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Kinetic resolution of a dihydrobenzofuran-type neolignan by lipase-catalysed acetylation

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Abstract—The kinetic resolution of 3,5′-dimethoxy-4′,7-epoxy-8,3′-neolignane-4,9,9′-triol **1** by lipase-catalysed acetylation in an organic solvent was investigated. Ten different lipases were screened for enantioselectivity in the reaction. The enantiomeric excess (e.e.) of the products was strongly dependent on the type of lipase used. After optimisation of the reaction conditions for *Candida cylindracea* lipase, the e.e. and yield of the reaction was improved greatly and, in some cases, the enantiomerically pure starting material **1** could be isolated, albeit in a low yield, with the acetylation affording predominantly (2*R*,3*S*)-**1** and the (2*S*,3*R*)-esters.

1. Introduction

Dihydrobenzofuran-type neolignans are known to possess hepatoprotective, hormone blocking, antibacterial, antifungal, antioxidant and wound healing properties.[1–3] Recently, the antitumour activity of several products of this class was demonstrated by Pieters et al.[4] This study also reveals that the (2*R*)-enantiomers are the active antitumoural compounds while the (2*S*)-enantiomers show only minimal activity.

The methods generally used for the synthesis of dihydrobenzofuran neolignans proceed via a bimolecular radical coupling of hydroxyphenylpropenoids,[5,6] yielding the desired *trans*-skeleton as a racemic mixture.[7] However, the results of the previously mentioned biological activity studies show the value of preparing enantiomerically pure or enriched neolignans.

One of the possible approaches to deal with this problem is the introduction of a chiral auxiliary in the

Table 1. Ratio of the enantiomers of **2** after the kinetic resolution with different lipases

Entry	Lipase from	Reaction time (h)	Conversion (%)	**2** (2*R*,3*S*)/(2*S*,3*R*) ratio
1	Porcine pancreas	3	52	33/67
2	*Pseudomonas cepacia*	0.5	55	35/65
3	*Mucor miehei*	6	52	42/58
4	*Candida antarctica*	0.5	46	54/46
5	*Pseudomonas fluorescens*	1.5	47	36/64
6	*Rhizopus arrhizus*	6.7	29[a]	51/49
7	*Rhizopus niveus*	22	34[a]	51/49
8	*Aspergillus niger*	5	46	44/56
9	*C. cylindracea*	33	47	25/75[b]
10	*C. antarctica*	23	25[a]	33/67[b]

[a] Denaturation of the enzyme prevented higher conversions.
[b] Ratio of the enantiomers of diacetate **3** (Scheme 2).

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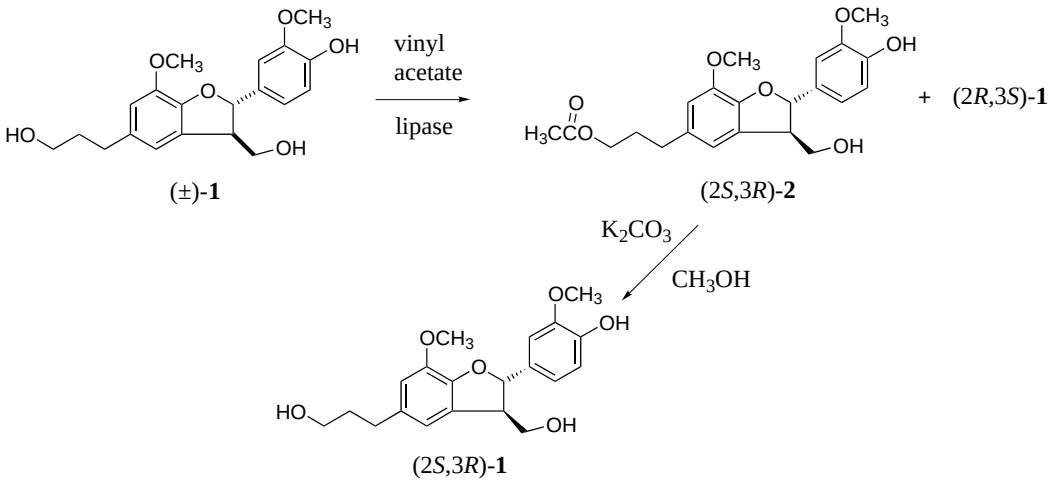
starting material.[6] In this way one enantiomerically enriched neolignan is prepared with e.e.s of 18–84% depending on the type of auxiliary and the reaction conditions employed.[8,9] Another approach is the enzymatic kinetic resolution of racemic synthetic dihydrobenzofuran neolignans.[10] An advantage of this route is that no specific chiral auxiliaries have to be introduced or removed. Until now no report of any method using enzymatic reactions to obtain optically pure dihydrobenzofuran neolignans can be found in the literature. Therefore, we decided to investigate the possibility of using lipases for this specific purpose.

