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A Chemoenzymatic Process to Achieve Optically Active Mandelic Acid Employing Continuous-Flow Resolution of Cyanohydrin as a Key Step

Síntese Químio-Enzimática do Ácido Mandélico Opticamente Ativo Empregando a Resolução em Fluxo Contínuo do Precursor Cianoidrina como Etapa Chave

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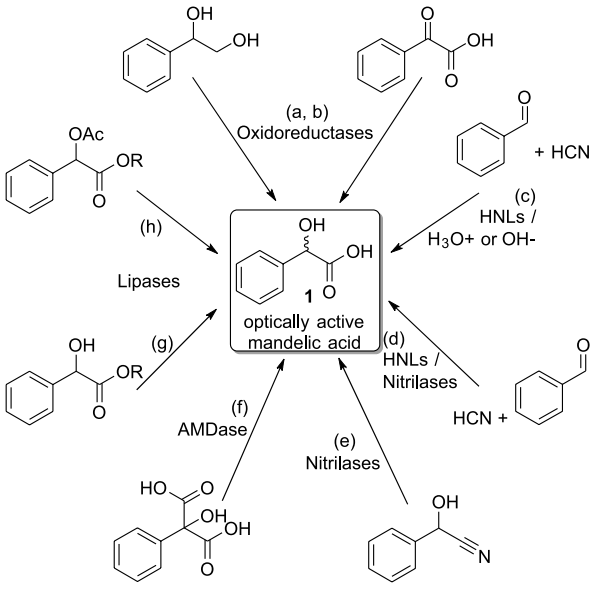
Optically active mandelic acid is important to organic synthesis in stereochemical investigations and as a precursor of different pharmaceuticals. Therefore, several synthetic methodologies have been developed for its synthesis, including biocatalytic approaches. Among the biocatalyzed reactions, lipase-mediated enzymatic kinetic resolution (EKR) reactions are advantageous, since both enantiomers can be achieved. However, limitations are observed in direct EKR of mandelic acid and its ester derivatives, related to enzyme inhibition and structural dependence to achieve high enantioselectivity. Herein, a chemo-enzymatic approach is proposed to achieve optically active mandelic acid from a continuous-flow lipase-mediated resolution of mandelonitrile followed by separation and chemical hydrolysis of nitrile moiety. Using this strategy, both mandelic acid enantiomers were obtained in good isolated yields and high enantiomeric excesses (*R* enantiomer: 70% yield; e.e. = 94%; *S* enantiomer: 80% yield; e.e. = 98%).

Keywords: Flow chemistry; kinetic resolution; cyanohydrins; chemoenzymatic synthesis; mandelic acid

1. Introduction

Optically active α-hydroxyacids are very important to enantioselective synthesis.[1] For instance, mandelic acid enantiomers are widely used in stereochemical investigations as resolution agents[2-5] and as precursors of several synthetic pharmaceuticals,[6, 7] such as oxybutynin,[8] cyclandelate,[9] pemoline,[10] besides several mandelate esters derivatives with important biological activity.[11] Due to importance of optically active mandelic acid and the high costs associated with buying enantiopure substances,[12] several synthetic methodologies have been developed over the years, including stereoselective biocatalyzed reactions,[13] to synthesize mandelic acid in its optically active form. Biocatalytic approaches include oxidoreductases-mediated oxidation of primary alcohol[14-18] or ketone reduction[19-23] (Scheme 1 – a, b); hydroxynitrile lyases-mediated (HNLs) addition of hydrogen cyanide to benzaldehyde, followed by chemical hydrolysis[24, 25] (Scheme 1 – c) or coupling with nitrilases[26-29] (Scheme 1 – d); classic and dynamic kinetic resolution of mandelonitrile employing nitrilases[30-38] (Scheme 1 – e); decarboxylation mediated by arylmalonate decarboxylase[39, 40] (AMDase; Scheme 1 – f); enzymatic kinetic resolution (EKR) mediated by lipases, via acylation[41-51] (Scheme 1 – g) or deacylation[52-57] (Scheme 1 – h) of mandelic acid or its ester derivatives.

