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Combined Biocatalytic Preparation of (*R*)-2-Ethylhexanol and 2-Ethylhexyl Laurate*

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Summary

*(R)-2-Ethylhexanol (R-**1**) is prepared in high optical purity (> 99 % enantiomeric excess, e.e.) by the Pseudomonas sp. lipase-catalyzed acylation of racemic **1** using vinyl laurate as the acylating agent that allows the isolation of (R)-**1** by distillation. Nonactivated lauric acid proved to be very reactive, affording (R)-**1** with > 99 % e.e., but difficulties in down-stream process disfavored its use. 2-Ethylhexyl laurate (**3**), the other product in this process, is also a commercially valuable material. Recycling of the enzyme and simple isolation of optically pure alcohol (R)-**1** makes the method amenable to the scale-up.*

Key words: Pseudomonas sp. lipase, kinetic resolution, (*R*)-2-ethylhexanol

Introduction

2-Ethylhexanol (*rac* **1**) is produced world-wide in quantities exceeding 2.5 billion tons/y, and thus represents the largest and the cheapest racemic industrial product (*1*). Since it has only one functionality per 8 C atoms, its application is limited to the use as the solvent alcohol, and in the production of surfactants, *e.g.* Aerosol OT, bis-2-ethylhexyl sodium sulfosuccinate (*2*). 2-Ethylhexyl laurate is used as lubricant for friction (*3*) and in paper industry (*4*), but also as activity enhancer for pesticides (*5,6*), and as ingredient in cosmetic creams (*7*). *Rac* **1** has recently found an important application in constructing electroluminescent conjugated polymers (*8*), and specific application in the production of 2-ethylhexyl-*para*-methoxycinnamate, a well-known cosmetic product used in sun-protection creams under the commercial name Parsol^{R}MCX (*9*). We have recently reported the preparation of (*S*)-2-ethylhexyl-*para*-methoxycinnamate by the lipase-catalyzed sequential kinetic resolution (*10*). This method was prompted by the observation that toxicology for two potential metabolites of (*R*)- and (*S*)-2-ethylhexanol are notably different. The oxidation product of the former, (*R*)-2-ethylhexanoic acid, is highly teratogenic while the acid derived from the (*S*)-enantiomer is not (*11,12*) However, from *R*-enantiomer of 2-ethylhexanol was prepared optically active Aerosol OT, following the lipase-catalyzed resolution of *rac* **1** (*13,14*).

Related to our ongoing study of organic liquid crystals (*15,16*), we needed larger quantities of enantiomerically pure 2-ethylhexanol (≥ 99 % enantiomeric excess, e.e.). None methods for the production of enantiomerically pure **1** described in the literature are amenable to any scale-up (*17–19*). We therefore entered on the study of a practical method for the production of this compound with high enantiomeric purity, that is a well-known prerequisite for materials with electroluminescent and ferroelectric properties, and its isolation by a simple down-stream process, that is prerequisite for any commercial enzyme-mediated process. We anticipated that biocatalytic approach balances both the enormous pool of commercially available *rac* 2-ethylhexanol and the versatility of the commercial lipases in kinetic resolution of *rac* alcohols.

* Dedicated to Professor Pavao Mildner for his 80th birdthday

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Materials and Methods

General

IR spectra were run on Perkin Elmer M 137 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL-GEM 300 spectrometer; shifts are given in ppm downfield from TMS. Optical rotations were measured on Optical Activity LTD automatic polarimeter AA-10.

Chemicals

Lipase *Pseudomonas fluorescens* (Fluka Co., 31.5 U/mg), *Pseudomonas* sp., *Candida cylindracea* and *Penicillium camembertii* (Amano, unknown activity) were used. Lauric acid was from Aldrich, vinyl acetate and vinyl laurate were from Fluka Co., 2-ethylhexanol was from Merck Co., and they were used as received. Dichloromethane and *n*-hexane were distilled before use. Molecular sieves type 4A were from Fluka Co. (powder) and from Aldrich (pellets, 2r = 3.2 mm) and were activated before use.

