Synthesis and Biological Activity of Enantiomeric Pairs of Phosphosulfonate Herbicides

Lori A. Spangler,*,† Marian Mikołajczyk,‡ Ernest L. Burdge,† Piotr Kiełbasiński,‡ Howard C. Smith,† Piotr Łyzwa,‡ James D. Fisher,† and Jan Omelańczuk‡

Agricultural Products Research, Rohm and Haas Company, 727 Norristown Road, Spring House, Pennsylvania 19477, and Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, 90-363 Łódź, Sienkiewicza 112, Poland

The phosphosulfonates are a new class of soil-active herbicides which control a variety of annual grass and broadleaf weeds. Chirality at the phosphorus atom afforded the opportunity to explore stereospecific requirements for herbicidal activity. Chiral (hydroxymethyl)phosphinate intermediates were enzymatically resolved (*Pseudomonas fluorescens* lipase) from the racemic mixtures and then used to prepare two pairs of enantiomeric phosphosulfonates. Biological testing of the enantiomeric phosphosulfonate herbicides demonstrated that, in each case, the herbicidal activity was attributed to the (+) enantiomer and that the (+) enantiomer is more active than the racemate.

Keywords: *Phosphosulfonate; herbicide; enantiomer; resolution; lipase*

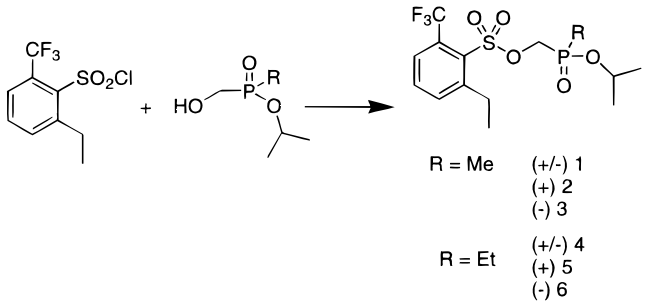
INTRODUCTION

Many agrochemicals have chiral structures, but for ease of synthesis, they are initially prepared and often marketed as racemic mixtures. It is desirable to prepare and test pure enantiomers since the biological systems with which the agrochemicals interact are chiral (Williams, 1996; Kurihara et al., 1997). Several single enantiomer herbicides and insecticides are currently used commercially. It is possible that the pure enantiomers will have very different levels of the desired biological activity, as well as varying levels of toxicity, crop selectivity, rates of metabolism, etc. Enantiomeric agrochemicals, in which one enantiomer is significantly more active than the other, have the potential for lower production costs, lower use rates, and reduced environmental impact.

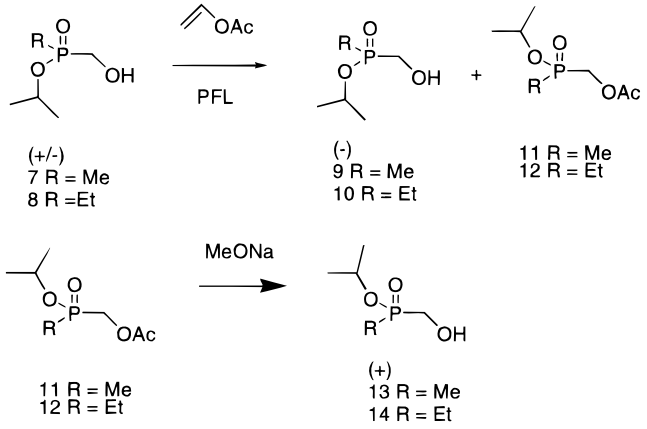
The phosphosulfonates (Scheme 1, **1**−**6**) are a new class of soil-applied herbicides with activity against a variety of grassy weeds (Rosen et al., 1996). The size and shape of the substituents on the phosphorus atom of the phosphosulfonates have an impact on the herbicidal potency because those phosphosulfonate molecules containing an unsymmetrically substituted phosphorus atom bearing one relatively large and one smaller group are most herbicidally active. The phosphorus atom is stereogenic in molecules with this substitution pattern. Individual enantiomers are necessary to explore the potential relationship between chirality at phosphorus and herbicidal activity.