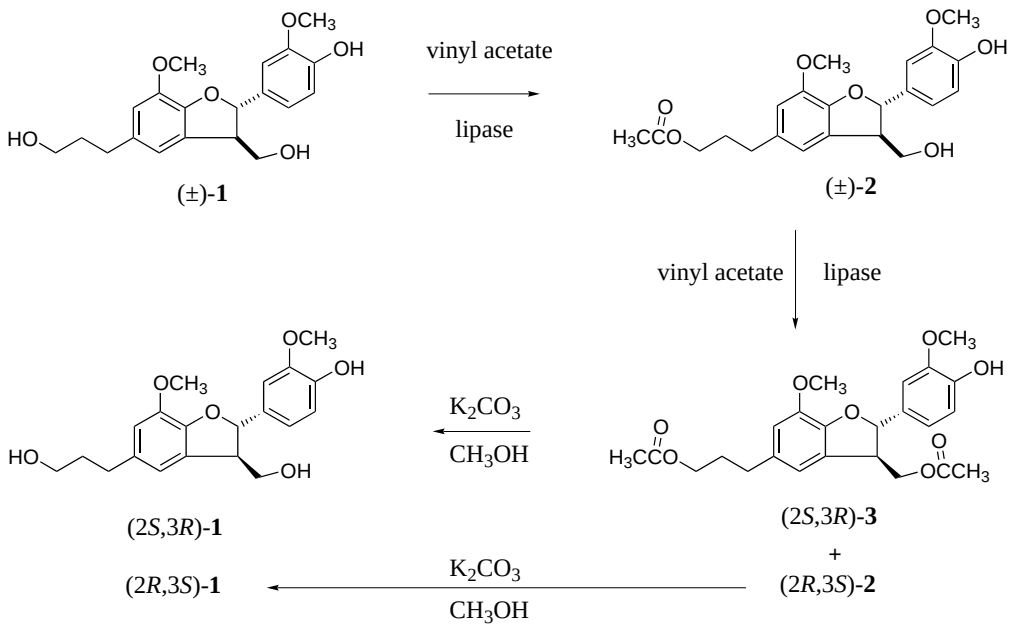
2. Results and discussion

Ten lipases were screened in order to investigate the influence of the type of the lipase on the enzymatic kinetic resolution of **1**. For these reactions the starting material was prepared from ferulic acid, as described by Lemière et al.[4,11] Subsequently, the enantioselective acetylation was performed by stirring a suspension of the chosen lipase in a solution of **1** in pure vinyl acetate. Samples were taken from the reaction mixtures at set intervals and were analysed by chiral analytical HPLC. The (*RS*)/(*SR*) ratios of the reaction products and the degrees of conversion of the starting material were calculated from the chromatograms. In order to compare the different lipases for their enantioselectivity and chemical yield the (*RS*)/(*SR*) ratios at approximately 50% conversion are reported in Table 1.

For all of the enzymes studied except for the *Candida* lipases only one alcohol function was acetylated (Scheme 1). The acetylation proceeded with high regioselectivity at the propanol side chain. The exact position of this newly introduced acetate group was elucidated by using 2D NMR techniques. In entries 9 and 10 (*Candida* lipases), the second alcohol function also reacted with the acetyl donor, but only after the



Scheme 1. Lipase (non-*Candida* type) catalysed acetylation.[12]



Scheme 2. Lipase (*Candida* type) catalysed acetylation.

