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Enzymatic kinetic resolution of tropic acid

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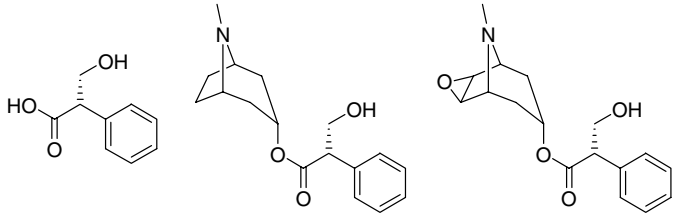
Abstract—A new strategy has been developed for the CAL-B catalysed kinetic resolution of tropic acid by which both enantiomers of tropic acid can be obtained in good enantiomeric excess. (*R*)-Tropic acid was synthesised with 90% ee and (*S*)-tropic acid butyl ester in 99% ee by the hydrolysis of tropic acid butyl ester. The other enantiomers were available through the enzymatically catalysed reaction of tropic acid lactone with butanol to give (*S*)-tropic acid lactone and (*R*)-tropic acid ester in >98% ee.

1. Introduction

(*S*)-Tropic acid (*S*)-**1** is an important building block for biologically active alkaloids such as hyoscyamine **2** and hyoscine **3** (Fig. 1), which are well-established pharmaceuticals. The synthesis of the enantiomers of tropic acid **1** is a big challenge, since this compound racemises under alkaline conditions. Analogously, hyoscyamine and hyoscine racemise easily into their racemates atropine and scopolamine. The enantiomers of tropic acid can not only be applied in alkaloid synthesis but are also building blocks in polyester synthesis. Poly-3-hydroxyacids are of great interest for bioerodable and biodegradable polymers,[1] while the enantiomeric ratio of the monomers has a dramatic effect on the crystallinity and solubility of the resulting polymer.[2,3]

The enzymatic kinetic resolution of 2-arylpropionic acids, mainly ibuprofen,[4–12] and related structures,[13–19] has been studied intensively, but any literature on the enzymatic kinetic resolutions of 2-aryl-3-hydroxycompounds such as tropic acid, is scarce. Only recently, was an enzymatic kinetic resolution of this hydroxy acid utilising lipase PS (*Pseudomonas cepacia* lipase) to target the primary alcohol reported.[20] The ethyl ester of tropic acid was resolved, to give (*S*)-acetoxy tropic acid ethyl ester in 39% yield and 87% ee and (*R*)-tropic acid ethyl ester in 46% yield and 94% ee. Until now, only a few reports on the resolution or enantioselective synthesis of tropic acid have been published. So far, the most convenient method for the preparation of enantiopure (*S*)-tropic acid is the hydrolysis of hyoscyamine and similar compounds isolated from plants. Racemic tropic acid can be resolved in various ways, for example, by employing quinine[21] or other co-crystallisation agents,[22] preparative LC[23] or electrophoreses.[24] A report on an elaborate synthesis of (*R*)-tropic acid appeared a few years ago.[25]



(*S*)-tropic acid **1** hyoscyamine **2** hyoscine **3**

Figure 1. Tropic acid and some tropic acid containing alkaloids.

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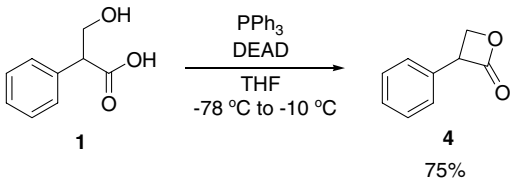
Herein, we report a successful strategy for the enzymatic kinetic resolution of tropic acid. In contrast to the enzymatic resolution described in the literature, this can be achieved through functionalisation of the carboxylate rather than of the primary alcohol group.

2. Results and discussion

2.1. Screening of enzymes for the hydrolysis of esters of tropic acid

A hydrolysis reaction was chosen as an enzyme screening experiment, since in this way the reaction could be followed in real time by monitoring the hydroxide consumption. Initially, tropic acid lactone **4** was studied as a test substrate. This β-lactone was synthesised via a Mitsunobu reaction in 75% yield (Scheme 1).[26] The major advantages of this compound are that both the hydroxyl and carboxylic acid groups are protected and that the ester is activated as a result of the ring tension in the four-membered lactone. The dual protection also blocks polyester formation. The hydrolysis reactions were carried out at 25 °C in a 10 mM phosphate buffer at pH 7.



Scheme 1. Synthesis of tropic acid lactone **4**.

The blank reaction, however, showed that **4** is too reactive in the buffer. The starting material was converted to racemic **1** within 24 h and therefore tropic acid butyl ester **5** was chosen as an alternative test substrate. The blank reaction now showed <2% conversion in 24 h. The hydrolysis (Scheme 2) was performed with the commercially readily available enzymes that were earlier used successfully in the kinetic resolution of phenylpropionic acids.

