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Enantioselective Reduction of Diethyl 2-Oxoalkylphosphonates by Baker's Yeast

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Abstract: Baker's yeast catalyses the asymmetric reductive biotransformation of diethyl 2-oxoalkylphosphonates yielding diethyl 2-hydroxyalkylphosphonates in good yields and with high enantiomeric excess (>97%). As indicated by spectral analyses these hydroxyphosphonates exist in conformation which is "frozen" by intramolecular hydrogen bonding between phosphonate oxygen and hydroxyl hydrogen.

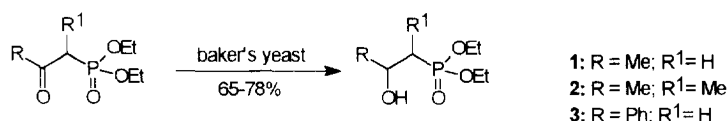
INTRODUCTION

Advances in biocatalysis have recently been focused on the growing number of applications of enzymes in organic synthesis as a consequence of the increased availability of new biocatalysts derived from various sources.[1-3] However, due to the significant economical convenience associated with the use of whole cells as the catalysts the former are preferentially used in some applications. On the other hand the bioconversions with the whole cells usually require detailed microbiological knowledge and access to fermentation facilities. In this context a special role is played by baker's yeast for the obvious reasons such as its low price, simplicity of growth and rich catalytic properties.[4,5] Baker's yeast (*Saccharomyces cerevisiae)* is indeed an extremely rich system containing hundreds of intracellular and extracellular enzymes, which are able to display their catalytic activity within the narrow experimental conditions and as such is widely used as catalyst in organic chemistry. Particularly it has been successfully exploited for the catalysis of stereospecific reductions of a wide variety of ketones and aldehydes.[1-5] In this paper we have studied how the introduction of a phosphonate group into the substrate influences the activity of this biocatalyst, and thus tested the usefulness of baker's yeast for the reduction of ketophosphonates. Resulting hydroxyalkylphosphonates may be considered as convenient substrates for the stereospecific synthesis of aminophosphonates via Mitsunobu reaction.[6,7]

Phosphonic acid analogues of amino and hydroxy acids display diverse and useful biological properties, possible applications of which range from medicine to agriculture.[8] For proper evaluation of their biological activity the availability of enantiomerically pure compounds of the defined configuration is indispensible and the search for new methods of their preparation is of great importance.

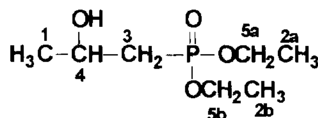
RESULTS AND DISCUSSION

The use of baker's yeast for the reduction of diethyl 1-oxoethylphosphonates failed because of instability of the substrates in aqueous solutions. This finding is in a good agreement with the literature.[9] Diethyl 2-oxoalkylphosphonates appeared to be good substrates and underwent reduction readily yielding the desired diethyl 2-hydroxyethylphosphonates (compounds **1**, **2** and **3**) in good yields and with high enantiomeric excess (>97%) as determined by means of capillary gc analysis on a column coated with chiral amide phase (valine bornyl amide bonded to a polydimethylsiloxane backbone). Considering the literature data on the similar reductions of β-ketoalkanoates the *R* configuration may be tentatively assigned for the obtained hydroxyphosphonates.[4,5,10,11]



Spectral data for **1** (Table 1 and Experimental) indicate that this hydroxyphosphonate exists, similarly as other representatives of this class of compunds,[12] in a conformation which is "frozen" by intramolecular hydrogen bonding between phosphonate oxygen and hydroxyl hydrogen (Figure 1).

