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Production of *l*-menthyl acetate through kinetic resolution by *Candida cylindracea* lipase: effects of alkaloids as additives

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Abstract

Enzymatic transesterification of *dl*-menthol with vinyl acetate in *tert*-Butyl methyl ether (TBME) catalyzed by *Candida cylindracea* lipase (CCL) was carried out in the presence of cinchona alkaloid as additive. The effects of various reaction parameters, such as lipase nature and loading, acylating agent, molecular sieves, solvents and various additives, on the reactivity as well as on the enantioselectivity were investigated. A significant improvement of CCL reactivity has been recorded after using cinchona alkaloid as additive in TBME. A high enantiomeric ratio (E = 80) was achieved when 30 mol% of quinidine was added, and *l*-(-)-menthyl acetate was obtained with 93% optical purity and 49% conversion. This process was easily applied to gram-scale quantities, using commercially inexpensive lipase, providing high yield optically active menthol under mild experimental conditions.

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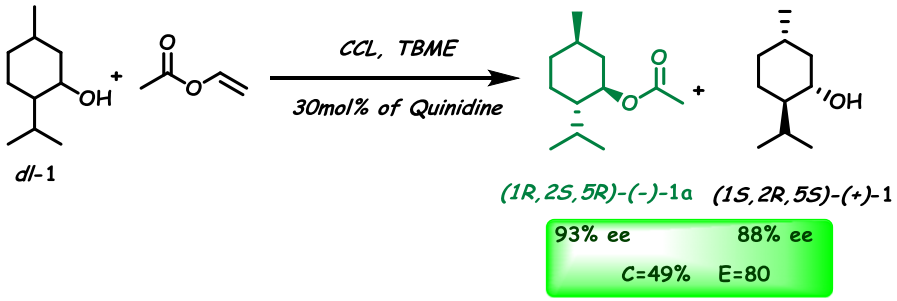
Graphical abstract



Keywords *l*-Menthyl acetate · Kinetic resolution · *Candida cylindracea* lipase · Alkaloids · Additives

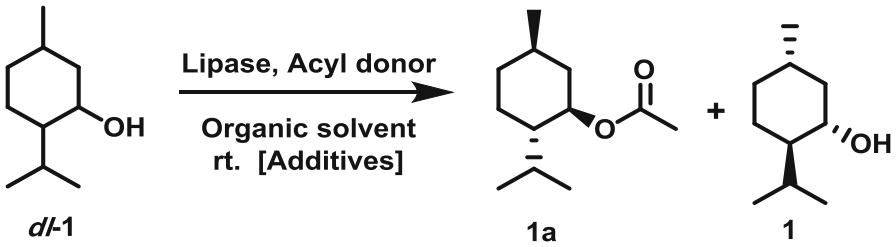
Introduction

Menthol is the world's most-sold flavor ingredient and can be found in countless products in common use. The worldwide demand of 25,000–30,000 metric tons per year already exceeds the available supply and is constantly growing [1]. *l*-(-)-Menthol has the best organoleptic performance, since it is widely used in flavor and fragrance, food, cosmetics and pharmaceutics industries due to its refreshing flavor and cooling effects, whereas *d*-(+) menthol has an undesirable taste [2–4]. The demand for the pure form of *l*-(-)-menthol is an important preoccupation at an industrial level, which explains the research efforts to establish efficient routes for yielding it at high purity [5–7]. One of the efficient routes to obtain it as optically active is the biocatalytic process [8–15]. The enzymatic kinetic resolution of racemates is one of the practical modes used for the separation of the two enantiomers, and lipases (E.C.3.1.1.3) are the most used biocatalysts with considerable industrial potential. Lipases can act under mild conditions, are cheap, stable, and efficient, and present a remarkable chemo-, regio- and enantioselectivity to a large panel of substrates, in particular secondary alcohols [16–19].