Analytical monitoring of the lipase-catalyzed acylation

Enzymatic reactions were monitored by GL chromatography on a Hewlett Packard instrument 5890 Series II, using HP-17 column under the following conditions; temperature was programmed for acetylation with vinyl acetate as follows: 50 °C for 2 min, 5 °C/min up to 200 °C; for acylation with vinyl laurate as follows: 80 °C, 5 °C/min up to 220 °C, and for acylation with lauric acid as follows; 70 °C for 3 min, 20 °C/min up to 260 °C; detector and injector temperature 300 °C, carrier gas N_2, pressure 25 kPa. Optical purity (e.e. %) was determined by GLC using column CP-Chirasil-DEX-CB with chiral stationary phase (from Chrompack Co.) under the following conditions: temperature program: 100 °C for 20 min, 10 °C/min up to 170 °C, pressure 60 kPa. Retention times were 19.7 min for (*R*)-**1** and 20.6 for (*S*)-**1**. *E*-values were calculated on the basis of conversion and e.e. values, according to Sih *et. al.* (*20*) using Selective Mac 1.0 program.

(R)-2-Ethylhexanol with vinyl acetate as acyl donor

Racemic **1** (13.4 g, 0.1 mol) and *Pseudomonas* sp. lipase (PSL) (500 mg, 0.037 g/g of *rac*-**1**) were slurried in distilled tetrahydrofuran (THF) (100 mL) at 30 °C. The reaction was started by the addition of vinyl acetate (34.7 g, 0.4 mol), and stirring at 220 r. min^{-1} on a thermostated shaker continued for 70 h. Samples (30 μL) were taken at regular time intervals, diluted by dichloromethane (300 μL), and 2 μL of the sample were analyzed. After 70 h, at 89 % conversion, > 99 % e.e. of remaining *R*-**1** was reached. The enzyme was filtered off, the filtrate evaporated, and the mixture applied on silica gel column (400 g). Eluating with dichloromethane/*n*-hexane (6:4) alcohol (*R*)-**1** (1.48 g, 11.0 %) was separated from the enantiomerically enriched (*S*)-**2**.

(R)-2-Ethylhexanol with vinyl laurate as acyl donor

a. Effect of the enzyme recycling

Acylation was performed as described for vinyl acetate, using vinyl laurate (105 mL, 0.4 mol). After 48 h and 88 % conversion, the remaining *R*-alcohol exhibited > 99 % e.e. The enzyme was filtered off, washed with THF, dried and deposited for reuse. The same lipase was reused three times without any loss of activity. The filtrate was distilled at 30–31 °C/0.15 mm Hg, affording 1.45 g (10.8 %) of (*R*)-**1** with 97 % chemical purity.

b. Effect of the molar excess of vinyl laurate

Rac **1** (1 g, 7.7 mmol) and PSL (150 mg, 0.15 g/g of *rac* **1**) were slurried in distilled THF (100 mL) at 30 °C and 220 r. min^{-1}. The reaction was started by the addition of vinyl laurate: 8 mL (30.8 mmol), 6 mL (23.1 mmol), 4 mL (15.4 mmol) and 2 mL (7.7 mmol). Samples (30 μL) were taken at regular time intervals, diluted by dichloromethane (300 μL), and 2 μL of the sample were analyzed. Time-dependent conversions are presented in Fig. 5.

c. Preparative method

Preparative reaction was performed with 10 g (0.077 mol) of *rac* **1**, and PSL (1.5 g, 0.15 g/g of *rac* **1**), slurried in distilled THF (75 mL) at 30 °C. The reaction was started by the addition of vinyl laurate (40 mL, 0.154 mol), and stirred at 220 r. min^{-1} on a thermostated shaker. After 14 h and 89 % conversion, the remaining *R*-alcohol exhibited > 99 % e.e. The enzyme was filtered off and the filtrate distilled at 30–32 °C/0.15 mmHg, affording 1.08 g (10.8 %) of (*R*)-**1** with 99.1 % chemical purity and 99.4 % e.e., $[\alpha]_D$ = –2.8 (c 1.08 CH_2Cl_2). The excess of vinyl laurate was distilled at 73–75 °C, and finally 2-ethylhexyl laurate was distilled at 130–132 °C.