Table 1. ^{1}H, and ^{13}C NMR Data for 1



H or C	δ ^{1}H, ppm (m, J in Hz)[a]	^{13}C, ppm (J in Hz)[b]
1	1.288 (dd, J_{HH}= 6.2, J_{PH}= 2.4)	24.32 (J = 17.8)
2a,2b	1.341 (t, J_{HH}= 7.1)	16.43 (J = 4.9)
	1.343 (t, J_{HH}= 7.1)	16.49 (J = 4.9)
	1.345 (t, J_{HH}= 7.1)	
	1.346 (t, J_{HH}= 7.1)	
3	1.927 (dd, J_{HH}= 7.8, J_{PH}= 16.3)	35.16 (J= 137.7)
	1.937 (dd, J_{HH}= 4.4, J_{PH}= 18,0)	
4	4.18 (m[c])	62.91 (J = 5.2)
5a,5b	4.14 (m[d])	61.89 (J = 6.3)
OH	3.536 (bs)	

[a] 250MHz and 300 MHz, $CDCl_3$. [b] 62,85 MHz in $CDCl_3$. [c] Covered by ester methylene peaks. [d] Can not be resolved.

This is clearly seen from ^{13}C and ^{1}H NMR spectra in which a very strong conformational fixation of the molecule by this bonding and presence of chiral center at C-4 induces a substantial anisochrony of the two diastereotopic phosphonate ethoxy groups. After suitable resolution enhancement, it is seen as doubling of the methyl signals of these groups (two doublets in ^{13}C and four triplets in ^{1}H NMR spectra). It is worth to note that spectrum of compound **1** obtained by baker's yeast mediated reduction is identical with this obtained for racemic mixture of **1** oibtained by the reduction of diethyl 2-oxopropylphosphonate with sobium borohydride. Additional structural proof comes from the comparison of EI mass spectra of the substrate (diethyl 2-oxopropylphosphonate) and the product of its reduction (compound **1**). The two fragmentation patterns (see Experimental) unequivocally demonstrate that both the McLafferty process and the cleavage of an ethoxy radical are not observed in the spectrum of diethyl 2-hydroxypropylphosphonate. It is most likely due to the existence of and extremely strong hydrogen bridge between P=O and O-H functions.

Assuming that the two possible conformers **1a** and **1b** are in rapidly-exchanging equilibrium the predominant one seems to be the conformer **1a**. This suggestion comes from a significant through-space couplings between phosphorus and C-1, and between phosphorus and H-1 atoms (17.8 and 2.4 Hz respectively). A similar conformational pattern was also found for diethyl 2-hydroxy-2-phenylethylphosphonate (compound **3**).

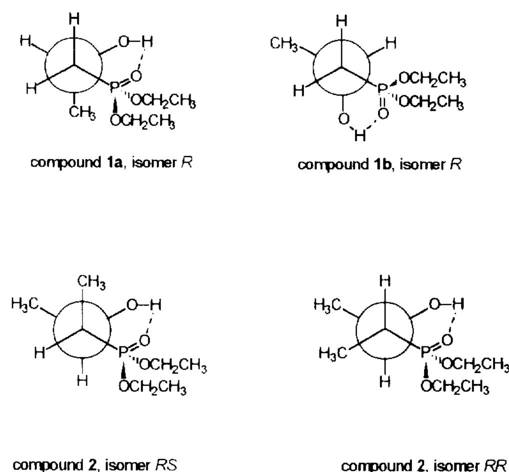


Figure 1. The proposed conformations of compounds **1** and **2** "frozen"by intramolecular hydrogen bonding.

The second substrate, diethyl 3-oxobut-2-ylphosphonate (compound **2**), already contains a stereogenic center at C-4, and was used as a racemic mixture. Although the reaction is still highly stereospecific its enantioselectivity is quite low and the mixture of two diastereoisomers in 2:1 molar ratio was obtained. If assuming that the forming asymmetric center is of *R* configuration, and the molecule is conformationally fixed by intramolecular six-member ring formation upon hydrogen bonding, the examination of ^{1}H NMR (Table 2) spectra justify the assignement of *R,S* configuration to the major isomer and *R,R* to the minor one (Figure 1). This assignement was based on the observation that orientation of H-4 and H-5 is antiperiplanar in major and gauche in minor diastereomer. This results in coupling constants between these protons being 8.3 Hz and 2.4

Hz respectively. A co-planar W-pathway found for the ^{4}J coupling between the H-2 methyl protons and H-4 tertiary hydrogen (1.1. Hz, see Table 2) also supports this assignment. An analysis of the ^{13}C NMR spectrum reveals that the OCH_2 resonance for the two ethoxyl groups may be resolved into eight lines. Four lines each, in roughly 1:2 intensity ratio, correspond to the two diasteroisomers. Since the two ethoxy groups once again are diasterotopic for each isomer, the respective resonances are doubled (Figure 1).

