Tetrahedron Vol 45 No 16 pp 5051 to 5058 1989
Printed in Great Britain

0040-4020/89 $3 00+ 00

PREPARATION OF (R)-VERATRYL- AND (R)-(3-METHOXYBENZYL)SUCCINATES

Jean-Pierre Barnier, Luis Blanco, Eryka Guibé-Jampel and Gérard Rousseau
Laboratoire des Carbocycles (Associé au C.N.R.S.), Institut de Chimie Moléculaire d' Orsay, Bât 420
Université de Paris-Sud, 91405 Orsay (France)

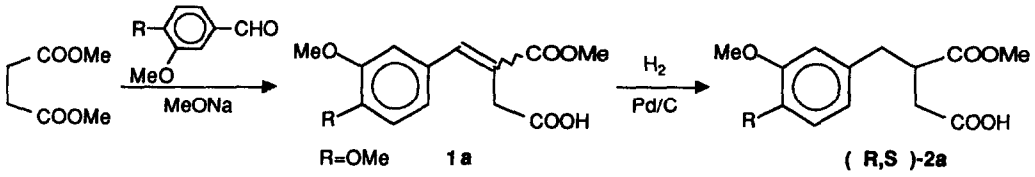
(*Received in Belgium* 8 *May* 1989)

Abstract *LP-catalyzed hydrolyses of 4-cyanomethyl 1-methyl veratryl- and (3-methoxy benzyl)succinates lead in high optical yield to the corresponding (R)-butanoic acids. HLE-catalyzed hydrolyses of various methyl and cyanomethyl veratrylsuccinates lead to mixtures of propanoic and butanoic acids with medium enantioselectivity*

We have reported previously that optically active methyl- and benzylsuccinates could be obtained by a Pig pancreatic lipase (PPL)-catalyzed hydrolysis of their racemates [1] Since optically active (R)-veratryl-, (R)-(3-methoxybenzyl)succinates and 3-aryl-γ-butyrolactones are useful intermediates for the preparation of lignans and alkaloids [2] we have tried the enzymatic resolution of the corresponding racemic succinates Up to now (S) and (R)-veratrylsuccinic acids 1-methyl ester have been obtained by crystallisation of their salts with (S)- or (R)-α–methylbenzylamine[3a] and (S)-2-veratrylsuccinic acid has been synthetized by asymmetric hydrogenation of veratrylidenesuccinic acid using a ruthenium-BINAP complex[3b] Optically active (3-methoxybenzyl)succinates derivatives are still unknown, however (R)-(+)-3-(3-methoxybenzyl)-γ–butyrolactone has been obtained by tedious procedures [2c, 4]

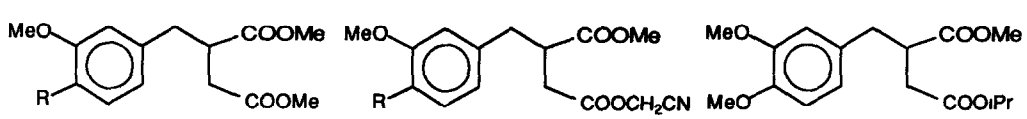
Preparation of the racemic substrates.

Racemic esters **3a,b** and **4a,b** have been prepared by methylation (MeOH, H^+) and cyanomethylation ($ClCH_2CN$, Et_3N) of hemiesters **2a** and **2b** obtained by a previously described sequence [2a] using Stobbe condensation of 3-methoxy- and 3,4-dimethoxybenzaldehydes with dimethyl succinate followed by a palladium-catalyzed hydrogenation of the unsaturated esters **1a** and **1b**.Isopropyl methyl diester **5** was obtained by reaction of hemiester **2a** with diazopropane[5]