R-2-Ethylhexanol with lauric acid as acyl donor

a. Effect of the molar excess of lauric acid

Rac **1** (100 mg, 0.77 mmol) and PSL (100 mg, 1g/g of *rac* **1**) were slurried in distilled *n*-hexane (25 mL) at 30 °C and 225 r. min^{-1}. The reaction was started by the addition of lauric acid: 154 mg (0.77 mmol), 308 mg (1.54 mmol), 924 mg (4.62 mmol) and 1.54 g (7.7 mmol). Samples (30 μL) were taken at regular time intervals, diluted by *n*-hexane (500 μL), and 2 μL of the sample were analyzed. Time dependent conversions are presented in Fig. 2.

b. Effect of the addition of molecular sieves

Rac **1** (100 mg, 0.77 mmol) and lauric acid (154 mg, 0.77 mmol) were dissolved in distilled *n*-hexane (25 mL). Water content was controled by the addition of molecular sieves type 4A, powder or 2r = 3.2 mm pellets (125 mg, 5 mg/mL of solvent). The reaction was started by the addition of PSL (100 mg, 1 g/g of *rac* **1**) at 30 °C and 225 r. min^{-1}. Samples (500 μL) were taken at regular time intervals and 2 μL of the sample were analyzed.

c. Preparative method for 2-ethylhexyl laurate

Acylation of *rac* **1** (2.5 g, 0.019 mol) was performed by *Candida cylindracea* lipase (2.5 g) in *n*-hexane (50 mL) with lauric acid (3.85 g, 0.019 mol) at 30 °C. After 2 h 6 g of molecular sieves type 4A (pellets) were added. After 40 h the enzyme and molecular sieves were filtered off, the filtrate evaporated and distilled affording 5.88 g (99.1 %) of *rac* **3** with 98.4 % chemical purity.

IR (neat): 2900, 1740, 1460, 1380, 1280, 1170 cm^{-1}.
^1H NMR. ($CDCl_3$) δ:0.85–0.91 (m, 9H), 1.20–1.69 (m, 27H), 2.29 (t, 7.5 Hz, 2H), 3.98 (d, 6 Hz, 2H) ppm.
^{13}C NMR. ($CDCl_3$) δ:10.7, 13.7, 13.8, 22.4, 22.7, 23.6, 24.8, 28.7, 28.9, 29.0, 29.1, 29.2, 29.3, 29.4, 30.2, 31.7, 34.2, 38.6, 66.4, 174.1 ppm.

Results and Discussion

Acetylation of *rac* **1** was performed by *Pseudomonas species* lipase, which was shown by others (*14, 17–19*), and by us (*10*) as the most effective in producing (*R*)-alcohol with very high (> 99 % e.e.) optical purity, if reaction is stopped at *ca.* 90 % conversion, Fig. 1.

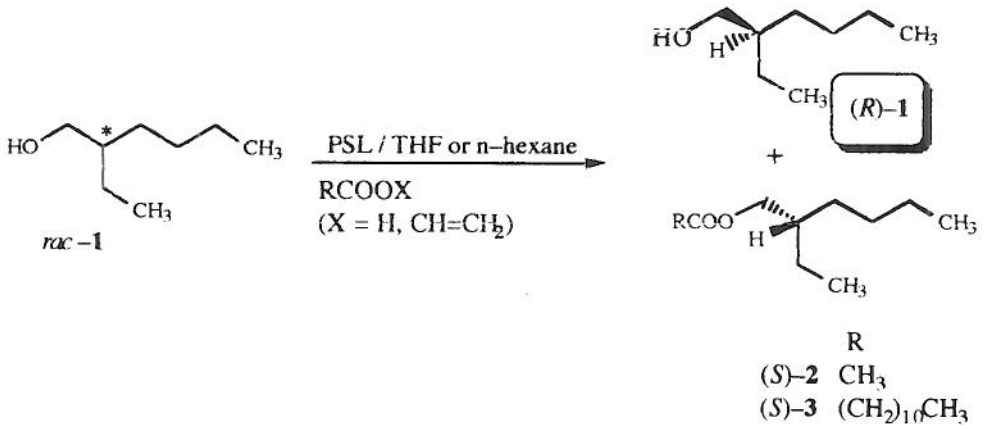

Fig. 1. Scheme of the PSL-catalyzed kinetic resolution of *rac* **1** to (*R*)-**1** and 2-ethylhexyl esters **2,3**

Continuing this project, we have first examined a series of other acylating agents: vinyl acetate, trifluoroethyl butyrate, trifluoroethyl laurate, succinic anhydride, glutaric anhydride and camphoric anhydride (chiral agent). The first four exhibited acylating properties in the presence of *Penicillium camembertii* lipase, but the *E*-values were uniformly low (Table 1).

