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Microbial Synthesis of (+)-(3R)-Ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate

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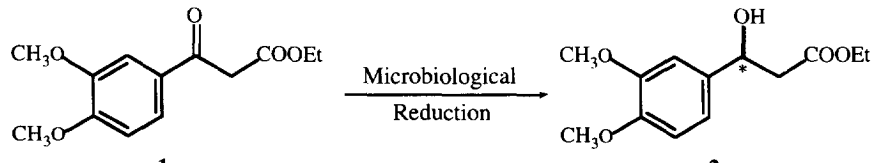
Abstract : From the microbiological reduction of ethyl 3-oxo-3-(3,4-dimethoxyphenyl)-propionate, (+)-(3R)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate was prepared on a quantitative scale. The absolute configuration was assigned by X-ray structural determination of the crystallized camphanate derivative. Copyright © 1996 Elsevier Science Ltd

Chiral β-hydroxyesters are useful chiral building blocks for the synthesis of biologically active compounds[1-5]. The importance of obtaining enantiomerically pure compounds hardly requires restatement since enantiomers may have different biological activities and be responsible for toxic sides effects. Biological methods in particular have been widely developed and used for this purpose. Many papers report the microbial reduction of aliphatic β-hydroxyesters[6, 7], the microorganism the most studied being bakers' yeast[4,5]. It is used as a reducing agent because it is easily available and cheap. According to the conditions of the bioconversion (fermentation or not, ratio of bakers' yeast to substrate...), the product obtained exhibits a wide range of yields and enantiomeric excesses[8].

In connection with natural products synthesis, we were interested in the preparation of optically active ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate **2**. Fewer studies deal with the microbiological reduction of 3-aryl-3-ketoesters[4]. Both enantiomers of **2** have been obtained by the resolution of the racemic mixture with a lipase PS in the presence of a fatty acid vinyl ester, with a chemical yield of 40 % and an enantiomeric excess of about 50 %[9].

We report here the microbiological reduction of ethyl 3-oxo-3-(3,4-dimethoxyphenyl)propionate **1** with several different microorganisms in order to prepare one or both enantiomers of the corresponding hydroxyester.



1 **2**

Results and Discussion

Ethyl 3-oxo-3-(3,4-dimethoxyphenyl)propionate **1** was prepared by reaction of lithium enolate of ethylacetate with 3,4-dimethoxybenzoyl chloride as described[10].

On the basis of our previous results for the microbiological reduction of carbonyl compounds[11, 12], we screened our strains to identify those which would reduce ethyl 3-oxo-3-(3,4-dimethoxyphenyl)propionate **1**. We selected the yeasts (Bakers' yeast and *Rhodotorula glutinis*), the fungi (*Aspergillus niger*, *Geotrichum candidum* and *Mortierella isabellina*) and the bacterium (*Lactobacillus kefir*). Bakers' yeast was used freeze-dried under fermenting and non-fermenting conditions. Bioconversions with the other microorganisms were carried out using washed resting cells. The results are reported in Table 1. The ratio of each compound present in the mixture after the bioconversion has been determined by ^{1}H NMR.

Table 1- Microbiological reduction of ethyl 3-oxo-3-(3,4-dimethoxyphenyl)propionate **1**.

Microorganism	**Reaction time**	**Ratio of each product present in the residue**		
		Ketoester	Hydroxy ester	3,4-dimethoxy acetophenone
Bakers' yeast (non-fermenting conditions[a])	48 h	95	5	0
Bakers' yeast (fermenting conditions[b])	24 h	57	32	11
	48 h	35	40	25
Rhodotorula glutinis	3h	0	0	100
Aspergillus niger	48h	100	0	0
Geotrichum candidum	6h	91	0	6
	24 h	50	0	50
	48 h	0	0	100
Mortierella isabellina	4h30	15	38	47
Lactobacillus kefir	48h	100	0	0

a - 1g bakers' yeast, 50 mL distilled water, 0.2 mL of a 1M ethanolic solution of **1**.

b - 2g bakers' yeast, 2g sucrose, 20 mL distilled water. After incubation during 30 min at 30°C, 0.2 mL of a 1M ethanolic solution of **1** was added.

Only two microorganisms (bakers' yeast and *Mortierella isabellina*) reduced ketoester **1** and gave hydroxyester **2**. The addition of sucrose with bakers' yeast (fermenting conditions) increased the rate of the reaction and allowed 32% of hydroxyester after 24 h to be obtained. It is recognized that the addition of sucrose improves the chemical yield[13]. With other microorganisms, either no reduction was observed or a by-product appeared in major proportion : this product was present in most bioconversions and was identified by NMR as 3,4-dimethoxyacetophenone. Even when the reaction time was very short (for example, 3 h with *Rhodotorula glutinis*), 3, 4-dimethoxyacetophenone was the only product.

This side reaction leading to the decarboxylated product has been already reported[4, 14, 15]. It probably occurs through hydrolysis and decarboxylation of ketoester **1**.

Quantitative assays were carried out with bakers' yeast under fermenting conditions and with *Mortierella isabellina*. Table 2 shows the results obtained.

Table 2 - Microbiological reduction of ethyl 3-oxo-3-(3,4-dimethoxyphenyl)propionate **1**.

	Reaction time	**Hydroxyester 2** $[\alpha]_D^{25}$	ee	Conf.	**Yield**[a]
Bakers' yeast	24 h	+ 24.5	80 %	(R)	30 % (46 %)
M. isabellina	4h30	+ 31	≥ 98 %	(R)	12 %

a - Yield of isolated compound **2** (Yield based on reacting starting material).