R=OMe **1a**
R=H **1b**

(R,S)-**2a**
(R,S)-**2b**

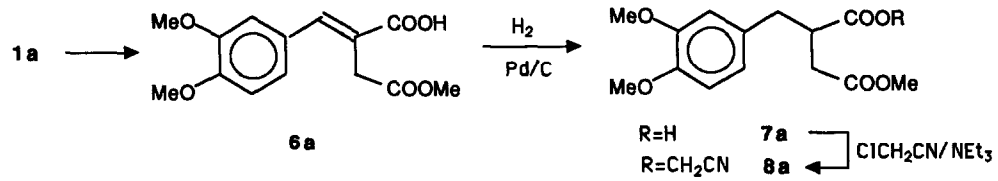


R=OMe (R,S)-**3a**
R=H (, R,S)-**3b**

(R,S)-**4a**
(R,S)-**4b**

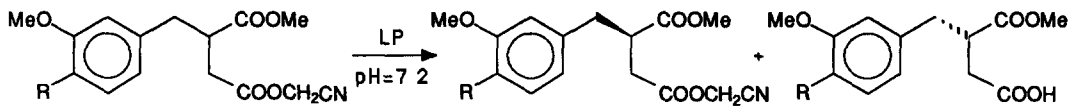
5

Transformation[6] of hemiester **1a** into its regioisomer **6a** allowed the preparation of the mixed ester **8a** via the hemiacid **7a**



Lipase-catalyzed hydrolyses of esters **3a** and **4a,b**

We found that ester **3a** was very poor substrate of PPL, α–chymotrypsin, lipase Amano P from Pseudomonas sp (LP) and Candida cylindracea lipase (CCL) However cyanomethyl esters **4a,b** underwent a fast hydrolysis in the presence of LP These results are in agreement with the increased hydrolysis rate observed by Sih and al [7] in the lipase-catalyzed reactions of cyanomethyl esters For less than 50% hydrolysis at pH=7 2 (pH stat technique) the acids **(R)-2a,b** were isolated with excellent enantiomeric excesses (ee's) and the remaining esters **(S)-4a,b** were obtained with 65% ee [8] Our results are reported in table 1 The enantiomeric excess of



(R,S)-4a,b **(S)-4a,b** **(R)-2a,b**

butanoic acid **(R)-2b** was improved after another cyanomethylation followed by a second LP-catalyzed hydrolysis this acid was isolated with an ee > 95% after 80% conversion

With PPL, only ester **4b,** unsubstituted at the para position of the aromatic ring, was substrate, but the hydrolysis rate and the ee of the products are lower than those observed with LP

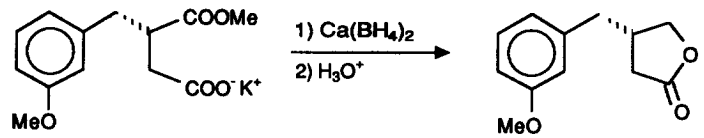
The ee and absolute configuration of compound **(R)-2a** were determined by comparison of its $[\alpha]_D$ with the value of the literature (lit [3a] $[\alpha]_D$= +27° (c= 1 2, EtOH)) The ee was confirmed after reaction with diazomethane, by ^{1}H-NMR of the dimethyl ester in the presence of (+)-tris[3-(heptafluoropropylhydroxymethylene) camphorato] europium (III) (Eu(hfc)3)

Table 1. Lipase hydrolyses of esters **4a, b**

	Reaction conditions			Esters (S)-4a, b				Acids (R)-2a, b			
Substrat	Enzyme	Time %	Conversion %	Yield %	ee	$[\alpha]_D$[a]	Conf. %	Yield %	ee	$[\alpha]_D$[a]	Conf
4a	LP	5 0	42	55	65	-5 0°	S	40	>95	+25 6°	R
4b	LP	4 0	47	42	68	-4 7°	S	35	82	+22.0°	R
4a	PPL	b									
4b	PPL	8 5	36	60	35	-2.2°	S	30	75	+20.6°	R

a) c=2, THF b) no hydrolysis after 2 hours.

