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Enantioselective Reduction of Dialkyl 4-(Dialkoxyphosphoryl)-3-Oxobutanoates by Baker's Yeast

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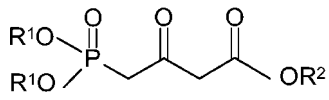
ABSTRACT: *Dialkyl 4-(dialkoxyphosphoryl)-3-oxobutanoates (**1**), upon yeast-mediated bioreduction, afforded chiral dialkyl 4-(dialkoxyphosphoryl)-3-hydroxybutanoates (**2**) in moderate to good yields and ee values. Significant improvement was reported for the preparation of dialkyl 4-(dialkoxyphosphoryl)-3-oxobutanoates (**1**), the key substrates of this bioreductive conversion.* © 2002 Wiley Periodicals, Inc. Heteroatom Chem 13:153–156, 2002; Published online in Wiley Interscience (www.interscience.wiley.com). DOI 10.1002/hc.10011

INTRODUCTION

Bioreduction of β-ketocarboxylates by baker's yeast (*Saccharomyces cerevisiae*) are probably the most extensively studied small molecule microbial transformations leading to chiral intermediates in asymmetric syntheses. Nevertheless, this biotransformation has been known for a long time [1–3]; there are only a few reports dealing with the enzymatic reduction of their phosphorus analogues, namely β-ketoalkanephosphonates [4]. On the other hand, chiral β-hydroxyalkanephosphonic acids have received much attention due to their unique physiological activities as well as their ability to mimic the corresponding hydroxy- or amino-alkanecarboxylic acids [5]. As a part of our systematic study of the biotransformation of organophosphorus compounds [6], we wish to report in this paper the bioreductive behaviors of dialkyl 4-(dialkoxyphosphoryl)-3-oxobutanoates (**1**), molecules containing both carboxylate and phosphonate functions sharing a ketomoiety in the β-position. In addition to this, an improvement has been made for the synthesis of dialkyl 4-(dialkoxyphosphoryl)-3-oxobutanoates (**1**), the key substrates in our study.

RESULTS AND DISCUSSION

As reported by Arbuzov et al. in the early fifties [7], thermal rearrangement accompanying the reactions of trialkylphosphites with 4-bromo-3-oxobutanonates gave diethyl 4-(dialkoxyphosphoryl)-3-oxobutanoates, contaminated with unsaturated impurities which consumed bromine up to 0.2%. It is now clear that Arbuzov's thermal rearrangement is usually accompanied by the Perkow reaction that leads to the formation of enol phosphate [8]. More recently, Bodalski et al. described the preparation of diethyl 4-(dialkoxyphosphoryl)-3-oxobutanoates (**1**) by the Michaclis-Beaker's method [9]. We reexamined the Arbuzov's approach for the synthesis of dialkyl 4-(dialkoxyphosphoryl)-3-oxobutanoates (**1**) and purified the reaction products by column chromatography on silicon gel; thus we were able to prepare compounds **1** in a pure state.



1

R^1=Me,Et,iPr,nBu
R^2=Me,Et

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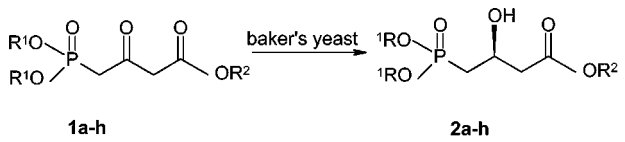

SCHEME 1

The carbonyl substrates (**1**) thus obtained are stable in water and undergo enantioselective reduction with baker's yeast to give chiral dialkyl 4-(dialkoxy phosphoryl)-3-hydroxybutanoates (**2**), as described in the experimental part (Scheme 1.) The identity and purity of each dialkyl 4-(dialkoxyphosphoryl)-3-hydroxybutanoate (**2**) was examined by spectroscopical methods, in addition to elemental analyses.

