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Stereospecific and selective enzymatic resolution of carboxylic acid derivatives in organic solvents

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Abstract. Enzymes such as lipases and proteases have been used as catalysts in nearly anhydrous organic solvents such as diisopropyl ether and hexane to carry out stereospecific and selective enzymatic resolutions of carboxylic acid derivatives such as 2-bromohexadecanoic acid, which may be used for prevention and treatment of bone loss-related diseases and β-phenyllactic acid, which has a role as a human metabolite.

Keywords: stereospecific and stereoselective resolution, lipases, proteases, organic solvents, carboxylic acid derivatives, substrate, stereo-, regio- and chemo-selectivity, active pharmaceutical ingredients.

Introduction

Since the early experiments reported by Klibanov[1], the use of enzymes in organic solvents rather than in their natural aqueous reaction media was widely recognized revealing that change in solvent from water to organic solvents is feasible and that in such allegedly hostile environment, enzymes can catalyze reactions that are impossible in water. A broad spectrum of experiments showed that enzymes exhibit higher stability and unexpected behaviors such as molecular memory[2,3,4,5]. In addition, it has been discovered that enzymatic selectivity, including substrate, stereo-, regio- and chemo-selectivity, can be affected and inverted by the solvent. Enzyme-catalyzed reactions in organic solvents, which can be named bio-transformations, have found some commercial applications. In some cases, bio-transformations can be good alternative to chemical synthesis for the production of optically active compounds by using non-aqueous enzymology, representing an important area of research and biotechnological development. This methodology is especially suitable for the modification of precursors of pharmaceutical compounds and fine chemicals, which, in most cases, are insoluble or poorly soluble in water. Thus, the purpose of the present article is demonstrating a number of the synthetic applications of enzymes in organic media, which can be scaled up from preparation of milligrams to ton-scale of active pharmaceutical ingredients.

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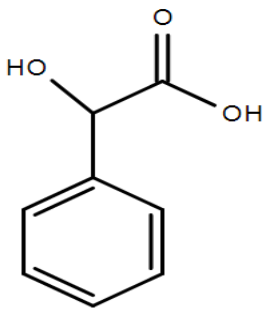
Materials and methods

Several enzymes have been used as catalysts, such as lipases selected from Aspergillus niger lipase, Candida antartica lipase, Candida cylindracea lipase, Candida rugosa lipase, Chromobacterium lipase, Mucor miehei lipase, Porcine pancreatic lipase, Pseudomonas fluorescence lipase, Pseudomonas cepacia and Wheat germ lipase and proteases selected from subtilisin, tripsin, pepsin and aspergillus oryzae protease, said enzymes were optionally immobilized on inorganic insoluble matrices selected from alumina, celite, calcium carbonate, diatomaceous earth, glass beads and silica gel, in a number of nearly anhydrous organic solvents such as diisopropyl ether and hexane, subjecting several carboxylic acid derivatives to stereospecific or selective esterification and hydrolysis; some of these reactions proceed to an appreciable extent only in non-aqueous solvents including stereo-selective and/or stereospecific enzymatic esterification of R,S-mandelic acid, DL-β-phenyllactic acid, (+/-)-2-bromohexadecanoic acid and hydrolysis of DL-β-phenyllactic acid methyl ester to afford the desired products in both high chemical yield and optical purity.

Results and Discussion

Enzymatic resolution of mandelic acid

Mandelic acid is a 2-hydroxy monocarboxylic acid that is acetic acid in which two of the methyl hydrogens are substituted by phenyl and hydroxyl groups. It has a role as an antibacterial agent and a human xenobiotic metabolite. The compound is a white crystalline solid that is soluble in water and polar organic solvents. The chiral molecule may be useful as precursor for the synthesis of drugs. The racemic mixture is known as DL-mandelic acid or R,S-mandelic acid:



R,S-mandelic acid

Resolution of R,S-mandelic acid by enzymatic esterification with n-butanol in organic solvent was carried out by dissolving 30 mg of the acid (0.2 mmol) in 1 ml of diisopropyl ether (DIPE) followed by addition of 45µl of n-butanol (0.5 mmol) employing the enzyme lipase immobilized on inert substrate (enzyme substrate ratio of 1:1) shaken in a benchtop shaker at 50°C for 12 hours. Four different immobilized enzyme preparations have shown significant reaction rates, as determined by HPLC. The reaction is depicted in Figure 1 below.