This finding alongside with the lack of McLafferty process and absence of the cleavage of an ethoxy radical in the mass spectra of both diesteroisomers of diethyl 3-hydroxybut-2-ylphosphonate (Experimental) once more support the existence of strong conformational fixation of these molecules by hydrogen bonding.

EXPERIMENTAL

General. Unless noted otherwise, all starting materials were obtained from commercial suppliers and were used without further purification. Diethyl 2-oxalkylphosphonates were prepared according to the literature [13,14] and were purified by column chromatography on Merck silica gel 60 (230-400 mesh) using ethyl ether or acetone-hexane (1:1, v/v) as eluents. IR spectra were recorded as films. ^{1}H, ^{13}C and ^{31}P NMR spectra were obtained in solutions in $CDCl_3$ and chemical shifts were reported in ppm (δ) downfield relative to TMS (internal standard) in proton spectra, center line of $CDCl_3$ triplet in carbon spectra and 80% phosphorus acid (external standard) in phosphorus spectra. Capillary gas chromatography was performed using three phases: achiral PS 086 carrier (to check the composition and purity of products), dimethylpolysiloxane modified with permethylated cyclodextrin (no separation of enantiomers was detected) and chiral amide phase (valine bornyl amide bonded to a polydimethylsiloxane backbone) on which a good separation of the racemic products, obtained by chemical reduction of diethyl 2-oxoalkylphosohonates with sodium borohydride, was achieved.

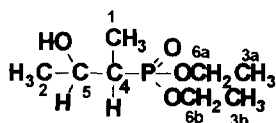
Baker's yeast were purchased from Wołczyn, Poland (strain PW-A-79002) and from ordinary bakery in Montpellier, France (DHW S-3 strain produced in Hamburg, Germany). Despite of the source of microorganism the results of biocatalysis were identical for all substrates.

Reduction of Diethyl 2-Oxoalkylphosphonates by Baker's Yeast. General Procedure. Baker's yeast (35 g) was suspended in water (200 ml), then ketophosphonate (1 mmole) was added and the mixture was shaken for 120 h at 28-30 ^{o}C. Biomass was removed by centrifugation (3000 rpm/20 min) and supernatant extracted with ethyl ether (3 x 20 ml) and then with chloroform (3 x 20 ml). The combined organic layers were dried over anhydrous magnesium sulfate and the solvents removed under reduced pressure. The product contained hydroxyphosphonate and starting material. They were separated by means of column chromatography on Merck silica gel 60 (230-400 mesh) using solvents indicated below.

Diethyl 2-hydroxypropylphosphonate (1): colorless oil; ee 97%; yield 78%; acetonitrile used as eluent in column chromatographic separation; $[\alpha]_{578} = +7.5^{o}$ (c=2, methanol); IR (film, cm^{-1}) 3410 (OH), 1245 (PO), 1050 and 1025 (POC); ^{1}H and ^{13}C NMR see Table 1; ^{31}P NMR (300 MHz) δ 30.51 ppm; mass spectrum (EI) *m/z* 197.05 ($C_7H_{17}O_4P$ + H requires 197.09), 195, 181, 152, 125 (base) and lack of 168 (Mc Lafferty fragmentation) and 151 (cleavage of ethoxy radical).

Diethyl 3-hydroxybut-2-ylphosphonate (2): colorless oil; ee 100%; yield 70%; acetone-hexane (1:1 v/v) as eluent; $[\alpha]_{578} = +5^{o}$ (c=2, methanol); IR (film, cm^{-1}) 3430 (OH), 1255 and 1235 (PO), 1055 and 1025 (POC); ^{1}H and ^{13}C NMR see Table 2; ^{31}P NMR (300 MHz) 30.65 (major isomer) and 30.34 (minor isomer)

