Pergamon

Tetrahedron: Asymmetry, Vol. 8, No. 7, pp. 999–1003, 1997

Printed in Great Britain
PII: S0957-4166(97)00075-X
0957-4166/97 $17.00 + 0.00

Determination of the regio- and enantioselectivity of the enzymatic hydrolyses of succinates based on H,C COLOC analysis and deuterium NMR in a chiral liquid crystal

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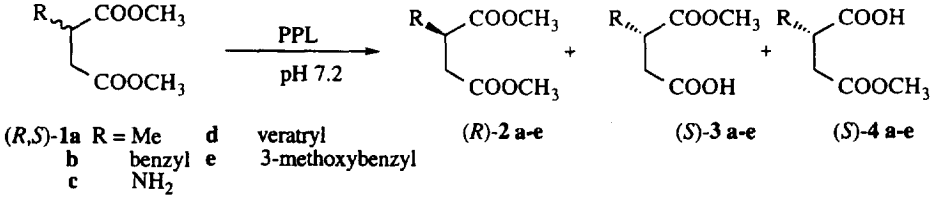
Abstract: The regio- and enantiomeric excess analyses of (*S*)-half esters resulting from enzymatic (lipase, esterase) hydrolyses of chiral dimethyl succinates, were accurately determined from H,C COLOC experiments and ^2H NMR in liquid crystal (PBLG, CH_2Cl_2). © 1997 Elsevier Science Ltd

We have previously reported that porcine pancreatic lipase (PPL) can provide enantiomerically pure succinates, aspartates and glutamates (95–100% ee) from the simple hydrolysis of their dimethyl racemates.[1] Thus, PPL catalyzed hydrolysis of the (*RS*)-dimethyl 2-methylsuccinate **1a** (R=Me), gave the non-hydrolyzed diesters (*R*)-**2a** (93% yield) with an enantioselectivity higher than 95%, determined by ^1H NMR spectroscopy (250 MHz) in the presence of 20–30% mol/mol of a shift reagent ($Eu(hfc)_3$) (Scheme 1).[2] Then, acidification of the aqueous phase provided the half-ester (*S*)-**3a** (R=Me) (76% yield), but with only 73% of enantiomeric excess. However, re-esterification of (*S*)-**3a** either by diazomethane or by acidic methanol ($SOCl_2$), followed by a second PPL catalyzed hydrolysis, allowed its enantiomeric purity to be improved to 96%.[1,3]



Scheme 1.

These asymmetric α-alkylsuccinates, which are now readily available on a preparative scale (~0.25 mole), have been alternatively prepared from the stereoselective alkylation of chiral imide enolates.[4] They are of great synthetic importance and constitute very convenient building blocks. For instance, they allowed the ready synthesis of optically active cyclopropanes,[3,5] which have been used as suitable precursors of homologous asymmetric C_4–C_6 rings,[3] as well as to prepare natural and non-natural γ-butyrolactones,[3,6] and 2,3-methanoamino acids.[7]

First of all, this enantioselective hydrolysis by PPL was considered to occur β to the stereogenic centre of (*RS*)-**1a–c** to produce the half-ester (*S*)-**3a–c**, regioselectively.[1,3] However, it appeared later that in the presence of esterases (HLE, PLE) the dimethyl succinates **1d, e** (R=veratryl, 3-methoxybenzyl) which are very poor substrates of lipases, underwent a rapid hydrolysis to produce a

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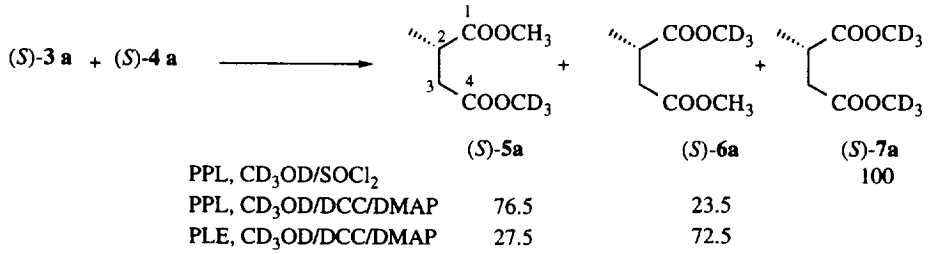
mixture of the two possible regioisomeric half esters (*S*)-**3d, e** and (*S*)-**4d, e**, resulting of hydrolysis both α- and β- to the stereogenic carbons.[8] Likewise, transesterification of these dimethyl esters by supported enzymes (alumina, silica gel, fluorisil) in the absence of solvent, appeared also non-regioselective.[9]

We report now that ^{1}H–^{13}C correlation in COLOC experiment and deuterium NMR in cholesteric lyotropic liquid crystal solvent allow us to determine with high accuracy the regio- and enantioselectivity of such enzymatic hydrolyses.