Mortierella isabellina gave the (R) enantiomer of hydroxyester **2** with an optical purity of at least 98%, but with a very low chemical yield. The same enantiomer was obtained with bakers' yeast under fermenting conditions. The yield was higher (30%, 46% based on consumed ketoester) and the enantiomeric excess was 80%. The percentage in weight of the residue, obtained after bioconversion and extraction, was relatively low (50%) compared to the initial weight of ketoester. A solution had to be found to avoid this loss of material. In order to extract some more organic compounds, the aqueous layer was dried out to check its content but no product was detected. The products of the reaction were either degraded totally into H_2O and CO_2 or stocked in the cells. After bioconversion, the cells were broken, suspended in ethanol and stirred for 24 h. From this ethanolic suspension, ketoester **1** and hydroxyester **2** were isolated in a ratio 65/35. By this treatment, chemical yield of hydroxyester **2** was increased to 62% based on consumed starting material, but optical purity of hydroxyester **2** thus obtained was lower ($[\alpha]_D^{25}$ = +17.5 (c = 0.61, $CHCl_3$); ee = 57%).

The enantiomeric excess was determined after converting the optically active and the racemic hydroxyester **2** to the corresponding MTPA esters by Mosher's method[16] either from the ^1H and ^{19}F NMR spectra of the diastereomers or by normal phase HPLC.

The assignment of the absolute configuration of (+)-hydroxyester **2** was determined by X-Ray analysis of the camphanate derivative. It was shown that it cristallizes in a monoclinic system with a space group C2 (crystallographic data : a = 23.728(7) Å, b = 6.269(4) Å, c = 15.633(3) Å, β = 95.33(2)°, V = 2315(2) $Å^3$, Z = 4, Reliability factor R = 0.045). The ORTEP stereoview is presented in Figure 1.

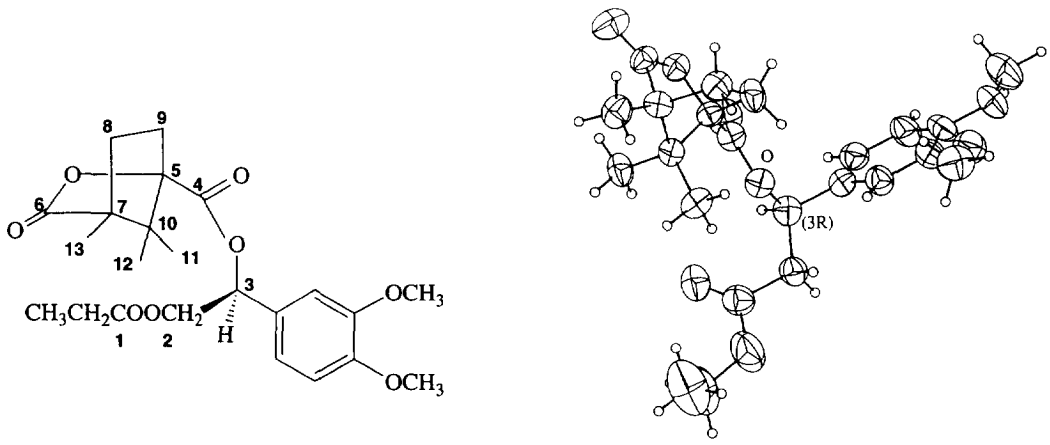


Figure 1. Absolute stereostructure and ORTEP view of the camphanate of (+)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate

This result shows that (+)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate **2** has a (R) absolute configuration.

Although the (R) enantiomer of **2** was obtained enantiomerically pure with *Mortierella isabellina*, the yield was too low (12%) to produce grams of the synthon for further synthesis. With bakers' yeast, the yield was higher but the hydroxyester obtained was not enantiomerically pure. The formation of 3,4-dimethoxyacetophenone as a by-product and the low percentage of conversion were the main reasons for this low yield.

In order to improve yield and enantioselectivity, we then explored the effect of reaction conditions. Various modifications of the bioconversion conditions have been reported with bakers' yeast such as immobilization[17] or addition of a third compound[18].

An assay was carried out with bakers' yeast immobilized on sodium alginate beads[19]. A slowing down of the reaction was observed. After 48 h, the mixture contained 72% of unreacted starting material **1**, 16% of hydroxyester **2** and 12% of 3,4-dimethoxyacetophenone. Hydroxyester **2** was isolated with an enantiomeric excess of 64% ($[\alpha]_D^{25}$ = +19.6 (c = 1.08, $CHCl_3$)).

Nakamura[20-22] has shown that the addition of specific inhibitors allows to control the stereochemical course of the reaction by inhibiting enzyme that gives one stereoisomer specifically while preserving the activity of the other one. It is then possible to inhibit specifically the D or the L enzyme. The use of these inhibitors for the reduction of aryl-β-ketoesters has not been studied. We decided to check the activity of several additives : ethyl chloroacetate, which is known to inhibit the D-enzyme and allyl alcohol, methylvinylketone and cyclohex-2-enone, which inhibit the L-enzyme.

As these different assays require a large quantity of substrate, our study was first carried out with the commercially available ethyl benzoylacetate, which was considered as a model.

The reduction of ethyl benzoylacetate has been studied either by microbiological (usually with bakers' yeast) or by chemical methods. These latter, using catalytic hydrogenation in the presence of BINAP[23] or chiral diphosphine Ru(II) complexes[24], give hydroxyester with an excellent enantiomeric excess. The results obtained with bakers'yeast show usually moderate yield (around 50%) and enantiomeric excesses reported are different according to the bakers' yeast and the conditions used (from 85 to 93%)[2, 4, 25].

Only freeze-dried bakers' yeast was used under fermenting and non-fermenting conditions to carry out quantitative assays with ethyl benzoylacetate. The yields indicated in Table 3 correspond to those obtained after purification.