From the enzymes tested (see Table 1), CAL-B showed the most promising results. After 18 h, (*R*)-tropic acid (*R*)-**1** and (*S*)-tropic acid butyl ester (*S*)-**5** were obtained in 90% and >99% ee, respectively. CAL-B is not generally known to resolve racemic acids, although a few examples are known in which a transesterification with amines[27,28] or alcohols[29,30] takes place. A successful application of the resolution of α-methylene β-lactones using this enzyme has also been reported.[31] Some of the other enzymes tested showed unexpectedly low activity towards this substrate. CRL was reported to be successful in the resolution of 2-aryl propionic acids,[9] but apparently the additional hydroxyl group of tropic acid renders this enzyme inactive towards ester **5**. The enzyme α-CT, which promoted the hydrolysis of 2-benzyl-3-hydroxypropanoic acid methyl ester, although not enantioselectively,[32] does not show any reactivity with tropic acid as the substrate. The lactone (3*R*,4*S*)-3-benzyl-4-(bromoethyl)oxetan-2-one, a similar compound to **4**, was reported to bind to and act as an inhibitor for this enzyme.[33] Inhibition of the enzyme with **4** or **5** in an analogous pathway (an S_N2 reaction forming an ether) is unlikely to take place, however, still no reaction was observed. Subtilisin showed some activity towards the hydrolysis of the tropic acid butyl ester, but the reaction took over 4 days to complete.

Table 1. Enzymes screened for the hydrolysis of **5** and their activity

Enzyme	Reaction time (h)[a]	Initial reaction rate (mmol/h)[b]
Acylase	—	—
α-Chymotrypsin	—	—
Achromobacter sp. lipase	—	—
Candida rugosa lipase	—	—
Candida antarctica lipase B	18	0.071
Humicola lanuginosa lipase	—	—
Pig liver esterase	—	—
Rhizomucor miehei lipase	—	—
Subtilisin	96	0.018

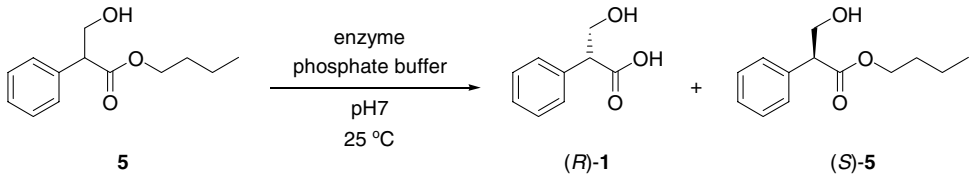
— Indicates no activity.
[a] Time needed for separation of the enantiomers.
[b] Initial reaction rate for 200 U enzyme.

Attempts at the hydrolysis of tropine acetate with CAL-B or subtilisin were unsuccessful, hence a coupling with tropic acid to synthesise hyoscyamine by these enzymes seemed to be unfeasible. The two parts are probably too bulky to be able to fit in the active site, preventing an enzymatic coupling. Based on the results described, CAL-B was selected as the enzyme for the enzymatic kinetic resolution of tropic acid.

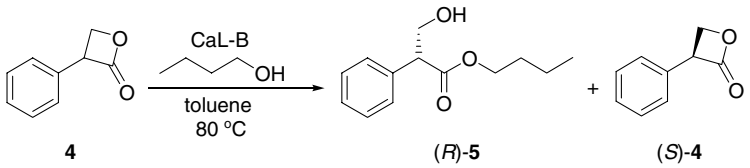
2.2. Enzymatic kinetic resolution in toluene

With an enzyme in hand that was proven to be very stable and selective in organic solvents, our attention next focussed on the esterification reactions in toluene. Therefore, β-lactone **4**, which is activated due to the ring strain, was selected as the starting compound.

The enzymatic resolution of **4** with butanol, employing CAL-B as the catalyst in toluene, gave a very fast and clean reaction towards butyl ester (*R*)-**5** (Scheme 3).



Scheme 2. Hydrolysis of tropic acid butyl ester **5**.



Scheme 3. Enzymatic resolution of tropic acid lactone **4**.