When the enzymatic hydrolysis of (*R,S*)-**1a**, performed in buffered water at pH 7.2, was completed as monitored by a pH-meter,[3] and after diethyl ether extraction of the non-hydrolyzed dimethyl 2-methylsuccinate (*R*)-**2a**, the sodium salts of the half-esters resulting of the PPL catalyzed hydrolysis were acidified to pH 2 and then extracted by Et_2O, continuously for 24 h. Re-esterification (Scheme 2) of this crude product by acidic deuteromethanol ($CD_3OD/SOCl_2$), produced the 1,4-bis(trideuteromethyl) 2-methylsuccinate (*S*)-**7a** exclusively, as shown by ^{1}H NMR and mass spectrometry. On the other hand, re-esterification by one equivalent of CD_3OD in dichloromethane in the presence of dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP),[10] under neutral conditions, led to a 76.5/23.5 mixture of the two regioisomeric (1 or 4)-trideuteromethyl, (1 or 4)-methyl 2-methylsuccinates (*S*)-**5a** and (*S*)-**6a**.



Scheme 2.

Hydrolysis of the succinate (*RS*)-**1a** by porcine liver acetone powder (PLE), followed by isolation of the half-esters mixture and analogous re-esterification by neutral CD_3OD,[10] gave reversely, a 27.5/72.5 mixture of these regioisomeric succinates. These ratios were determined from ^{1}H NMR (400.13 MHz) spectroscopy, which showed two methoxy signals at 3.610 and 3.628 ppm,[11] and by isotope ^{2}H NMR (61.40 MHz) spectroscopy, which displayed also two trideuteromethoxy signals.

In order to assign unambiguously the exact structures of the succinates (*S*)-**5a** and (*S*)-**6a**, we have then performed H,C COLOC (COrrelation spectroscopy via LOng range Coupling).[12] This technique allowed us to distinguish the two carboxylic carbons C_1 and C_4 of this succinate mixture through their long range couplings. ^{13}C NMR showed only two carboxylic carbon signals at 172.20 and 175.60 ppm; proton spectra gave a signal at 1.15 ppm for the methyl on carbon C_2, a signal at 2.36 ppm for the methine proton on carbon C_2, a signal at 2.67 ppm for the methylene protons at C_3 and two signals at 3.61 and 3.63 ppm for the two methoxy on carbon C_1 and C_4.

The H,C COLOC spectrum recorded for the (*S*)-succinate mixture arising from PLE hydrolysis followed by neutral deuteromethanol re-esterification,[10] displayed a correlation between the carboxylic carbon C_1 and the methyl protons on carbon C_2 ($^{3}J_{C-H}$), and between the carboxylic carbon C_4 and methoxy protons on C_4; while no correlation was observable between carboxylic carbon C_4 and the methyl on C_2 ($^{4}J_{C-H}$) and only a weaker correlation between the carboxylic carbon C_1 and the methoxy protons on C_1 (Figure 1). It can be concluded, that the major regioisomer was the succinate (*S*)-**6a** and therefore that the half-ester (*S*)-**4a** was the major product from the PLE catalyzed hydrolysis. Inversely, it can be concluded that the half-ester (*S*)-**3a** was the major product from the PPL catalyzed hydrolysis.

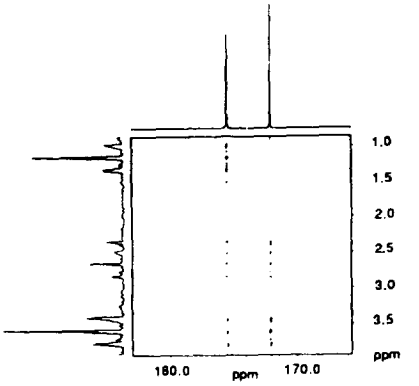


Figure 1. H,C COLOC analysis of succinates (*S*)-**5a** and (*S*)-**6a** from PLE-catalyzed hydrolysis.