As shown in Table 1, all 4-phosphoryl-3-oxobutanoates can be transferred conveniently to the corresponding 4-phosphoryl-3-hydroxybutanoates by the reaction of baker's yeast in moderate to good yields and ee values. The enatioselectivity of each reaction was determined by means of ^{31}P NMR spectroscopy using quinine as a chiral disciminating agent, and this method may be applied to the tentative estimation of the configuration of each product [10,11]. According to the general experimental observations, bioreduction of ketones by baker's yeast usually obeys Prelog's rule [12]. In our case, the phosphonate group is the larger substituent, while the ester group is the smaller one. Thus, the R configuration may be tentatively assigned for each of the dialkyl 4-(dialkoxy phosphoryl)-3-hydroxybutanoates (**2**). In the meantime, under the same conditions, stereoselectivity of the reduction is strongly dependent on the chemical structure of the 4-phosphoryl-3-oxobutanoates. As shown in Table 1, the ee values of the 4-phosphoryl-3-oxobutanoic acid methyl esters are much larger than those of the corresponding 4-phosphoryl-3-oxobutanoic acid ethyl esters (**a,b, c,d** vs. **e,f,g,h** respectively). These results illustrate that a minor modification of the number of carbon atoms in the ester alkyl group will bring about a significant change of the enantioselectivity of yeast mediated reductions. Moreover, the ee value of this bioreduction is also influenced remarkably by the presence of the phosphoryl group.

TABLE 1 Reduction of **1a–h** with Baker's Yeast[a]

Substrate	*R*^{1}	*R*^{2}	*Yield (%)*	*ee (%)*[b]
1a	Me	Me	61	68
1b	Et	Me	70	82
1c	*i*-Pr	Me	46	55
1d	*n*-Bu	Me	77	85
1e	Me	Et	50	51
1f	Et	Et	65	50
1g	*i*-Pr	Et	41	32
1h	*n*-Bu	Et	63	70

[a]Bioreduction was carried on in aqueous medium with baker's yeast at 30°C for 72 h.
[b]ee% was determined by the use of quinine as a chiral solvating agent.

EXPERIMENTAL

IR spectra were recorded on a Shimadzu IR-440 spectrometer. EI mass spectra (MS) were run on an Hp-5989A mass spectrometer. ^{1}H and ^{31}P NMR spectra were recorded on a Bruker AMX-330 (300 MHz) spectrometer in $CDCl_3$ solutions and chemical shifts (δ) were reported in ppm downfield relative to TMS (internal standard) and 80% phosphoric acid (external standard) in phosphorus spectra.

Spots in TLC monitoring were visualized by dipping the plate into a solution of 24 g of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ and 1 g of $Ce(SO_4)_2{\cdot}4H_2O$ in 500 ml of 10% H_2SO_4 in water, followed by heating with a hot gun. Baker's yeast was purchased from Sigma Co. Int. Methyl 4-bromo-3-oxobutanonate and ethyl 4-bromo-3-oxobutanonate were prepared according to the literature [13].

*4-(Dimethoxyphosphoryl)-3-oxobutanioic Acid Methyl Ester (***1a***)*

To a dried 10 ml flask fitted with a stirrer, was added 1.94 g (0.010 mol) of methyl 4-bromo-3-oxobutanonate, and the mixture then warmed to 100°C. Trimethyl phosphite 1.24 g (0.010 mol) was then added dropwise within 5 min. The reaction mixture was stirred for an additional 20 min at 100°C, then the mixture was cooled to room temperature rapidly and the low boiling product, bromomethane, was removed under reduced pressure. The reaction products was separated by column chromatography on silica gel. (hexane/acetone as eluent, $v/v = 2/3$).

Yellow oil; 1.62 g (yield 72%); IR (ν): 2961, 1749, 1720, 1440, 1260, 1032 cm^{-1}. ^{1}H NMR: 3.80 (d, 6H,$POCH_3$), 3.65 (s, 2H, CH_2CO), 3.85 (s, 3H, OCH_3).^{31}P NMR: 29.3; MS (*m/e*, %): 224, 192, 164, 124, 109, 94; Anal. Calcd. for $C_7H_{13}O_6P$: C, 37.51; H, 5.85; Found: C, 37.62; H, 5.88.

