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Enzymatic Resolution of 2-Hydroxy-4-Phenylbutanoic Acid and 2-Hydroxy-4-Phenylbutenoic Acid

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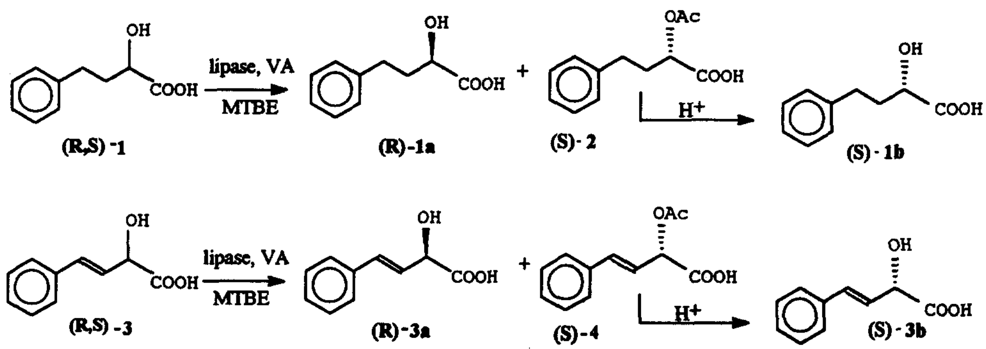
Abstract: Racemic 2-hydroxy-4-phenylbutanoic acid and 2-hydroxy- 4-phenyl-butenoic acid have been resolved using a lipase. In each case, the (R)-2-hydroxy and the (S)-2-acetoxy acids were isolated with high enantiomeric excess and yield.

Introduction: α-hydroxycarboxylic acid derivatives are important building blocks for many biologically active compounds[1]. (R)-2-hydroxy-4-phenylbutanoic acid (**1a**) is a precursor for the production of Angiotensin Converting Enzyme (ACE) inhibitors e.g. enalapril[2,3]. The synthesis of (**1a**) has been reported by enantioselective reduction of the corresponding α-keto acid - both, enzymatically and by chemical enantioselective hydrogenation[4]. Enzymatic resolution of the phenylpropionaldehyde cyanohydrin with the subsequent synthesis of ethyl (R)-2-hydroxy-4-phenylbutyrate is known[5]. The enantioselective hydrolysis of ethyl 2-hydroxy-4-phenylbutanoate has also been reported[6]. The resolution of 2-hydroxy-4-phenylbutanoic acid (**1**) using hexanoic anhydride has been published[7]. In this paper we report a simpler and less expensive procedure. Racemic 2-hydroxy-4-phenylbutanoic acid (**1**) has been resolved using a lipase in t-butyl methyl ether with vinyl acetate as the acylating agent. The enantiomeric excess and yield of the resulting enantiomers are higher than those reported earlier[7].

We also report the lipase catalysed resolution of the related 2-hydroxy-4-phenyl-3-butenoic acid (**3**). The case of the 2-hydroxy-3-enoic acids is special. These are allylic alcohols and at the same time unsaturated carboxylic acids. This is the reason for them to be considered useful for chiral synthesis. They have been prepared from the corresponding 2-oxo acids in enantiomerically pure form by the cells of *Proteus vulgaris* under anaerobic conditions[8]. Another procedure uses an enzyme (Lactate dehydrogenase) along with expensive cofactors[4]. To the best of our knowledge, the enzymatic resolution of the racemic 2-hydroxy-4-phenyl butenoic acid has not been reported so far. In this paper, we report a simple, straightforward lipase catalysed resolution of racemic 2-hydroxy-4-phenyl- butenoic acid with high enantiomeric excess and good yield.



