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LETTERS TO THE EDITOR

Biocatalytic Separation of α-Hydroxyphosphonates with Lipase of *Burkholderia cepacia*

O. O. Kolodyazhnaya and O. I. Kolodyazhnyi

Institute of Bioorganic Chemistry and Petroleum Chemistry, National Academy of Sciences of Ukraine, ul. Murmanskaya 1, Kiev, 02094 Ukraine
fax: 38(044)573-2555
e-mail: olegkol321@rambler.ru

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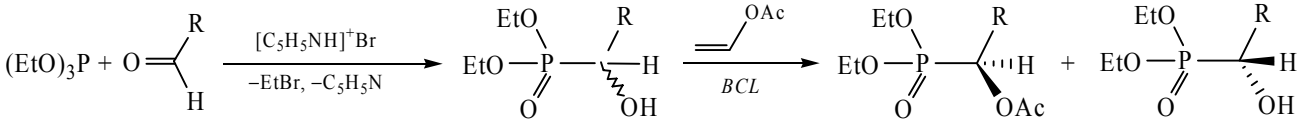
In recent years enzymes are with increasing frequency used to obtain the enantiomerically pure organophosphorus compounds [1–4]. In this work we found that lipase of *Burkholderia cepacia* (*BCL*) immobilized on diatomite [5, 6] is effective biocatalyst, which makes possible a kinetic separation of racemic α-hydroxyphosphonates into enatiomers. In the presence of this lipase vinyl acetate esterifies only (*S*)-enantiomer of racemic α-hydroxyphosphonates **Ia**–**Ic** to give a mixture containing 50% of α-acylphosphonate (*S*)-**IIa**–**IIc** and 50% of hydroxyphosphonate (*R*)-**Ia**–**Ic**. The latter can be separated by column chromatography. It was established that the esterification rate and optical purity of products little depend on the solvent (THF, toluene, vinyl acetate), but they strongly depend on temperature and lipase excess. When temperature is raised to 40 or 60°C, the reaction rate enhances approximately 1.5 and 2 times, respecttively. The time it takes to reach 50% esterification of α-hydroxyphosphonates, i.e. to the complete esterification of (*S*)-enantiomer, decreases as the biocatalyst amount increases. For example, for compound **Ia** in THF at 20°C 50% conversion time (depending on the weight ratio hydroxyphosphonate:lipase) was changed as follows: 48 h (1:0.05), 36 h (1:0.1), 24 h (1:0.2), 16 h (1:0.5), 12 h (1:1). The rate of 50% conversion for compounds **Ia**–**Ic** became slower as increased the length of the alkyl substituent R: 12 h (R = Me) and 60 h (R = Pr) in THF at 25°C, 300 h (C_5H_{11}) in THF at 45°C.

Racemic hydroxyphosphonates were obtained by the earlier reported method using the reaction of triethylphosphite with aldehydes in the presence of pyridinium bromide [7].



(R^+S)-**Ia–Ic** (*S*)-**IIa–IIc** (*R*)-**Ia–Ic**

R = Me (**a**), Pr (**b**), *n*-C_5H_{11} (**c**).

Optical purity of compounds (*S*)-**IIa–IIc** and (*R*)-**Ia–Ic** was established by means of ^{31}P NMR spectrometry in a chiral solvating agent (cinchonidine) medium [8], which showed high (≥ 99%) optical purity of the separated enantiomers. Optical purity and absolute configuration of the compounds were confirmed by derivatization with Mosher's acid [9]. Furthermore, we compared the optical rotation angles for the obtained hydroxyphosphonates **Ia** and **Ib** with published data [4].

(*R*)-Diethyl 1-hydroxyethylphosphonate (Ia). To a solution of 1.5 g (0.076 mol) of racemic (*S/R*)-**Ia** in 3 ml of THF and 3 ml of vinyl acetate was added

0.15 g of lipase. The mixture was stirred for 48 h at room temperature. The lipase was filtered off, the solution was concentrated, and the residue was subjected to chromatography on a silica gel to form two fractions, one of which (R_f 0.25, hexane–acetone 2:1) was optically pure alcohol (*R*)-**Ia**. Yield 48%, bp 85°C (0.1 mm Hg), $[\alpha]_D^{20}$ –7.0 (*c* 3, $CHCl_3$). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J,* Hz): 1.36 t (6H, C*H*$_3$CH$_2$O, *J* 6); 1.45 d.d (3H, CH_3CP, *J* 7, *J* 18); 3.61 br (1H, OH); 4.05 m (1H, CHP); 4.19 m (4H, CH_3CH_2O). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 16.2 d (CH_3, *J* 6); 17.28 s (CH_3); 61.63 d (CH_2O, *J* 6); 62.4 d (CH_2O, *J* 6); 62.8 d (PC, *J* 162.5). ^{31}P NMR spectrum ($CDCl_3$),δ_P, ppm:25.8 [2, 4].