EKR approaches are well established in literature since they allow to isolate both enantiomers in the process.[58] Moreover, several advantages related to the use of lipases can be observed, such as high chemo, regio and stereoselectivity, they do not need any cofactor and display a broad substrate specificity, among others.[59, 60] Lipase-mediated EKR of mandelic acid and derivatives is not unheard, but several drawbacks are associated to it. Reactions involving the hydroxyl moiety (Scheme 1 – g and h) in mandelate esters are strongly dependent of ester moiety, taking to products, sometimes, with low stereoselectivity.[61, 62] Only few data are available concerning to the use of carboxylic functional group of mandelic acid in esterification[63, 64] reactions or its ester derivatives in hydrolysis[65, 66] and amination[67] reactions. Furthermore, it is known that mandelic acid oneself can cause lipase inhibition in high concentrations.[68] An alternative and less exploited approach to synthesize optically active mandelic acid is lipase-mediated EKR of its cyanohydrin derivatives. Although EKR of cyanohydrins usually requires long reaction times to achieve high enantiomeric excesses, we



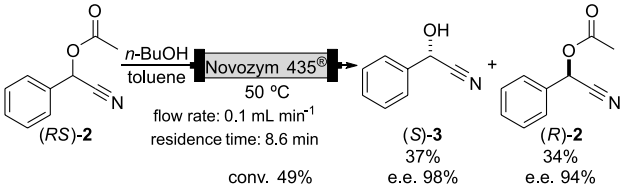
Scheme 1. Biocatalytic routes to directly achieve optically active mandelic acid (**1**)

had developed a protocol to perform EKR in continuous-flow mode,[69] which can aggregate many advantages when combined with biocatalysis, as well as fitting in some principles of green chemistry.[70-73] Employing this protocol, cyanohydrins could be obtained with high productivity, presenting short reaction times and without any loss of lipase enantioselectivity.[69] Now, as a proof of concept, we have expanded our approach towards the synthesis of optically active mandelic acid, employing two sequential reactions: the EKR of a mandelonitrile precursor via deacylation in continuous-flow mode followed by an acidic hydrolysis, presenting good enantioselectivity and yields.

2. Results and Discussion

2.1. Enzymatic kinetic resolution in continuous-flow mode

The first step was a preparative EKR reaction of mandelonitrile acetate ((*RS*)-**2**) in continuous-flow mode. For this, a continuous-flow reactor was filled with the supported biocatalyst (Novozym 435®) and reagents were pumped into it employing a flow rate of 0.1 mL min^{-1} for 2 cycles (total residence time 8.6 min), as previously optimized,[69] resulting in 49% conversion and yielding (*S*)-**3** and (*R*)-**2** in high optical purity (98% and 94% enantiomeric excess) (see Experimental Section and Supplementary Material for details) (Scheme 2).



Scheme 2. Continuous-flow EKR of mandelonitrile acetate ((*RS*)-**2**)

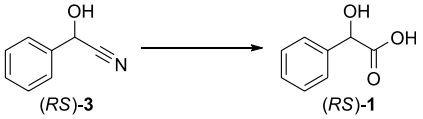
Compounds (*S*)-**3** and (*R*)-**2** were purified using column chromatography, and could be isolated in high yields (37% and 34%). These compounds were employed as optically active starting materials in the synthesis of mandelic acid enantiomers.

2.2. Synthesis of optically active mandelic acid

After the enzymatic step, we believed nitrile moiety of enantioenriched (*S*)-**3** would be easily hydrolyzed to produce the corresponding (*S*)-mandelic acid. However, very low yields and cyanide retro-addition reaction were observed employing that optically active starting material. Thus, we decided to optimize the reaction conditions for nitrile hydrolysis employing racemic mandelonitrile (*RS*-**3**) as starting material. For this, different acid sources for hydrolysis were investigated, as well as the extraction procedure (Table 1).

