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Asymmetric resolution of diastereomeric 4-ethoxycarbonyl-5-pentyl-γ-butyrolactones by crude PLE-mediated hydrolysis

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Abstract

Chemical reduction of diethyl 1-oxo-hexylsuccinate resulted in the formation of the corresponding *cis* and *trans* disubstituted γ-butyrolactones. Both racemic diastereomers were resolved by means of lipolytic enzymes leading to the precursors of interesting natural products such as (−)-methylenolactocin and (−)-phaseolinic acid.

Keywords: Lipase; Esterase; Asymmetric hydrolysis; γ-lactones

1. Introduction

The use of pure enzymes or whole cells of animal plants or microorganisms in organic synthesis has become a routine procedure for the preparation of enantiomerically pure compounds which find a wide application as building blocks in the synthesis of natural products [1–9].

Among the biocatalysts used by organic chemists, hydrolytic enzymes such as lipases and esterases have appeared to be particularly attractive for synthetic purposes, owing to their low cost, thermal stability and wide applicability to the asymmetric transformation of a broad variety of substrates [3,10].

Recently we focused our attention on the use of baker's yeast for the obtainment of optically pure polyfunctionalized γ-butyrolactones [11]. Our interest in these compounds arose from the fact that many γ-lactones with important biological activities are present in nature [12]. We also reported on the asymmetric resolution of racemic *trans*-4-ethoxycarbonyl-γ-butyrolactone derivative **2** by the use of porcine pancreas lipase (PPL) (Scheme 1) [13]. The resulting enantiomeric *trans*-4-carboxy-γ-butyrolactones (+)- and (−)-**4** are precursors of (+)- and (−)-methylenolactocin **6** respectively, this latter known as an antitumour antibiotic [14–16].

Since in the synthesis of the racemic lactone **2** (Scheme 1), also the diastereomeric *cis* lactone **3** was formed, we have taken into account its optical resolution. This in view of the fact

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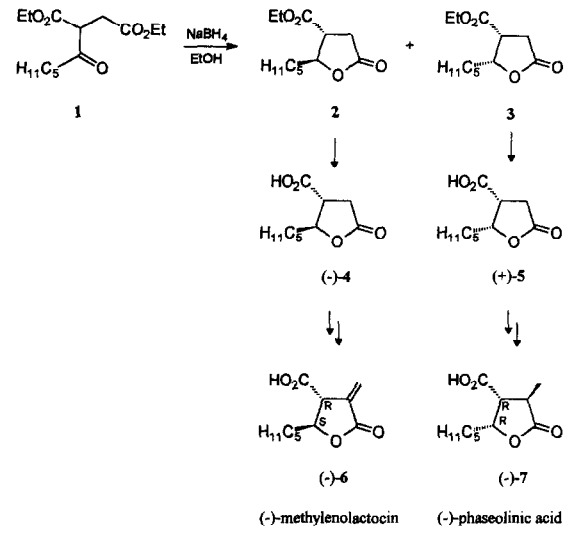


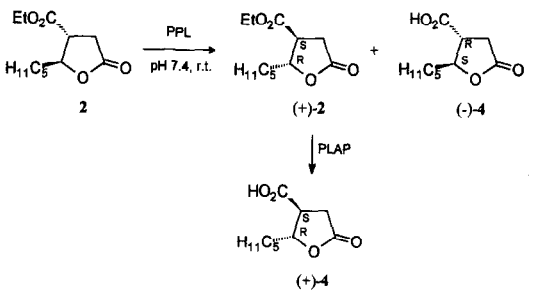
Scheme 1. Synthesis of the racemic diastereoisomeric lactones **2** and **3**.

that *cis*-4-carboxy-γ-butyrolactone derivative (+)-**5** is a precursor of another natural lactone of interest, namely (−)-phaseolinic acid **7** [17].