*4-(Diethoxyphosphoryl)-3-oxobutanoic Acid Methyl Ester (***1b***)*

1b was prepared analogously to the method described for **1a.** Yellow oil; 1.71 g (yield 68%); IR (ν):

2970, 1750, 1730, 1150, 1020, 980 cm^{-1}. ^1H NMR(δ): 4.20 (m, 4H, OCH_2CH_3), 3.87 (s, 3H, OCH_3), 3.77 (s, 2H, CH_2CO), 3.32 (d, 2H, PCH_2), 1.48 (t, 6H, OCH_2CH_3). MS (*m/e*, *%*): 252, 192, 164, 124, 109. Anal. Calcd. for $C_9H_{17}O_6P$: C, 42.86; H, 6.80; Found: C, 42.75; H, 6.85.

*4-(Diisopropoxyphosphoryl)-3-oxobutanoic Acid Methyl Ester (***1c***)*

1c was prepared analogously to the method described for **1a**. Column chromatography on silica gel using EtOAc:petroleum ether 4:3 as eluent afforded **1c**. Colorless oil; 1.54 g (yield 55%); IR (ν): 2984, 1752, 1720, 1388, 1377, 1253, 991 cm^{-1}. ^1H NMR (δ): 4.69 (m, 2H, $POCH(CH_3)_2$), 3.70 (s, 3H, OCH_3), 3.66(s, 2H, CH_2CO), 3.16 (d, 2H, PCH_2), 1.29 (d, 12H, $POCH(CH_3)_2$). MS (*m/e*, *%*): 280, 252, 192, 124. Anal. Calcd. for $C_{11}H_{21}O_6P$: C,47.14; H,7.56; Found: C, 47.12; H, 7.60.

*4-(Dibutoxyphosphoryl)-3-oxobutanoic Acid Methyl Ester (***1d***)*

1d was prepared analogously to the method described for **1a.** Column chromatography on silica gel using EtOAc:petroleum ether 3:1 as eluent afforded **1d**. Yellow oil; 1.17 g (yield 38%); IR (ν): 2963, 1753, 1721, 1257, 1023 cm^{-1}. ^1H NMR (δ): 4.07 (m, 4H, OCH_2), 3.27 (d, 2H, PCH_2), 1.67 (m, 4H, $OCH_2CH_2CH_2CH_3$), 1.44 (m, 4H, $OCH_2CH_2CH_2CH_3$), 0.95 (m, 4H, $OCH_2CH_2CH_2CH_3$). MS (*m/e*, *%*): 309, 277, 253, 221, 197, 165, 123. Anal. Calcd. for $C_{13}H_{25}O_6P$: C, 50.64; H, 8.18; Found: C, 50.41; H, 8.15.

*4-(Dimethoxyphosphoryl)-3-oxobutanoic Acid Ethyl Ester (***1e***)*

1e was prepared analogously to the method described for **1a**. Column chromatography on silica gel using EtOAc as eluent afforded **1e**. Yellow oil; 1.67 g (yield 70%); IR (ν): 2962, 1745, 1719, 1260, 1030 cm^{-1}. ^1H NMR (δ): 4.21 (q, 2H, OCH_2CH_3), 3.80 (d, 6H, $POCH_3$), 3.66 (s, 2H, CH_2CO), 3.30 (d, 2H, PCH_2), 1.29 (t, 3H, OCH_2CH_3). MS (*m/e*, *%*): 238, 192, 164, 151, 124, 109, 94, 79. Anal. Calcd. for $C_8H_{15}O_6P$: C, 40.34; H, 15.13; Found: C, 40.39, H, 15.20.