In kinetic resolution involving lipases, various parameters have to be taken into account for controlling both reactivity and enantioselectivity. For instance, in the kinetic resolution of racemic alcohols with lipases, several parameters such as the nature of the enzyme [20, 21], the solvent [22–24], the effect of additives [25, 26], the residual water in the reaction media [27, 28], the amount of lipase [29, 30] and the nature of the acetylating agent [31–34] are often examined for the optimization of selectivity and conversion. Some research is still dedicated to the study of the kinetic resolution of *dl*-menthol using lipases. In all the reported literature data, the use of free lipases exhibits no good reactivity or selectivity,

and it is the use of immobilization techniques that generally improve the efficiency of the various commercially available free lipases. The majority of the described kinetic resolutions exploit *Candida rugosa* lipase (CRL) immobilized on several supports in esterification, transesterification or hydrolysis reactions. The recombinant CRL was used for the enantioselective hydrolysis of *dl*-menthyl benzoate with high selectivity as opposed to the free lipase which showed a low selectivity [35]. Another immobilization of *Candida rugosa* lipase (AYL) was found to be the most appropriate in transesterification of *dl*-menthol with vinyl acetate; the conversion recorded was of at least 34% [36]. The lipase from *Candida cylindracea* (CCL), from the same source as CRL, was also investigated in an immobilized form: in kinetic resolution of *dl*-menthol via esterification [37] and transesterification [38], more recently for esterification reaction between 1-octanol and three butyric acid derivatives in low-water solvent-free systems [39]. Few studies have been carried out on the resolution of *dl*-menthol with free CCL and the enantio-discrimination displayed by lipases from *Candida cylindracea* (Sigma Type VII) and *Candida rugosa* (Sigma Type VII and Amano AY) was generally moderate [40]. Moreover, these yeast hydrolytic lipases are the most commonly used due to their high activity and non-genotoxic or cancerogenic effects on human health [41]. Hence, the species is generally regarded as safe (it has GRAS status), and classified as a biologically safe level [42, 43]. The above-mentioned lipases are most popular lipases and but, to date, no studies have focused on the effects of some additives on the reactivity and selectivity of these lipases.

In this paper, we report the optimization of the reactivity and selectivity of free commercially available lipases by modulation of several parameters influencing the catalytic process during the enzymatic kinetic resolution via acylation of *dl*-menthol. The reaction parameters, such as the amount and hydrolytic activity of the lipase sources, the nature of the acyl donors, solvent hydrophobicity, lipase amount and introduction of several additives, were optimized to achieve the ideal conversions and enantioselectivities during the enzymatic acylation of *dl*-menthol (Scheme 1). The use of additives to improve the selectivity of the lipases for the kinetic resolution of menthol is the innovative aspect in this work.



Scheme 1 Enzymatic acylation reactions

Materials and methods

Chemicals and materials

All reagents and solvents were of analytical grade and were used as purchased from Sigma-Aldrich. Lipases of different sources were used as purchased without any pre-treatment. CRL type VII (specific activity = 1170 U/mg), lipase from *Porcine pancreatic* type II (PPL) (specific activity ≈ 100–500 U/mg) and *Candida antarctica* lipase immobilized on acrylic resin (CAL-B) (specific activity > 10,000 U/g) were purchased from Sigma-Aldrich. The *Pseudomonas cepacia* lipase (PCL) was purchased from Amano (specific activity > 30,000 U/g). The CCL was purchased from Fluka (specific activity = 2.8 U/ g). CRL is an extra-purified form of CCL, but these forms have very distinctive hydrolytic activities. The outcome of the reactions was monitored using TLC on Silica gel $60F_{254}$ plates (type MERCK5179), 250 mesh. After stirring for the appropriate time, the lipase was removed by simple filtration. The separation of the resulting acetates and the remaining alcohols was performed by column chromatography using silica gel 60 Å, 70–230 mesh, 63–200 μm using petroleum ether/ethyl acetate (v/v: 80/20) as eluent.

Instrumentation

The spectroscopic characterisation was performed with Brüker spectrometers (300 MHz for ^{1}H, 75 MHz for ^{13}C). Chemical shifts were reported in δ ppm from tetramethylsilane with solvent resonance as an internal standard for ^{1}H NMR and chloroform-d (δ 77.0 ppm) for ^{13}C NMR. Enantiomeric excesses were measured by gas chromatography on a ThermoFinnigan Trace GC, equipped with an automatic auto sampler and using a CHIRALSIL-DEX CB column (25 m; 0.25 mm; 0.25 μm). Retention times are reported in minutes. Optical rotations were determined using a Perkin-Elmer 241 Polarimeter at room temperature using a cell of 1 dm length and λ = 589 nm.