Table 1. Acylation of *rac* **1** catalyzed by *Penicillium camembertii* lipase

Acyl donor	E [+] ((*S*)-**1** remained)
Vinyl acetate	2.0
Trifluoroethyl butyrate	1.8
Trifluoroethyl laurate	1.5
Succinic anhydride	1.7
Glutaric anhydride	‡
Camphoric anhydride	‡
Vinyl acetate*	4.7
Vinyl laurate*	4.7

* Catalyzed by PSL; ‡ (*R*)-**1** remained
[+] *E* was calculated according to (*20*)

Having the ultimate proof of the preferred activity of *Pseudomonas* lipase at hand, we repeated acetylation of *rac* **1** by vinyl acetate catalyzed by *Pseudomonas fluorescens* lipase of known activity, according to the method of Larpent *et al.* (*14*). Since the authors had determined the e.e. (%) by relatively inaccurate NMR method, we have monitored the reaction more accurately by GLC using chiral column. After 18.5 h conversion of 82 % was reached, and only 92 % e.e. of the remaining alcohol was obtained. The authors had stopped the reaction after 25 h, at 77 % conversion, and claimed 100 % e.e., as determined by NMR. Our repeated experiments have revealed that ca 90 % conversion is needed in order to obtain over 99 % e.e. of the remaining (*R*)-**1**.

In the preparative experiment acetylation was catalyzed by *Pseudomonas species* lipase (PSL), and the reaction was stopped after 70 h at 89 % conversion; the e.e. of the remaining (*R*)-**1** was >99 %. Alcohol was isolated by chromatography on silicagel in 11 % yield. Since chromatographic separation of the product is not a method of choice for any large-scale production, a simpler separation method was sought. All attempts to separate alcohol from acetate by distillation failed; distillation at high temperature was accompanied by acid catalyzed transesterification, which results with net racemization. Due to relatively close boiling points (b.p.), 185 °C for alcohol **1** and 199 °C for acetate **2**, fractionation at lower temperatures and pressures failed.

Prompted by the encountered difficulties in the down-stream process, we selected commercially available lauric acid and its esters as the acylating agent, expecting that high b.p. of 2-ethylhexyl laurate (124–126 °C/0.1 mm Hg) will assure separation of the alcohol from the ester by distillation at low pressure.

Free lauric acid turned out to be a very good substrate for PSL; complete acylation was achieved after 41 h at 30 °C, using equimolar quantitites of reagents in *n*-hexane. In view of various commercial applications of 2-ethylhexyl laurate (*3–7*) this result *per se* has definite importance. Generally, higher carboxylic acids are more reactive than lower ones in lipase-catalyzed acylation of alcohols (*21–23*), for the production of ethyl esters the optimum reactivity is observed for decanoic acid (*23*).

In order to get (*R*)-**1** with high optical purity this reaction was performed at the high molar excess of lauric acid (Fig. 2). Conversion of up to 96 % was achived with-

out elimination of water; in a separate experiment water was eliminated by the addition of 4A molecular sieves and ≥ 99 % conversion was obtained.

Fig. 3 reveals an interesting phenomenon of this process: at the low *rac* **1**/lauric acid ratio (1:6) small but reproducible diminishing of the e.e. in the final stage was observed. This effect was even more pronounced at higher (1:2, 1:1) *rac* **1**/lauric acid ratios, indicating that selectivity in the final stage of acylation is not only affected by depleting of (*S*)-enantiomer but also by the water formed during reaction. This observation differs from the recent result in *n*-hexane for two model reactions: esterification of 1-butanol with 2-methyl pentanoic acid, and irreversible transesterification between 2-methyl pentanol and vinyl acetate (*24*). Medium e.e.'s were reached and were not affected by the water content of the medium.

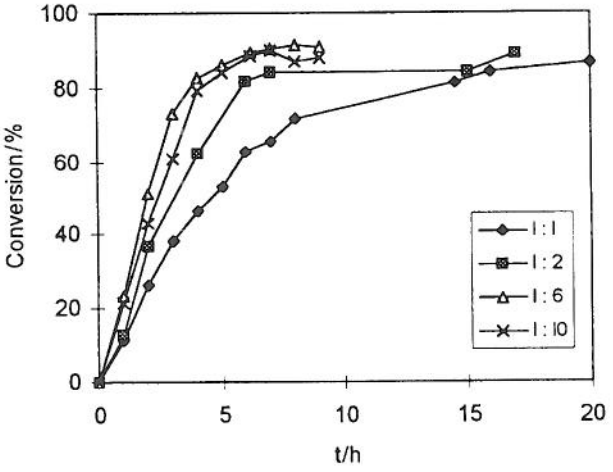

Fig. 2. Progress of acylation of *rac* **1** to (*S*)-**3** by lauric acid in the presence of PSL for different *rac* **1**/lauric acid ratios