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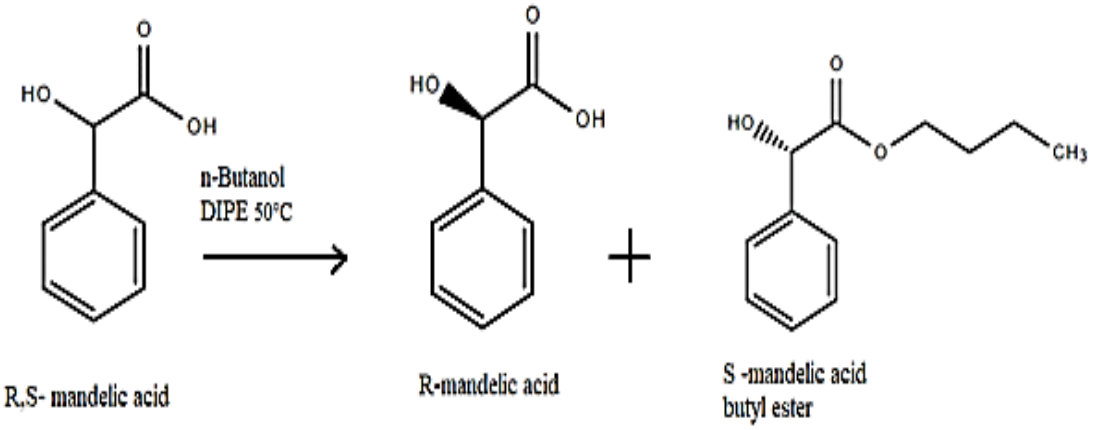
Figure 1



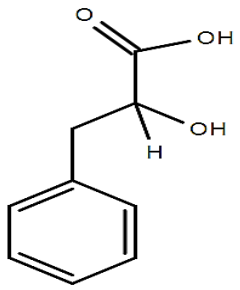
R-mandelic acid may be separated from S-mandelic butyl ester by methods of chromatography such as preparative HPLC or by extraction. S-mandelic acid may be obtained by changing the enzyme in the same reaction conditions or by isolating S-mandelic butyl ester by extraction and subjecting it to mild hydrolysis. The product R-mandelic acid was obtained having enantiomeric excess (ee) of about 90%.

Enzymatic esterification of DL-β-phenyllactic acid

DL-β-phenyllactic acid is a natural product found, e.g., in Aruncus dioicus (Goat's Beard), which is a perennial plant growing in the temperate northern hemisphere and belonging to the family Rosaceae.

β-phenyllactic acid, which has a role as a human metabolite, is a 2-hydroxy monocarboxylic acid that is lactic acid in which one of the methyl hydrogens is substituted by a phenyl group.

DL-β-phenyllactic acid, which is also named 2-hydroxy-3-phenylpropanoic acid or 3-phenyllactic acid, has the following structure:



DL-β-phenyllactic acid

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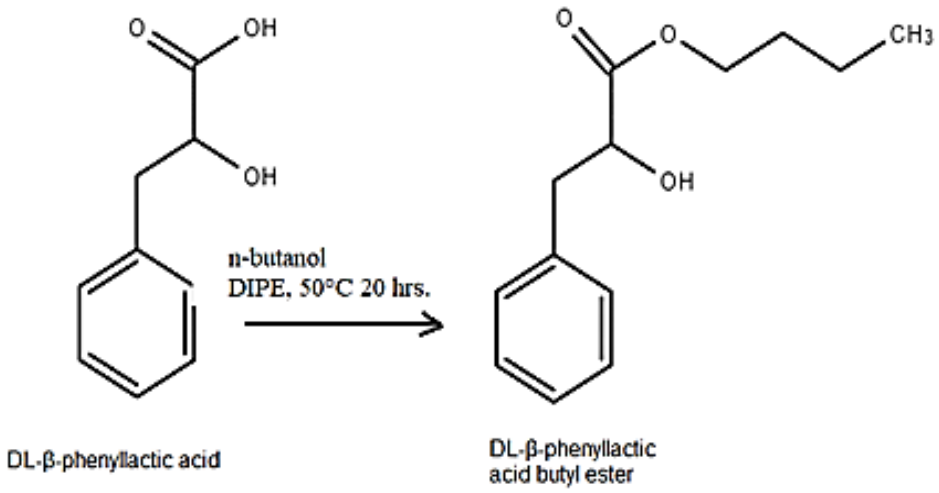
Patent Application CN-113388604 recites a resolution method of racemic 3-phenyllactic acid using the enzyme pseudomonas fluorescence covalently attached to an amino-functionalized magnetic metal–organic-framework (MOF) composite (Fe_3O_4@NH_2-MIL-88B(Fe)). However, covalently attaching an enzyme to a MOF is cumbersome and it is easier to use a physically immobilized enzyme.