The structures of the racemic herbicides (**1**, **4**) possess no reactive group for asymmetric functionalization or resolution; consequently, we chose to resolve the (hydroxymethyl)phosphinate intermediates (Scheme 2, **7** and **8**) first and use the resolved (hydroxymethyl)-phosphinates to prepare the desired chiral herbicide molecules. Mikołajczyk et al. (1995) reported the synthesis of chiral (α-hydroxyalkyl)phosphonates and related derivatives using the Abramov reaction of aldehydes with the appropriate chiral *H*-phosphonates or phosphinates. However, in contrast to our synthetic targets, Mikołajczyk et al. primarily used cyclic phosphorus compounds occasionally bearing additional chiral auxiliaries. Considering the synthetic difficulty in preparing the required starting *H*-phosphinates, *O*-isopropyl methylphosphinate, and *O*-isopropyl ethylphosphinate (for difficult syntheses of enantiomerically enriched

Scheme 1. Phosphosulfonate Herbicides



Scheme 2. Resolution of Racemic *O*-Isopropyl *P*-Alkyl-*P*-(hydroxymethyl)phosphinates



† Rohm and Haas Co. [phone (215) 619-5439; fax (215) 619-1617; e-mail rselas@rohmhaas.com].

‡ Polish Academy of Sciences.

10.1021/jf980527j CCC: $18.00
Published on Web 12/24/1998

H-phosphinates, see the following: Benschop and Van den Berg, 1970; Reiff and Aaron, 1970; Szafraniec et al., 1982), we have explored the possibility of using enzymatic procedures to resolve racemic isopropyl alkyl(hydroxymethyl)phosphinates **7** and **8**. Lipase-mediated acetylation was selected as the most promising transformation because racemic alcohols are considered good substrates for this type of reaction (for recent reviews, see the following: Jones, 1986; Chen and Sih, 1989; Klibanov, 1990; Faber, 1996, Sih et al., 1992; Fukui et al., 1990). Lipase-mediated acetylation has proven successful for the resolution of C-chiral (α-hydroxyalkyl)-phosphonates (Li and Hammerschmidt, 1993; Khushi et al., 1993); however, in this paper, we describe the first successful lipase-mediated resolution of racemic, *P*-alkyl(hydroxymethyl)phosphinates in which the phosphorus atom is the sole stereogenic center. In addition, we report the synthesis and biological testing of individual enantiomers of the phosphosulfonate herbicides.

PROCEDURES

The general synthetic strategy is summarized below and specific experimental details follow.

***O*-Isopropyl Alkyl(hydroxymethyl)phosphinates 7 and 8.** Racemic *O*-isopropyl alkyl(hydroxymethyl)phosphinate starting materials were prepared as described by Rosen et al. (1996) by reacting the appropriate alkyldichlorophosphine with 2-propanol and pyridine to form an *O*-isopropyl alkylphosphinate. Reaction of this material with formaldehyde and base resulted in the formation of the desired racemic *O*-isopropyl alkyl(hydroxymethyl)phosphinate.

Resolution of Racemic *O*-Isopropyl Alkyl(hydroxymethyl)phosphinates 7 and 8: General Strategy. The racemic *O*-isopropyl alkyl(hydroxymethyl)phosphinates **7** and **8** were resolved into enantiomers via enzyme-promoted acetylation. Thus, racemates of **7** and **8** (Scheme 2) were individually acetylated using vinyl acetate in the presence of *Pseudomonas fluorescens* lipase (PFL). The reaction was performed under kinetic resolution conditions; that is, it was stopped after ca. 50% conversion. In each case, the *O*-acetylated product, **11** or **12**, was then separated from the remaining unreacted alcohol chromatographically. Each was enaniomerically enriched, and ee was determined using ^{1}H NMR with (−)-*tert*-butylphenylphosphinothioic acid as a chiral solvating agent (Drabowicz et al., 1997) and by ^{31}P NMR using (−)-ephedrine as a chiral complexing agent. Since the first operation led to the desired products with ee 60−80% only, the procedure was repeated on each enantiomerically enriched sample. The levorotatory enantiomers **9** and **10** were those which remained unreacted during the acetylation. Methanolysis of the acetylated derivatives, **11** and **12**, gave in both cases the dextrorotatory enantiomers **13** and **14**.