Table 3 - Microbiological reduction of ethyl benzoylacetate by bakers' yeast

	Reaction	**Hydroxyester**			**Yield**
	time	$[\alpha]_D^{25}$	ee	Conf.	
Bakers' yeast (non-fermenting cond.)	24 h	- 26	53%	(S)	37%
Bakers' yeast (fermenting cond.)	24 h	- 35	71%	(S)	75%

As in the previous case, the best results were obtained with bakers' yeast under fermenting conditions. The yields were much higher than those obtained with ketoester **1**, because the reaction goes

quicker. After 24 h and under fermenting conditions, ethyl benzoylacetate was completely consumed. The addition of sucrose seemed to inhibit the decarboxylating pathway yielding to acetophenone (7% is detected in comparison with 43% under non-fermenting conditions). The hydroxyester exhibited an enantiomeric excess of only 71%.

The absolute configuration was determined by comparison of the sign of specific rotation of the alcohol with that given in the literature[25]. The enantiomeric excess was established by ^1H NMR analysis of the Mosher's derivative. The results are in agreement with the values given in the literature[25] ($[\alpha]_D^{25}$ = - 51 (c = 1.5, CH_3Cl); ee ≥ 98%).

Nakamura[21] has shown that the concentration of the additive can affect the stereoselectivity. So, assays were carried out with different concentrations of inhibitors and with or without presence of sucrose. The bioconversion reaction was stopped after 24 h. The ratio of each compound found in the mixture was measured by GC on a Carbowax column. The results are summarized in Tables 4, 5, 6.

Addition of ethyl chloroacetate

The addition of ethyl chloroacetate (Table 4) decreased the rate of the reduction but the formation of acetophenone seemed to be avoided too, in particular under non-fermenting conditions (entry 2). If the quantity of inhibitor was too important (entry 3), the reaction stopped and the ketoester was not reduced at all.

Table 4 - Microbiological reduction of ethyl benzoylacetate in the presence of ethyl chloroacetate

Entry		Ethyl chloroacetate (mmol / g bakers' yeast)	Ratio			Hydroxyester			Yield
			Ketoester	Hydroxy ester	Aceto phenone	$[\alpha]_D^{25}$	ee	Conf.	
1	n.f.	0	0	56	43	- 26	53%	D (S)	37%
2	n.f.	0.2	12	72	16	- 19	38%	D (S)	57%
3	n.f.	1	100	0	0	-	-	-	-
4	f.	0	0	93	7	- 35	71%	D (S)	75%
5	f.	0.1	37	60	3	- 16	32%	D (S)	35%
6	f.	0.1[a]	37	49	14	- 3	6%	D (S)	22%
7	f.	0.2	44	49	7	- 7	14%	D (S)	33%

n.f. : non-fermenting conditions; f. : fermenting conditions.

a - Quantity of sucrose added was divided by two (i.e. 1g of sucrose for 20 mL distilled water).

The presence of ethyl chloroacetate induced a decrease of the enantiomeric excess. Its action was not strong enough to invert completely the absolute configuration of the hydroxyester. Ethyl chloroacetate is known to inhibit the D-enzyme allowing the expression of the L-enzyme[20], which is in agreement with the results obtained (entries 6 and 7).

Other assays were necessary with inhibitors of the L-enzyme to improve the optical purity of ethyl 3-hydroxy-3-phenylpropionate.

Addition of methylvinylketone, allyl alcohol and cyclohex-2-enone

With methylvinylketone, the reaction slowed down in such an extent that the percentage of hydroxyester formed was always very low (between 0 and 15%), even with a low concentration of the inhibitor.

The two following tables give some of the results of the reduction in the presence of allyl alcohol and cyclohex-2-enone.

Table 5 - Microbiological reduction of ethyl benzoylacetate in the presence of allyl alcohol

Entry		Allyl alcohol (mmol / g bakers' yeast)	Ratio Ketoester	Hydroxy ester	Aceto phenone	**Hydroxyester** $[\alpha]_D^{25}$	ee	Conf.	Yield
1	n.f.	0	0	56	43	- 26	53%	D (S)	37%
2	n.f.	0.2	22	51	27	- 40	80%	D (S)	25%
3	n.f.	0.5	24	41	35	- 38	76%	D (S)	23%
4	f.	0	0	93	7	- 35	71%	D (S)	75%
5	f.	0.1	15	85	0	- 46	92%	D (S)	37%
6	f.	0.5	27	73	0	- 50	≥ 98%	D (S)	38%
7	f.	0.5[a]	38	20	42	- 50	≥ 98%	D (S)	19%

n.f. : non-fermenting conditions; f. : fermenting conditions

a - Quantity of sucrose added was divided by two (i.e. 1g of sucrose for 20 mL distilled water).

Table 6 - Microbiological reduction of ethyl benzoylacetate in the presence of cyclohex-2-enone

Entry		Cyclohex-2-enone (mmol / g bakers' yeast)	Ratio Ketoester	Hydroxy ester	Aceto phenone	**Hydroxyester** $[\alpha]_D^{25}$	ee	Conf.	Yield
1	n.f.	0	0	56	43	- 26	53%	D (S)	37%
2	n.f.	0.2	0	83	16	- 43	86%	D (S)	73%
3	n.f.	0.5	8	79	13	- 43	86%	D (S)	59%
4	f.	0	0	93	7	- 35	71%	D (S)	75%
5	f.	0.1	0	92	8	- 45	90%	D (S)	71%
6	f.	0.5	0	83	17	- 50	≥ 98%	D (S)	65%
7	f.	0.5[a]	0	80	20	- 50	≥ 98%	D (S)	59%

n.f. : non-fermenting conditions; f. : fermenting conditions

a - Quantity of sucrose added was divided by two (i.e. 1g of sucrose for 20 mL distilled water).

