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Regio- and Enantioselective Bioreduction of Ethyl 2,4-Dioxoalkanoates and γ-Keto-α-enamino Esters with Fermenting Baker's Yeast

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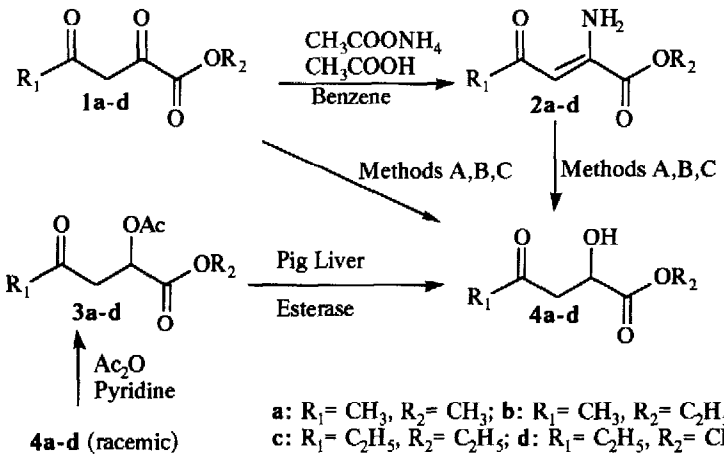
Abstract: *2,4-Dioxoalkanoates **1a-d** and the parent compounds γ-keto-α-enamino esters **2a-d** are regioselectively reduced by baker's yeast to (R)-α-hydroxy-γ-ketoesters **4a-d** in moderate to good chemical yield and appreciable enantioselectivity. Pig liver esterase enantioselective hydrolysis of the acetyl derivatives **3a-d**, easily obtained by treatment of racemic α-hydroxy-γ- keto esters **4a-d**, produced the optically active (S) **4a-d** in good chemical (60-92%) and optical (53-92%) yield.*

Ethyl 2,4-dioxoalkanoates, the ethoxyoxalyl derivatives of methyl ketones, are a class of compounds which have been known for a long time,[1] but their application as synthetic intermediates in organic synthesis still remains underdeveloped, due principally to the difficulty in discriminating the reactivity of the carbonyl functions.

Our previous investigations in this area have demonstrated that ethyl 2,4-dioxoalkanoates react regiospecifically with hydroxylamine hydrochloride or ammonium acetate at the more electrophilic C-2 carbonyl function to form the corresponding 3,5-disubstituted isoxazoles or γ-keto-α-enamino esters respectively.[2] Moreover, 2,4-dioxoalkanoates react chemoselectively with pyrrolidine acetate to form α-enamino-γ-ketoesters, which can be transformed into γ-oxoacrylates through reduction with $NaCNBH_3$ followed by pyrrolidine elimination.[3]

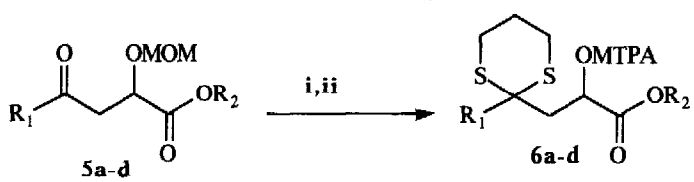
Although baker's yeast (B.Y.) reduction of β-keto esters is one of the most extensively studied microbial transformations for the production of chiral building blocks,[4] investigations concerning B.Y. reduction of the structurally related 2,4-dioxoalkanoates are limited to α-2-dioxocycloalkaneacetates, which are reduced regio- and enatioselectively by fermenting B.Y.[5] As a part of a synthetic program aimed to expand the utility of 2,4-dioxoalkanoates as synthetic intermediates, we here describe the preliminary results of our studies concerning B.Y. reduction of compounds **1a-d** as well as of the parent γ-keto-α-enamino esters **2a-d**, as a new approach to the regio- and enantioselective synthesis of α-hydroxy-γ-keto esters **4a-d**. Reduction of 2,4-dioxoalkanoates **1a-d** with fermenting B.Y. was initially examined in two different culture conditions namely, in the presence of ethanol and methanol in the medium, or without added solvents. While reduction in the presence of ethanol and methanol progressed smoothly to furnish racemic α-hydroxy-γ-keto esters **4a-d** in moderate yields (30-53%), interestingly, in the absence of the added alcohols, the reduction proceeded in low chemical yields (22-42%) but with appreciable enantioselectivity (0-62%).

SCHEME I



Determination of the enantiomeric excess of the α-hydroxy-γ-keto esters **4a-d** was particularly troublesome. ^{1}H NMR experiments in the presence of the chiral shift reagent Eu(hfc)3 were unsatisfactory, and the direct conversion of **4a-d** into α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters by treatment with (+)-MTPA chloride[6] was precluded by the concomitant β-elimination. The procedure required a two-step sequence consisting in: a) protection of the hydroxyl group as the MOM-derivatives **5a-d** in essentially quantitative yield, under standard conditions; b) masking of the carbonyl group by treatment with equimolar amount of 1,3-propanedithiol in the presence of BF_3-etherate (60% yield), with concomitant removal of the MOM protecting group. The sequence gave rise to the corresponding alcohol derivatives, easily converted to the MTPA esters **6a-d** by known methods.[7]

SCHEME II



Moreover, performing the B.Y.reduction of **1a-d** in the presence of allyl alcohol,[8] α-hydroxy-γ-keto esters **4a-d** can be obtained with higher enantiomeric excess in all but one of the substrates examined, together with an uniform lowering of the chemical yields. The *R* configuration of the reduction products, tentatively assigned in analogy with B.Y. enantioselective reductions of α-oxo esters,[9,10] has been previously established for **4a**.[7]

Having demonstrated that B.Y. could regiospecifically attack the more electrophilic α-carbonyl group of ethyl 2,4-dioxoalkanoates, we were interested to investigate its behaviour towards the free γ-carbonyl group of the parent enaminones **2a-d**. Surprisingly, their reduction with fermenting B.Y. still produced good chemical yields (48-82%) of **4a-d**, as racemic compounds in the presence of ethanol or methanol, and with acceptable

enantiomeric excess (50-65%) under the experimental conditions of methods B and C, paralleling the results obtained in the reduction of 2,4-dioxoalkanoates, apart from better chemical yield.