Table 2. ^{1}H, and ^{13}C NMR Data for 2



H or C	δ ^{1}H, ppm (m, J in Hz)[a]	^{13}C, ppm (J in Hz)[b]
	major isomer	
1	1.160 (dd, J_{HH}= 7.4, J_{PH}= 18.6)	7.16 (J = 4.2)
	1.164 (dd, J_{HH}= 7.4, J_{PH}= 18.6)	
2	1.236 (dd, J_{HH}= 6.4, J_{PH}= 2.0)	20.27 (J = 13.8)
	1.240 (dd, J_{HH}= 6.4, J_{PH}= 2.0)	
3a, 3b	1.335 (t, J_{HH}= 7.1)	16.49 (J = 5.7)
4	1.963 (4xqq, $J_{(1H4H)}$= 7.4, $J_{(4H5H)}$= 2.4, $J_{(2H4H)}$= 1.1, J_{PH}= 17.4)	37.42 (J = 136.8)
5	4.208 (qq-qq, $J_{(2H5H)}$= 6.4, $J_{(4H5H)}$= 2.4, J_{PH}= 8.0)	65.88 (J = 5.1)
6a, 6b	4.13 (m[d])	61.91 (J = 4.1)
		62.02 (J = 4.1)
	minor isomer	
1	1.110 (dd, J_{HH}= 7.4, J_{PH}= 17.9)	10.90 (J = 6.4)
	1.113 (dd, J_{HH}= 7.4, J_{PH}= 17.9)	
2	1.241 (dd, J_{HH}= 6.3, J_{PH}= 2.4)	20.91 (J = 13.9)
	1.244 (dd, J_{HH}= 6.3, J_{PH}= 2.4)	
3a, 3b	1.337 (t, J_{HH}= 7.1)	16.44 (J = 6.8)
4	1.913 (qq-qq, $J_{(1H4H)}$= 7.4, $J_{(4H5H)}$= 8.3, J_{PH}= 20.6)	39.12 (J = 135.8)
5	3.930 (qq-qq, $J_{(2H5H)}$= 6.3, $J_{(4H5H)}$= 8.3, J_{PH}= 11.3)	67.52 (J = 3.5)
6a, 6b	4.13 (m[d])	61.87 (J = 6.5)
		62.07 (J = 7.0)

[a] 250MHz and 300 MHz, $CDCl_3$. [b] 62,85 MHz in $CDCl_3$. [c] Since this part of spectrum was covered by ester methylene peaks J_{PH} was found from HETEROCOSY spectrum. [d] Can not be resolved.

ppm; mass spectrum identical for both diastereoisomers (CI) *m/z* 211.10 ($C_8H_{19}O_4P$ + H requires 211.11), 195, 166, 139 (base), 111; lack of 182 (McLafferty process) and 165 (ethoxy radical cleavage).

Diethyl 2-hydroxy-2-phenylethylphosphonate (3): colorless oil; ee 99%; yield 65%; ethyl acetate used as eluent; $[\alpha]_{578}$ = +3° (c=2, methanol); IR (film, cm^{-1}); ^1H NMR (250 MHz) 1.196 and 1.229 (t, J_{HH}= 7.1 Hz, 3H, **CH_3** each), 2.141 (d-d, J_{HH}= 9.0 Hz,J_{PH}= 15.7 Hz, 1H, **PCH_2**), 2.111 (d-d, J_{HH}= 3.8 Hz, J_{PH}= 18.3 Hz, 1H, **PCH_2**), 3.96-4.26 (m, 4H, **OCH_2**), 4.142 (bs, 1H, **OH**), 5.016 (d-d,d-d, J_{HH}= 3.8 Hz, J_{HH}= 9.0 Hz, 1H **PCH**), 7.13-7.33 (m, 5H, C_6**H_5**) ; ^{13}C NMR (250 MHz); 16.84 and 16.68 (**CH_3** each), 36.33 (J = 136.2 Hz, PCH_2), 62.42 (J = 6.3 Hz, **$POCH_2$**), 62.26 (J = 6.7 Hz, **$POCH_2$**), 69.17 (J = 5.0 Hz, **PCH**), 125.94, 128.06 and 128.86 (aromatic carbons); ^{31}P NMR (250 MHz) 30.23 ppm; mass spectrum (CI) *m/z* 259.05 ($C_{12}H_{19}O_4P$ + H requires 259.11), 258, 152, 125 (base), 97, 77.

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