's reagent in dry toluene for several hours afforded racemic **5** in 80% yield after chromatographic purification (Scheme 1).

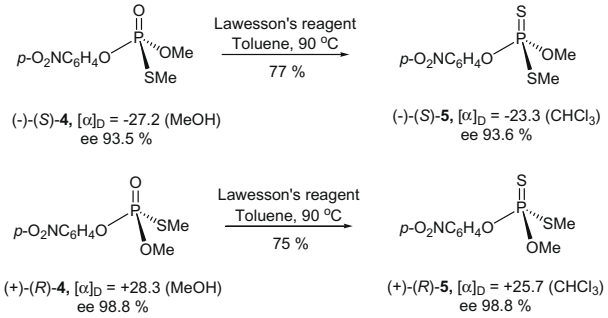


Scheme 1. Synthesis of racemic dithiophosphate **5**.

2.2. Stereoselective synthesis of enantiomeric *O*,*S*-dimethyl *O*-*p*-nitrophenyl phosphorodithioates 5

The efficient and clean conversion of racemic isoparathion-methyl **4** into its thiophosphoryl analogue **5** prompted us to use the enantiomeric forms of **4** for the thionation with Lawesson's reagent. The latter can be prepared in two ways. Hilgetag and Lehmann[13] obtained both optically active isomers from parathion-methyl **1** in the following sequence of reactions: demethylation with (−)-strychnine, diastereomer salt separation and S-methylation. In an alternative approach, Thompson et al.[14] synthesized (+)-(*R*)-**4** and (−)-(*S*)-**4** by BF_3-catalyzed methanolysis of the resolved diastereomeric phosphorus amides derived from L-proline ethyl ester. Herein, the required enantiomers of **4** were prepared according to the first method. Their optical rotations and enantiomeric purities were determined by chiral HPLC and are shown in Scheme 2.

The thionation reaction of (−)-(*S*)-isoparathion-methyl **4** with Lawesson's reagent was carried out under the conditions elaborated for a racemic form affording the levorotatory dithiophosphate **5** in 77% yield. In a similar way, (+)-(*R*)-**4** was converted efficiently



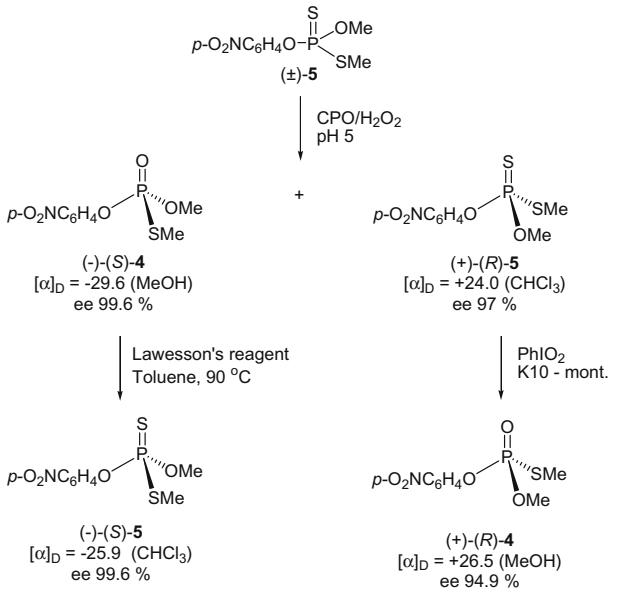
Scheme 2. Synthesis of enantiomeric dithiophosphates **5**.

(75% yield) into the dextrorotatory enantiomer **5**. An inspection of the enantiomeric excess values (chiral HPLC assay) of the starting oxons **4** and the thionation products **5** indicates that the stereochemical integrity is fully preserved in this reaction. With regard to the absolute configuration of (−)-**5** and (+)-**5** as obtained above, it is reasonable to assume at this stage that the reaction under discussion occurs with retention of configuration at the phosphorus atom. There is a piece of literature as evidence for the stereoretentive mechanism in the thionation reaction of phosphoryl compounds using phosphorus pentasulfide,[15] boron trisulfide,[16] and Lawesson's reagent.[17] Hence, the (*S*)-configuration was assigned to dithiophosphate (−)-**5** while the absolute stereochemistry of (+)-**5** should be (*R*). Our results discussed below provide unequivocal evidence for retention of configuration in the **4**→**5** conversion.

