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Absolute configurations of monoesters produced by enzyme catalyzed hydrolysis of diethyl 3-hydroxyglutarate

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Abstract—Biocatalytic asymmetrizations of diethyl 3-hydroxyglutarate furnish a route to the enantiomers of ethyl 4-cyano-3-hydroxybutanoate. The enantiopreference of different enzymes has been established by chiral chromatography. Conclusive evidence for absolute configurations has been provided by X-ray crystallographic structure determination of co-crystals of the predominant monoester with (*R*)-phenylethylamine. The predominant enantiopure monoester produced by ammonolysis of diethyl 3-hydroxyglutarate catalyzed by immobilized lipase B from *Candida antarctica* (Novozym 435) was ethyl (3*S*)-4-carbamoyl-3-hydroxybutanoate. This was converted to ethyl (3*S*)-4-cyano-3-hydroxybutanoate in high yield and enantiomeric excess. Growing cells of *Acinetobacter lwoffii* gave low ee and predominance of the (*S*)-enantiomer when used for hydrolysis of diethyl 3-hydroxyglutarate as opposed to previous reports. When Novozym 435 was used for hydrolysis it could be re-used 10 times without loss of activity and selectivity.

1. Introduction

Lipitor has for several years been one of the best selling drugs. It is used to reduce the natural synthesis of cholesterol in patients who suffer from high cholesterol level in blood. Lipitor acts by inhibiting the enzyme hydroxymethyl co-enzyme A reductase, which is essential for the biosynthesis of cholesterol.[1] The active ingredient in lipitor, atorvastatin (Fig. 1), is a member of a family of statins, which also comprises lovastatin,[2] compactin, simvastatin, fluvastatin, cerivastatin and rosuvastatin.[1] These all contain a C-7 side chain with two stereocentres. This C-7 moiety has, for the synthesis of atorvastatin, been made from ethyl (3*R*)-4-cyano-3-hydroxybutanoate **1** and has been the focus of developing efficient syntheses for this chiral building block in its enantiopure (*R*)-form. A frequently used way of producing chiral building blocks is starting with enantiopure natural products. Starting with iso-vitamin C,[3] enantiopure nitrile **1** was synthesized using a series of non-'Green chemistry' steps. Based on lactose as the starting material a range of chiral building blocks are commercially available; among them starting materials for **1**.[4] Biocatalysis may also offer a greener way to many, in particular chiral, building blocks. This has been demonstrated in recent years both in the production of bulk chemicals such as acryl amide and in the fine chemicals and drug industry.[5]

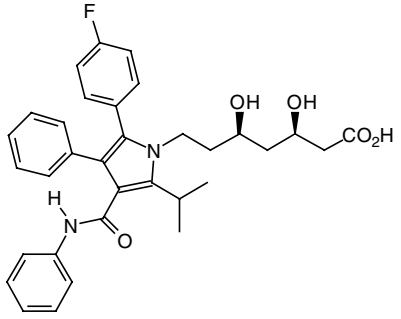

Figure 1. Atorvastatin.

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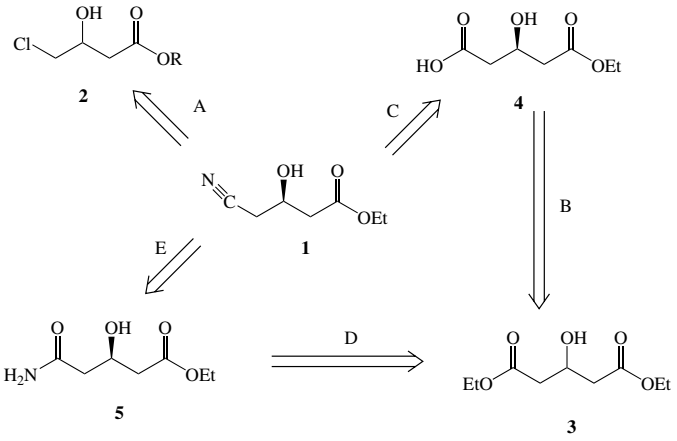

Figure 2. Retrosynthetic analysis for the synthesis of enantiopure target molecule **1** by biocatalysis. Route A, resolution of racemic chlorohydroxy ester **2**; route B, enzyme catalyzed asymmetrization by 'half'-hydrolysis of diethyl ester **3**, followed by route C, conversion of the enantiopure monoester **4** to nitrile **1**; route D ammonolysis of prochiral diester **3** to give enantiopure amide **5**, followed by route E, conversion of amide **5** to target nitrile **1**.