Results showed that with allyl alcohol and cyclohex-2-enone, the enantiomeric excess of β-hydroxyester was increased. Both compounds inhibited the L-enzyme. The addition of allyl alcohol retarded

the reaction and gave lower yield. With cyclohex-2-enone, the whole ketoester was reduced. The yields were relatively constant between the experiments. It was surprising to note that the decarboxylation reaction under non-fermenting conditions (Table 6 : entries 1 and 2) was inhibited by cyclohex-2-enone, whereas the mixture cyclohex-2-enone-sucrose enhanced the formation of acetophenone (Table 6 : entries 4 and 6). The role of the different enzymes and the way the inhibition occurs are particularly difficult to control.

Our results show that the best conditions to obtain enantiomerically pure ethyl 3-hydroxy-3-phenylpropionate have been found (Table 6, entry 6).

As the inhibitors of Nakamura have improved the enantiomeric excess of the model molecule (ethyl 3-hydroxy-3-phenylpropionate), the best conditions with each inhibitor were tried with ketoester **1**. With these concentrations, we found an important slowing down of the reduction of **1**. So other assays were carried out with lower quantities of inhibitors under fermenting conditions during 24 h.

Table 7 - Effects of the inhibitors of Nakamura on the reduction of ethyl 3-oxo-3-(3,4-dimethoxyphenyl)propionate **1**

	Ratio			**Hydroxyester 2**			Yield
	Keto ester	Hydroxy ester	Aceto phenone	$[\alpha]_D^{25}$	e.e.	Conf.	
No additive	57	32	11	+ 24.5	80%	L (R)	30%
Ethyl chloroacetate (0.2 mmol / g bakers' yeast)	88	12	0	+ 20	61%	L (R)	8%
Allyl alcohol (0.15 mmol / g bakers' yeast)	72	17	11	+ 17	51%	L (R)	9%
Cyclohexenone (0.15 mmol / g bakers' yeast)	100	0	0	-	-	-	0%

Contrary to the results obtained with ethyl benzoylacetate, the addition of an inhibitor had not a great effect on the enantiomeric excess of the β-hydroxyester. The reduction was very slow, so the formation of hydroxyester was low. We can notice that the two inhibitors, ethyl chloroacetate and allyl alcohol, lead to hydroxyester **2** with a lower enantiomeric excess. The use of these inhibitors can not be extended to whatever substrate.

The best method to obtain great quantity of (+)-(3R)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)-propionate is the first described : bakers' yeast under fermenting conditions and rupture of the cells after the reaction. This procedure was applied on a quantitative scale. Hydroxyester **2** (7g) will be used as starting material for further syntheses of natural products.

Acknowledgement : We gratefully acknowledge Martine Sancelme for growing the microorganisms, Alain Carpy for the crystal structure of the derivative of (+)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate

and Tibor Liptaj for the ^{19}F NMR spectra of the Mosher's esters.

EXPERIMENTAL SECTION

1 - GENERAL METHODS

CHROMATOGRAPHY : Gas chromatography (GC) was carried out using an instrument fitted with a flame ionisation detector and a 30 m x 0.32 mm capillary column coated with Carbowax 20 M. The carrier gas was hydrogen (65 KPa). Oven temperature was 150°C. Reaction progress was sometimes monitored using thin layer chromatography (TLC) with Kieselgel 60 PF plates using the same eluents as for column chromatography. Plates were developed directly using UV light and iodine, a pulverised vanilin solution or a solution of *p*-anisaldehyde. With the latter two, the plates were passed in an oven at 140°C. Column chromatography was performed on a silicagel 60 Merck (70-230 mm). Eluents varied and are indicated for each product. HPLC experiments for enantiomeric excess determination were performed using a Waters 600E liquid chromatograph fitted with a Nucleosil 5µm column (25 cm x 6.2 mm) at room temperature and were monitored at 254 nm.

SPECTROSCOPY AND ANALYTICAL METHODS : After bioconversions, crude mixtures were analysed by ^{1}H NMR or TLC for ethyl 3-oxo-3-(3,4-dimethoxyphenyl)propionate, and by GC or TLC for ethyl benzoylacetate. Retention times of the reduction products were compared with those of chemically obtained racemates. NMR analyses were carried out on purified compounds in $CDCl_3$. For ^{1}H (400.13 MHz) and ^{13}C (100.61 MHz) NMR spectra, the chemical shifts were relative to chloroform. For ^{19}F (376.48 MHz), they were relative to $CFCl_3$. Microanalyses were performed by the Service Central d'Analyses du CNRS, Vernaison (France).

MICROBIOLOGICAL METHODS : The microorganisms were all laboratory-grown with the exception of freeze-dried bakers' yeast. This was a commercial product (ANCEL S.A. Strasbourg). Preculture and culture conditions for fungi *Aspergillus niger* ATCC 9142, *Mortierella isabellina* NRRL 1757, *Geotrichum candidum* CBS 144-88, for bacterium *Lactobacillus kefir* DSM 20587 and for yeast *Rhodotorula glutinis* NRRL Y 1091 have been described elsewhere[11, 12].

BIOCONVERSION CONDITIONS :

• General case: Bioconversions with microorganisms in metabolic resting phase were carried out as previously described[12].

• Commercial freeze-dried bakers' yeast under non-fermenting conditions : 1 g of freeze-dried bakers' yeast was placed in a 500 mL conical flask containing 50 mL of distilled water and 50 µL of ethyl benzoylacetate or 0.2 mL of an ethanolic solution of ethyl 3-(3,4-dimethoxyphenyl)-3-oxopropionate (50 mg/0.2 mL). After incubation at 27°C on a rotating table set at 200 rpm, the mixture was spun for ten min at 8000 rpm. The liquor was then continuously extracted with ether for 24 h. The ether phase was dried on $MgSO_4$ and the solvent evaporated off under vacuum.