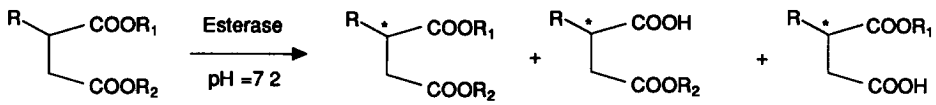
Optically pure butanoic acid **(R)-2a** was cyanomethylated without racemization ($ClCH_2CN$, NEt_3,CH_2Cl_2) to give **(R)-4a** the antipodal isomer of **(S)-4a** ($[\alpha]_D$=+7 7°, c=2, THF) [9] The resulting $[\alpha]_D$ allowed to calculate **(S)-4a**'ee [10] The enantiomeric excesses of esters **(S)-4b** were determined by ^{1}H-NMR in the presence of Eu(hfc)$_3$ and those of the corresponding acid **(R)-2b** by the same method after transformation into its dimethyl ester (CH_2N_2) The absolute configuration of these monomethoxy derivatives were determined by chemical correlation butanoic acid **(R)-2b** (ee=82%) was reduced, via its potassium salt **(R)-2c**, by calcium borohydride [2a] and lactonized in acidic conditions into the known (R)-(+)-3-(3-methoxybenzyl)-γ-butyrolactone **9** (yield=80%) ($[\alpha]_D$=+5 1°, c=2, $CHCl_3$, ee=82%) This preparation of optically active lactone **9** appears to be easier than those already published [2c, 4]



(R)-2c **9**

Esterase-catalyzed hydrolyses.

Ester **3a** was not substrate of lipases, but in the presence of Horse liver esterase (HLE) or Pig liver esterase (PLE) a fast hydrolysis was observed However these reactions were not regioselective and we have obtained the two possible regioisomeric acids (see table 2)



α-acid β-acid

R = veratryl				
R_1=R_2=Me	**(R,S)-3a**	**(R or S)-3a**	**(S or R)-7a**	**(S or R)-2a**
R_1=Me,R_2=CH_2CN	**(R,S)-4a**	**(S)-4a**	**(R)-10**	**(R,S)-2a**
R_1=CH_2CN,R_2=Me	**(R,S)-8a**	**(R,S)-8a**	**(S)-7a**	**(R)-11**
R_1=Me,R_2=iPr	**(R,S)-5**	**(S)-5**	**(R)-12**	**(R)-2a**

Table 2 Esterase hydrolyses of esters **3a**,**4a**,**5** and **8 a**

Substrat	Reaction conditions			Remaining diester			Products					
									α-acid		β–acid	
	Enzyme	Time (h)	Conversion (%)	Yield (%)	ee (%)	Conf	Yield (%)	α/β	ee (%)	Conf	ee (%)	Conf
(R,S)-3a	PLE	2 0	60	35	40	R	55	37/63	24	S	18	S
(R,S)-3a	HLE	2 5	40	55	40	S	35	46/54	66	R	48	R
(R,S)-4a	HLE	2 5	70	25	25	S	65	23/77	25	R	0	-
(R,S)-8a	HLE	3 0	60	35	0	-	55	33/67	25	S	13	R
(R,S)-5	HLE	4 3	80	20	61	S	75	80/20	24	R	19	R

Determinations of absolute configurations and ee's are explained in the experimental section

HLE hydrolyzed mainly the R-isomer of **3a** and PLE the S-isomer In this HLE-catalyzed reaction, each acid results indiscriminately from the hydrolysis of the two enantiomers since the hydrolysis of **(R)-3a** (prepared by reaction of diazomethane with **(R)-2a** obtained in the LP-hydrolysis) gave the same ratio of the two regioisomers **(R)-2a** and **(R)-7a** With these two esterases, the enantioselectivity for hydrolysis of the ester function is greater in α than in β of the chiral center