2.3. Chemo-enzymatic synthesis of the enantiomeric pairs of dithiophosphate 5 and oxon 4

Although the enantiomers of **5** have successfully been obtained as above, their chemical synthesis required several steps including laborious resolution of the diastereomeric thioacid strychnine salts. Looking for a shorter and more efficient route, we focused our attention on the chloroperoxidase catalyzed oxidation of racemic dithiophosphate **5.** This thionoester was subjected to oxidation with hydrogen peroxide in the presence of chloroperoxidase (CPO) in a mixture of citrate buffer, pH 5, and ethanol. The extent of the oxidation was monitored by HPLC. After 22 days the reaction was stopped and two compounds present in the reaction mixture in a 1:1 ratio were detected by ^{31}P NMR. The first resonance signal at δ_P = 27.3 ppm corresponds to the oxidation product, oxon **4**, while the second resonance signal at 95.7 ppm is due to the unreacted dithiophosphate **5**. This indicates that complete kinetic resolution took place in this reaction. Traditional work-up and separation by chromatography gave in a somewhat lower yield, oxon **4** exhibiting a specific rotation of $[\alpha]_D = -29.6$ (this value corresponds to 99.6% ee as determined by chiral HPLC), and dithiophosphate **5** with $[\alpha]_D = +24.0$ (97% ee). As the absolute configuration of (−)-**4** is (*S*), the unoxidized enantiomer of **5** must have the opposite configuration at the phosphorus, that is, (*R*) (Scheme 3).

With almost enantiomerically pure oxon (−)-(*S*)-**4** and dithiophosphate (+)-(*R*)-**5** from the complete biocatalytic kinetic resolution, we decided to prepare the opposite enantiomers of these compounds. Thus, the thionation reaction of (−)-(*S*)-**4** with Lawesson's reagent gave dithiophosphate (−)-(*S*)-**5** with full stereoselectivity. On the other hand, the oxidation reaction of (+)-(*R*)-**5** with iodoxybenzene in the presence of montmorillonite K10 as an activator[18] resulted in the formation of oxon (+)-(*R*)-**4** with a very high stereoselectivity. An inspection of the interrelationship between absolute configurations of **4** and **5** depicted in Scheme 3 led to the conclusion that the thionation reaction with Lawesson's reagent as well as enzymatic and chemical oxidation must occur



Scheme 3. Chemo-enzymatic synthesis of enantiomeric dithiophosphates **5** and their oxons **4**.

with retention of configuration at the phosphorus. In this context, it should be noted that the oxidation of the enantiomers of fonofos (*O*-ethyl *S*-phenyl ethylphosphonothionate) to the corresponding oxons in the presence of mouse liver mixed-function oxidase predominantly occurs with retention of configuration as reported by Fukuto.[19]

3. Conclusion

We have developed a new, one-pot biocatalytic method for the synthesis of the enantiomers of parathiolothion-methyl **5** and its oxon **4** based on the almost complete kinetic resolution taking place in the chloroperoxidase catalyzed oxidation of the thiophosphoryl group in the substrate. It has been shown that the biocatalytic oxidation in combination with the thionation reaction using Lawesson's reagent and oxidation with iodoxybenzene allowed both enantiomeric pairs of a thiophosphoryl substrate and oxon product to be easily prepared. Enzymatic and chemical oxidation, as well as the thionation reaction, have been demonstrated to occur with retention of configuration at the phosphorus and with almost full stereoselectivity. The described chemo-enzymatic synthesis of chiral enantiomers of thiophosphoryl and phosphoryl compounds has a general character and may be applied to other classes of organophosphorus compounds. Studies in this direction are currently underway.

4. Experimental

4.1. General

Chloroperoxidase (CPO) from *C. fumago* was purchased from Sigma. Other chemicals were purchased from Sigma–Aldrich. NMR spectra were recorded on a Bruker instrument at 200 MHz for ^{1}H, 50.32 MHz for ^{13}C and 81 MHz for ^{31}P with $CDCl_3$ as solvent. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter at 20 °C. Column chromatography was carried out using Merck 60 silica gel. TLC was performed on Merck 60 F_{254} silica gel plates. The enantiomeric excess (ee) values were determined by chiral HPLC (Varian Pro Star 210, Chiralpak AS).