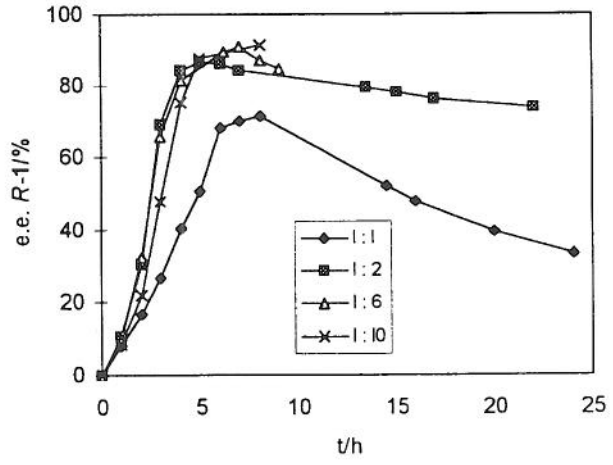

Fig. 3. Effect of the molar ratio *rac* **1** / lauric acid on the e.e. (%) of (*R*)-**1** in the reaction catalyzed by PSL

Although nearly 100 % e.e. of (*R*)-**1** was reached at 1:10 *rac* **1**/lauric acid molar ratio, the separation of (*R*)-**1** from the reaction mixture was difficult. Since direct distillation of the alcohol was found to rise transesterification, separation of lauric acid as an aqueous solution of the sodium salt was attempted. However, the expected difficulites caused by the detergent effect were encountered.

We therefore turned to the use of active vinyl laurate as acylating agent, expecting its higher reactivity and fewer difficulties in the down-stream process. The first experiments revealed that PSL catalyzed acylation with vinyl laurate is faster than with vinyl acetate but significantly slower than with lauric acid.

In order to reduce the quantity of vinyl laurate, acylation was repeated with *rac* **1**/acyl donor ratios from 1:1 to 1:4 (Fig. 4). The curves indicate a significant effect of the ratio on the conversion and optical purity and confirmed that optimal conditions are with *rac* **1**/acyl donor 1:2 at conversion of *ca.* 90 %, affording with 99.4 % e.e. (*R*)-alcohol (Table 2.)

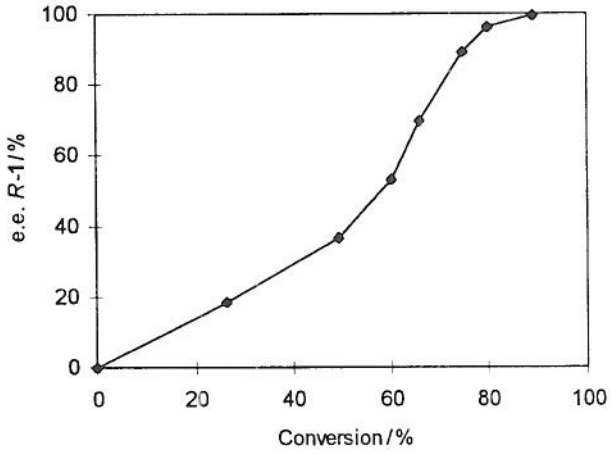

Fig. 4. Effect of the molar ratio vinyl laurate/*rac* **1** on the acylation rate of *rac* **1** by PSL

Table 2. Effect of molar ratio on the conversion rate and the e.e. of (*R*)-**1**

Molar ratio *Rac*-**1**/vinyl laurate	(*R*)-**1**/%	t/h	e.e/%
1 : 4	11.2	10	100.0
1 : 2	11.2	13	99.4
1 : 1	10.8	48	96.5

Correlation between the degree of conversion and the e.e. of the remaining non-acylated (*R*)-alcohol was then determined (Fig. 5.) The sigmoid correlation was observed, as already reported for the e.e. of (*S*)-**2** during acetylation of *rac* **1** in various solvents (*10*). Preparative reaction was stopped at 89 % conversion after 14 h and (*R*)-alcohol was isolated by distillation in 10.8 % yield and 99.4 % e.e.

In the final stage of this study we briefly examined the recycling of the lipase: after three cycles, comprising filtration and washing, there was no observable loss of the activity or e.e (Fig. 6.)