Results And Discussion: Racemic 2-hydroxy-4-phenylbutanoic acid (**1**) was stirred with Lipase PS (Amano) and vinyl acetate in t-butyl methylether. The reaction was monitored on HPLC and after ~50% conversion (in 30h) the enzyme was filtered off, the products separated as reported earlier[7] and purified by silica chromatography. The (R)-2-hydroxy-4-phenylbutanoic acid (**1a**) was recovered in ~45% yield and >99% ee, while the acetate (**2**) was recovered in 35% yield. The acetate was hydrolysed to the (S)-hydroxy acid (**1b**), 84% ee. Sugai and Ohta[7], have obtained the R(-)-2-hydroxy-4-phenylbutanoic acid from the racemic hydroxy acid in the presence of lipase, using hexanoic anhydride as the acylating agent. The acylating agent used by us is the more commonly used, less expensive vinyl acetate. The enantiomeric excess and the yields of the products are high and, in fact, better than that reported earlier[7]. The enzymatic reduction of the α-keto acid, 2-oxo-4-phenylbutanoic acid has been achieved by many workers[4] but all these procedures need cofactors. In the case of microbial reduction, an ee of 99% is achieved. However, this method, as reported by Simon et. al[4] requires stringent anaerobic conditions. Our attempts to reduce the 2-oxo acid and ester with bakers yeast did not yield the required reduced product. Resolution of the racemic 2-hydroxy-4-phenylbutanoic acid using a lipase obviates the need for cofactors.

Immobilised enzymes have been known to be more stable with the distinct advantage of reusability. Even though many commercial processes use immobilised enzymes[9], reports of immobilised lipases are few[10]. We have immobilised the lipase on celite. This immobilised lipase displays a decrease in activity intially which shows signs of picking up after four batch cycles. After immobilisation on Eupergit C, a similar trend is seen. Further experiments on immobilisation are in progress and will be reported elsewhere.

In a related study, 2-hydroxy-4-phenylbutenoic acid (**3**) was also resolved enzymatically. Earlier reports of the formation of (R)-2-hydroxy-4-phenylbutanoic acid[8], indicate the use of whole cells of *Proteus vulgaris*. This organism requires anaerobic conditions under which quantitative reduction of the corresponding 2-oxo acid is carried out, in the presence of electron donors. The (S)-isomer is not formed. In our procedure, transacylation is done in the presence of a lipase to get the (R)-2-hydroxy acid (**3a**) and the (S)-2-acetoxy acid (**4**), which is hydrolysed to give the (S)-2-hydroxy acid (**3b**). The (R)-2-hydroxy-4-phenylbutenoic acid was recovered in ~42% yield and >99% ee, while the acetate was recovered in 34% yield and hydrolysed to give (S)-2-hydroxy-4-phenylbutenoic acid in 94% ee.

Experimental: To racemic 2-hydroxy-4-phenylbutanoic acid (1g) in MTBE was added vinyl acetate (5 ml) and lipase PS (500 mg) from Amano. The mixture was stirred at 25°C for 30h. HPLC (ODS column, $CH_3CN:H_2O:THF$, 20:65:15 @ 1 ml/min analysis showed a conversion of 51%. The reaction mixture was filtered and the recovered lipase was dried and reused. The filtrate and washings were concentrated and treated with cold hexane to obtain crystalline material (**1a**) (0.45g), m.p. 114°, $[\alpha]_D^{25}$= -9 (c=1, EtOH). Reported $[\alpha]_D^{25}$= -8.75 (c=1.04, EtOH). The IR and NMR were identical to the data reported earlier. The mother liquor was chromatographed to give the 2-acetoxy compound (**2**) (0.44g), $[\alpha]_D^{25}$ = -11 (c=0.92, EtOH). This compound was hydrolysed in the presence of acid to give (S)(+)-2-hydroxy-4-phenylbutanoic acid (**1b**) (0.28g), $[\alpha]_D^{25}$ = +7.5, (c=0.5, EtOH), 84% ee. The R(-)-2-hydroxy-4-phenylbutanoic acid was converted to its acetate. The $[\alpha]_D^{25}$ of this (R)-2-acetoxy-4-phenylbutanoic acid was found to be +11.76 (c=0.51, EtOH). IR(neat) cm^{-1}: 2910, 1740, 1370, 1240. NMR (60 MHz, $CDCl_3$) δ: 6.95 - 7.15 (aromatic 5H), 4.95 (t, 1H), 2.75 (m, 2H), 2.05 (s, m & 3H, 2H). Chiral chromatography on a Chiracel OD column (Hexane:IPA:TFA, 100:2:0.1, 3 ml/min), showed an ee of >99% for each of the two enantiomers, R(-) and S(+) 2-hydroxy-4-phenylbutanoic acids.