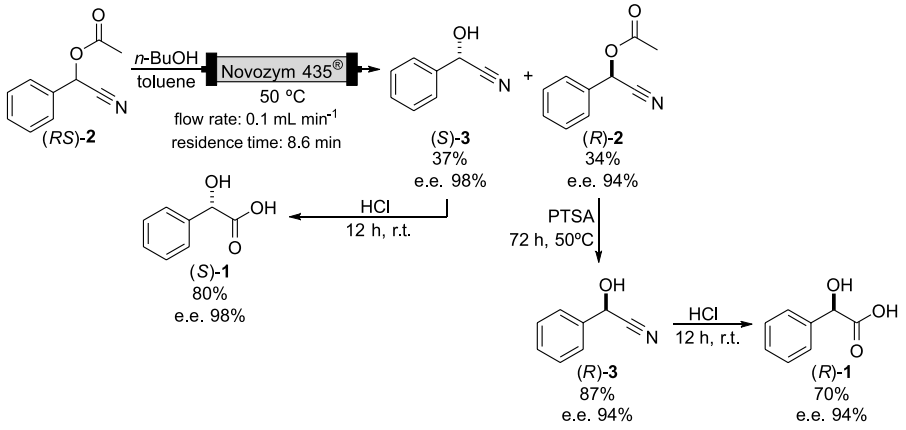
Sulphuric acid was tested as an acid source for hydrolysis (Table 1 – entry 1), resulting in a very low conversion rate and low yield (<10%). When *p*-toluenesulphonic acid (PTSA. H_2O) was tested, no conversion of mandelonitrile (*RS*-**3**) into mandelic acid (*RS*-**1**) was observed, neither at room temperature nor when heated to 80 °C. Only by using of concentrated HCl as acid source mandelic acid formation

Table 1. Hydrolysis of *RS*-**3** to mandelic acid (*RS*-**1**)



Entry	Reagents	Note	Yield	Ref.
1	H_2SO_4[a,b]	Liquid-liquid extraction[d]	<10	-
2	PTSA and H_2O in toluene	Liquid-liquid extraction[d]	-	-
3	Hot HCl[a,b]	Liquid-liquid extraction[d]	37	74
4	HCl[a,c]	Liquid-liquid extraction[d]	41	75
5	HCl[a,c]	Solvent evaporation	74	75

[a] 12 mol L^{-1}; [b] 80 °C; [c] Room temperature; [d] Organic solvent: Et_2O



Scheme 3. Chemo-enzymatic route to synthesize optically active mandelic acid

was observed. Reaction employing HCl at room temperature resulted in higher yield (41%) than when hot HCl (80 °C) was used, since reactions were carried out in open vessels and high temperature caused some loss by evaporation (Table 1 – entries 3 and 4). It is worth comment that, due to mandelic acid high solubility in water, extractions using ethyl acetate, dichloromethane or diethyl ether were considered not efficient. Among the cited organic solvents, diethyl ether was the best one resulting in 40.6% yield (Table 1 – entry 4).

The switch of extraction procedure to evaporation of reaction media (HCl 12 mol L^{-1}) in a water steam bath resulted in a significant increase of reaction isolated yield to 74% (Table 1 – entry 5).

The optimized reaction conditions for the whole processes were: HCl 12 mol L^{-1} as acid source and solvent, room temperature, solvent evaporation and purification by recrystallization in benzene (Table 1 – entry 5). After the optimization for racemic standard, reaction conditions were applied in the synthesis of optically active mandelic acid.

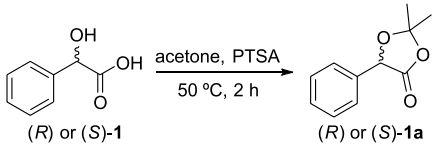
It is important to highlight that, for mandelonitrile acetate ((*R*)-**2**), nitrile hydrolysis employing previously optimized conditions led to very low yields. Due to this fact, we first hydrolyzed acyl moiety of (*R*)-**2** to give (*R*)-**3** and then employed it as start material for nitrile hydrolysis.