Other fraction (R_f 0.55, hexane–acetone 2:1) was **(*S*)-diethyl 1-acetyloxyethylphosphonate (IIa)**. Yield 49%, $[\alpha]_D^{20}$ +25.0 (*c* 2, $CHCl_3$). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J,* Hz): 1.3 t (6H, C*H*$_3$CH$_2$O, *J* 6); 1.45 d.d (3H, C*H*$_3$CHP, *J* 7, *J* 17); 2.1 s (3H, CH_3CO); 4.15 m (4H, CH_2O); 5.1 d.q (1H, CHP, *J* 7, *J* 8). ^{31}P NMR spectrum ($CDCl_3$),δ_P, ppm: 21.1 [2].

(*R*)-Diethyl 1-hydroxybutylphosphonate (Ib). Yield 47%, $[\alpha]_D^{20}$ –25.0 (*c* 3, $CHCl_3$), bp 120 (0.1 mm Hg). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J,* Hz): 0.9 t (3H, CH_3, *J* 7); 1.1–1.3 m (2H, CH_2); 1.36 t (3H, C*H*$_3$CH$_2$O, *J* 7); 1.38 t (3H, C*H*$_3$CH$_2$O, *J* 7); 1.6 m (2H, CH_2); 3.3 br (1H, OH); 3.8 m (1H, PCH); 4.2 m (4H, OCH_2). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 13.6 s (CH_3); 16.5 s (C*H*$_3$CH$_2$O, *J* 8.5); 18.5 d (CH_2, *J* 7.5); 33.5 d (CH_2, *J* 5); 62 d (OCH_2, *J* 7); 70.5 d (PC, *J* 165). ^{31}P NMR spectrum ($CDCl_3$), δ_P: 26.

(*S*)-Diethyl 1-acetoxybutylphosphonate (IIb). Yield 47%. $[\alpha]_D^{20}$ +30 (*c* 3, $CHCl_3$). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J,* Hz): 0.89 t (3H, CH_3, *J* 7); 1.28 t (3H, C*H*$_3$CH$_2$O, *J* 7); 1.29 t (3H, C*H*$_3$CH$_2$O, *J* 7); 1.40 m (2H, CH_2); 1.77 m (2H, CH_2); 2.08 s (3H, $COCH_3$); 4.10 m 4H, CH_2O); 5.25 d.t (lH, PCH, *J* 4.9, *J* 8.4). ^{31}P NMR spectrum ($CDCl_3$), δ_P, ppm: 21 [1, 2].

(*R*)-Diethyl 1-hydroxyhexylphosphonate (Ic). Yield 40%, bp 160°C (0.1 mm Hg). $[\alpha]_D^{20}$ –20.0 (*c* 3, $CHCl_3$). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J,* Hz): 0.88 t (3H, CH_3, *J* 6.5); 1.315 t (3H, C*H*$_3$CH$_2$O, *J* 6); 1.32 t (3H, C*H*$_3$CH$_2$O, *J* 6); 1.4 m (2H, CH_2); 1.6–1.8 m (4H, CH_2CH_2); 4.5 br (1H, OH); 3.85 d.t (1H, PCH, *J* 4.5, *J* 9); 4.1.5 m (4H, CH_3CH_2O). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.01 (CH_3); 16.20 d (CH_3CH_2O, *J* 6.0); 16.30 d (CH_3CH_2O, *J* 5.8); 22.6 (CH_2); 25.99 d (CH_2, *J* 10); 30.3 (CH_2); 30.5 (CH_2); 61.9 d (CH_2O, *J* 6.0); 62.62 d (CH_2O, *J* 7.1); 66.9 d (PCH, *J* 165). Found, %: P 13.12. $C_{10}H_{23}O_4P$. Calculated, %: P 13.00.

(*S*)-Diethyl 1-acetoxyhexylphosphonate (IIc). Yield 40%, $[\alpha]_D^{20}$ +30.0 (*c* 2, $CHCl_3$). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J,* Hz): 0.85 t (3H, CH_3, *J* 7); 1.26 t (3H, CH_3CH_2O, *J* 7); 1.28 t (3H, CH_3CH_2O, *J* 7); 1.2–1.5 m (6H, CH_2); 1.77 m (2H, CH_2); 2.06 s (3H, $COCH_3$); 4.1 m (4H, CH_3CH_2O); 5.20 m (1H, PCH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 13.9 (CH_3); 16.3 d (CH_3CH_2, *J* 6.0); 16.4 d (CH_3CH_2, *J* 6); 20.9 ($COCH_3$); 22.8 (CH_2); 25.9 (CH_2); 29.3 (CH_2); 31.0 (CH_2); 62.0 d (CH_2O, *J* 6.5); 62.5 d (CH_2O, *J* 7.0); 71.0 d (PCH, *J* 165); 170.0 (C=O, *J* 6). Found, %: C 51.21; H 9.00; P 10.92. $C_{12}H_{25}O_5P$. Calculated, %: C 51.42; H 8.99; P 11.05.

The NMR spectra were recorded on a Varian-300 instrument relative to internal TMS (^1H, ^{13}C) and 85% H_3PO_4 in D_2O (^{31}P).

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