2. Results and discussion

2.1. Asymmetric hydrolysis of (±)-trans-4-ethoxycarbonyl-5-pentyl-γ-butyrolactone 2

As already reported [13], we obtained both enantiomers (+)- and (−)-**4** with a high degree of enantioselectivity, by using porcine pancreatic lipase (PPL) as the hydrolytic enzyme. When hydrolysis of the ester function at C-4 of racemic lactone **2** was run with 150 mg/mmol of PPL for 6 h, (−)-*trans*-4-carboxy-5-pentyl-

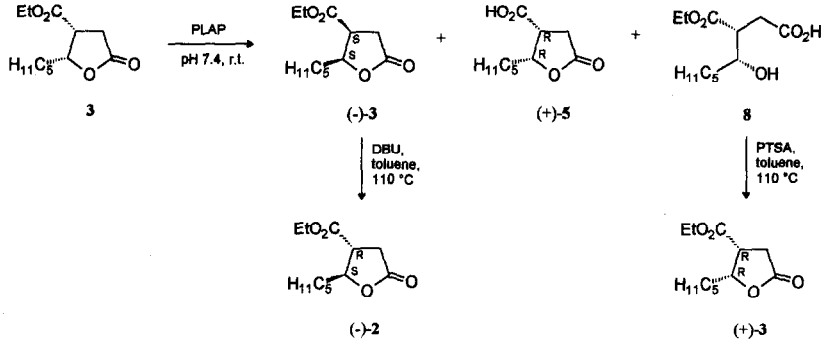


Scheme 2. Asymmetric resolution of the *trans* lactone **2**.

γ-butyrolactone **4** was isolated with 19% yield and 92% ee (Scheme 2). On the contrary, running the hydrolysis with 300 mg/mmol of PPL for 42 h, the unreacted ester (+)-**2** was recovered with 29% yield and 99% ee. The ester (+)-**2** was converted into the corresponding acid (+)-**4** with the same optical purity (determined by chiral HRGC of its ethyl ester derivative [18]) by hydrolysis catalyzed by porcine liver acetone powder (PLAP). Chemical hydrolysis of (+)-**2** (99% ee) under acidic and basic conditions also furnished the corresponding acid (+)-**4**, however with loss of optical purity (88% and 87% ee respectively) [13]. Yield of the biotransformation however was lower than yields of chemical hydrolyses (39% versus 98%).

2.2. Asymmetric hydrolysis of (±)-cis-4-ethoxycarbonyl-5-pentyl-γ-butyrolactone 3

Asymmetric hydrolysis of (±)-*cis*-4-ethoxycarbonyl-5-pentyl-γ-butyrolactone **3** was performed with PLAP under the conditions reported in the Section 3 (Scheme 3). When hy-



Scheme 3. Asymmetric resolution of the *cis* lactone **3**.

drolysis was stopped after 5 h, the unreacted ester (−)-**3** was obtained in 66% yield and 25% ee, by extraction of the neutral water solution. After acidification and extraction of the mother liquors, a 1:2 mixture of the 4-carboxy-γ-butyrolactone derivative (+)-**5** and the γ-hydroxy acid derivative **8** was isolated, this latter compound deriving from hydrolysis of the lactone ring of the system (+)-**3**. Compound **8** could not be isolated as a pure state owing to its high tendency towards lactonization. Therefore the mixture of (+)-**5** and **8** was refluxed in toluene for few minutes in the presence of a catalytic amount of *p*-toluenesulphonic acid (PTSA) to induce complete cyclization of **8** into the corresponding ester (+)-**3**. The 2:1 mixture of the ester (+)-**3** and the acid (+)-**5** thus obtained was separated by the work-up described above. The ester (+)-**3** derived by cyclization of **8** resulted to have 87% ee, while the ee of the acid (+)-**5** was 48% (determined by chiral HRGC of its ethyl ester derivative [18]).

The absolute configuration of the *cis* isomer (−)-**3** was determined by conversion into the *trans* isomer (−)-**2** of known absolute configuration [16] under refluxing toluene for a few minutes, in the presence of DBU in equimolar ratio (Scheme 3). Since equilibration of (−)-**3** proceeds with inversion of configuration at C-4 and the absolute configuration of (−)-**2** is 4R,5S, the absolute configuration of (−)-**3** is 4S,5S. Thus also the absolute configuration of (+)-**3** is proved to be 4R,5R.