In order to increase the regio and the enantioselectivity of the HLE-catalyzed hydrolysis we have examined the behaviour of esters **4a**, **8a**and **5**, where the two ester functions are different (see table 2) The hydrolysis of 4-cyanomethyl 1-methyl succinate **(R,S)-4a** in the presence of HLE occurred faster on the cyanomethyl ester No enantioselectivity was observed in the cleavage of this cyanomethyl ester function On the other hand, the hydrolysis of the more hindered methyl ester group was enantioselective The hydrolysis of 1-cyanomethyl 4-methyl succinate **(R,S)-8a** led to the remaining diester devoid of optical activity This is the result of the low regioselectivity of the reaction and of the *unusual reverse in absolute configuration during the hydrolysis of the cyanomethyl ester function* Despite the presence of the carbocyanomethoxy group, the carbomethoxy ester group was preferentially hydrolyzed The presence of the larger isopropyl group in 4-isopropyl 1-methyl succinate **5** favored the reaction at the hindered 1-ester function but the regioselectivity was low The enantioselectivity of the hydrolysis of the two ester functions was low and no significant improvement of the ee of the remaining ester was observed.

In conclusion the absence of regioselectivity in the esterase-catalyzed hydrolyses of veratryl- and (3-methoxybenzyl)succinates make these reactions synthetically useless However for the first time we have observed that the substitution of a methyl group by a cyanomethyl one inverses the HLE enzymatic recognition of the chiral center Besides a good enantioselective resolution was obtained by Pseudomonas lipase-catalyzed hydrolysis of the corresponding 4-cyanomethyl 1-methyl esters leading to the R-hemiesters necessary to synthesize chiral natural products

Experimental section

Nuclear magnetic resonance spectra were recorded on a Brucker AM 250 All chemical shifts were reported in ppm in deuterochloroform. IR spectra were recorded on a Perkin-Elmer 682 spectrometer GC-MS was carried out on a Nermag R10-10 (70 eV). Rotations were determined on a Perkin-Elmer 240 polarimeter The lipase P from Pseudomonas species was obtained from Amano (30 units per mg). Porcine pancreas lipase, used in crude form (steapsin), Horse liver esterase and Pig liver esterase, used as acetone powders, were obtained from Sigma. All reactants and solvents were purified and distilled before use

Preparation of racemic dimethyl (3,4-dimethoxybenzyl)succinate **3a**.

To a methanolic solution (50 mL) of veratrylsuccinic acid 1-methyl ester **2a** [2b] (5g, 17 7 mmol) were added five drops of thionyl chloride After one night at reflux the methanol was distilled Water (30 mL) was added to the residue and the mixture was extracted with ether (3 x 30 mL) The organic phase was washed with aqueous 5% $NaHCO_3$ (20 mL)

and dried (Na_2SO_4) The solvent was removed and the crude product distilled. bp 154 °C (0.05 mm Hg). Yield: 4.0 g (76%)

Anal Calcd. for $C_{15}H_{20}O_6$, % C, 60 78, H, 6.81 Found %. C, 60.93; H, 6 75

^{1}H NMR($CDCl_3$), δ (ppm) · 6.75 (d, J = 7Hz, 1H), 6.66 (d, J = 7 Hz, 1H), 6.63 (d, J = 2Hz, 1H), 3.72 (s, 3H); 3 71 (s, 3H), 3.63 (s, 3H), 3.60 (s, 3H), 3 15-2.88 (m, 2H), 2 72-2.52 (m, 2H), 2.36 (dd, J = 13.5 and 4.5 Hz, 1H)

IR (neat) (cm^{-1}) 1740 (vs), 1610 (w), 1600 (m), 1520 (s)

MS m/e (rel int %) · 296 (M^+, 31); 236 (8); 233 (7), 222 (14), 191 (8), 177 (6), 152 (10); 151 (100), 107 (8), 91 (8)

Preparation of racemic 4-cyanomethyl 1-methyl 2-(3,4-dimethoxybenzyl)succinate **4a**.