Experimental setup

Synthesis of racemic 2-isopropyl-5-methylcyclohexyl acetate (1a)

The menthyl acetate (**1a**) was synthesized by classical chemical acylation via the corresponding racemic menthol (1 equiv.), using 1.5 equiv. of acetic anhydride, 1.2 equiv. of Et_3N, and a catalytic amount of 4-dimethylaminopyridine (0.2 equiv.) in 5 mL of diethyl ether. The final products were obtained pure after standard work-up in excellent yield. The structure was confirmed by ^{1}H and $1^{3}C$ NMR spectra. Molecular formula: $C_{12}H_{22}O_2$. Crude oil. yield = 78%. R_f = 0.72. Eluent (*v,v*): petroleum ether/ethyl acetate (90/10).

^{1}H NMR (300 MHz, $CDCl_3$): **δ (ppm)** = 0.74–0.76 (m, 3H, cycl-C$\underline{H_3}$), 0.79–0.83 (m, 1H, CH_2cyclic), 0.87–0.90 (m, 6H, 2C$\underline{H_3}$), 0.91–0.98 (m, 1H,

CH_2cyclic), 1.01–1.10 (m, 1H, CH_2cyclic), 1.30–1.39 (m, 1H,CHcyclic), 1.41–1.53 (m, 1H,CHcyclic), 1.62–1.64 (m, 1H, CH_2cyclic), 1.66–1.69 (m, 1H, CH_2cyclic), 1.81–1.90 (m, 1H,CH), 1.94–2.00 (m, 1H, CH_2cyclic), 2.02 (s, 3H,O–C–CH_3), 4.62–4.71 (m, $1H_{cycl}$, O-CH_{cycl}).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.82, 74.2, 47.14, 41.06, 34.39, 31.50, 26.25, 23.63, 22.15, 21.47, 20.87, 16.52.

General procedures of enzymatic kinetic resolutions

Enzymatic acylation with enol esters

To 1 mmol of racemic alcohol (**1**), 3 mmol of the appropriate enol ester (isopropenyl acetate or vinyl acetate) and a catalytic amount of lipase were dissolved in 2 mL of organic solvent. The suspension was stirred at room temperature for the indicated time. The reaction mixture was filtered on Celite and concentrated in vacuum. The remaining alcohol and the produced acetate were separated by chromatography on silica gel (petroleum ether/ethyl acetate: 95/5) and analyzed by chiral GC. The same procedure was followed for the reactions in the presence of 30 mol% of additives.

Enzymatic acylation with succinic anhydride

A dry Schlenk tube was charged with rac-alcohol (1 equivalent) and succinic anhydride (1 equiv.) dissolved in 2 mL of diethyl ether. The reaction was initiated by the addition of 100 mg of CCL. The reaction mixture was shaken at room temperature for 24 h. After removal of the lipase by filtration, the filtrate was shaken with 1 M Na_2CO_3 solution, and the remaining alcohol and the produced monoester succinate were separated by liquid–liquid extraction. The remaining enantiomer was obtained from the organic layer and the aqueous phase was washed with an organic solvent and treated by adding 1 M NaOH solution to obtain the other enantiomer. The enantiomeric excesses values were quantified by chiral GC analyses.

Chiral GC: Chiralsil-DEX CB: (T_{column} = 120 °C. flow: 1,2 mL/min); dl-(±)-menthyl acetate: $t_{d\text{-}(+)}$ = 9.58 min; $t_{l\text{-}(-)}$ =l0.85 min. dl-(±)-menthol: $t_{d\text{-}(+)}$ = 13.25 min; $t_{l\text{-}(-)}$ = 13.69 min.

Results and discussion

First, we have examined the influence of hydrolytic activity of some lipase's source commercially available, as well as the catalytic loading during the acylation of *dl*-menthol **1** using isopropenyl acetate (IA) as acyl donor. All the experiments were carried out in diethyl ether as organic solvent, both in the presence and the absence of molecular sieves 4 Å, in order to regulate the water in the reaction medium. The conversions and the enantiomeric excesses of obtained acetates **1a** and the remaining alcohols **1** were quantified by chiral gas chromatography after their separations by flash chromatography. The results are summarized in Table 1.