A recent and very accurate method for the determination of the enantiomeric purity of benzylic alcohols,[13] of α-amino acids[14] or of substrates which do not contain any functional group to coordinate with lanthanides[15] is based on deuterium NMR spectroscopy in cholesteric lyotropic liquid crystals formed from poly-γ-benzyl L-glutamate (PBLG) and dichloromethane.[13] Thus it is possible to determine the enantiomeric excesses through the analysis of the different quadrupolar splittings displayed by enantiomers. The discrimination originates from the fact that in such an anisotropic chiral medium, the averaged molecular ordering parameters are different for each enantiomer.[16]

^2H NMR in liquid crystal (PBLG, CH_2Cl_2) of succinate (*S*)-**7a** displayed such a quadrupolar splitting:

- a broad doublet with a quadrupolar splitting ($\Delta\nu_q$) of 230 Hz for the deuteromethyl on C_4,
- two doublets with quadrupolar splittings of 129 Hz and 99.5 Hz for the deuteromethyl on C_1: one doublet for each enantiomer. From simple integration of these two doublets, it can be concluded that the enantiomeric excess of (*S*)-**7a** is 76.5% (Figure 2A).

Acidic re-esterification (methanol/$SOCl_2$) of the half-ester (*S*)-**3a** (76.5% ee) containing 25% of its regioisomer (*S*)-**4a**, followed by a second PPL catalyzed hydrolysis gave, as previously reported, (*S*)-**3a** with an improved enantiomeric excess.[1,3] Thus the ^2H NMR spectrum in liquid crystal of the corresponding succinate, obtained after re-esterification ($CD_3OD/SOCl_2$), exhibited again a broad doublet for the deuteromethyl at C_4, and two doublets for the deuteromethyl at C_1, from which signals integration revealed 91.5% enantiomeric excess (Figure 2B).

On the other hand, PPL catalyzed hydrolysis of (*RS*)-**1a** followed by neutral CD_3OD re-esterification[10] produced a mixture of succinates (*S*)-**5a** and (*S*)-**6a**, with ratios and enantioselectivities, highly dependent on the experimental conditions. As shown in Scheme 2, uncompleted PPL hydrolysis led again to a 76.5/23.5 mixture of (*S*)-**5a** and (*S*)-**6a**, but with only 13% enantiomeric excess (Figure 2C). Otherwise further hydrolysis monitored by a pH meter, produced a 71/29 mixture with 61% ee (Figure 2D) or a 68/32 mixture with 77.4 enantiomeric excess (Figure 2E). Re-esterification of this last mixture ($CH_3OH/SOCl_2$) and second PPL hydrolysis gave, after re-esterification by neutral CD_3OD,[10] a 61/39 regioisomeric mixture of these half-esters with 85.4% enantiomeric excess (Figure 2F). However, following the same procedure but using PLE catalyzed hydrolysis led, reversely to a 27.5/72.5 regioisomeric mixture of (*S*)-**5a** and (*S*)-**6a** with 48% enantiomeric excess (Figure 2G).

In conclusion, it appeared clearly from the ^2H NMR analyses in PBLG that successive PPL-catalyzed hydrolysis of dimethyl succinates can improve the enantioselectivity of the resulting (*S*)-half-esters, however to the detriment of the regioselectivity of the reaction. Moreover, this regioselectivity appeared also to depend on the nature of the enzyme. From a pratical point of view, this ^2H NMR spectroscopy in chiral liquid crystal of readily available deuterated succinates, provide a convenient and often more feasible alternative to the ^1H NMR spectroscopy recorded in the presence of chiral

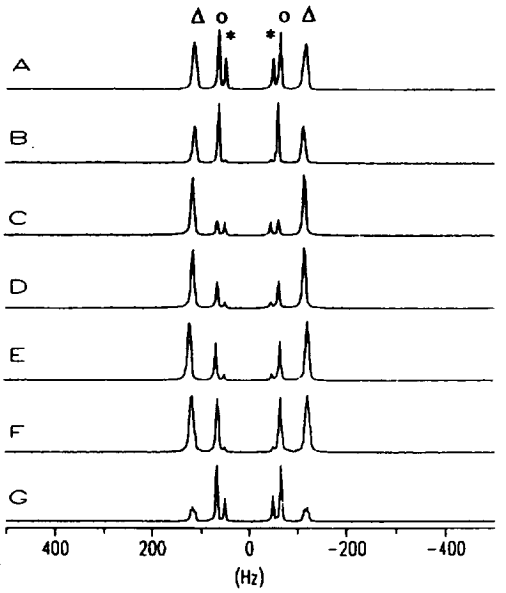


Figure 2. Selected proton decoupled deuterium NMR spectra of products in the lyotropic liquid crystal solvent PBLG/CH_2Cl_2 (Δ: ^2H doublet associated to the deuteromethyl on C_4; o: ^2H doublet of the *S* enantiomer associated to the deuteromethyl on C_1; *: ^2H doublet of the *R* enantiomer associated to the deuteromethyl on C_1).

shift reagents;[2] it must be underlined that the same experiment allows one to determine both the regio- and enantioselectivity of the reaction with great accuracy.

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(Received in UK 31 January 1997)