4.2. Synthesis of enantiomeric *O,S*-dimethyl *O*-*p*-nitrophenyl phosphorothiolates (isoparathion-methyl) 4

Caution! Isoparathion-methyl is toxic. One should not attempt to handle this compound without proper training and adequate laboratory facilities.

To a stirred solution of *N*-methostrychnium salt of *O*-methyl *O*-*p*-nitrophenyl phosphorothioic acid, $[\alpha]_D = -17$ (MeOH) (400 mg, 0.67 mmol) in acetonitrile (6 mL) was added methyl iodide (140 mg, 1 mmol) and stirring was continued overnight. The precipitated salt was filtered off and washed with ether. The solution was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, 230–400 mesh, CH_2Cl_2) to give the pure title compound (−)-(*S*)-**4** (140 mg, 79%), $[\alpha]_D = -27.2$ (*c* 0.84, MeOH), 93.5% ee, as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 2.36 (d, J = 16.1 Hz, SMe), 3.95 (d, J = 12.9 Hz, OMe), 7.42–7.43 and 8.24–8.27 (arom).^{31}P NMR ($CDCl_3$): δ 27.3. ^{13}C NMR: δ 12.67, 54.46, 121.05, 125.64, 144.96, 154.87.

The enantiomeric purity of (−)-(*S*)-**4** was determined chromatographically on Chiralpak AS column (250 × 4.6) using a hexane–(*iso*-PrOH–EtOH–THF, 3:1:1) 90:10 solvent system at a flow rate of 0.7 mL/min with detection at 254 nm. For (+)-(*R*)-**4** and (−)-(*S*)-**4,** retention time is 26.9 and 30.0 min, respectively.

According to the procedure described above, from the other diastereomeric thioacid salt, $[\alpha]_D = +19$ (MeOH), (+)-(*R*)-isoparathion-methyl **4** was obtained in 75% yield, $[\alpha]_D = +28.3$ (*c* 2.0, MeOH), 98.8% ee.

4.3. Synthesis of (±)-*O,S*-dimethyl-*O*-*p*-nitrophenyl phosphorodithioate 5

A magnetically stirred suspension of (±)-**4** (65 mg, 0.25 mmol) and Lawesson's reagent (64 mg, 0.16 mmol) in dry toluene (5 mL) was heated at 90 °C overnight. After solvent evaporation, the residue was purified by column chromatography (SiO_2, 230–400 mesh, $CHCl_3$) to give (±)-**5** (55 mg, 80% yield) as an oil. Chiral HPLC [Chiralpak AS; hexane–(*iso*-PrOH–EtOH–THF, 3:1:1) 9:1; fl 0.7 mL/min] reveals two peaks of equal integration which are ascribed to the particular enantiomers (R_{T1} = 11.45 min, R_{T2} = 12.50 min) ^{1}H NMR ($CDCl_3$): δ 2.41 (d, J = 17.3 Hz, SMe), 3.91 (d, J = 15.4 Hz, OMe), 7.38–8.28 (arom). ^{31}P NMR ($CDCl_3$): δ 95.7. ^{13}C NMR: δ 15.57, 54.49, 121.99, 125.39, 145.04, 155.13.

MS(CI): *m/z* 280.1 (M+1). HRMS(CI): calcd for $C_8H_{10}NO_4PS_2$ 278.9790. Found 278.9792. Anal. Calcd for $C_8H_{10}NO_4PS_2$: C, 34.40; H, 3.58; P, 11.11; S, 22.94. Found: C, 34.52; H, 3.67; P, 11.26; S, 22.91.

4.4. Synthesis of (−)-(*S*)- and (+)-(*R*)–*O,S*-dimethyl-*O*-*p*-nitrophenyl phosphorodithioate 5

A mixture of (−)-(*S*)-**4,** $[\alpha]_D = -27.2$ (MeOH), 93.5% ee, (60 mg, 0.22 mmol), and Lawesson's reagent (53 mg, 0.12 mmol) in dry toluene (6 mL) was stirred at 90 °C overnight. After evaporation of the solvent, the crude mixture was subjected to column chromatography (SiO_2, 230–400 mesh, $CHCl_3$) to afford (−)-(*S*)-**5,** $[\alpha]_D = -23.2$ (*c* 0.50, $CHCl_3$), 93.6% ee, as a pale yellow oil (49 mg, 77% yield).