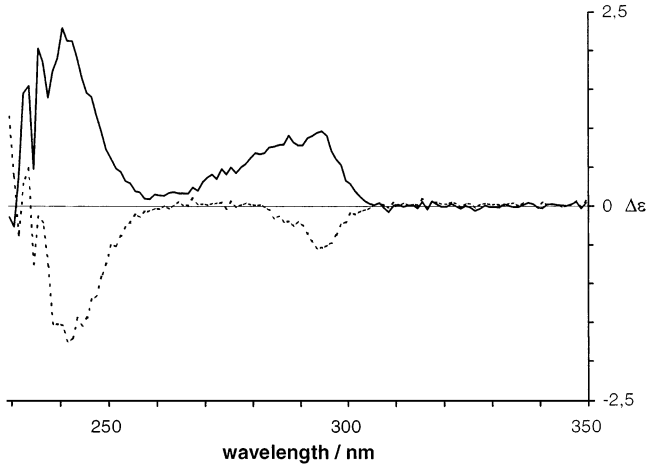


Figure 1. CD spectra of the two pseudo-enantiomers: (2*R*,3*S*)-**2** (—) and (2*S*,3*R*)-**3** (- - - -).

first acetate was introduced (Scheme 2). The absolute configuration of the reaction products was assigned on the basis of CD spectroscopic evidence. Compounds with (2*R*,3*S*)-configuration showed two positive Cotton effects, one at 235–245 nm and one at 285–295 nm. In the enantiomers with (2*S*,3*R*)-configuration both Cotton effects were negative (Fig. 1). It has been shown in the literature that these CD observations are reliable for determination of the absolute configuration of dihydrobenzofuran-type neolignans.[13,14]

As can be concluded from Table 1, in this first screening the reaction with *Candida cylindracea* lipase (entry 9) gave the best e.e. For this enzyme the introduction of a first acetate group was rapid and the reaction was complete in less than 15 min without any noticeable enantioselectivity (Scheme 2). The racemic monoacetate produced underwent a second enzyme-mediated acetylation which proceeded less rapidly and gave product with a reasonable e.e. at approximately 50% conversion. Also entries 1, 2, 5 and 10 showed better e.e. than the other reactions. As could be expected, at lower conversions the best e.e. of the products was observed. We next examined the e.e. of the unreacted substrate **1** (Table 2). The best e.e. of the unreacted substrate was observed at conversions of greater than 50% (entries 11–14). In some cases the (2*S*,3*R*)-enantiomer of **1** reacted quantatively with vinyl acetate and enantiomerically pure (2*R*,3*S*)-alcohol remained. Optimisation of the initial reaction conditions turned out to be necessary since the yields of the reactions with better e.e. were unsatisfactory. Porcine pancreatic lipase (PPL) and *C. cylindracea* lipase (CCL) were selected for more detailed study since these enzymes gave the best selectivities in the initial screening.

Several parameters were varied such as temperature, concentration, the use of co-solvents, the type of acetyl donor and immobilisation of the enzymes in sol-gel-AK. The effect of changes in the reaction temperature and enzyme loading were not significant. When the acetylation was performed in other solvents with the use of only 10% v/v of vinyl acetate or another acetyl donor often a strong effect on the reaction rate was observed, but the e.e. did not improve and in many cases decreased.

In contrast, the use of immobilised enzymes showed a pronounced change in e.e. for the immobilised *C. cylindracea* lipase, while for immobilised PPL only a small improvement was seen (Table 2, entries 15 and 16). In the reaction using the immobilised *C. cylindracea* lipase again the first acetate group was introduced rapidly with no selectivity. The second acetylation proceeded more slowly giving the diacetate **3** in good e.e. However, the best enantiomeric purity was observed for the remaining monoacetate **2** with an excess of the desired (2*R*,3*S*)-enantiomer. During the optimisation process the initial enantiomeric ratio of $E=4.1$ for the *C. cylindracea* lipase was more then doubled resulting in an E-value of 9.0 for the immobilised enzyme.

3. Conclusions

In summary, these results showed that optimised enzymatic kinetic resolution could be used to synthesise dihydrobenzofuran neolignans in workable yields with significant e.e.s. The observed enantioselectivity using this method was of the same magnitude as the only other reported method for preparing enantiomerically enriched neolignans which requires the use of chiral auxiliaries.[9] Additional advantages for these enzymatic resolutions were the very mild reaction conditions, good reaction rates and the commercial availability of the mentioned lipases. Therefore this methodology is a useful approach to valuable neolignans.