2. Results and discussion

We analyzed the possibilities of making **1** by biocatalysis based on the retrosynthetic scheme shown in Figure 2. Our first approach was to make **1** from racemic chlorohydroxy ester **2** by enzyme catalyzed kinetic resolution (route A).[6] The drawback with this resolution is that only 50% of the right enantiomer could be obtained. This may not be serious since quantitative conversion to one single enantiomer is possible using the technique of stereoinversion.[7,8] We started a systematic approach by varying the R-groups, solvents, acyl donors and enzymes. The highest *E*-value of >100 was obtained for the *tert*-butyl ester with vinyl propanoate as the acyl donor and lipase from *Rhizomucor miehei* as the catalyst. However, the synthesis of the ester substrate was not trivial starting with the diketene. Moreover, target nitrile **1** is an ethyl ester and a transesterification would be necessary in order to convert the *tert*-butyl ester to the ethyl ester. Hence we looked for an alternative method. By asymmetric synthesis instead of resolution, a theoretical yield of 100% of one enantiomer may be obtained directly, however, with enzyme catalysis, it is not guaranteed that this will be the wanted enantiomer.

An obvious starting material for the asymmetric synthesis would be diethyl 3-hydroxyglutarate **3**, a prochiral substrate. Enantiopure monoester **4** could be obtained by route B using enzymatic hydrolysis and this in turn could be converted to the nitrile using chlorosulfonylisocyanate (route C).[9] An even simpler method would be enzyme catalyzed ammonolysis via route D to the enantiopure monoamide **5**, which in turn could be converted to the target nitrile **1** using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (route E).[10]

All of the mentioned strategies were tried using a range of enzymes.[11] By far the highest ee-values were obtained using lipase B from *Candida antarctica* (CALB). Indeed, the ammonolysis reaction gave ee = 98% and 95% yield. Chymotrypsin catalyzed hydrolysis gave much lower ee-values (≈50%). It has previously been reported that the (*R*)-monoethyl ester is predominant in hydrolysis catalyzed by chymotrypsin, and moreover, that the reaction gives the product with very high ee.[12] This reported high ee-value and configuration is based on comparison with a previously reported specific rotation value for the monomethyl ester produced by classical resolution via the cinchonidine salt.[13] It has been reported that growing cells of *Acinetobacter lwoffii* gives predominance of the (*R*)-enantiomer when used for hydrolysis of diethyl 3-hydroxyglutarate with an ee of 80%.[14] In our hands the same organism (ATCC-17925) gave the (*S*)-enantiomer and with low ee (56%).

Since the specific rotation of the monoethyl ester **4** is a very small numerical value, we wanted to check the absolute configuration of the monoester produced by CALB not only by comparison of the $[\alpha]_D$ values, but with a more reliable method. The monoethyl ester produced by CALB was co-crystallized with (*R*)-phenylethylamine with the crystal structure determined to be as shown in Figure 3. This structure determination shows conclusively that the monoester produced by CALB is the (*S*)-enantiomer and so we consider it safe to assume that amide **5** has the same configuration. Based on very reliable chiral GLC analyses we also find that chymotrypsin gives a predominance of the (*R*)-enantiomer **4**. However, in our hands the ee values obtained were much lower than previously reported[12] both for the diethyl and dimethyl esters.[11] Recently, a patent for a process for producing the monoethyl ester of diethyl 3-acetoxyglutarate using chymotrypsin, has been reported. The ee-values are very high and the configuration (*R*) as wanted for synthesis of **1**.[15]

After use in the first hydrolysis of diethyl and dimethyl 3-hydroxyglutarate, the immobilized catalyst CALB was re-used more than 10 times with retention of high activity and enantioselectivity. In fact, the activity of the enzyme increased significantly from the first to the second batch of hydrolysis of **3** (Fig. 4).