Under similar experimental conditions (R) (-)-2-hydroxy, 4-phenylbutenoic acid (**3a**) was obtained rom (**3**) in 42% yield and >99% ee, m.p =104 °, $[\alpha]_D^{25}$ = -91.5; reported $[\alpha]_D^{25}$ = -90.6 (c= 1.9, MeOH). The (S) (+)-2-hydroxy-4-phenylbutenoic acid (**3b**) (94% ee) $[\alpha]_D^{25}$ = + 85.2 (c=0.55, MeOH) was obtained from the (S) 2-acetoxy, 4-phenylbutenoic acid (**4**) $[\alpha]_D^{25}$ = +108 (c=0.36, EtOH), m.p. 82°. NMR (60 MHz) $CDCl_3$(δ): 7.2-7.6 (arom, 5H), 6.9 (d, Ph-CH_a=CH_b, J_{ab} = ~16 Hz), 6.3 (dd, Ph-CH_a=CH_b-CH_cOAc-J_{ab}=16 Hz, J_{bc}=6 Hz), 5.65(d, Ph-CH_a=CH_b-CH_cOAcCOOH, J_{bc}=6 Hz), 2.2 (s,-O-CO-CH_3, 3H). IR (KBr) (cm^{-1}): 3400, 2920, 1690, 1720, 1380, 1280, 1250, 1230, 1100.

Since our final product of interest has an ethyl ester group, we tried the enzymatic resolution of 2-hydroxy-4-phenylbutanoic acid ethyl ester. The lipase PS (Amano) was used in the presence of vinyl acetate and methyl t-butyl ether to give ethyl 2-hydroxy-4-phenylbutanoate $[\alpha]_D^{25}$ = -7.14 (c=1.12, EtOH) Reported $[\alpha]_D^{25}$ = 7.8 (c=1, EtOH)[11] and ethyl 2-acetoxy-4-phenyl butaonate, $[\alpha]_D^{25}$ = -15.3 (c=1.5, EtOH). The R(-) - ethyl - 2-hydroxy-4-phenylbutanoate can thus be used directly for further reactions.

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References

1. Parker, D.; J.Chem. Soc., Perkin Trans., **1983**, 2, 83.
2. Urbach, H.; Henning, R.; Tetrahedron Lett., **1984**, 25, 1143.
3. Lawton, G.; et al, US patent **1988**, 4, 757, 069.
4. Schmidt, E.; Blaser, H. U.; Fauquex, P. F; Sedelmeier, G.; Spindler, F.; 'Microbial Reagents in Organic Synthesis', Ed. Servi, S.; Ed. **1992**; 377. Kluwer Academic Publishers, The Netherlands.
5. Wang, Y. -F.; Chen, S. -T.; Kevin, K. C. Liu Wong, C. -H, Tetrahedron Lett., **1989**, 30, 1917.
6. Kalaritis, P.; Regenye, R. W.; Partridge, J. J.; Coffen, D. L.; Jour. Org. Chem. **1990**, 55, 812.
7. Sugai, T.; Ohta, H.; Agric. Biol. Chem., **1991**, 55, 293.
8. Yu. H.; Simon, H.; Tetrahedron, **1991**, 47, 9035; Schummer. A.; Yu. H; Simon. H; Tetrahedron, **1991**, 47, 9019.
9. Katchalski-Katzir, E.; Trends in Biotechnology, **1993**, 11, 471.
10. Hsu, S. H.; Wu, S. S.; Wang, Y. F.; Wang, C. H.; Tet.Lett., **1990**, 31, 6403; Maleata, F. X.; Hill Jr., C. G.; Amundson, C. H.; Biotech and Bioeng., **1991**, 38, 853; Bosley, J. A.; Clayton, J.C.. Biotech and Bioeng., **1994**, 43, 934.
11. Atwood, M. R., Hassall, C. H., Krohn, A., Lawton, G., Redshaw, S., Jour. Chem. Soc. Perkin, I., **1986**, 1011.

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