• Commercial freeze-dried bakers' yeast under fermenting conditions : 2 g of freeze-dried bakers' yeast was placed in a 500 mL conical flask containing 20 mL of distilled water and 2g of sucrose. After fermentation at 30°C for 30 min, 50 µL of ethyl benzoylacetate or 0.2 mL of an ethanolic solution of ethyl 3-oxo-3-(3,4-dimethoxyphenyl)propionate (50 mg/0.2 mL) was added. After incubation at 30°C, the work-up was the same as in the previous case.

• Bakers' yeast under fermenting conditions in the presence of an additive : The procedure was the

same as that described in the previous case. The additive was added simultaneously to the substrate.

2 - SYNTHESIS OF ETHYL 3-OXO-3-(3,4-DIMETHOXYPHENYL)PROPIONATE 1

To a solution of N-isopropylcyclohexylamine (11.3 g; 13.2 mL; 80.0 mmol) in THF (80 mL) was added a solution of *n*-butyllithium (53.3 mL; 80 mmol) in hexane while stirring and cooling at -78°C. The mixture was stirred at -78°C for 30 min. Ethyl acetate (3.52 g; 40.0 mmol) was added dropwise over a period of 5 min followed after 15 min, by a solution of 3,4-dimethoxybenzoyl chloride (8.0 g; 40.0 mmol) in THF (40 mL). The reaction mixture was stirred for 15 min and then quenched with 20 % hydrochloric acid (24 mL). The solution was allowed to reach room temperature. The aqueous layer was extracted with ether. The combined extracts were washed with a saturated sodium bicarbonate solution. The organic phase was dried on $MgSO_4$. After evaporation, the residue was purified by flash chromatography (ethyl acetate/hexane 70/30) to give a solid. Yield: 96%. mp = 42-43 °C. TLC : R_f (ethyl acetate/hexane : 50/50) : 0.48. IR (CCl_4) cm^{-1}: 1680; 1750. ^1H NMR, δ : 1.23 (t, 3H, J = 7.1 Hz, $CH_2\underline{CH_3}$); 3,90 (s, 2H, $\underline{CH_2}COOEt$); 3.93 (s, 3H, OCH_3); 3.94 (s, 3H, OCH_3); 4.18 (q, 2H, J = 7.1 Hz, $\underline{CH_2}CH_3$); 6.87 (d, 1H, J = 8.3 Hz, Ar$\underline{H}$); 7.48-7.54 (m, 2H, Ar$\underline{H}$). ^{13}C NMR, δ : 14.0 ($CH_2\underline{CH_3}$); 45.6 ($\underline{CH_2}COOEt$); 55.9 ($O\underline{C}H_3$); 56.0 ($O\underline{C}H_3$); 61.3 ($\underline{CH_2}CH_3$); 110.0, 110.3, 123.5, 129.2, 149.1 and 153.8 (Ar$\underline{C}$); 167.7 ($\underline{C}OOEt$); 191.0 ($\underline{C}O$). Anal. Calcd for $C_{13}H_{16}O_5$: C: 61.90; H: 6.39. Found : C: 62.14; H: 6.47.

3 - SYNTHESIS OF (±)-ETHYL 3-HYDROXY-3-(3,4-DIMETHOXYPHENYL)PROPIONATE 2

The racemic hydroxyester **2** was prepared from veratraldehyde and ethyl acetate according to the procedure described for the synthesis of **1**. TLC : R_f (ethyl acetate/hexane : 50/50) : 0.36. IR (CCl_4) cm^{-1} : 3610; 3530; 1720. ^1H NMR, δ : 1.26 (t, 3H, J = 7.1 Hz, $CH_2\underline{CH_3}$); 2.67 (dd, 1H, J = 3.8 Hz, J = 16.2 Hz, $\underline{CH_2}COOEt$); 2.75 (dd, 1H, J= 9.2 Hz, J = 16.2 Hz, $\underline{CH_2}COOEt$); 3.30 (d, 1H, J = 3.4 Hz, OH); 3.86 (s, 3H, OCH_3); 3.88 (s, 3H, OCH_3); 4.18 (q, 2H, J = 7.1 Hz, $\underline{CH_2}CH_3$); 5.07 (td, 1H, J = 3.4 Hz, J = 9.2 Hz, $\underline{C}HOH$); 6.85-6.95 (m, 3H, Ar$\underline{H}$). ^{13}C NMR, δ : 14.1 ($CH_2\underline{CH_3}$); 43.6 ($\underline{CH_2}COOEt$); 55.7 ($O\underline{C}H_3$); 55.8 ($O\underline{C}H_3$); 60.6 ($\underline{CH_2}CH_3$); 70.1 ($\underline{C}HOH$); 109.0, 111.2, 117.9, 135.7, 148.5 and 149.0 (Ar$\underline{C}$); 172.0 ($\underline{C}OOEt$). Anal. Calcd for $C_{13}H_{18}O_5$: C: 61.40; H: 7.14. Found : C: 61.24; H: 7.11.

4 - MICROBIOLOGICAL REDUCTION OF ETHYL 3-OXO-3-(3,4-DIMETHOXYPHENYL)PROPIONATE

Incubation times varied and are indicated for each microorganism. The bioconversion conditions are described in General Methods. The residual products were separated on a silicagel column with ethyl acetate/hexane 70/30 as eluent.