The resolution of lactone **4** towards (*R*)-**5** and (*S*)-**4** proceeds within 70 min with an initial reaction rate of 2.6 mmol/h/200U (Scheme 3, Fig. 2). Both compounds were obtained in an enantiomeric excess of >98% in almost quantitative yields. With longer primary alcohols such as octanol, the results and reaction times were similar. The inversion of the stereoselectivity of the enzyme by the long-chain alcohols which was shown for CRL in the esterification of 3-hydroxybutyric acid,[34] was not observed for CAL-B. Remarkably, CAL-B does not attack the primary alcohol that is released upon ring opening of the lactone, no dimers or oligomers were formed during the experiment. It is also striking that in the resolution of the similar 2-phenylbutyric acid vinyl ester with butanol, the product is also the (*R*)-butyl ester.[29] This means that the relative stereochemistry is opposite from that observed with lactone **4**. Apparently, substitution of the hydroxyl group in tropic acid by a methyl group causes the enzyme to change its preference for the different groups in the molecule.

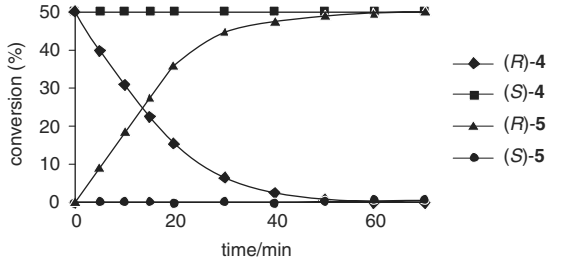


Figure 2. Reaction progress of the resolution of tropic acid lactone **4**.

Since an enzymatic coupling of tropic acid and tropine was not possible, a chemical coupling of (*S*)-**4** and *endo*-tropine was attempted. Due to the alkaline properties of the tertiary amine in tropine, the lactone was easily opened in this way. However, the stereogenic centre in tropic acid is also readily racemised under these conditions. The classical syntheses of atropine by refluxing the two components in hydrochloric acid[35,36] or by treating tropic acid subsequently with acetyl chloride, thionyl chloride and tropine hydrochloride[21] do however offer an alternative route, starting from enantiopure (*S*)-tropic acid and will give enantiopure hyoscyamine.

3. Conclusion

The enzymatic resolution of tropic acid with CAL-B was very successful using the strategies described. Both (*R*)- and (*S*)-tropic acid butyl esters can be obtained in >98% ee and high yields. The complementary (*S*)-tropic acid and (*R*)-tropic acid lactone were also obtained in high yields in 90% and >98% ee, respectively. As described earlier, the primary alcohol group of tropic acid is not a substrate for CAL-B[20] and therefore, no polyesters were formed.

CAL-B is an outstanding enzyme for the resolution of this β-hydroxy acid and it can be expected that it is also a good enzyme for the resolution of 3-hydroxy-2-aryl-propionic acids in general.

4. Experimental

4.1. General remarks

All experiments were performed in dried glassware under a nitrogen atmosphere unless stated otherwise. All chemicals were purchased from Aldrich or Acros. Anhydrous solvents and solids were used as received. Enzymes were purchased from Sigma, immobilised *Candida antarctica* lipase B (CAL-B) as Chirazyme L2,c-f.C2, lyo was a gift from Roche Diagnostics. Enantiomeric excesses were determined and reactions were followed by gas chromatography using a Shimadzu GC-17A gas chromatograph, equipped with a 25 m × 0.32 mm chiral column Chrompack™ Chirasil-Dex CB, split injector (1/97) at 220 °C, a Flame Ionisation Detector at 220 °C and He as carrier gas. Retention times (min) at 120 °C isotherm: (*R*)-3-phenyl-oxetan-2-one **4** (9.9), (*S*)-3-phenyl-oxetan-2-one **4** (10.7), (*S*)-3-hydroxy-2-phenyl-propionic acid butyl ester **5** (56.3), (*R*)-3-hydroxy-2-phenyl-propionic acid butyl ester **5** (58.4), (*R*)-3-hydroxy-2-phenyl-propionic acid methyl ester (63.9), (*S*)-3-hydroxy-2-phenyl-propionic acid methyl ester (70.0). NMR spectra were recorded on a Varian Unity Inova-300 spectrometer at 25 °C. For column chromatography, Fluka silica gel 60 was used and Merck aluminium sheets with silica gel 60 F_{254} were used for TLC. Elution was carried out with mixtures of petroleum ether 40–65 °C (PE) and ethyl acetate (EtOAc).

4.2. 3-Hydroxy-2-phenyl-propionic acid butyl ester 5

Tropic acid (3.3 g, 20 mmol) and butanol (1.8 mL, 20 mmol) were dissolved in toluene (50 mL). The mixture was refluxed in a Dean–Stark set-up for 6 h and the volatiles evaporated. After column chromatography (PE/EtOAc 17:3), 3.8 g (17 mmol, 85%) of the ester **5** was obtained as a liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, $J = 7.20$ Hz, CH_3), 1.25 (sextet, 2H, CH_2–CH_3), 1.56 (quintet, 2H, $J = 7.20$ Hz, CH_2–CH_2–

CH_3), 2.38 (br s, 1H, O*H*) 3.83 (t, 2H, $J = 5.70$ Hz, CH_2–CH_2–CH_2–CH_3), 4.04–4.18 (m, 3H, C*H*–CH_2–OH), 7.22–7.40 (m, 5H, Ar*H*).