The enantiomeric purity was determined chromatographically on a Chiralpak AS Column (250 × 4.6) using hexane–(*iso*-PrOH–EtOH–THF, 3:1:1) 90:10; as an eluent system at a flow rate of 0.7 mL/min with detection at 254 nm for (+)-(*R*)-**5,** R_T = 11.4 min; for (−)-(*S*)-**5,** R_T = 12.50 min.

Similarly, starting from (+)-(*R*)-**4,** $[\alpha]_D = +28.3$ (MeOH), 98.8% ee, (+)-(*R*)-**5,** $[\alpha]_D = -25.7$ (*c* 0.80, $CHCl_3$), 98.8% ee, 74.5% yield was obtained.

4.5. Kinetic resolution of racemic dithiophosphate 5 by oxidation with CPO/H_2O_2 system

To a magnetically stirred (±)-**5** (20 mg, 0.074 mmol) and CPO (300 U) in a mixture of citrate buffer, pH5 (19 mL), and EtOH (1 mL), H_2O_2 (0.082 mmol) in 1 mL of buffer was added in portions. The reaction was monitored by HPLC. After 22 days, the reaction was quenched with sodium sulfite. The aqueous layer was extracted with $CHCl_3$ and the organic extract dried over $MgSO_4$. After removal of the solvent, the crude material (15 mg, 77% yield) was separated by chromatography (SiO_2, 230–400 mesh, $CHCl_3$) to give unreacted (+)-(*R*)-**5** (5 mg); $[\alpha]_D$ = +24.0 (*c* 0.25, $CHCl_3$), 97% ee [Chiralpak AS; hexane–(*iso*-PrOH–EtOH–THF, 3:1:1) 9:1; fl 0.7 mL/min, R_T = 11.10 min] and oxon (−)-(*S*)-**4** (5 mg); $[\alpha]_D$ = −29.6 (*c* 0.25, MeOH), 99.6% ee [Chiralpak AS; hexane–(*iso*-PrOH–EtOH–THF, 3:1:1) 9:1; fl 0.7 mL/min, R_T = 28.88 min].

4.6. Oxidation of (−)-(*S*)-dithiophosphate 5 with CPO/H_2O_2 system

To a magnetically stirred solution of (−)-(*S*)-**5** (20 mg, 0.074 mmol, $[\alpha]_D$ = −23.2 ($CHCl_3$), 93.1% ee) and CPO (300 U) in a mixture of citrate buffer, pH 5 (10 mL), and acetonitrile (10 mL), hydrogen peroxide (0.1 mmol in 1 mL of buffer, pH 5) was added. Stirring was continued at room temperature for ca one month. Products were extracted with CH_2Cl_2. The solution was concentrated under reduced pressure and the residue was separated by column chromatography (SiO_2, 230–400 mesh, CH_2Cl_2) to give unreacted (+)-(*R*)-**5** (0.87 mg, 4.7%, 76% ee) and (−)-(*S*)-**4** (10 mg, 51.3%), $[\alpha]_D$ = −29.6 ($CHCl_3$), 98.5% ee).

Oxidation of (+)-(*R*)-**5**, $[\alpha]_D$ = +23.1 ($CHCl_3$, 98.8% ee) under the above conditions led to the recovery of this unchanged enantiomer.

4.7. Oxidation of dithiophosphate (+)-(*R*)-5 with iodoxybenzene

To dithioester **5,** $[\alpha]_D$ = +25.0 (*c* 1.35, $CHCl_3$), (27 mg, 0.097 mmol) in $CHCl_3$ (3 mL) iodoxybenzene (24 mg, 0.1 mmol) and a few mg of K10-montmorillonite as an activator were added. The suspension was stirred for 20 h and after filtration of the solid material, the filtrate containing product was separated by column chromatography to give pure oxon (+)-(*R*)-**4** $[\alpha]_D$ = +26.9 (*c* 0.55, MeOH), (11 mg, 43% yield).

Acknowledgment

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