Finally, mandelonitrile enantiomers ((*R*)- and (*S*)-**3**) were hydrolyzed and optically active mandelic acid could be obtained in good yields (70% for *R* and 80% for *S* enantiomer). After all reaction steps, mandelic acid enantiomers could be synthesized in 21% (for *R* enantiomer) and 30% (for *S* enantiomer) global yield.

The entire synthetic route is presented in Scheme 3.

Since mandelic acid detection was not possible via gas chromatography, in order to determine the enantiomeric excesses of (*R*) and (*S*) mandelic acid, they were converted to their corresponding 2,2-dimethyl-5-phenyl-1,3-dioxolane-4-one (**1a**) derivative (Scheme 4) (see Experimental Section for details), resulting in 94% and 98% enantiomeric excess, respectively, indicating that there was no loss of optical purity in the hydrolysis step.

In summary, a successfull chemo-enzymatic process was applyied to synthesize optically active mandelic acid.



Scheme 4. Derivatization of mandelic acid (**1**) to compound **1a**

In this process, mandelonitrile was produced by a previsouly developed continuous-flow resolution protocol in high optically purity and, after an extensive study of nitrile moiety hydrolysis, mandelic acid was then synthesized in good yields and high optically purity.

3. Conclusions

A chemo-enzymatic protocol to synthesize optically active mandelic acid has been developed. Continuous-flow EKR step was pivotal to achieve high optically purity mandelonitrile and its acetate derivative. These process can be easily scaled-up to provide high amounts of these optically active compounds. Subsequent chemical hydrolysis of nitrile moiety employing optimized conditions resulted in reasonable yields and no optical purity was lost during this reaction. Employing this protocol, optically active mandelic acid could be obtained in good isolated yield and optically purity.

4. Experimental

4.1. Synthesis of mandelonitrile acetate (*RS*-2) and mandelonitrile (*RS*-3)

Benzaldehyde (2.122 g, 20 mmol) was added dropwise to a solution of $Na_2S_2O_5$ (2.282 g, 12 mmol) in water (10 mL), which was previous cooled in an ice bath and maintained under continuous stirring. After 10 min, a solution of NaCN (0.490 g, 20 mmol) in cold water (10 mL) was added dropwise and the mixture was stirred at room temperature for 24 h. The reaction media was extracted with CH_2Cl_2 (3 x 15 mL), dried with $MgSO_4$, filtered and

the solvent was evaporated under reduced pressure giving mandelonitrile (*RS*-3). For the synthesis of mandelonitrile acetate, previously synthesized cyanohydrin was dissolved in CH_2Cl_2 (20 mL) and acetic anhydride (2.84 mL, 30 mmol) and DMAP (one crystal) were added. The mixture was stirred at room temperature overnight. Then, the reaction media was washed with a solution of $NaHCO_3$, dried with $MgSO_4$, filtered and solvent was evaporated under reduced pressure.[75] Remaining aldehyde was removed via crystallization as its bisulfide salt. Yields and spectroscopic characterization are in accordance to our previous work.[69]

4.2. Enzymatic kinetic resolution of *RS*-2 in continuous-flow mode

Mandelonitrile acetate (*RS*-**2**) (2.628 g, 15 mmol) and *n*-butanol (5.50 mL, 60 mmol) were dissolved in toluene (150 mL), and the solution was eluted through a reactor (74.0 x 4.6 mm, internal volume 4.3 mL) filled with Novozym 435® (200 mg) with a flow rate of 0.1 mL min^{-1} for two cycles (total residence time = 8.6 min) at 50 °C, resulting in (*R*)-**2** (94% e.e.) and (*S*)-**3** (98% e.e.). Enantiomeric excesses were determined by chiral GC analysis. Then, solvent was removed under reduced pressure and the mixture was separated by column chromatography (hexanes/ethyl acetate 10:1). The solvent was removed and (*R*)-**2** and (*S*)-**3** were obtained in 34% and 37% yield, respectively.[69]

(*R*)-Mandelonitrile acetate. $[\alpha]_D^{20}$ = +3.7 (c = 0.5, $CHCl_3$; e.e. 94%). Ref.[76] $[\alpha]_D^{25.8}$ = 4.1 (c = 0.5, $CHCl_3$; e.e. 85%).