*4-(Diethoxyphosphoryl)-3-oxobutanoic Acid Ethyl Ester (***1f***)*

1f was prepared analogously to the method described for **1a**, Column chromatography on silica gel using EtOAc : petroleum ether 1:1 as eluent afforded **1f**. Yellow oil; 1.46 g (yield 55%); IR (ν): 2960, 1748, 1721, 1260, 1020 cm^{-1}. ^1H NMR (δ): 4.50 (q, 4H, OCH_2CH_3), 3.95 (s, 2H, CH_2CO), 3.65 (d, 2H, PCH_2), 1.60 (m, 9H, OCH_2CH_3). Anal. Calcd. for $C_{10}H_{19}O_6P$: C, 42.86; H, 6.80; Found: C, 42.75; H, 6.85.

*4-(Diisopropoxyphosphoryl)-3-oxobutanoic Acid Ethyl Ester (***1g***)*

1g was prepared analogously to the method described for **1a**, Column chromatography on silica gel using EtOAc:petroleum ether 1:1 as eluent afforded **1g**. Yellow oil; 1.08 g (yield 37%); IR (ν): 2980, 1752, 1718, 1378, 1250, 1100 cm^{-1}. ^1H NMR (δ): 4.69 (m, 2H, $POCH$), 4.20 (m, 2H, OCH_2CH_3), 3.65 (s, 2H, CH_2CO), 3.15 (d, 2H, PCH_2), 1.28 (d,12H, $POCH(CH_3)_2$). MS (*m/e*, *%*): 294, 253, 235, 193, 123, 96. Anal. Calcd. for $C_{12}H_{23}O_6P$: C, 48.97; H, 7.88; Found: C, 48.82; H, 7.57.

*4-(Dibutyloxyphosphoryl)-3-oxobutanoic Acid Ethyl Ester (***1h***)*

1h was prepared analogously to the method described for **1a**, Column chromatography on silica gel using EtOAc:petroleum ether 2:1 as eluent afforded **1h**. Yellow oil; 1.06 g (yield 33%); IR (ν): 2964, 1748, 1721, 1257, 1027 cm^{-1}. ^1H NMR (δ): 4.20 (M, 2H, OCH_2CH_3), 4.10 (m, 4H, $OCH_2CH_2CH_2CH_3$), 3.68 (s, 2H, CH_2CO), 3.27 (d, 2H, PCH_2), 1.67 (m, 4H, $OCH_2CH_2CH_2CH_3$), 1.42 (m, 4H, $OCH_2CH_2CH_2CH_3$), 1.28 (m, 3H, OCH_2CH_3), 0.94 (m, 6H, $OCH_2CH_2CH_2CH_3$). MS (*m/e*, *%*): 323, 277, 267, 221, 165, 123, 97. Anal. Calcd. for $C_{14}H_{27}O_6P$: C, 52.16; H, 8.45; Found: C, 52.03; H, 8.41.

General Procedure for the Bioreduction of **1a–h** *with Baker's Yeast*

To 200 ml of tap water, warmed to 30°C, was added 20 g of dried active baker's yeast and the mixture was then shaken for 30 min. After that, each dialkyl 4-(dialkoxy phosphoryl)-3-oxobutanoate (**1a–h)** (1 mmol) was added and subjected to continuous stirring for about 72 h at 30°C, the progress of the reaction being monitored by TLC. The biomass was removed and extracted with ethyl ether, and with chloroform (20 ml × 3). The combined organic layers were dried over anhydrous $MgSO_4$ and the solvents removed under reduced pressure. The product was separated by column chromatography (hexane/acetone = 1/2).

*4-(Dimethoxyphosphoryl)-3-hydroxybutanoic Acid Methyl Ester (***2a***)*

Colorless oil; 0.134 g (yield 61%); ee 68%; IR (ν): 3350, 2970, 1735, 1380, 1220, 1005 cm^{-1}. ^1H NMR

(δ): 4.40 (1H, OH), 3.80 (d, 6H, PO$\underline{CH_3}$), 2.65 (d, 2H, $\underline{CH_2}$). MS (*m/e*, *%*): 227, 209, 179, 124, 109. Anal. Calcd. for $C_7H_{15}O_6P$: C, 37.20; H, 6.70; Found: C, 37.15; H, 6.36.