A mixture of triethylamine (3.4 g, 30 mmol) and chloroacetonitrile (2.54 g, 40 mmol.) was added to a methylene chloride solution (10 mL) of veratrylsuccinic acid 1-methyl ester **2a** [2b] (2.82 g, 10 mmol.) maintained at 0 °C After one night at room temperature the mixture was poured into water (20mL) After ether extraction (3 x 20 mL), the organic phase was washed with 0.5 M hydrochloric acid solution (10 mL), 10% aqueous sodium bicarbonate solution (10 mL) and water (10 mL) After drying (Na_2SO_4) and evaporation the residue was purified by silica gel column chromatography (ether) Yield 2 7 g (96%)

Anal Calcd for $C_{16}H_{19}O_6N$, % C, 59.81, H, 5.92, O, 29.90, N, 4.36 Found % C, 59.60, H, 5.93, O, 30 17, N, 4.30

^{1}H NMR($CDCl_3$), δ (ppm) 6.81 (d, J = 10 Hz, 1H), 6.69 (dd, J = 10 and 3 Hz, 1H), 6.67 (d, J = 3 Hz, 1H), 4.69 and 4.67 (AB syst., J = 16 Hz, 2H), 3.90 (s, 3H), 3.85 (s, 3H); 3 72 (s, 3H), 3.22-3.12 (m, 2H), 2.82-2.66 (m, 2H), 2.55-2 40 (m, 1H)

IR (neat) (cm^{-1}) 1760 (s); 1740 (s); 1610 (w), 1595 (m), 1520 (s)

MS m/e (rel int %) 321 (M^+, 19), 181 (15), 151 (100), 106 (6), 91 (7)

Preparation of 2-(3-methoxybenzylidene)succinic acid 1-methyl ester **1b**.

The previously described procedure for the preparation of 2-(3,4-dimethoxybenzylidene)succinic acid 1-methyl ester was used [2b] Starting from 0 1 mole of 3-methoxybenzaldehyde we obtained **1b** as an oil Yield 11.5 g (84%)

^{1}H NMR($CDCl_3$), δ (ppm) 11.3 (broad s, 1H), 7.80 (s, 1H), 7 40-7 15 (m, 1H), 6.90-6 75 (m, 3H), 3.80 (s, 3H), 3 75 (s, 3H), 2.60 (m, 2H)

IR (CCl_4) (cm^{-1}) 1740 (s), 1720 (s), 1640 (m), 1610 (m)

MS m/e (rel int %) 250 (M^+, 23), 218 (11), 206 (16), 174 (30); 147 (38), 146 (100), 145 (36), 131 (22), 115 (34), 103 (39), 102 (19), 91 (19); 77 (41)

Preparation of racemic 2-(3-methoxybenzyl)succinic acid 1-methyl ester **2b**.

The butenoic acid **1b** was hydrogenated in methanol in the presence of Palladium-charcoal (10%) (5 h under atmospheric pressure) using the procedure published for the preparation of acid **2a** [2b] Yield 95% This hemiester was a solid (prisms), m.p. = 77-78 °C

^{1}H NMR($CDCl_3$), δ (ppm) 8 70 (broad s, 1H), 7.30-7 10 (m, 1H), 6.85-6.65 (m, 3H), 3.80 (s, 3H), 3 70 (s, 3H), 3.20-3 00 (m, 2H), 2.84-2.60 (m, 2H), 2 45 (dd, J = 20 and 6 Hz, 1H)

IR (CCl_4) (cm^{-1}) 1745 (s) 1720 (s)

MS m/e (rel int %) 252 (M^+, 72), 193 (38), 192 (100), 175 (83), 161 (69), 147 (55), 121 (83), 91 (66)

Preparation of racemic dimethyl (3-methoxybenzyl)succinate **3b.**

The procedure described for the preparation of succinate **3a** was used. Ester **3b**, obtained from hemiester **2b**, was purified by silica gel column chromatography (ether-hexane). Yield 80% (oil).

^{1}H NMR ($CDCl_3$), δ (ppm) 7.29-7.16 (m, 1H), 6.80-6.68 (m, 3H), 3.80 (s, 3H), 3.70 (s, 3H), 3.65 (s, 3H); 3.23-2.98 (m, 2H), 2.82-2.59 (m, 2H), 2.43 (dd, J = 16 and 4 Hz, 1H).