(*S*)-Mandelonitrile. $[\alpha]_D^{20}$ = -27.3 (c = 0.5, $CHCl_3$; e.e. 98%). Ref.[77] $[\alpha]_D^{20}$ = -8.6 (c = 0.5, $CHCl_3$; e.e. 81%).

4.3. Synthesis of (*R*)-mandelonitrile ((*R*)-3)

(*R*)-Mandelonitrile acetate ((*R*)-**2**) (1.051 g, 6 mmol) was dissolved in ethanol (10 mL) and PTSA (5% w/w) was added. The reaction media was maintained under magnetic stirring at 50 °C for 72 h. After that, ethanol was removed under reduced pressure, the crude material was dissolved in CH_2Cl_2 (10 mL) and washed with a solution of $NaHCO_3$, dried with $MgSO_4$ and solvent was evaporated under reduced pressure.[78] (*R*)-Mandelonitrile ((*R*)-**3**) was obtained in 87% yield, 94% e.e. and spectroscopic data are in accordance to racemic standard.

4.4. Synthesis of (*R*) and (*S*) mandelic acid (1)

Optically active (*R*)- or (*S*)-mandelonitrile (0.666 g, 5 mmol) and HCl (36%, 5.8 mL) were added to a mortar. The reaction media was left without stirring at room temperature for 12 h. Then, solvent was removed via evaporation with water steam bath and left drying overnight at room temperature. The crude residue was transferred to a beaker and dissolved in ethyl acetate (35 mL), under magnetic stirring for 5 min. This solution was filtered to remove NH_4Cl (remaining as a solid) and the solvent was removed under reduced pressure. After that, the solid residue was powdered, washed twice with cold benzene (2 x 4 mL, 5-10 °C) and then recrystallized in benzene (22 mL), filtered off in a sintered glass funnel (5-10 °C) and washed with cold benzene (8 mL, 5-10 °C) to give (*RS*)-**1**, (*R*)-**1** and (*S*)-**1** in 74%, 70% and 80% yield, respectively.[75]

(*RS*)-Mandelic Acid. White solid. MP: 118 °C. Yield: 74%. ^{1}H NMR (200 MHz, MeOD, TMS), δ 5.14 (s, 1H); 7.30–7.48 (m, 5H). ^{13}C NMR (50 MHz, MeOD), δ 72.8; 126.5; 127.8; 128.0; 139.4; 174.8. IR (KBr) ν/cm^{-1} 3400, 3029, 2967, 2716, 2628, 1717, 1452, 1299, 1190, 1059, 938, 888, 732, 696.

(*R*)-Mandelic Acid. White solid. MP: 118.0-120.5 °C. $[\alpha]_D^{20}$ = -116.3 (c = 0.25, H_2O; e.e. 94%). Ref.[79] $[\alpha]_D^{20}$ = -121.6 (c = 1.0, H_2O; e.e. 80%).

(*S*)-Mandelic Acid. White solid. MP: 130.5 °C. $[\alpha]_D^{20}$ = +143.2 (c = 0.25, H_2O; e.e. 98%). Ref.[80] $[\alpha]_D^{20}$ = +151.0 (c = 1.0, H_2O; e.e. 99%).

4.5. Determination of enantiomeric excess of optically active mandelic acid (1)

In a 4 mL sealed vial, optically active mandelic acid (0.045 g, 0.25 mmol) was dissolved in acetone (2 mL) and PTSA was added (10 mg). The reaction media was left stirring at 50 °C for 3 h.[81] After that, one aliquot (200 µL) was taken from reaction media, washed three times with a solution of $NaHCO_3$ (3 x 500 µL), dried over $MgSO_4$, filtered and analyzed via gas chromatography. Compounds (*R*)-**1a** and (*S*)-**1a** were obtained in 94% and 98% enantiomeric excess, respectively.

Supporting Information

Supporting information for this article is available free of charge at https://rvq.sbq.org.br/

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