4.3. 3-Phenyl-oxetan-2-one 4

DEAD (diethyl azodicarboxylate) (1.89 mL, 12 mmol) was added dropwise to a stirred solution of triphenylphosphine (3.18 g, 12 mmol) in THF (40 mL) at −78 °C. After about 30 min, the suspension became white and then a solution of tropic acid (2.00 g, 12 mmol) in THF (40 mL) was added dropwise. The resulting mixture was stirred and warmed in about 2 h to −10 °C, at which temperature the solution was homogeneous. After concentration at room temperature and purification by column chromatography (PE/EtOAc 17:3), the product was isolated as a clear liquid in 1.30 g yield (9.00 mmol, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 4.34$ (dd, 1H, $J = 4.76$, 5.12 Hz, CH_2), 4.64 (dd, 1H, $J = 5.12$, 6.78 Hz, CH_2), 4.92 (dd, 1H, $J = 4.76$, 6.78 Hz, CH), 7.26–7.42 (m, 5H, Ar-H), ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 56.91$ (*C*H), 66.38 (CH_2), 127.15 (2 × Ar-*C*), 128.31 (Ar-*C*), 129.19 (2 × Ar-*C*), 132.63 (Ar-*C*), 169.54 (*C*O).

4.4. Enzyme activity test, general procedure[37]

In the enzymatic resolution experiments, 200 U of enzyme was added to the reaction mixture. The activity of the enzymes was determined in an experiment using an automatic burette. Tributyrin (1.47 mL, 5.0 mmol) was added to a 10 mM potassium phosphate buffer of pH 7 (48.5 mL). The mixture was stirred at 25 °C and the enzyme was added. The pH was kept constant by the addition of 0.1 M NaOH solution. The activity was determined by the addition of NaOH per minute when the reaction rate was constant. 1 unit (1 U) corresponds to 1 μmol of NaOH solution added per minute.

4.5. General procedure for the hydrolysis of 3-hydroxy-2-phenyl-propionic acid butyl ester 5

3-Hydroxy-2-phenyl-propionic acid butyl ester (222 mg, 1 mmol) was added to a 10 mM phosphate buffer at pH 7 (10 mL) at 25 °C. The enzyme (200 U) was added and the pH kept constant by the addition of a 0.1 M NaOH solution with an automatic burette. Then, using CAL-B (60 mg) as the enzyme, the reaction was stopped after 18 h. At neutral pH, ester (*S*)-**5** was extracted with ether and after decreasing the pH to about 2 with a few drops of concentrated hydrochloric acid, the acid (*R*)-**1** was extracted with toluene yielding (*S*)-tropic acid butyl ester (*S*)-**5** (95 mg, 0.43 mmol, 43%, ee >99%) and (*R*)-tropic acid (*R*)-**1** (105 mg, 0.47 mmol, 47%, ee 90%). The ee of (*R*)-**1** was determined by GC analysis after conversion with diazomethane into its methyl ester.

4.6. (*R*)-3-Hydroxy-2-phenyl-propionic acid butyl ester (*R*)-5 and (*S*)-3-phenyl-oxetan-2-one (*S*)-4

3-Phenyloxetan-2-one **4** (296 mg, 2 mmol) and 1-butanol (0.18 mL, 2 mmol) were dissolved in dry toluene (4 mL). The temperature was raised to 80 °C and CAL-B (50.0 mg, 160 U) added. After 70 min, the reaction was stopped by filtering off the enzyme. The filtrate was concentrated. The products were separated by column chromatography (PE/EtOAc 17:3) giving 204 mg 0.94 mmol, 47%, ee >98%, $[\alpha]_D^{25} = +40.0$ (*c* 3.4, $CHCl_3$) of (*R*)-3-hydroxy-2-phenyl-propionic acid butyl ester (*R*)-**5** as a colourless liquid and 139 mg 0.92 mmol, 46%, ee >98%, $[\alpha]_D^{25} = +24.0$ (*c* 4.0, $CHCl_3$), mp: 45.6 °C of (*S*)-3-phenyl-oxetan-2-one (*S*)-**4** as a white crystalline solid. Hydrolysis of (*S*)-**4** yielded (*S*)-**1** with $[\alpha]_D^{25} = -63.3$ (*c* 0.3, acetone); Lit.[21] $[\alpha]_D^{15} = -83.3$ (*c* 1.8, acetone).

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