IR (CCl_4) (cm^{-1}) 1735 (vs), 1590 (s).

MS m/e (rel. int. %): 266 (M^+, 76), 206 (98), 203 (33), 193 (44), 192 (25), 175 (69), 161 (100); 147 (53), 91 (57), 77 (31).

Preparation of racemic 4-cyanomethyl 1-methyl 2-(3-methoxybenzyl)succinate **4b.**

4b was prepared according to the procedure described for **4a**. Starting from hemiester **2b**, ester **4b** was isolated as an oil after silica gel column chromatography (ether). Yield 90%.

^{1}H NMR ($CDCl_3$), δ (ppm) 7.32 (m, 1H), 6.95-6.75 (m, 3H), 4.68 and 4.66 (AB syst., J = 16 Hz, 2H); 3.81 (s, 3H), 3.72 (s, 3H); 3.28-2.98 (m, 2H), 2.85-2.62 (m, 2H), 2.55-2.40 (m, 1H).

IR (CCl_4) (cm^{-1}) 1740 (s), 1720 (s), 1605 (m), 1590 (m).

MS m/e (rel. int. %) 291 (M^+, 100), 231 (50), 175 (46), 161 (47), 121 (99), 91 (75).

Preparation of racemic 4-isopropyl 1-methyl 2-(3,4-methoxybenzyl)succinate **5.**

To veratrylsuccinic acid 1-methyl ester **2a** (1 g, 3.54 mmol) was added slowly an etheral solution of diazopropane 5 until persistence of the red color. The solution was dried (Na_2SO_4) and after complete decolorization, was concentrated under vacuum. The mixed ester **5** was used without purification for the enzymatic reaction. Yield 98%.

Anal. Calcd for $C_{17}H_{24}O_6$, % C, 62.96, H, 7.40, O, 29.63. Found % C, 63.14, H, 7.29, O, 29.52.

^{1}H NMR ($CDCl_3$), δ (ppm) 6.80 (d, J = 8 Hz, 1H), 6.70 (d, J = 8 Hz, 2H), 5.00 (septuplet, J = 5 Hz, 1H), 3.98 (s, 3H), 3.97 (s, 3H), 3.68 (s, 3H), 3.28-2.94 (m, 2H), 2.78-2.58 (m, 2H), 2.38 (dd, J = 15 and 5 Hz, 1H).

IR (neat) (cm^{-1}) 1735 (vs), 1650 (w), 1595 (w).

MS m/e (rel. int. %) 324 (M^+, 62), 265 (18), 222 (28), 205 (11), 191 (13), 152 (11), 151 (100).

Preparation of 2-(3,4-dimethoxybenzylidene)succinic acid 4-methyl ester **6a**

The unsaturated monoester **1a** (10 g, 35 mmol) was treated 24 hours at room temperature with 1N NaOH (100 mL) and ethanol (100 mL). The solution was extracted with ether, acidified and extracted with ether (4 x 60 mL). After concentration the residue was treated with boiling acetic anhydride (150 mL) and concentrated. A methanolic solution (150 mL) of the crude anhydride was refluxed 6 hours and concentrated. The residue was taken up in 10% $NaHCO_3$, acidified and extracted with ether (3 x 50 mL) leading to monoester **6a** after concentration. Yield. 9 g, 90%.

^{1}H NMR ($CDCl_3$), δ (ppm) 7.98 (s, 1H), 7.08-6.90 (m, 3H), 3.95 (s, 3H), 3.91 (s, 3H), 3.77 (s, 3H), 3.65 (s, 2H).

^{13}C NMR ($CDCl_3$), δ (ppm) 173.5, 171.2, 150.2, 148.8, 144.2, 127.5, 123.0, 122.9, 112.5, 111.0, 55.9, 52.2, 33.5.

IR (neat) (cm^{-1}) 1740 (s, ν_{CO} ester), 1686 (s, ν_{CO} acid).