*4-(Diethoxyphosphoryl)-3-hydroxybutanoic Acid Methyl Ester (**2b**)*

Colorless oil; 0.177 g (yield 70%); ee 82%; IR (ν): 3365, 2985, 1739, 1440, 1226, 1029 cm^{-1}. ^1H NMR (δ): 4.50 (1H, OH), 4.20 (m, 4H, O$\underline{CH_2}CH_3$), 3.80 (s, 3H, O$\underline{CH_3}$), 2.55 (d, 2H, CH_2), 2.05 (m, 2H, P$\underline{CH_2}$), 1.40 (t, 6H, $OCH_2\underline{CH_3}$). MS (*m/e*, *%*): 255, 238, 199, 149, 97. Anal. Calcd. for $C_9H_{19}O_6P$: C, 42.52; H, 7.48; Found: C, 42.10; H, 7.32.

*4-(Diisopropoxyphosphoryl)-3-hydroxybutanoic Acid Methyl Ester (**2c**)*

Colorless oil; 0.129 g (yield 46%); ee 55%; IR (ν): 3300, 2970, 1732, 1380, 1372, 1220, 1030 cm^{-1}. ^1H NMR (δ): 4.30 (1H, OH), 4.71 (m, 2H, PO$\underline{CH}$), 3.70 (s, 3H, O$\underline{CH_3}$), 2.65 (d, 2H), 2.05 (m, 2H), 1.28 (d, 12H, POCH($\underline{CH_3}$)$_2$). MS (*m/e*, *%*): 283, 266, 199, 181, 149. Anal. Calcd. for $C_{11}H_{23}O_6P$: C,46.81; H, 8.21; Found: C, 46.73; H, 8.30.

*4-(Dibutoxyphosphoryl)-3-hydroxybutanoic Acid Methyl Ester (**2d**)*

Colorless oil; 0.119 (yield 77%), ee 85%. IR (ν): 3361, 2962, 1741, 1465, 1438, 1250, 1026 cm^{-1}. ^1H NMR (δ): 4.50 (1H, OH), 4.10 (m, 4H, OCH_2), 3.69 (s, 3H, OCH_3), 2.60 (d, 2H, CH_2), 2.00 (m, 2H, CH_2P), 1.65 (m, 4H, $OCH_2\underline{CH_2}CH_2CH_3$), 1.45 (m, 4H, $OCH_2CH_2\underline{CH_2}CH_3$), 0.96 (m, 6H, $OCH_2CH_2CH_2\underline{CH_3}$). Anal. Calcd. for $C_{13}H_{27}O_6P$: C, 50.16; H, 8.71; Found: C, 49.98; H, 8.78.

*4-(Dimethoxyphosphoryl)-3-hydroxybutanoic Acid Ethyl Ester (**2e**)*

Colorless oil; 0.122g(yield 50%); ee 51%; ^1H NMR (δ): 4.19 (q, 2H, OCH_2CH_3), 3.83 (d, 6H, $POCH_3$), 2.70 (d, 2H, CH_2),1.25 (m, 3H, OCH_2CH_3). MS (*m/e*, *%*): 241, 224, 205, 81, 149. Anal. Calcd. for $C_8H_{17}O_6P$: C, 40.0; H ,7.08; Found: C, 39.7; H, 7.22.

*4-(Diethoxyphosphoryl)-3-hydroxybutanoic Acid Ethyl Ester (**2f**)*

Colorless oil; 0.174 g (yield 65%); ee 50%; IR (ν): 3369, 2985, 1736, 1229, 1027 cm^{-1}. ^1H NMR (δ): 4.42 (OH), 4.12 (m, 6H, O$\underline{CH_2}CH_3$), 2.60 (d, 2H, $\underline{CH_2}$CO), 2.03 (m, 2H, P$\underline{CH_2}$), 1.33 (m, 9H, $OCH_2\underline{CH_3}$). MS (*m/e*, *%*): 269, 252, 223, 205, 181, 149. Anal. Calcd. for $C_{10}H_{21}O_6P$: C, 44.77; H, 7.90; Found: C, 44.29; H, 8.19.