Preparation of Optically Active 10 and 14. Racemic **8** (1.5 g, 9 mmol) was dissolved in diisopropyl ether (30 mL) and *P. fluorescens* lipase (100 mg, 44.9 U/mg, Fluka) was added. Vinyl acetate (3.6 mL) was added, and the mixture was stirred at room temperature. The progress of the reaction was followed by ^{31}P NMR (for **10**, δ = 55 ppm and, for **12**, δ = 48 ppm in diisopropyl ether). When the conversion reached 50% (2 h), the enzyme was removed by filtration and the solvent and excess vinyl acetate were removed by evaporation at room temperature. The residue was chromatographed on a silica gel column using a gradient of chloroform to chloroform/acetone (5:1) to elute **12** (0.89 g) and then chloroform/methanol (20:1) to elute (−)-**10** (0.58 g). Optical rotations were obtained and ee measured for these enantiomerically enriched samples.

12: $[\alpha]_D$ +13.33 (c = 1.0, chloroform), 64% ee from ^{1}H NMR using (−)-*tert*-butylphenylphosphinothioic acid as a chiral solvating agent.

10: $[\alpha]_D$ −13.55 (c = 1.2, chloroform), >86% ee (from ^{1}H NMR as above and ^{31}P NMR using (−)-ephedrine as a chiral agent.

To obtain enantiomerically pure products, enzymatic acetylations were repeated using enantiomerically enriched samples. In the case of **10**, the same procedure was applied, but the reaction was stopped after 40% conversion and only the fractions containing **10** (0.31 g) were collected.

10: $[\alpha]_D$ −14.5 (c = 1.0, chloroform), ee > 95%. No trace of the (+) enantiomer was detected by ^{1}H or ^{31}P NMR as described above.

Preparation of 14. The sample of **12** (0.9 g) from above was dissolved in anhydrous methanol (4 mL) and treated with a solution of sodium methoxide in methanol (0.1 mL of a 1 M solution). After 5 min, thin-layer chromatography of the reaction mixture on silica gel using chloroform/methanol as solvent (10:1) showed an absence of **12**. A sufficient amount of nonhydrated ion-exchange resin (Dowex 50 H^+) was added to neutralize the solution. When the solution reached pH 7, the resin was removed by filtration and the methanol solvent was evaporated under vauum. The residue was used in a subsequent enzymatic acetylation and stopped at 50% conversion. After workup as described above, pure **12** (0.34 g) was isolated.

12: $[\alpha]_D$ +18.43 (c = 1.3, chloroform).

Deacetylation followed by column chromatography on silica gel as described above gave **14** (0.54 g).

14: $[\alpha]_D$ +14.0, (c = 1.4, chloroform), ee > 95% by ^{1}H and ^{31}P NMR.

Preparation of Optically Active 9 and 13. 9 and **13** were prepared from racemic **7** using the procedures described above for **10** and **14**.

11: $[\alpha]_D$ +15.5 (c = 1.84, chloroform).

9: $[\alpha]_D$ −18.4 (c = 0.9, chloroform), ee > 95%.

13: $[\alpha]_D$ +19.64 (c = 1.14, chloroform), ee > 95%.