Preparation of racemic 2-(3,4-dimethoxybenzyl)succinic acid 4-methyl ester **7a.**

Starting from the unsaturated monoester **6a**, the procedure described for the preparation of hemiester **2b** was used. Yield 93%.

Anal Calcd for $C_{14}H_{18}O_6$, %. C, 59.57, H, 6.42, O, 34.00. Found % C, 59.97, H, 6 10, O, 33.72

^{1}H NMR ($CDCl_3$), δ (ppm) 6.84-6.67 (m, 3H), 3.88 (s, 3H), 3 87 (s, 3H), 3.67 (s, 3H), 3.23-3.04 (m, 2H), 2.82-2.60 (m, 2H), 2.50-2.38 (m, 1H)

^{13}C NMR ($CDCl_3$), δ (ppm) 180.0, 172.3, 149.0, 147.9, 130.4, 121.2, 112.1, 111.3, 55.8, 51 7; 42.8, 38 9, 34.3

IR (neat) (cm^{-1}) 1740 (s, ν_{CO} ester), 1715 (s, ν_{CO} acid)

MS m/e (rel int %) 191 (3), 178 (2), 152 (11), 151 (100)

Preparation of racemic 1-cyanomethyl 4-methyl 2-(3,4-dimethoxybenzyl)succinate **8a**.

The procedure described for the synthesis of ester **4a** was used Starting from the monoester **7a**, ester **8a** was isolated as an oil after silica gel column chromatography (ether) Yield 85%

Anal Calcd for $C_{16}H_{19}O_6N$, % C, 59.81, H, 5.92, O, 29.90, N, 4.36 Found % C, 59.85, H, 5.96, O, 29 76, N, 4.60

^{1}H NMR ($CDCl_3$), δ (ppm) 6.85-6 77 (m, 1H), 6 73-6.64 (m, 2H), 4 73 and 4 70 (AB syst., J = 16 Hz, 2H), 3.90 (s, 3H), 3.88 (s, 3H), 3 68 (s, 3H), 3.22-2.95 (m, 2H); 2.84-2.62 (m, 2H), 2 60-2 40 (m, 1H)

IR (CCl_4) (cm^{-1}) 1735 (vs), 1590 (s)

MS m/e (rel int %) 291 (100), 231 (53), 192 (32), 161 (70), 121 (94), 91 (86)

Preparation of (R)-(+)-3-(3-methoxybenzyl)-γ-butyrolactone **9**.

The potassium salt **(R)-2c** of 2-(3-methoxybenzyl)succinic acid 1-methyl ester **(R)-2b** (ee = 82%) was prepared by addition of an aqueous potassium hydroxide solution (40%) until basic to phenolphtalein Evaporation of the solvent under high vacuum gave an oil

Calcium chloride (0 43 g, 3.9 mmol) was dissolved in methanol (40 mL) and cooled to -10 °C Sodium borohydride (0.36 g, 9.36 mmol) was added in 30 minutes at this temperature followed by the solution of the potassium salt **2c** (prepared from 1 g of **2b**, 3 9 mmol) in 5 mL of ethanol (in 30 minutes) The mixture was stirred 3 h at -10 °C and 2 h at room temperature After acidification (pH 2) the solvents were evaporated, the residue was taken up by water (50 mL) and the product extracted with ether (5 x 20 mL). The organic phase was dried (Na_2SO_4) and concentrated to give the lactone **9** which was purified by silica gel column chromatography (ether) Yield 0 660 g (80%) $[\alpha]_D$=+5.2° (c= 2, $CHCl_3$, ee = 82%), lit.[4] $[\alpha]_D$= +6 4° (c= 1.2, $CHCl_3$, ee = 100%)

^{1}H NMR ($CDCl_3$), δ (ppm) 7.25 (m, 1H), 6.68 (m, 3H), 4 15 (dd, J = 9 and 6.3 Hz, 1H), 4.04 (dd, J = 9 and 6.2 Hz, 1H), 3.02 (s, 3H), 2 95-2 80 (m, 1H), 2 75 (m, 2H), 2.62 (dd, J = 18.6 and 7.5 Hz, 1H), 2.30 (dd, J = 16 7 and 7.5 Hz, 1H)

IR (neat) (cm^{-1}) 1770 (s, ν_{CO} lactone)

General procedure of enzyme-catalyzed hydrolysis of esters.