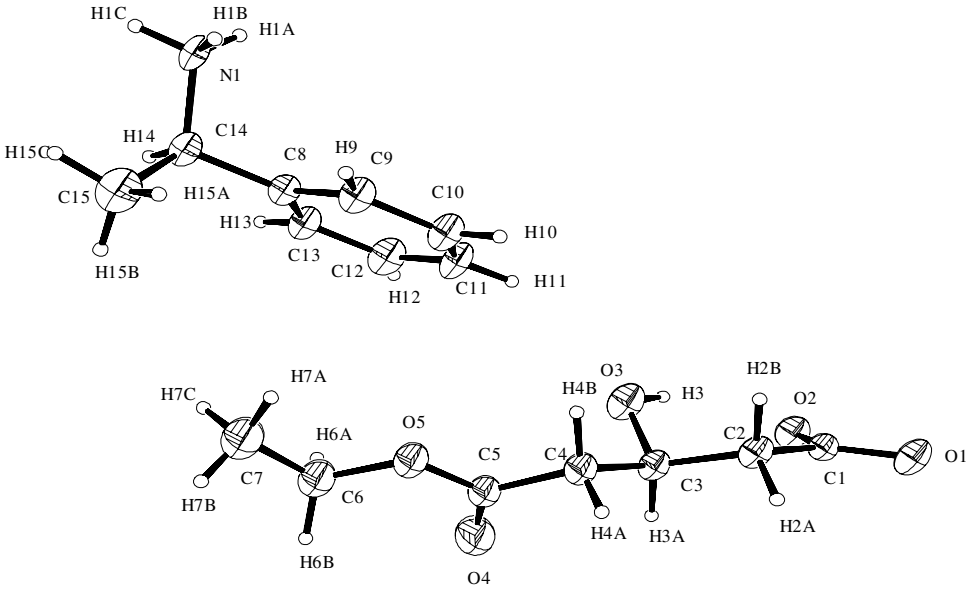

Figure 3. X-ray crystal structure of the co-crystal of (*R*)-phenylethylamine and the monoethyl ester produced by hydrolysis catalyzed by CALB. The stereocentre of (*R*)-phenylethylamine is labelled C-14 and it is inferred that it has (*R*)-configuration. The carboxylate group of the monoester is labelled C-1 and O-1, O-2; the ethyl ester group is C-6, C-7. From the X-ray structure it is clear that the stereocentre at C-3 has an (*S*)-configuration.

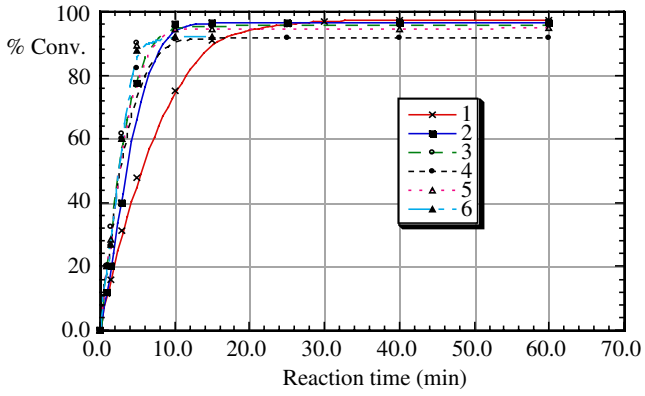

Figure 4. Conversion in six subsequent CALB catalyzed hydrolyses of diethyl 3-hydroxyglutarate **3**. The reaction time increased from reaction 1 to 6.

3. Experimental

3.1. Ethyl (3*S*)-4-cyano-3-hydroxybutanoate 1

Ethyl (3*S*)-4-carbamoyl-3-hydroxybutanoate (1.0 g, 5.7 mmol) was added to CH_2Cl_2 (15 mL), pyridine (0.89 g, 11.4 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, EDCI (1.78 g, 11.4 mmol). The mixture was stirred at room temperature for 4 days and the reaction monitored using GLC. After the addition of water (10 mL) and shaking, the water layer was removed and the solution dried over $MgSO_4$. The solvent was removed under vacuum, yield: 0.81 g (90%) MS: M+1 158, *m/z* 130, 112, 88, 84, 43; ^{1}H NMR (Bruker DPX 300, 300 MHz; $CDCl_3$): 1.22 (t, 3H), 4.15 (q, 2H), 4.30 (m, 1H), 2.60 (m, 4H), ^{13}C NMR (Bruker DPX 300, 75 MHz, $CDCl_3$): 174.71, 41.40. 64.41, 25.65, 117.73, 61.53, 14.5; enantiomeric excess by chiral GLC 98% (see below), same as amide **5**, $[\alpha]_D^{20} = +31.5$ (*c* 1.0, $CHCl_3$), for the (*R*)-enantiomer $[\alpha]_D^{20} = -31.3$ (*c* 1.0, $CHCl_3$).[16]