Preparation of Optically Active Phosphosulfonate Herbicides. A mixture of methylene chloride (20 mL) and sodium hydroxide (0.21 g of a 50% aqueous solution) was cooled to 0 °C and treated with **9** (0.37 g) and benzyltriethylammonium chloride (50 mg). After 5 min, a solution of 2-ethyl-6-(trifluoromethyl)benzenesulfonyl chloride (0.60 g) (Spangler, 1996) was added and the mixture stirred for 2 h at 0 °C. The reaction mixture was poured into water, the phases were separated, and the organic phase was washed with brine. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo to yield a pale oil which was then chromatographed on a flash silica gel column (2 × 10 cm) with 50:50 ethyl acetate/hexane. The resulting oil (0.68 g) was identified as **3** by GC and NMR characterization relative to a known sample of the racemic material, **1** (Rosen et al., 1996).

3: $[\alpha]_D$ −13.20 (c = 2.0, methanol).

The other herbicides were prepared in a similar fashion (**2** from **13**; **5** from **14**; **6** from **10**).

2: $[\alpha]_D$ +13.69 (c = 2.4, methanol).

5: $[\alpha]_D$ +13.92 (c = 2.3, methanol).

6: $[\alpha]_D$ −13.83 (c = 2.6, methanol).

Greenhouse Methods. The compounds described above were evaluated preemergence under greenhouse conditions on five monocot weeds: green foxtail (*Setaria viridis*), Hungarian millet (*Setaria italica*), large crabgrass (*Digitaria sanguinalis*), green sprangletop (*Leptochloa dubia*), and barnyardgrass (*Echinochloa crus-galli*).

A silt loam soil (Lawrenceville series) having about 2% organic matter was amended with washed sand (2 parts soil to 1 part sand). The amended soil was placed in 16 × 25 × 7 cm plastic trays to within 2 cm of the top. On top of the soil was placed the appropriate number of seeds of each weed species after which an additional 1 cm of amended soil was layered over the seeds. A known amount of test compound was dissolved in acetone and applied directly to the soil surface. Herbicide applications equaled 19, 38, 75, 150, 300, and 600 g/ha and were done with a laboratory track sprayer calibrated to deliver 234 L/ha using a 8002E nozzle (TeeJet) which delivered a spray pressure of 200 kPa.

After treatment, the trays were placed in a greenhouse and immediately watered overhead; subsequent watering was also overhead. Visual ratings of weed control were made 3 weeks after treatment. Results of the greenhouse evaluation were

Table 1. Herbicidal Activity of Phosphosulfonate Enantiomers[a]

	weed control rating				
compd	SET VI[b]	SET IT	DIG SA	LEF DU	ECH CG
1	20	50	50	100	0
2	100	100	100	100	40
3	0	0	0	0	0
4	0	0	85	0	0
5	50	100	90	100	0
6	0	0	0	0	0

[a] Herbicides applied to the soil at 38 g of ai/ha. [b] SET VI (*Setaria viridis*), SET IT (*Setaria italica*), DIG SA (*Digitaria sanguinalis*), LEF DU (*Leptochloa* dubia), ECH CG (*Echinochloa crus-galli*).

expressed on a 0−100 rating scale. The scale is based on visual observation of plant stand, vigor, malformation, size, and overall plant appearance as compared to an untreated control. With this scale, 0 represents no injury and 100 represents complete kill.