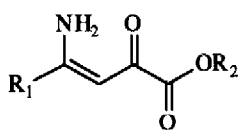
Table. Baker's Yeast Reduction of 2,4-Dioxoalkanoates **1a-d** and γ-Keto-α-enamino Esters **2a-d.**

Starting materials	Products	Yield (%) Method			[α, $CHCl_3$] Method		ee Method	
		A	B	C	B	C	B	C
1a		30	22	0	+ 1.84	-	62	-
2a	**4a**	48	30	4	+ 1.92	+ 1.72	65	59
1b		42	34	20	- 0.58	- 1.86	28	88
2b	**4b**	73	49	19	- 1.04	- 1.5	49	70
1c		53	43	23	- 3.0	- 3.7	45	55
2c	**4c**	82	78	21	0	- 3.23	0	48
1d		46	34	28	- 0.88	- 1.68	47	90
2d	**4d**	71	62	20	- 0.97	- 1.47	52	79

Typical experiment conditions (**Method B**): a suspension of baker's yeast (7g) in 1000 ml of water containing $(NH_4)_2SO_4$ (0.7 g), KH_2PO_4 (0.075 g) and $MgSO_4$ (0.1 g) was stirred at 30 °C for 30 min.* The substrate (7 mmol) and glucose (14 g) were added and stirring was continued at the same temperature. After 24 h, ethyl acetate was added and the mixture filtered through celite. The celite was washed with ethyl acetate and the combined filtrates were extracted with ethyl acetate. The organic portion was washed with water an dried with anhydrous sodium sulfate, concentrated and purified by flash chromatography.

***Method A**: methanol and ethanol were added (3.5 and 2.2 mL respectively). **Method C**: allyl alcohol was added (1 g).

The interpretation of these results is not easy: since both 2,4-dioxoalkanoates or enaminones are reduced with different chemical but practically identical optical yields, it is likely that the multitude of enzymes present in B.Y. may mediate a two step sequence involving hydrolysis of enaminones to the corresponding dicarbonyl compounds, which are subsequently reduced to α-hydroxy-γ-keto esters. We may advance the hypothesis that the presence of the α-enamino-ester moiety seems to be necessary for enzyme recognition, as supported by the fact that B.Y. failed to reduce enaminones such as 7 and 8 , which were recovered unchanged in the conditions above described.



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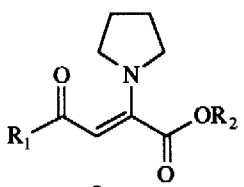

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The flexibility of our approach is further illustrated by the pig liver esterase (EC 3.1.1.1, Fluka) enantioselective hydrolysis of the acetyl derivatives **3a-d**, easily obtained by treatment of racemic α-hydroxy-γ-keto esters **4a-d** with acetic anhydride in pyridine solution in the presence of catalytic amount of DMAP (90% yield), to the optically active *(S)* **4a-d**.[11] Enzymic hydrolysis was performed in 0.1 M phosphate buffer solution (pH 7.25) at 33 °C for 48 h with good chemical (60-92%) and optical (53-92%) yields.[12]

In summary application of enzymatic methodologies to the readily available 2,4-dioxoalkanoates has opened a simple and short route to α-hydroxy-γ-keto esters, which is amenable for scale preparation. The versatility of the proposed method is further enhanced by the possibility of obtaining both enantiomers of the α-hydroxy-γ-keto esters.

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11. ^{1}H NMR of compounds **4a-d** are reported: **4a:** 2.21 (s, 3 H); 2.96 (dd, 2 H, J=4.5 and 5.6 Hz); 3.30 (d, 1 H, J= 5.0 Hz); 3.79 (s, 3 H); 4.5 (m, 1 H). **4b**: 1.29 (t, 3 H, J=7.2 Hz); 2.20 (s, 3 H); 2.95 (dd, 2 J=4.7 and 5.8 Hz); 3.2 (d, 1 H, J=5.4 Hz); 4.26 (q, 2 H, J=7.2 Hz); 4.46 (m, 1 H). **4c:** 1.07 (t, 3 H, J=7.3 Hz); 1.29 (t, 3 H, J=7.2 Hz); 2.45 (q, 2 H, J=7.28 Hz); 2.90 (dd, 2 H, J=4.8 and 5.8 Hz); 3.45 (br, 1 H); 4.22 (q, 2 H, J=7.2 Hz); 4.48 (dd, 1 H, J=4.8 and 5.8 Hz).**4d**: 1.07 (t, 3 H, J=7.2 Hz); 2.49 (q, 2 H, J=7.3 Hz); 2.94 (dd, 2 H, J=4.3 and 5.8 Hz); 3.25 (br, 1 H); 3.81 (s, 3 H); 4.51 (dd, 1H, J=4.3 and 5.8 Hz).
12 Yields, ee and α ($CHCl_3$) of compounds **4a-d** obtained by enzymic hydrolysis (PLE) of acetyl derivatives **3a-d** are reported: **4a**, 67; 55; - 1.63. **4b**, 60; 53; + 1.11. **4c**, 78; 92; + 6.2. **4d**, 92; 81; + 1.32.

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