*4-(Diisopropoxyphosphoryl)-3-hydroxybutanoic Acid Ethyl Ester (**2g**)*

Colorless oil; 0.121 g (yield 41%); ee 32%; IR (ν): 3300, 2980, 1732, 1380, 1372, 1220, 1030 cm^{-1}. ^1H NMR (δ): 4.30 (1H, OH), 4.20 (m, 2H, OCH_2CH_3), 4.71 (m, 2H, PO$\underline{CH}$), 3.70 (s, 3H, O$\underline{CH_3}$), 2.65 (d, 2H), 2.05 (m, 2H), 1.28 (m, 15H, $\underline{OCH_2CH}$ + POCH(CH_3)$_2$). MS (*m/e*, *%*): 297, 280, 255, 237, 193, 123, 96. Anal. Calcd. for $C_{12}H_{23}O_6P$: C, 48.60; H, 8.50; C, 48.44; H, 8.17.

*4-(Dibutyloxyphosphoryl)-3-hydroxybutanoic Acid Ethyl Ester (**2h**)*

Colorless oil; 0.203 g (yield 63%); ee 70%; IR (ν): 3369, 2962, 2985, 1736, 1229, 1027 cm^{-1}.^1H NMR (δ): 4.30 (1H, OH), 4.10 (m, 4H, O$\underline{CH_2}$), 2.60 (d, 2H, $\underline{CH_2}$), 2.10 (m, 2H, P$\underline{CH_2}$), 1.65 (m, 4H, $OCH_2\underline{CH_2}CH_2CH_3$), 1.45 (m, 4H, $OCH_2CH_2\underline{CH_2}CH_3$), 1.28 (m, 3H, $OCH_2\underline{CH_3}$), 0.96 (m, 6H, $OCH_2CH_2CH_2\underline{CH_3}$). MS (*m/e*, *%*): 325, 308, 266, 199, 181, 149. Anal. Calcd. for $C_{14}H_{29}O_6P$: C, 51.79; H, 9.01; Found: C, 51.68; H, 8.75.

REFERENCES

[1] Csuk, R.; Glanzer, B. I. Chem Rev 1991, 91, 49.
[2] Csuk, R.; Glanzer, B. I. In Stereoselective Biocatalysis; Patel, R. N. (Ed.); Marcel Dekker: New York, 2000, p 527.
[3] Servi, S. Synthesis 1990, 8, 1.
[4] Zymanczyk-Duda, E.; Lejczak, B.; Kafarski, P.; Grimaud, J.; Fischer, P. Tetrahedron 1995, 51, 11809.
[5] Kitamura, M.; Tokunaga, M.; Noyori, R. J Am Chem Soc 1995, 117, 2931.
[6] Yuan, C. Y.; Wang, K.; Li, Z. Y. Heteroat Chem 2001, 12, 551–556.
[7] Arbuzov, B. A. Zh Obshch Khim 1950, 20, 1468.
[8] Perkow, W. Naturwissenschaften 1952, 39, 353.
[9] Bodalski, R.; Pietrusiewicz, K. M.; Monkiewicz, J.; Koszuk, J. Tetrahedron Lett 1980, 21, 2287.
[10] Zymanczyk-Duda, E.; Skwarczynski, M.; Lejczak, B; Kafarski, P. Tetrahedron:Asymmetry 1996, 7, 1277.
[11] Skwarczynski, M.; Lejczak, B.; Kafarski, P. Chirality 1999, 11, 109.
[12] Prelog, V. Pure Appl Chem 1964, 9, 179.
[13] Duthaler, R. O. Helv Chim Acta 1983, 66, 1475.