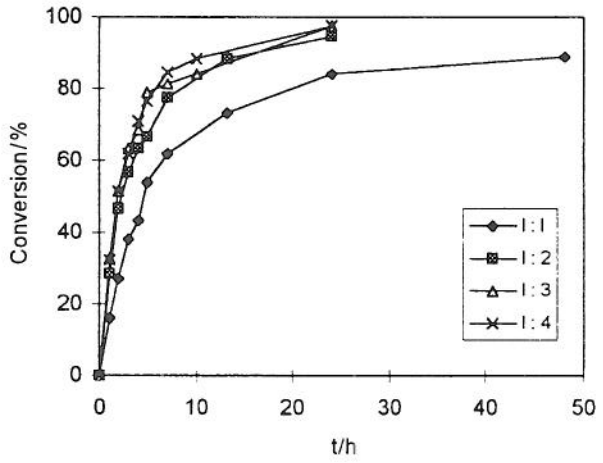

Fig. 5. Dependence of e.e. (%) of (*R*)-**1** on the conversion of *rac* **1** by vinyl laurate, catalyzed by PSL and molar ratio *rac* **1**/vinyl laurate 1:2

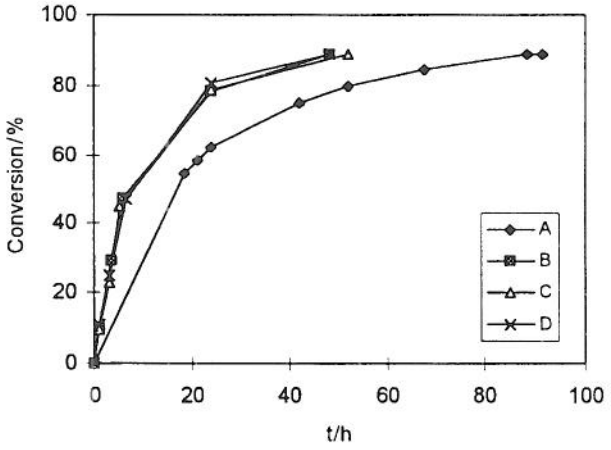

Fig. 6. Progress of acetylation of *rac* **1** by PSL; (**A**) to (*S*)-**2** with vinyl acetate, (**B**) to (*S*)-**3** with vinyl laurate, (**C**) to (*S*)-**3** by PSL once recycled, (**D**) by PSL twice recycled

The separation of the product (*R*)-**1** by distillation at 0.15 mmHg was quantitative, even when performed without any particularly effective distillation column, and without any racemisation. Bath temperature was maintained bellow 80 °C, and the vapors of (*R*)-alcohol had cca 30 °C. Such a large ΔT between the bath and the vapor temperature is convenient when the minor, more volatile component is distilled from the large volume of the reaction mixture. The excess of vinyl laurate is recovered at ca 75 °C/0.15 mm Hg, and finally (enantiomerically slightly enriched) ester **3** is isolated by distillation at cca 130 °C/ 0.15 mm Hg.

In conclusion, we have reported a practical preparation of (*R*)-2-ethylhexanol by the lipase-catalyzed acylation of racemic alcohol with vinyl laurate. This process affords > 99 % optically pure product on simple distillation, allows the use and recycling of the enzyme in organic solvent, coproducing 2-ethylhexyl laurate, a valuable product for cosmetic and agrochemical application.

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Povezana biokatalitička priprava *(R)*-2-etilheksanola i 2-etilheksil laurata

Sažetak

*(R)-2-etilheksanol (R-**1**) pripravljen je s velikom optičkom čistoćom (>99 % enantiomernog viška, e.e.) iz racemičnog **1** acilacijom, kataliziranom lipazom u Pseudomonas sp., koristeći vinil laurat kao sredstvo za acilaciju, što omogućava izolaciju (R)-**1** destilacijom. Neaktivirana laurinska kiselina bila je vrlo reaktivna dajući (R)-**1** s > 99 % e.e., ali se zbog teškoća u nastavku procesa odustalo od njezine primjene.*

*Drugi proizvod u tom procesu, 2-etilheksil laurat (**3**), također je komercijalno vrijedan. Ponovna uporaba enzima i jednostavna izolacija optički čistog alkohola (R)-**1** omogućavaju da se postupak može primijeniti i u industrijskom mjerilu.*