Other enzymes were used to perform the asymmetric hydrolysis of the racemic *cis*-4-ethoxycarbonyl-γ-butyrolactone derivative **3**, however with unsatisfactory results. In fact pure PLE (500 u/mmol under the usual conditions of temperature and pH) furnished the desired acid (+)-**5** with 19% yield and 19% ee. No cleavage of the lactone ring was observed in this case, contrarily to what found by using crude PLE, as described above. PPL and *pseudomonas fluorescens* lipase (PFL) were inactive and immobilized *Lipozyme*® did not prove enantioselective. Crude horse liver acetone powder (HLAP) and *Candida Cilindracea* lipase (CCL) gave the desired products in low yields and very low enantiomeric excess.

3. Experimental

3.1. Materials

Lactone (±)-**3** was synthesized as previously described [13]. Lipase (EC 3.1.1.3) from porcine pancreas (type II, crude, No. L-3126), from *Candida Cilindracea* (No. L-1754), esterase from pig liver (EC 3.1.1.1.) in 3.2 M $(NH_4)_2SO_4$ suspension (No. E-2884), porcine liver acetone powder (No. L-8251) and horse liver acetone powder (No. L-9627), were supplied from Sigma Chemicals Co.; Lipase from *Pseudomonas Fluorescens* and immobilized *Lipozyme*® (from *Mucor miehei*) were supplied from Fluka BioChemica.

3.2. General

IR spectra were recorded on a Jasco FT-IR 200 spectrophotometer. ^1H NMR and ^{13}C NMR were run on a Jeol EX-400, at 400 MHz and 100.4 MHz respectively, using deuteriochloroform as a solvent and tetramethylsilane as internal standard. Optical rotations were determined on a Perkin Elmer Model 241 polarimeter, at 25°C. CD spectra were obtained on a Jasco J-700A spectropolarimeter (0.1 cm cell) in acetonitrile. Mass spectra (EI, positive ions) were obtained with a VG 7070 spectrometer at 70 eV. Chiral HRGC analyses were obtained on a Carlo Erba GC 8000 instrument, using a Chiraldex™ type G-TA, trifluoroacetyl γ-cyclodextrin (40 m × 0.25 mm), at 150°C (isotherm).

3.3. Enzymatic hydrolysis of (+)-trans-4-ethoxycarbonyl-5-pentyl-γ-butyrolactone 2

To a suspension of (+)-**2** (100 mg, 0.43 mmol) in 5 ml of 0.1 M KH_2PO_4/Na_2HPO_4 buffer (pH 7.4) were added 80 mg of PLAP

(180 mg/mmol) under stirring. The reaction was monitored by chiral HRGC until complete disappearance of the ester, then the solution was acidified to pH 2, added with ether and centrifuged (3 times). The ethereal phases were combined and dried over Na_2SO_4. Evaporation of the solvent left (+)-**4** [13] which was crystallized from light petroleum-ethyl acetate (9:1), mp: 105–6°C (30 mg, 35% yield), 99% ee, $[\alpha]_D^{25}$ +58.6 (c 0.15, $CHCl_3$) (lit. [13] for the enantiomer (−)-**4**, $[\alpha]_D^{25}$ −54.5 (c 0.5, $CHCl_3$)).

3.4. Enzymatic hydrolysis of (±)-cis-4-ethoxycarbonyl-5-pentyl-γ-butyrolactone 3

To 1.1 g (4.8 mmol) of (±)-*cis*-4-ethoxycarbonyl-5-pentyl-γ-butyrolactone **3** suspended in 10 ml of 0.1 M KH_2PO_4/Na_2HPO_4 buffer (pH 7.4), was added 1.0 g of PLAP (250 mg/mmol) at room temperature and with vigorous magnetic stirring. The pH value was kept within 7.4 and 7.8 by addition of 1 M NaOH. The course of the reaction was monitored by chiral HRGC. After 5 h and addition of 0.25 eq of base, diethyl ether was added, the mixture was centrifuged and the ethereal phase separated. This procedure was repeated 4 times, the ethereal extracts were dried over Na_2SO_4. Removal of the solvent gave the unreacted (−)-**3** (740 mg, 66% yield, 25% ee). The neutral aqueous mother liquors were concentrated in vacuo at room temperature, acidified to pH 2 with 1 M HCl and extracted with diethyl ether, by centrifugation. The organic phase was washed with water, brine and dried (Na_2SO_4). Evaporation of the solvent gave a red oily residue which was identified as a 1:2 mixture of (+)-**5** and **8** (180 mg, 19% total yield).