Bakers' yeast : Incubation time : 24h. The residue from twenty flasks consisted of 57% of ethyl 3-oxo-3-(3,4-dimethoxyphenyl)propionate, 32% of (+)-(3R)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate and 11% of 3,4-dimethoxyacetophenone. Yield : 30 % (46% based on consumed starting material).

(+)-(3R)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate (0.300 g). TLC : R_f (Ethyl acetate/Hexane : 1/1) : 0.36. IR (CCl_4) cm^{-1} : 3618; 3532; 1720. ^1H NMR, δ : 1.23 (t, 3H, J = 7.1 Hz, $CH_2\underline{CH_3}$); 2.64 (dd, 1H, J = 16.1 Hz, J = 3.8 Hz, $\underline{CH_2}COOEt$); 2.73 (dd, 1H, J = 16.1 Hz, J = 9.2 Hz, $\underline{CH_2}COOEt$); 3.45 (s broad, 1H, OH); 3.88 (s, 3H, OCH_3); 3.90 (s, 3H, OCH_3); 4.19 (q, 2H, J = 7.1 Hz, $\underline{CH_2}CH_3$); 5.04 (dd, 1H, J = 3.8 Hz, J = 9.2 Hz, $\underline{C}HOH$); 6.77-6.93 (m, 3H, Ar$\underline{H}$). ^{13}C NMR, δ : 14.1 ($CH_2\underline{CH_3}$); 43.4 ($\underline{CH_2}COOEt$); 55.7 ($O\underline{C}H_3$); 55.8 ($O\underline{C}H_3$); 60.7 ($\underline{CH_2}CH_3$); 70.1 ($\underline{C}HOH$); 108.8, 110.9, 117.8, 135.3, 148.4 and 148.9 (Ar$\underline{C}$); 172.3

(COOEt). $[\alpha]_D^{25}$ = +24.5 (c = 0.7, $CHCl_3$); ee = 80 %. Anal. Calcd for $C_{13}H_{18}O_5$: C: 61.40; H: 7.14. Found : C: 60.89; H: 7.22.

Treatment of cells : The cells were broken, suspended in ethanol and stirred for 24 h. The ethanolic suspension was filtered, concentrated. After purification, 65% of ethyl 3-oxo-3-(3,4-dimethoxyphenyl)propionate and 35% of (+)-(3R)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate were isolated.

(+)-(3R)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate (0.049 g). $[\alpha]_D^{25}$ = +17.5 (c = 0.61, $CHCl_3$); ee = 57%.

The overall yield after treatment was 35% (62% based on consumed starting material).

Mortierella isabellina : Incubation time : 4h30. The residue from twenty flasks consisted of 15% of ethyl 3-oxo-3-(3,4-dimethoxyphenyl)propionate, 38% (+)-(3R)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate and 47% of 3,4-dimethoxyacetophenone. Yield : 12 %.

(+)-(3R)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate (0.120 g). $[\alpha]_D^{25}$ = +31 (c = 3.75, $CHCl_3$); ee ≥ 98%.

5 - DETERMINATION OF THE ENANTIOMERIC EXCESS OF ETHYL 3-HYDROXY-3-(3,4-DIMETHOXYPHENYL)PROPIONATE 1

Mosher's ester of (±)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate

The enantiomeric excess is determined by HPLC analysis or ^1H NMR or ^{19}F NMR after converting an aliquot of the product to the α-methoxy-α-trifluoromethylphenyl acetate (MPTA ester). To a solution of (±)-β-hydroxyester (0.100 g; 0.394 mmol) in dichloromethane (1.5 mL) were added (-)-(R)-MPTACl (0.139 g; 0.104 mL; 0.552 mmol) and pyridine (0.29 mL). The mixture was stirred at 20°C for 20 h. To this were added ether (5.0 mL) and water (2.0 mL) and the mixture was vigourously stirred for 15 min. The aqueous layer was extracted with two 5.0 ml portions of ether and the combined organic layers were successively washed with 1 N hydrochloric acid (8.0 mL), sodium hydroxyde (8.0 mL), water (8.0 mL) and brine (8.0 mL). Drying over $MgSO_4$, evaporation of the solvent under reduced pressure and purification by flash chromatography afforded MPTA ester. Yield : 87 %. TLC : R_f (ethyl acetate/hexane : 70/30) : 0.61. ^1H NMR, δ : 1.19 (t, 1.5 H, J = 7.2 Hz, CH_2CH_3, (S,S)-diastereomer); 1.24 (t, 1.5 H, J = 7.2 Hz, CH_2CH_3, (S,R)-diastereomer); 2.75 (dd, 0.5 H, J = 16.4 Hz, J = 3.9 Hz, CH_2COOEt, (S,R)-diastereomer); 2.76 (dd, 0.5 H, J = 16.3 Hz, J = 4.4 Hz, CH_2COOEt, (S,S)-diastereomer); 3.04 (dd, 1 H, J = 10.0 Hz, J = 16.4 Hz, CH_2COOEt, (S,R) and (S,S)-diastereomers); 3.42 (s, 1.5 H, OCH_3, (S,S)-diastereomer); 3.54 (s, 1.5 H, OCH_3, (S,R)-diastereomer); 3.70 (s, 1.5 H, OCH_3, (S,R)-diastereomer); 3.84 (s, 1.5 H, OCH_3, (S,S)-diastereomer); 3.88 (s, 1.5 H, OCH_3, (S,R)-diastereomer); 3.90 (s, 1.5 H, OCH_3, (S,S)-diastereomer); 4.07 (q, 1 H, J = 7.1 Hz, CH_2CH_3, (S,S)-diastereomer); 4.16 (q, 1 H, J = 7.1 Hz, CH_2CH_3, (S,R)-diastereomer); 6.32 (dd, 0.5 H, J = 3.9 Hz, J = 10.1 Hz, ArCHOCOO, (S,R)-diastereomer); 6.40 (dd, 0.5 H, J = 10.0 Hz, J = 4.4 Hz, ArCHOCOO, (S,S)-diastereomer); 6.65-7.45 (m, 8H, ArH, (S,R)- and (S,S)-diastereomers). ^{13}C NMR, δ : 14.1 (CH_2CH_3, (S,R)-diastereomer); 14.2 (CH_2CH_3, (S,S)-diastereomer); 40.8 (CH_2COOEt); 41.1 (CH_2COOEt, (S,R)-diastereomer); 55.4 (OCH_3); 55.7 (OCH_3, (S,R)-diastereomer); 55.9 (OCH_3, (S,R)-diastereomer); 56.0 (OCH_3); 61.0 (CH_2CH_3, (S,R)-diastereomer); 61.1 (CH_2CH_3); 74.5 (ArCHOCO); 74.7 (ArCHOCO, (S,R)-diastereomer); 109.4, 110.8 and 119.7 (ArCH, (S,R)-diastereomer); 110.0, 111.0, 119.8