Table 1 Transesterification of *dl*-menthol with various lipases in the presence and the absence of molecular sieves 4 Å

Entry	Lipase[a] (mg)	MS4 Å (mg)	Acyl donor	ee_s % [f](Yield %)[h]	ee_p %[f](Yield %)[h]	C(%)[g]	*E*[g]
1	PCL (80)	Without[b]	IA	–	–	NR	–
2	PPL (100)			–	–	NR	–
3	CAL-B (200)			–	–	NR	–
4	CAL-B (150)			–	–	NR	–
5	CAL-B (100)			–	–	NR	–
6	CRL (200)	Without[b]		20 (69)	> 99 (10)	17	> 200
7		With[c]		31 (61)	98 (18)	24	> 100
8	CRL (150)	Without[b]		**68 (42)**	> **99 (33)**	**41**	> **200**
9		With[c]		31 (62)	97 (20)	24	89
10	CRL (100)	Without[b]		**62 (45)**	> **99 (30)**	**38.5**	> **200**
11		With[c]		9 (ND)	> 99 (ND)	8.3	> 200
12	CCL (200)	Without[b]		54 (35)	98 (28)	35.5	> 100
13		With[c]		56 (30)	98 (28)	36.4	> 100
14	CCL (150)	Without[b]		44 (53)	98 (25)	31	> 100
15		With[c]		43 (54)	98 (23)	31	> 100
16	CCL (100)	Without[b]		39 (64)	> 99 (14)	28	> 200
17[d]				**66 (38)**	> **99 (32)**	**40**	> **200**
18		With[c]		39 (64)	> 99 (14)	28	> 200
19[d]				51 (40)	> 99 (19)	34	> 200
20		Without[b]	VA	64.4 (42)	95.2 (32)	40.3	80
21		With[c]		63.7 (38)	95.2 (30)	40	80
22[e]		Without[e]	SA	25 (39)	53 (19)	32	4

NR no reaction, *ND* not determined

Bold is to highlight the most important results

[a]*Pseudomonas cepacia* lipase from Amano (> 30,000 U/g), *Candida rugosa* lipase type VII (1170 U/mg), *Porcine pancreatic* lipase type II (100–500 U/mg), *Candida antarctica* lipase immobilized on acrylic resin (> 10,000 U/g) from Sigma-Aldrich. *Candida cylindracea* lipase from Fluka (2.8U/g)

[b]Reaction conditions : 1 mmol*dl*-menthol, 3 mmolenol ester (IA or VA), 2 mL diethyl ether with lipase for 24 h at room temperature

[c]With molecular sieve (4 Å) (20 mg)

[d]48 h at room temperature

[e]1 mmol*dl*-menthol, 1 mmol succinic anhydride, 2 mL diethyl ether with lipase for 24 h at room temperature

[f]Enantiomeric excess measured by chiral GC

[g]Conversion: $C = ee_s/ee_p + ee_s$; Selectivity: $E = \mathrm{Ln}\,[(1 - C)\,(11 - ee_{(S)})]/\mathrm{Ln}\,[(11 - C)\,(1 + ee_{(S)})]$ [44, 45]

[h]Isolated yield quantified after separation by flash chromatography

Among all the lipases screened, CRL and CCL gave the best conversions and high enantiomeric excesses as shown in Table 1. The transesterification of *dl*-menthol with IA in diethyl ether by employing lipases from sources such as PCL, PPL, CAL-B showed no progress even after prolonged reaction time (Table 1, entry 1–5). However, the lipases from CRL and CCL provided moderate to good enantioselectivity for *l*-menthyl acetate. Experiments carried out with and without molecular sieves illustrated its effect on the catalytic process, and this effect is particularly interesting. An increase in the amount of CRL was clearly in disfavor of the lipase reactivity without any perturbation of selectivity factor ($E > 200$) (Table 1, entries 6, 8 and 10), and the conversion decreased from $c = 41\%$ to $c = 17\%$ when the loading amount of the CRL was 150 and 200 mg, respectively (Table 1, entry 8 vs. 6). The same observation was made in our previous investigation on the influence of CCL amount during the acylation of ferrocenylethanol [29]. A drastic decrease of CRL reactivity by the introduction of the molecular sieves was observed when amounts of 150 and 100 mg were used (Table 1, entry 9 vs. 8 and 11 vs. 10). This effect strongly declined at 200 mg of CRL (Table 1, entry 7 vs. 9 and 11). In the case of CCL, no significant effects were observed in the presence of the molecular sieves either on reactivity or on selectivity. The decrease of the lipase amount exhibits optimization of the acylation advancement without affecting the selectivity (Table 1, entries 12, 14, 16 vs. entries 13, 15, 18).