In Vitro Methods. Phosphosulfonate herbicides were dissolved in DMSO which was subsequently diluted into 20 mL of a nutrient solution comprised of 0.131 g/L KH_2PO_4, 0.506 g/L KNO_3, 1.181 g/L $Ca(NO_3)_2{\cdot}4H_2O$, 0.241 g/L $MgSO_4$, 2.5 mg/L H_3BO_3, 0.5 mg/L $ZnCl_2$, 0.05 mg/L $CuCl_2{\cdot}2H_2O$, 0.084 mg/L $NaMoO_4{\cdot}2H_2O$, 0.5 mg/L $MnCl_2{\cdot}4H_2O$, and 2.5 mg/L sodium ferric diethylenetriamine pentaacetate (Sequestrene 330, Ciba Geigy). Test solutions were obtained by 1:1 serial dilutions with nutrient solution. Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) seeds were germinated and grown on filter paper (Whatman No. 1) in 100 × 20 mm Petri dishes containing 10 mL of test solution under four 48 in. fluorescent lamps providing a light intensity of 50 μE m^{-2} s^{-1} of photosynthetically active radiation with a day−night cycle of 16 h light and 8 h dark. After 7 days, shoot length was measured to the nearest millimeter and was used as an index of herbicidal activity. The concentration of herbicide resulting in a 50% reduction in shoot length (I_{50}) was estimated by fitting the data to the dose−response equation (Streibig et al., 1993) using nonlinear regression.

$$y = C + (D - C)/(1 + (x/A)^b)$$

In this equation, y is shoot length, x is herbicide concentration, A is the I_{50}, b is a parameter which describes the slope of the curve, and C and D are the minimum and maximum responses, respectively.

RESULTS AND DISCUSSION

The enantiopure and racemic phosphosulfonate herbicides were evaluated in both greenhouse and laboratory tests. Table 1 shows the results from a typical greenhouse test where the herbicidal activity was evaluated on several grass weeds in preemergence applications at 38 g of active ingredient (ai)/ha. In both analogue sets, the (+) enantiomers (**2**, **5**) were significantly more herbicidal than were the corresponding (−) enantiomers (**3**, **6**) or racemic mixtures (**1**, **4**). The (+)-methyl phosphinate analogue was slightly more active than the (+)-ethyl phosphinate, as was observed for the corresponding racemates. With either phosphosulfonate, the (−) enantiomers had no herbicidal activity even when tested as high as 150 g/ha (data not shown).

The herbicide analogues were also tested for inhibition of shoot elongation in a soilless laboratory assay (Table 2). Similar to the greenhouse test results, the (+) enantiomers were the most active of the enantiomeric pairs in the laboratory tests. The (+) enantiomers were two times more active than their corresponding racemic mixtures, and the (+)-methyl phosphinate was approximately 1.5 times more active than the (+)-ethyl phosphinate. The (−) enantiomers were inactive at the highest concentrations tested (5.0 μM, **3**; 2.5 μM, **6**).

Table 2. Herbicidal Activity of Phosphosulfonate Enantiomers on Barnyardgrass in a Soilless Bioassay

compd	rotation	I_{50} (μM)
1	(±)	0.26
2	(+)	0.11
3	(−)	>5.0
4	(±)	0.32
5	(+)	0.16
6	(−)	>2.5

Taken together, these greenhouse and laboratory tests demonstrate that the herbicidal action of the phosphosulfonates possesses a stereospecific character. Although the mechanism which underlies this stereospecificity is unknown, there are several possibilities which could explain the results. First, it may be that the phosphorus portion of the molecule is involved in binding to the unknown target site enzyme for the phosphosulfonate herbicides. Alternatively, the (−) enantiomers could be taken up by the plant tissue more slowly than the (+) enantiomers or the two enantiomers could be metabolized at different rates in vivo. It is unlikely that this stereospecificity involves selective detoxification in the soil because similar results were obtained using both the soilless laboratory assay and the greenhouse test.

Currently, we cannot differentiate between these possible explanations for stereoselectivity; however, we have shown that the use rate of a typical phosphosulfonate herbicide can be reduced, potentially by a factor of 2, by using the active enantiomer rather than the racemic mixture.

Supporting Information Available: NMR spectra (5 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

Benschop, H. P.; Van den Berg, G. R. Stereospecific inclusion in cycloamyloses: partial resolution of isopropyl methylphosphinate and related compounds. *J. Chem. Soc, Chem. Commun.* **1970**, 1431−1432.

Chen, C.-S.; Sih, C. J. General aspects and optimization of enantioselective biocataysis in organic solvents: the use of lipases. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695−707.