(ArCH, (S,S)-diastereomer); 121.7 (CF_3, (S,S)-diastereomer); 121.8 (CF_3, (S,R)-diastereomer); 124.7 (CCF_3, (S,R)-diastereomer); 124.8 (CCF_3, (S,S)-diastereomer); 127.3, 128.2 and 129.5 (ArCH, (S,R)-diastereomer); 127.5, 128.3 and 129.6 (ArCH, (S,S)-diastereomer); 130.2, 132.2, 149.0 and 149.3 (ArC, (S,R)-diastereomer); 130.3, 132.2, 149.1 and 149.5 (ArC, (S,S)-diastereomer); 165.3 and 169.1 (C=O, (S,R)-diastereomer); 165.5, 169.3 (C=O, (S,S)-diastereomer). ^{19}F NMR, δ : -12.79 (s, CF_3, (S,R)-diastereomer); -12.72 (s, CF_3, (S,S)-diastereomer). Diastereomeric mixture was analyzed by HPLC (Nucleosil column, hexane/ethyl acetate : 90/10, 0.7 mL/min) : t_R = 32 min (S,R)-diastereomer, t_R = 35 min (S,S)-diastereomer.

Mosher's ester of (+)-(3R)-ethyl 3-hydroxy-3-(3,4-dimethoxyphenyl)propionate

A sample of (+)-**2** isolated from bakers' yeast reduction of **1** was esterified with (-)-(R)-MPTACl as described previously.

HPLC analysis, ^1H NMR and ^{19}F NMR spectra showed signals for (S,R)- and (S,S)-diastereomers in a ratio of 90/10 respectively, indicating 80% ee. $[\alpha]_D^{25}$ = +6.5 (c = 0.645, $CHCl_3$).

^1H NMR of the Mosher's ester from **2** prepared by reduction of **1** with *M. isabellina* showed only signals attributed to the (S,R)-diastereomer, thus indicating an optical purity of at least 98%.

6 - DETERMINATION OF THE ABSOLUTE CONFIGURATION OF ETHYL 3-HYDROXY-3-(3,4-DIMETHOXYPHENYL)PROPIONATE

A mixture of (+)-β-hydroxyester **2** (0.20 g; 0.79 mmol) and (-)-(1S)-camphanic acid chloride (0.600 g; 2.77 mmol) in pyridine (6.0 mL) was stirred for 24 h at room temperature. Ether was added and the organic phase was washed with a solution of saturated sodium bicarbonate, 1N hydrochloric acid and brine. Evaporation of the solvent gave a syrup which was taken up in water/ethanol (70/30) to give a solid which was purified by recristallisation. Yield : 35 %. mp 102-103 °C (water/etanol 70/30). $[\alpha]_D^{25}$ = + 30.2 (c = 0.195, $CHCl_3$). IR (CCl_4) cm^{-1} : 1798, 1741. ^1H NMR, δ : 0.80 (s, 3H, CH_3 camph); 1.02 (s, 3H, CH_3 camph); 1.10 (s, 3H, CH_3 camph); 1.25 (t, 3H, J = 7.1 Hz, CH_2CH_3); 1.62-1.72 (m, 1H camph); 1.86-2.05 (m, 2H camph); 2.36-2.47 (m, 1H camph); 2.80 (dd, 1H, J = 16.2 Hz, J = 4.7 Hz, CH_2COOEt); 3.07 (dd, 1H, J = 16.2 Hz, J = 9.5 Hz, CH_2COOEt); 3.85 (s, 3H, OCH_3); 3.90 (s, 3H, OCH_3); 4.15 (q, 2H, J = 7.1 Hz, CH_2CH_3); 6.29 (dd, 1H, J = 4.7 Hz, J = 9.5 Hz, ArCH); 6.82-6.95 (m, 3H, ArH). ^{13}C NMR, δ : 9.7 (CH_3 camph); 14.2 (CH_2CH_3); 16.6 (CH_3 camph); 16.7 (CH_3, camph); 28.9 (CH_2 camph); 30.7 (CH_2 camph); 41.1 (CH_2COOEt); 54.4 (C camph); 54.9 (C camph); 56.0 (OCH_3); 61.0 (CH_2CH_3); 73.3 (ArCH); 91.0 (C camph); 109.7, 111.0, 119.3, 131.0, 149.0 and 149.3 (ArC); 166.5, 169.7 and 178.3 (C=O).