3.2. Determination of enantiomeric excesses

The product from the above reaction, predominantly ethyl (3*S*)-4-cyano-3-hydroxybutanoate **1**, was derivatized with trifluoroacetic anhydride and analyzed using Varian 3400 gas chromatograph equipped with a chiral CP-Chirsil Dex CB column (25 m, 0.32 mm i.d., 0.25 μm film thickness), temperature program 90–95 °C, 0.2 °C/min, 95–180 °C, 15 °C/min, column pressure 7.5 psi and split flow 60 mL/min. Retention times (*R*) = 15.4 min, (*S*) = 15.9 min, resolution $R_S = 1.6$. Chiral GLC of 4-ethoxycarbonyl-3-hydroxybutanoic acid and ethyl 4-carbamoyl-3-hydroxybutanoate have been described earlier.[11]

Cells of *A. lwoffii* (ATCC-17925) were grown and used as described previously.[14] The resulting monoester was analyzed as described earlier[11] with the results showing that the (*S*)-enantiomer was formed with an ee of 56%.

3.3. Determination of the absolute configuration of monoethyl ester

Monoethyl ester produced by CALB hydrolysis (0.5 g, 2.8 mmol) was dissolved in Et_2O (15 mL) and (*R*)-phenylethylamine (0.34 g, 2.8 mmol) was added. After cooling on ice, crystals were formed. The X-ray crystallographic structure is shown in Figure 3 and proves the identity as being (3*S*)-3-hydroxypentanedioic monoethyl ester.

3.4. X-ray crystallography

Diffraction data were collected on a CAD-4 diffractometer ($\theta_{max} = 23°$) using graphite monochromated Mo-K_α radiation ($\lambda = 0.71069$ Å). The structure was solved by direct methods and refined using the integrated program package OSCAIL.[17]

3.5. X-ray data

Orthorhombic space group $P2_12_12_1$ with $a = 5.9796(16)$, $b = 14.546(5)$, $c = 19.477(6)$ Å, $V = 1694.1(9)$ Å^3 and one molecule in the asymmetric unit. The structure was refined to $R = 0.086$ and $R_w = 0.27$ (the crystal was of poor quality) using 1394 unique reflections. H-atom parameters were not refined. CCDC 235996.[18]

3.6. Re-use of Novozym 435

To diethyl 3-hydroxyglutarate **3** (3.0 g, 14.7 mmol) was added buffer (15 mL) and CALB (0.5 and 0.05 g) and the reaction monitored using a pH-stat. The amount of added base (1 M NaOH) expressed the rate of reaction. The catalyst was separated by filtration and re-used 10 times under the same conditions.

Acknowledgements

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References and notes

1. Istvan, E. S.; Deisenhofer, J. *Science* **2002**, *292*, 1160–1164.
2. Henwood, J. M.; Heel, R. C. *Drugs* **1988**, *36*, 429–454.
3. Andelic, I.; Fure, R.; Leivestad, T.; Weiseth, M.; Danielsen, K.; Aarum, P.; Josefsen, S. *Abstract* 15th Norwegian Organic Wintermeeting, Norwegian Chemical Society, Geilo, Norway, 2000; p 46.
4. Synthon Chiragenics Corp., New Jersey, USA.
5. Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolts, M. C.; Witholt, B. *Nature* **2001**, *409*, 258–268.
6. Hoff, B. H.; Anthonsen, T. *Tetrahedron: Asymmetry* **1999**, *10*, 1401–1412.
7. Vänttinen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1995**, *6*, 1779–1786.
8. Liu, H.-L.; Anthonsen, T. *Chirality* **2002**, *14*, 25–27.
9. Luhaus, G. *Org. Synth.* **1988**, *6*, 304–305.
10. Bose, D. S.; Sunder, K. S. *Synth. Commun.* **1999**, *29*, 4235–4239.
11. Jacobsen, E. E.; Hoff, B. H.; Riise Moen, A.; Anthonsen, T. *J. Mol. Catal. B* **2003**, *21*, 55–58.
12. Cohen, S. G.; Khedouri, E. *J. Am. Chem. Soc.* **1961**, *83*, 4228–4233.
13. Serck-Hanssen, K. *Arkiv Kemi* **1956**, *10*, 135–149.
14. Gopalan, A. S.; Sih, C. J. *Tetrahedron Lett.* **1984**, *25*, 5235–5238.
15. Öhrlein, R.; Baisch, G.; End, N.; Burkhardt, S.; Studer, M. Patent WO 03/004450 A, 1-55, Germany, 2003.
16. Wang, G.; Hollingsworth, R. I. *Tetrahedron: Asymmetry* **1999**, *10*, 1895–1901.
17. McArdle, P. *J. Appl. Cryst.* **1993**, *26*, 752.
18. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].