Drabowicz, J.; Dudzinski, B.; Mikołajczyk, M.; Colonna, S.; Gaggero, N. Chiral *tert*-butylphenylphosphinothioic acid in NMR analysis of tertiary amine oxides with stereogenic nitrogen atom. *Tetrahedron*: *Asymmetry*. **1997**, *8*, 2267−2270 and references therein.

Faber, K. *Biotransformations in Organic Chemistry*, 3rd ed. Springer-Verlag: Berlin, 1996.

Fukui, T.; Kawamoto, T.; Sonomoto, K.; Tanaka, A.. Long-term continuous production of optically active 2-(4-chlorophenoxy)propanoic acid by yeast lipase in an organic solvent system. *Appl. Microbiol. Biotechnol.* **1990**, *34*, 330−334.

Jones, J. B. Enzymes in organic synthesis. *Tetrahedron* **1986**, *42* (13), 3351−3403.

Khushi, T.; O'Toole, K. J.; Sime, J. T. Biotransformation of phosphonate esters. *Tetrahedron Lett.* **1993**, *34*, 2375−2378.

Klibanov, A. M. Asymmetric transformations catalyzed by enzymes in organic solvents. *Acc. Chem. Res.* **1990**, *23*, 114−120.

Kurihara, N.; Miyamoto, J.; Paulson, G. D.; Zeeh, B.; Skidmore, M. W.; Hollingworth, R. M.; Kuiper, H. A. Chirality in synthetic agrochemicals: bioactivity and safety considerations. *Pure Appl. Chem.* **1997**, *69* (6), 1335−1348.

Li, Y.-F.; Hammerschmidt, F. Enantioselective hydrolysis of α-(acyloxy)phosphonates by esterolytic enzymes. *Tetrahedron: Asymmetry* **1993**, *4*, 109−120.

Mikołajczyk, M.; Drabowicz, J.; Kiełbasinski, P. Formation of C-P Bonds. In *Methods of Organic Chemistry, Houben-Weyl*; Helmchen, G., Hoffmann, R. W., Mulzer, J., Schaumann, E., Eds.; G. Thieme Verlag: Stuttgart, Germany, 1995, Vol. 21e, pp 5701−5731.

Reiff, L. P.; Aaron, H. S. Stereospecific synthesis and reactions of optically active isopropyl methylphosphonate. *J. Am. Chem. Soc.* **1970**, *92*, 5275−5276.

Rosen, R. E.; Weaver, D. G.; Cornille, J. W.; Spangler, L. A. Phosphosulfonate Herbicides. U.S. Patent 5,500,405, 1996.

Sih, C. J.; Gu, Q. M.; Holdgrun, X.; Harris, K. Optically-active compounds via biocatalytic methods. *Chirality* **1992**, *4*, 91−97.

Spangler, L. A. A novel method for the preparation of 2,6-disubstituted benzenesulfonates and benzenesulfonyl chlorides using the powerful alkyl sulfonate ortho directing group. *Tetrahedron Lett.* **1996**, *37* (21), 3639−3642.

Streibig, J. C.; Rudemo, M.; Jensen, J. E. Dose-response curves and statistical models. In *Herbicide Bioassays*; Streibig, J. C., Kudsk, P., Eds.; CRC Press: Boca Raton, FL, 1993.

Szafraniec, L. J.; Szafraniec, L. L.; Aaron, H. S. Reaction of (*R*)−(+)-isopropyl methylphosphinate with methyl triflate. Stereospecific synthesis of (*R*)−(+)-isopropyl methyl methylphosphonite. *J. Org. Chem.* **1982**, *47*, 1936−1939.

Williams, A. Opportunities for chiral agrochemicals. *Pestic. Sci.* **1996**, *46*, 3−9.

Received for review May 18, 1998. Revised manuscript received November 3, 1998. Accepted November 4, 1998.

JF980527J