7 - MICROBIOLOGICAL REDUCTION OF ETHYL BENZOYLACETATE:

Incubation times varied and are indicated for each microorganism. The residual products were separated on a silicagel column with pentane/ether 80/20 as eluent.

a - Microbial reduction of ethyl benzoylacetate

Bakers' yeast : • Non-fermenting conditions : Incubation time : 24h. The residue from 6 flasks consisted of : 56% of (-)-(3S)-ethyl 3-hydroxy-3-phenylpropionate and 43% of acetophenone. Yield : 37 %.

(-)-(3S)-ethyl 3-hydroxy-3-phenylpropionate (0.110 g). Same ^1H NMR spectrum as that described in the literature[25]. $[\alpha]_D^{25}$ = -26 (c = 0.034, $CHCl_3$); ee = 53 %.

• Fermenting conditions : Incubation time : 24h. The residue from 6 flasks consisted of : 93% of (-)-(3S)-ethyl 3-hydroxy-3-phenylpropionate and 7% of acetophenone. Yield : 75 %.

(-)-(3S)-ethyl 3-hydroxy-3-phenylpropionate (0.225 g). $[\alpha]_D^{25}$ = -35 (c = 0.052, $CHCl_3$); ee = 71 %.

b - Determination of the enantiomeric excess of ethyl 3-hydroxy-3-phenylpropionate

The Mosher's ester was synthetized according to the method described previously. The enantiomeric excesses were determined from the integrals of the following peaks : ^{1}H NMR δ = 3.44 (s, OCH_3, (S,S)-diastereomer); 3.54 (s, OCH_3, (S,R)-diastereomer); 6.38 (dd, J = 10.1 Hz, J = 3.9 Hz, ArC<u>H</u>OCOO, (S,R)-diastereomer); 6.45 (dd, J = 9.7 Hz, J = 4.5 Hz, ArC<u>H</u>OCOO, (S,S)-diastereomer).

c - Use of Nakamura's inhibitors

The results are reported in Tables 4, 5 and 6. Assays were carried out from 3 flasks with a 24 h incubation time.

REFERENCES

1) - Mori, K. and Ikunaka, M., *Tetrahedron*, **1984,** *40*, 3471.

2) - Kumar, A., Ner, D.H. and Dike, S., *Indian J. Chem.*, **1992**, 803.

3) - Seebach, D., Sutter, M.A., Weber, R.H. and Züger, M.F., *Org. Synth.*, **1985**, *63*, 1.

4) - Csuk, R.C. and Glänzer, B.I., *Chem. Rev.*, **1991**, *91*, 49.

5) - Servi, S., *Synthesis*, **1990**, *1*, 1.

6) - Buisson, D., Azerad, R., Sanner, C. and Larchevêque, M., *Biocatalysis*, **1992**, *5*, 249.

7) - Azerad, R. and Buisson, D. in *Microbial Reagents in Organic Synthesis*; Servi, S. Ed. NATO ASI Series; Kluwer Acad. Press; Netherlands, **1992**, pp 421-440.

8) - Manzocchi, A., Casati, R., Fiecchi, A. and Santaniello, E., *J. Chem. Soc. Perkin Trans I,* **1987**, 2753.

9) - Miyazawa, K. and Yoshida, N., Eur. Pat. Appl., **1991**, EP 451,668 A2; *CA*, **1991**, *116*, 57578n.

10) - Rathke, M.W. and Deitch, J., *Tetrahedron Lett.*, **1971**, 2953.

11) - Belan, A., Bolte, J., Fauve, A., Gourcy, J.G. and Veschambre, H., *J. Org. Chem.*, **1987**, *52*, 256.

12) - Besse, P., Renard, M.F. and Veschambre, H., *Tetrahedron : Asymmetry*, **1994**, *5*, 1249.

13) - Besse, P., Bolte, J., Fauve, A. and Veschambre, H., *Bioorganic Chem.*, **1993**, *21*, 342.

14) - Buisson, D., Azerad, R., Sanner, C. and Larchevêque, M., *Tetrahedron : Asymmetry,* **1991**, *2*, 987.

15) - Cabon, O., Larchevêque, M., Buisson, D. and Azerad, R., *Tetrahedron Lett.*, **1992**, *33*, 7337.

16) - Dale, J.A., Dull, D.L. and Mosher, H.S., *J. Org. Chem.*, **1969,** *34*, 2543.

17) - Nakamura, K., Higaki, M., Ushio, K., Oka, S. and Ohno, A., *Tetrahedron Lett.*, **1985**, *26*, 4213.

18) - Nakamura, K., Kawai, Y., Nakajima, N. and Ohno, A., *J. Org. Chem.*, **1991**, *56*, 4778.

19) - Takeda, A. *et al.* and Kierstan, M. *et al.* in Preparative Biotransformations; Roberts, S.M. Ed, John Wiley and Sons, Chichester-New York-Brisbane-Toronto-Singapore, **1994**, Update 3, pp 2:9.1.

20) - Nakamura, K., Kawai, Y. and Ohno, A., *Tetrahedron Lett.*, **1990**, *31*, 267.

21) - Nakamura, K., Inoue, K., Ushio, K., Oka, S. and Ohno, A., *Chem. Lett.*, **1987**, 679.

22) - Nakamura, K., Kawai, Y., Oka, S. and Ohno, A., *Bull. Chem. Soc. Jpn,* **1989**, *62*, 875.

23) - Noyori, R., Ohkuma, T. and Kitamura, M., *J. Am. Chem. Soc.*, **1987**, *109*, 5856.

24) - Genêt, J.P., Ratovelomanana-Vidal, V., Cano de Andrade, M.C., Pfister, X., Guerreiro, P. and Lenoir, J.Y., *Tetrahedron Lett.*, **1995**, *36*, 4801.

25) - Chênevert, R., Fortier, G. and Bel Rhlid, R., *Tetrahedron*, **1992**, *48*, 6769.

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