PPL, HLE and PLE were used as received Lipase Amano P (1 75 g) was suspended in water (10 mL) containing 0 45 M $CaCl_2$ The pH was adjusted to 7.2 by addition of 2 M NaOH aqueous solution After 5 minutes stirring, the heterogeneous mixture was centrifugated and the solution was taken for hydrolyses.

The mixture of the ester (0.5 g) in water (10 mL) containing 0 45 M $CaCl_2$ and the enzyme (1 75 g for LP, 0.5 g for PPL and 1 g for HLE and PLE) was maintained at pH 7.2 by addition of 2 M NaOH until one equivalent of base was consumed (pH stat) Then 1 g of Celite was added and the mixture was filtered Ether extraction of the aqueous phase and of the Celite cake (3 x 20 mL) removed the remaining ester The aqueous phase and the Celite were acidified (pH 2) and extracted with ether (3 x 20 mL) The etheral phases were dried (Na_2SO_4) and respectively evaporated to give the ester and the acid fractions.

Determination of ee's and absolute configurations in the esterase-catalyzed hydrolyses

The enantiomeric excesses of the esters were determined by ^{1}H NMR (solvent: $CDCl_3$) in the presence of Eu(hfc)$_3$. We needed ~ 40% (in mole) of Eu(hfc)$_3$ for all esters studied but esters **5a**,**8a** for which 100% Eu(hfc)$_3$ were used. In the case of ester **5a**, determinations were made in C_6D_6.

The α/β acid ratios were determined on the mixture by ^{1}H-NMR. The absolute configuration of the major enantiomer of ester (**R** or **S**)-**3a** was deduced from its optical rotation sign. Methylation (CH_2N_2) of the acid **2a**,**7a** mixtures gave the dimethyl esters whose ee's and absolute configurations, determined by NMR, were respectively 52% (R) for HLE and 20% (S) for PLE-catalyzed reactions these mean values indicate in each case a similar configuration for the acids **2a** and **7a** which is obviously opposite to that of the remaining ester **3a**. Moreover the ee's of acids **2a** and **7a** were determined, after reaction with 2-diazopropane, on the corresponding mixture of isopropyl methyl esters by ^{1}H-NMR (Eu(hfc)$_3$;C_6D_6).

The enantiomeric excesses and absolute configurations of acids **2a**, **3a** , **7a** , **(R)-10** and **(R)-11** were determined by ^{1}H-NMR after transformation into the dimethyl ester (CH_2N_2). The remaining ester of the HLE-catalyzed hydrolysis of cyanomethyl ester **(R,S)-8a** was racemic: this implies that the S configuration of acid **7a** is opposite to the configuration of acid **11**. The absolute configuration of ester **5** was attributed by comparison of its ^{1}H-NMR spectrum, in the presence of Eu(hfc)$_3$, with that of the ester obtained from a sample of acid **2a** of known configuration (diazopropane). The same procedure was used with the acid **12** after reaction with diazomethane.

Acknowledgement: We thank Amano Co. for a generous grit of Lipase P.

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8) The cyanomethyl ester groups of **(S)-4a**,**b** could be selectively removed by PLE-catalyzed hydrolyses *without any change of ee*. The hemiesters so obtained could be used for further transformations or racemization.[3a]

9) After a second LP-catalyzed hydrolysis of ester **(S)-4a** ($[\alpha]_D$= -5.0°) run to 80% conversion, the $[\alpha]_D$ of the remaining ester was risen to -7.7°.

10) We have not been able to determine the ee of **(S)-4a** by ^{1}H-NMR in the presence of Eu(hfc)$_3$.