The CRL and CCL lipases used are commercial and acquired from two different suppliers, and they are supposed to be differentiated solely by their hydrolytic activity; however, we found that only CRL was strongly influenced by the addition of the molecular sieves. This is probably due to the perturbation of the molecular water partition as well as to the reduction of its presence in the organic media which seems necessary for CRL. This result confirms the results of previous studies [46, 47] which note the need to add water to improve CRL conversion, and this even in the case of immobilized lipase. Both lipases are specific to (1R,2S,5R)-**1a**; the absolute configuration of these compounds is well known and *l*-menthyl acetate enantiomer is obtained. Due to the sensitivity of the free CRL, we have selected CCL as being less expensive and more stable as well as modestly studied for the resolution of the *dl*-menthol [40], with the appropriate catalytic load of $m = 100$ mg (280U). All the enzymatic reactions dedicated to the study of several parameters affecting the catalytic process were stirred for 48 h at room temperature (Table 1, entry 17). Other acetylating agents were used under optimized conditions: vinyl acetate (VA) and succinic anhydride (SA). With the first one, (-)-menthyl acetate **1a** was recovered with 95% ee_P at 40% of conversion with and without molecular sieves, but with a slight diminution of selectivity in the first case (Table 1, entries 20–21). On the other hand, a drastic decrease in the selectivity was shown using succinic anhydride as acetyl donor $E = 4$, regardless of the reactivity $c = 32\%$ (Table 1, entry 22). Since it is well known that the enantioselectivity of enzymes depends essentially on the polarity of the solvents, we have envisaged studying the dependence of both enol esters to the hydrophobicity of the employed organic solvent, and its impact on the CCL-catalyzed resolution of this α-substituted cycloalkanol. For that, the same optimum conditions were undertaken, using five

organic solvents with different LogP values: tetrahydrofuran (THF), diethylether (Et_2O), diisopropylether (DIPE), *tert*-butylmethylether (TBME) and heptane. All experiments were conducted without molecular sieves for 48 h. The obtained results are summarized in Table 2.

The results in Table 2 show very good enantioselectivity for *l*-(-)-menthyl acetate. The conversion and selectivity factors recorded depend on the nature of the enol ester and the solvent used (Fig. 1). The CCL maintains its high selectivity in the five solvents used using IA as acetyl donor ($E > 200$), the best conversion being achieved in diethylether $c = 40\%$ (Table 2, entry 7). Unfortunately, a denaturation effect of this yeast was noted in THF, TBME and heptane, where the conversion rate does not exceed 4% (Table 2, entries 6, 8 and 10), and a loss of reactivity is noted in DIPE, $c = 26\%$ (Table 2, entry 9).

We can conclude here that in some cases the nature of the organic solvent acts as an inhibitor factor of the CCL-catalyzed acylation using IA, whereas, a significant effect of the solvent hydrophobicity on the CCL reactivity and selectivity was revealed when vinyl acetate was used. In diethyl ether, TBME, DIPE and heptane, the CCL reactivity was still stable and the conversion varied between $33\% < c < 44\%$ (Table 2, entries 2–5). Moderate advancement was noted in THF $c = 12\%$ (Table 2, entry 1), while a drastic decrease of the selectivity factor was obtained in DIPE, $E = 44$ (Table 2, entry 4). It is to be underlined that the best results in terms of reactivity and selectivity are obtained with diethyl ether, TBME, DIPE as solvents during the acylation of *dl*-menthol by means of the free CCL. In