Table 2. Ratio of the enantiomers of the remaining alcohol **1** after the kinetic resolution with different lipases

Entry	Lipase from	Reaction time (h)	Conversion (%)	**1** (2*R*,3*S*)/(2*S*,3*R*) ratio	Yield of **1** (%)[a]
11	Porcine pancreas	9	75	82/18	41
12	*P. cepacia*	1.75	87	100/0	26
13	*P. fluorescens*	4	85	100/0	30
14	*C. cylindracea*	33	47	71/29[b]	75
15	*C. cylindracea* (immobilised)	20	57	89/11[b]	77
16	PPL (immobilised)	20	52	72/28	70

[a] Since these reactions started from the racemic mixture containing 0.5 equiv. of each enantiomer one should note that a synthesis of 0.5 equiv. of enantiomerically pure product corresponds with a 100% yield.
[b] Ratio of the enantiomers of the remaining monoacetate **2** (Scheme 2).

4. Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 400 spectrometer in the specified solvent with TMS as internal standard. Additional COSY, HETCOR and long-range HETCOR measurements were performed on the same spectrometer. Chemical shifts are reported in ppm and coupling constants in Hz. The atoms are numbered as before and in agreement with a recent recommendation concerning the nomenclature of lignans and neolignans of IUPACs Joint Commission on Biochemical Nomenclature.[15]

DCI mass spectra were obtained on a Ribermag R-10-10B mass spectrometer. Column chromatography was performed on Merck silica gel 60, 0.040–0.063 mm, 230–400 mesh ASTM. Precoated silica gel plates (kieselgel 60, F_{254}, 0.2 mm) were used for TLC analysis.

Chiral analytical HPLC was performed using a Spectra-Physics Spectraseries P200 pump equipped with a Spectra-Physics Spectraseries UV100 detector (range 0.01 AUFS, rise time 0.3 s) and using a Chirobiotic V™ column (5 μm, 150×4.6 mm) purchased from Alltech. 20/80 Methanol/20 mM ammonium acetate at pH 5 was used as the mobile phase. The flow rate was kept at 0.8 cm^3/min and UV detection occurred at 254 nm.

All enzymes were purchased from Fluka, while all other chemicals were purchased from Aldrich.

UV–vis spectra were recorded with a Perkin–Elmer Lambda 5 instrument, while CD spectra were recorded with an Aviv (Lakewood, NJ, USA) 62 A DS circular dichroism spectrometer, equipped with an Oxford Instruments DN 1714 (static) liquid nitrogen cryostat. All solvents used were of spectrograde quality.

4.1. General procedure for the enzymatic acetylation

In a 50 cm^3 flask **1** (60 mg) was dissolved in vinyl acetate (15 cm^3). The reaction mixture was kept at a constant temperature of 37°C in an oil bath. After 5 min the (immobilised) lipase was added and the resulting suspension was gently stirred with a magnetic stirrer.

Samples (0.5 cm^3) of these suspensions were taken with a syringe equipped with an Alltech syringe filter (13 mm, 0.2 μm PTFE) in order to filter off the enzyme. Vinyl acetate was evaporated and the remaining product was evaporated in vacuo. The product was dissolved in HPLC-grade methanol (1.5 cm^3) and was used for HPLC analysis.

When the desired e.e. was reached, the mixture was filtered through a sintered glass filter and the filtrate was evaporated under reduced pressure. All compounds were separated using column chromatography with ethyl acetate/heptane 1:1 as the initial eluent. After the elution of the acetate(s), the eluent was changed to ethyl acetate/heptane 1:1 containing 5% methanol. Analytical data for the products formed by this procedure are as follows.