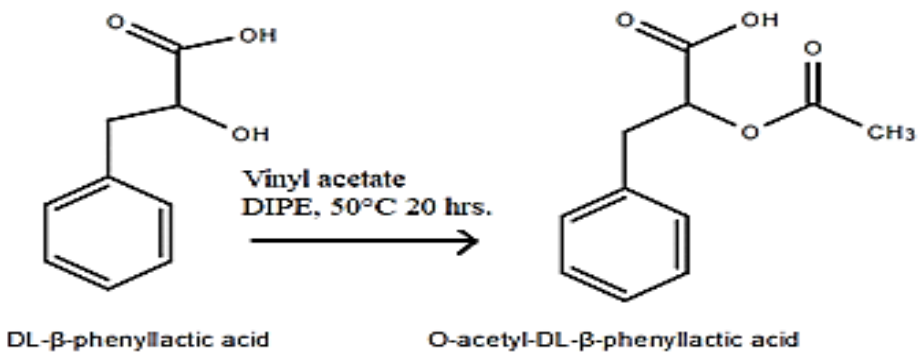
Immobilized lipase shows different selectivities in enzymatic esterification of β-phenyllactic acid depending on the reaction conditions. Esterification of β-phenyl-lactic acid with n-butanol in diisopropyl ether (DIPE) catalyzed by immobilized lipase shaken for 20 hours in a benchtop shaker at 50°C and 450 RPM afforded 28% of ester as sole product, as depicted in Figure 2 below.

Figure 2



However, esterification of β-phenyllactic acid with vinyl acetate in DIPE catalyzed by immobilized lipase shaken for 20 hours in a benchtop shaker at 50°C and 450 RPM afforded the o-acetyl ester in 46% yield as sole product, as depicted in Figure 3 below:

Figure 3



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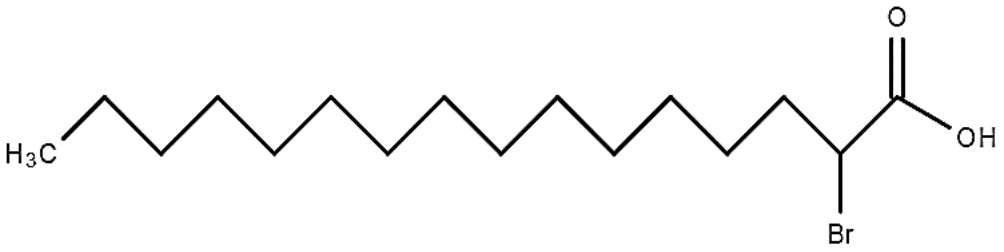
Enzymatic resolution of (+/-)-2-bromohexadecanoic acid

2-bromohexadecanoic acid is a 2-bromocarboxylic acid compound deriving from hexadecanoic (palmitic) acid, carrying a single bromo substituent at position 2. It is a long straight-chain fatty acid having the following structure:



(R,S) or (+/-)-2-bromohexadecanoic acid

2-bromohexadecanoic acid may be used as an Active Pharmaceutical Ingredient (API) as described in patent application WO2022/041311, which recites the application of 2-bromohexadecanoic acid in the preparation of a drug for prevention and treatment of a bone loss-related disease. By constructing an ovarian customized mouse osteoporosis model, the inventors of application WO2022/041311 showed that the compound inhibited osteoclast differentiation and activation in vivo, and alleviated the pathological process of a large bone loss caused by estrogen deficiency, thus 2-bromohexadecanoic acid may be used for prevention and treatment of bone loss-related diseases.

According to one approach, resolution of racemic 2-bromohexadecanoic acid can be carried out by converting the starting material to racemic 2-bromohexadecanoic acid methyl ester by refluxing the fatty acid dissolved in methanol in the presence of sulfuric acid. In the second step the isolated racemic 2-bromohexadecanoic acid methyl ester can be reacted in a mixture of an organic solvent miscible with water and phosphate butter using the enzyme lipase to form a mixture of the enzymatically hydrolyzed optically active R-(+)-2-bromohexadecanoic acid and the other enantiomer of 2-bromohexadecanoic acid methyl ester which is left intact. The desired product R-(+)-2-bromohexadecanoic acid may be obtained by separating the two compounds using column chromatography.