Table 2 Influence of enol ester and solvent hydrophobicity on the CCL-catalyzed resolution of *dl*-menthol

Entry	Enol ester[a]	Solvent (log *P*)	ee_S %[b] (Yield %)[d]	ee_P %[b] (Yield %)[d]	*C* (%)[c]	*E*[c]
1	VA	THF (0.48)	14 (55)	> 99 (7)	12	> 200
2		**Et_2O (0.85)**	**64 (42)**	**95 (32)**	**40**	**76**
3		**TBME (1.35)**	**54 (36)**	**> 99 (29)**	**35**	**> 200**
4		DIPE (1.4)	69 (46)	91 (36)	43	44
5		Heptane (4)	48 (40)	> 99 (25)	33	> 200
6	IA	THF (0.48)	3 (ND)	> 99 (ND)	3	> 200
7		**Et_2O (0.85)**	**66 (38)**	**> 99 (32)**	**40**	**> 200**
8		TBME (1.35)	4 (ND)	> 99 (ND)	4	> 200
9		DIPE (1.4)	34 (ND)	> 99 (ND)	26	> 200
10		Heptane (4)	4 (ND)	> 99 (ND)	4	> 200

ND not determined

Bold is to highlight the most important results

[a]Reaction conditions: 1 mmol*dl*-menthol, 3 mmolenol ester, 2 mL organic solvent with *CCL* (280U) for 48 h at room temperature

[b]Enantiomeric excess are measured by chiral GC

[c]Conversion : $C = ee_S/ee_P + ee_{s;}$ selectivity: $E = \text{Ln}\ [(1 - C)\ (1 - ee_{(S)})]/\text{Ln}\ [(1 - C)\ (1 + ee_{(S)})]$ [44, 45]

[d]Isolated yield quantified after separation by flash chromatography

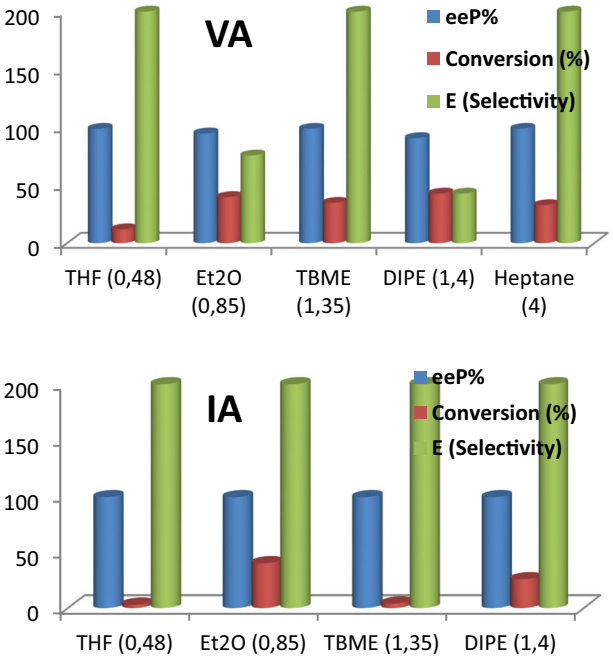


Fig. 1 Effect of the solvent on the CCL performance in kinetic resolution of *dl*-menthol

addition, it was found that the highest selectivity and reactivity were obtained with vinyl acetate as the acylating agent. To the best of our knowledge, these results are the first to have been described.

Finally, an attempt at optimization of the CCL-catalyzed resolution of menthol was carried out by the introduction of some additives directly into the organic medium. This alternative used in our previous works has shown that the activity of some lipases in kinetic resolution could be highly influenced by the alkaloid additives [25]. Therefore, we selected the acylation conditions, using vinyl acetate as acetyl donor and as additives: crown ether (18-C-6), a mineral base (Na_2CO_3) and organic base (NEt_3), as well as two chiral Lewis bases, alkaloids type (cinchonidine and quinidine). A catalytic amount of those additives were introduced directly following the previously described procedures [25, 26]. The obtained results are summarized in Table 3.