4.1.1. 4,9-Dihydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neolignane-9′-yl acetate 2. Colourless oil; ^{1}H NMR ($CDCl_3$): δ 6.93 (d, $J=1.84$ Hz, 1H, H-2), 6.90 (dd, $J=8.08$, 1.84 Hz, 1H, H-6), 6.86 (d, $J=8.08$ Hz, 1H, H-5), 6.65 (s, 2H, H-2′,H-6′), 5.62 (s, 1H, 4-OH), 5.52 (d, $J=7.32$ Hz, 1H, H-7), 4.10 (m, $J=4.21$ Hz, 2H, H-9′), 3.96 (dd, $J=10.99$, 5.86 Hz, 1H, H-9a), 3.90 (s, 3H, 3′-OCH_3), 3.89 (dd, $J=10.99$, 4.77 Hz, 1H, H-9b), 3.85 (s, 3H, 3-OCH_3), 3.58 (m(b), $J=5.86$ Hz, 1H, H-8), 2.63 (t, $J=7.63$ Hz, 2H, H-7′), 2.05 (s, 3H, 9-$OCOCH_3$), 1.95 (m, $J=7.63$, 4.21 Hz, 2H, H-8′), 1.82 (s, 1H, 9-OH); ^{13}C NMR ($CDCl_3$): δ 171.31 (9′-CO), 147.03 (C-3), 146.95 (C-4′), 145.94 (C-4), 144.48 (C-3′), 134.84 (C-1′), 133.46 (C-1), 128.27 (C-5′), 119.58 (C-6), 116.38 (C-6′), 114.60 (C-5), 113.11 (C-2′), 109.17 (C-2), 88.03 (C-7), 64.27 (C-9), 63.94 (C-9′), 56.39 (3′-OCH_3), 56.24 (3-OCH_3), 54.05 (C-8), 32.18 (C-7′), 30.70 (C-8′); DCI-MS (NH_3) m/z: 403 (MH^+), 420 (MNH_4^+); IR (KBr): 1734.43 cm^{-1} (CO); e.e. = 78%; $[\alpha]_D^{20}=-3.2$ ($c=0.0348$ M, ethanol). Anal. found for $C_{22}H_{26}O_7$: C, 65.43; H, 6.47; O, 27.89%

4.1.2. 4-Hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neolignane-9,9′-diyl diacetate 3. Colourless oil; ^{1}H NMR ($CDCl_3$): δ 6.91 (s(b), 1H, H-2), 6.87 (d(b), 2H, H-5,H-6), 6.65 (s, 2H, H-2′,H-6′), 5.61 (s, 1H, 4-OH), 5.48 (d, $J=7.47$ Hz, 1H, H-7), 4.43 (dd, $J=5.49$, 11.14 Hz, 1H, H-9a), 4.29 (dd, $J=7.63$, 11.14 Hz, 1H, H-9b), 4.09 (t, $J=6.56$ Hz, 1H, H-9′), 3.88 (s, 3H, 3′-OCH_3), 3.80 (s, 3H, 3-OCH_3), 3.75 (m, $J=5.49$, 7.47, 7.63 Hz, 1H, H-8), 2.64 (t, $J=7.71$ Hz, 2H, H-7′), 2.06 (s, 3H, 9-$OCOCH_3$), 2.01 (s, 3H, 9′-$OCOCH_3$), 1.93 (m, $J=7.71$, 6.56 Hz, 2H, H-8′); ^{13}C NMR ($CDCl_3$): δ 171.10 (9′-CO), 170.78 (9-CO), 146.75 (C-3), 146.44 (C-4′), 145.86 (C-4), 144.25 (C-3′), 134.90 (C-1′), 132.67 (C-1), 127.51 (C-5′), 119.57 (C-6), 116.26 (C-6′), 114.34 (C-5), 112.83 (C-2′), 108.81 (C-2), 88.53 (C-7), 65.52 (C-9), 63.81 (C-9′), 56.16 (3′-OCH_3), 56.05 (3-OCH_3), 50.64 (C-8), 32.07 (C-7′), 30.61 (C-8′); DCI-MS (NH_3) m/z: 445 (MH^+), 462 (MNH_4^+); e.e. = 61%; $[\alpha]_D^{20}=+12.2$ ($c=0.026$ M, ethanol). Anal. found for $C_{24}H_{28}O_8$: C, 64.94; H, 6.44; O, 28.71%.

4.2. General procedure for removal of the acetates

The mono- or diacetate **2** or **3** were dissolved in methanol and K_2CO_3 (20 equiv.) was added. The mixture was heated under reflux for 30 min, after which time the solution was poured into water and extracted with ethyl acetate. The organic extracts were concentrated under reduced pressure and purified through a short plug of silica gel with ethyl acetate as the eluent. Compound **1** was obtained in 92% yield and the spectroscopic data corresponded with those reported previously in the literature.[4]

4.3. Immobilisation of the enzymes

Although the immobilised enzymes are readily available from Fluka, they can easily be prepared using the method of Reetz et al.[16–18]

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