However, R-(+)-2-bromohexadecanoic acid was obtained in one-step stereospecific enzymatic esterification of racemic (+/-)-2-bromohexadecanoic acid with n-butanol in hexane using a lipase enzyme.

Figure 4 below depicts the one-step reaction scheme:

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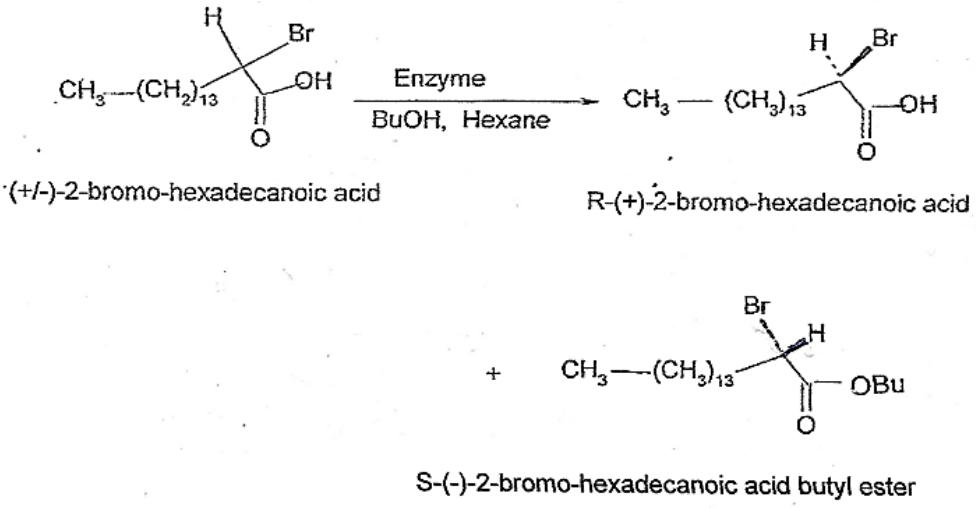
Figure 4



The one-step preparation of R-(+)-2-bromohexadecanoic acid was carried by adding 251.5 mg (0.75 mmol) of the racemic acid to a small glass flask followed by addition of 2 ml of hexane and 151 µl (1.65 mmol) of n-butanol and 160 mg of lipase enzyme. The mixture was shaken in a benchtop shaker at 40°C and samples were occasionally withdrawn, filtered to separate the enzyme, diluted and injected to GC to check the conversion rate. A calibration curve for determining the degree of conversion was developed in order to terminate the reaction at about 60% conversion. Six samples of mixtures of different molar combinations of the racemic acid as well as the butyl ester, which relate to different conversion rates, were injected to GC and the percent of the GC peak relative areas were correlated to the known molar concentrations of the two components to obtain the calibration curve. The predominant product of the stereospecific enzymatic esterification was S-(-)-2-bromohexadecanoic acid butyl ester, while the other enantiomer R-(+)-2-bromohexadecanoic acid did not react and was left intact. The two compounds were separated by extraction. R-(+)-2-bromohexadecanoic acid was isolated in 50% yield and enantiomeric excess (ee) of about 80%, having optical rotation of $[\alpha]D,25$ (5.0, $CHCl_3$) = + 10.7.

S-(-)-2-bromohexadecanoic acid butyl ester was obtained in 35% yield. Hydrolysis of S-(-)-2-bromohexadecanoic acid butyl ester in 1:3 (v/v) solution of 1M KOH: methanol afforded (-)-2-bromohexadecanoic acid.

Enzymatic hydrolysis of DL-β-phenyllactic acid methyl ester

15 mg of R,S-β-phenyllactic acid methyl ester were dissolved in a mixture of 10% ethanol and 90% phosphate buffer and 30 mg of protease enzyme was added and the mixture was shaken for 4 hours in a benchtop shaker at 42°C and 450 RPM. After 4 hours of reaction the desired product S-β-phenyllactic acid was obtained in nearly quantitative yield, according to HPLC.

This search was conducted in the years 2000-2001.

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Conclusion

Enzymes such as lipases and proteases optionaly immobilized on inorganic insoluble matrices are capable of subjecting several pharmaceutically important carboxylic acid derivatives to stereospecific or selective esterification in non-aqueous solvents including stereo-selective and/or stereospecific enzymatic esterification of R,S-mandelic acid, DL-β-phenyllactic acid and (+/-)-2-bromohexadecanoic acid to afford the desired products in both high purity and yield.

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[5] The Jerry March Textbook of Advanced Organic Chemistry, 6th Edition, page 1431, including a reaction scheme, Ref. 16-1793.