3.4.1. Syn-3-ethoxycarbonyl-4-hydroxynonanoic acid 8

Although it was not separated from (+)-**5**, the spectroscopic data of **8** are given separately for sake of clarity. ν_{max} (neat), cm^{-1} 3508 (br, OH), 1738 (CO_2Et), 1720 (CO_2H); δ_H (400 MHz; $CDCl_3$), 5.85 (2H, br. signal, CO_2H and OH), 4.19 (2H, q, CH_3CH_2O), 3.82 (1H, m, $CHOH$), 2.96 (1H, m, $CHCO_2Et$), 2.85 (1H, dd, *J* 6.8, 16.6, CH_2COOH), 2.70 (1H, dd, *J* 3.4, 16.6, CH_2COOH), 1.42 (2H, m, CH_2), 1.27 (5H, m and t, CH_2 and CH_3CH_2O), 0.87 (3 H, t, CH_3); δ_C 177.2 (s), 174.9 (s), 71.8 (d), 61.7 (t), 46.5 (d), 34.7 (t), 32.9 (t), 32.5 (t), 25.4 (t), 22.5 (t), 14.0 (q), 13.9 (q).

The 1:2 mixture of (+)-**5** and **8** was heated in benzene in the presence of a catalytic amount of PTSA giving a 2:1 mixture of (+)-**3** and (+)-**5**. In order to separate the products, the mixture was dissolved in ether and washed (3 times) with a saturated solution of $NaHCO_3$. The ethereal phase was dried with Na_2SO_4 and evaporated to give (+)-**3** (90 mg, 9% yield), 87% ee; $[\alpha]_D^{25}$ +73.0 (c 1.1, CH_3CN).

The aqueous phase was acidified to pH 2, extracted 3 times with diethyl ether, dried and the solvent removed *in vacuo* to give (+)-**5**.

3.4.2. (+)-cis-4-carboxy-5-pentyl-γ-butyrolactone 5

(45 mg, 4.5% yield), mp: 102°C (from light petroleum–ethyl acetate); ν_{max}, cm^{-1} ($CHCl_3$): 3500 (br, OH), 1782 (O–C=O), 1710 (CO_2H); δ_H 8.55 (1H, br, COOH), 4.66 (1H, q, H-5), 3.47 (1H, dt, *J* 4.9 and 8.3, H-4), 2.89 (1H, dd, *J* 4.9 and 17.6, H-3), 2.70 (1H, dd, *J* 8.3 and 17.6, H-3), 1.42 (2H, m, CH_2), 1.56 (1H, m), 1.43 (1H, m), 1.30 (4H, m), 0.89 (3H, t, CH_3CH_2O). δ_C: 175.8 (s), 174.8 (s), 80.2 (d), 44.1 (d), 31.8 (t), 31.3 (t), 31.2 (t), 25.5 (t), 22.4 (t), 13.9 (q); *m/z* (electron impact 70 eV) 200 (M+, 0.8%), 182 (14), 154 (24), 140 (16), 129 (92), 101 (100), 100 (48), 73 (39), 55 (83); 48% ee; $[\alpha]_D^{25}$ +35.0 (c 0.5, CH_3CN), CD: $\Delta\varepsilon_{230}$ = −0.03.

The compound (−)-**3** (740 mg, 3.24 mmol), having 25% ee, isolated from the reaction described above, was hydrolysed with PLAP (750 mg) under the same conditions as above for 50 h. After the usual work-up, the unreacted (−)-**3** (102 mg, 14% yield after purification on column chromatography) had 96% ee, $[\alpha]_D^{24}$ −79.8 (c 0.5, CH_3CN); CD: $\Delta\varepsilon_{224}$: +0.13. The re-

maining products were (+)-**5** and **8**, as a 1:1 mixture (255 mg, 39% total yield). They were separated as described above to give (+)-**3** (102 mg, 14% yield), 46% ee and (+)-**5** (85 mg, 14% yield), 22% ee.

3.5. Measurements of enantiomeric excess

The enantiomeric excess of the products (+)-**3** and (−)-**3** was determined by chiral HGRC. R_t of (−)-**3** and (+)-**3** were 48 min and 52 min respectively. The ee of the product (+)-**5** was determined by chiral HRGC of its ethyl ester (+)-**3**, obtained by esterification of (+)-**5** with ethyl iodide and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [18].

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