Introduction of quinidine (30 mol%) in CCL-catalyzed acylation of *dl*-menthol in TBME, exhibited a large activation of the catalytic process. The conversion rate achieve the threshold of 49% and *l*-(-)-menthyl acetate **1a** was obtained at high enantiomeric excess, ee_P = 93% (Table 3, entry 3) and E = 80. In diethyl ether, the recorded conversion was 56% with a drastic decrease of selectivity, E = 30, and the remaining alcohol, *d*-(+)-menthol was recovered at ee_S = 97% (Table 3, entry 1). The use of heptane as an organic solvent causes a significant enhancement of the CCL reactivity during the acylation of *dl*-menthol, as the enantiodiscrimination of CCL disappears and the reaction velocity of both menthol enantiomers are quasi-

Table 3 Influence of the additives on the outcome of the CCL-catalyzed resolution of *dl*-menthol

Entry	Additive[a]	Solvent (log *P*)	ee_S %[b](Yield %)[d]	ee_P %[b](Yield %)[d]	*C* (%)[c]	*E*[c]
1	Quinidine	**Et_2O (0.85)**	**97 (42)**	**75 (48)**	**56**	**30**
2		Heptane (4)	27 (20)	9 (55)	75	1
3		**TBME (1.35)**	**88 (38)**	**93 (39)**	**49**	**80**
4	Cinchonidine	Et_2O (0.85)	–	–	NR	–
5		Heptane (4)	40 (44)	> 99 (20)	30	> 200
6		TBME (1.35)	45.5 (40)	98 (22)	32	> 100
7	Na_2CO_3	TBME (1.35)	42 (40)	98 (20)	30	> 100
8	Et_3N		24 (57)	98 (12)	19.6	> 100
9	18-C-6	Et_2O (0.85)	61 (52)	> 99 (21)	38	> 200
10		TBME (1.35)	24 (52)	98 (12)	20	> 100

Bold is to highlight the most important results

[a]Reaction conditions: 1mmol*dl*-menthol, 3 mmol vinyl acetate, 2 mL organic solvent with *CCL* (280U), 30 mol% additive for 48 h at room temperature

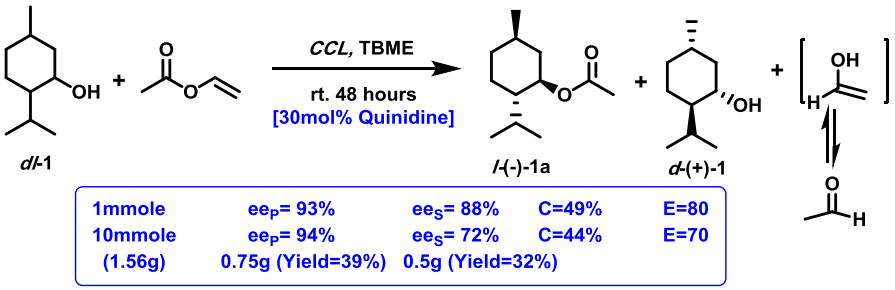
[b]Enantiomeric excesses are measured by chiral GC

[c]Conversion : $C = ee_S/ee_P + ee_{s;}$ selectivity: $E = \text{Ln}\ [(1 - C)\ (1 - ee_{(S)})]/\text{Ln}\ [(1 - C)\ (1 + ee_{(S)})]$ [44, 45]

[d]Isolated yield quantified after separation by flash chromatography

equal (c = 75% and E = 1) (Table 3, entry 2). On the other hand, no effect was observed on the CCL performance when cinchonidine was added to both heptane and TBME (Table 3, entry 5, 6), whereas the catalytic activity was totally inhibited in diethyl ether (Table 3, entry 4). The addition of Na_2CO_3, a weak mineral base, led to a slight diminution of the conversion from c = 35% without additive to c = 30% with it (Table 3, entry 7), and this effect was amplified by the addition of Et_3N, an organic base, c = 19% (Table 3, entry 8). Moreover, the introduction of an ether crown-type additive, 18-C-6, led to a more drastic decrease of the conversion rate with TBME as solvent, c = 20% than in diethylether, c = 38% (Table 3, entries 9, 10). Thus, the direct introduction of a catalytic amount of a natural additive, such as quinidine, significantly optimizes the catalytic performance of CCL during the acylation of *dl*-menthol using vinyl acetate in TBME, so that this solvent is an alternative to diethyl ether in terms of environmental exigencies [48, 49]. To the best of our knowledge, this result is the first described using such an additive to access *l*-menthol quantitatively at high enantiomeric purity. In order to valorize this simple and easy methodology under mild conditions, we have applied it at a larger scale (Scheme 2).

Optimal conditions were undertaken and applied to resolve 10 mmol (1.5 g) of racemic menthol using 30 mmol of vinyl acetate and 1 g of CCL in the presence of 30 mol% (0.97 g) of quinidine as additive and dissolved in 20 mL of TBME. The suspension was stirred at room temperature for 48 h. After filtration, the remaining alcohol and the produced acetate were separated by chromatography on silica gel (petroleum ether/ethyl acetate: 95/5) and analyzed by chiral GC. The reaction



Scheme 2 Multigram scale of acylation of *dl*-menthol with quinidine

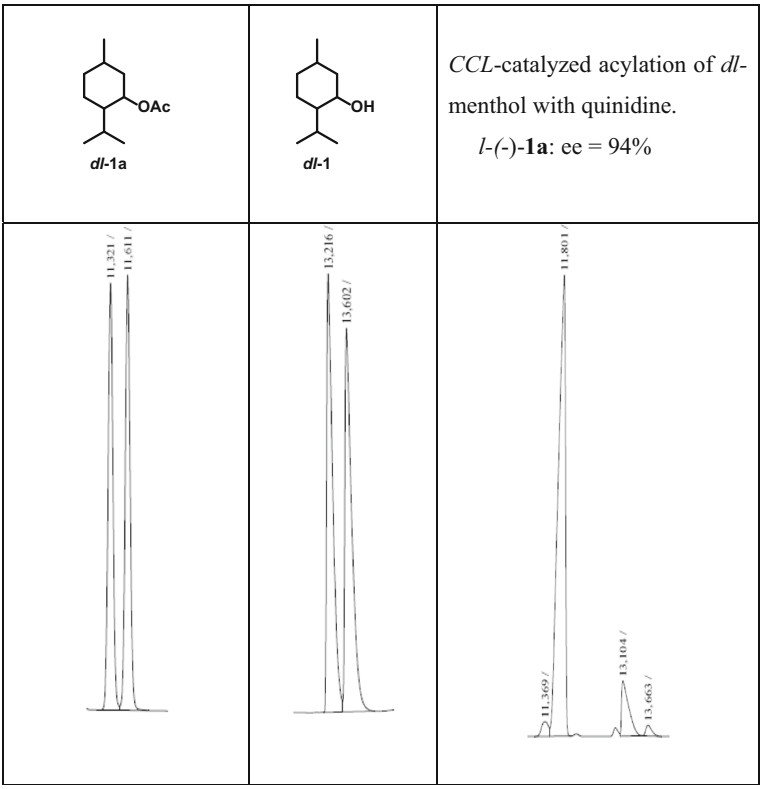


Fig. 2 Chromatograms of scale-up acylation of *dl*-menthol

results were reproduced, and the most important *l*-(-)-**1a** enantiomer was obtained quantitatively with ee_p = 94% under mild conditions (Fig. 2).

Conclusion

The kinetic resolution of *dl*-menthol by CCL-catalyzed acylation was optimized with vinyl acetate and quinidine as additives in TBME as solvent. Optimal conversion, $c = 49\%$,and high selectivity, $E = 80$, were obtained under optimized conditions with quinidine as additive. The reaction parameters such as enzyme load, the nature of enol esters and organic solvents and water activity were found to have profound effects on the conversion and enantioselectivity. The comparison of the behavior of CRL and CCL for the enantioselective acylation of *dl*-menthol showed a greater stability of CCL activity and did not depend on the water content. The gram-scale reaction was efficient and constitutes a new approach to the enantioselective transesterification of *dl*-menthol. Another interesting aspect of these results is the use of TBME as a substitute for diethyl ether, which is considered a highly hazardous solvent. We can expect to find this alternative in a preparative production of optically active *l*-menthol enantiomers which constitutes a simple protocol for the production of the enantiomerically enriched *l*-menthyl acetate.

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Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

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