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Enzymatic kinetic resolution of 1-(3-furyl)-3-buten-1-ol

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Abstract—The enzymatic kinetic resolution of 1-(3-furyl)-3-buten-1-ol was investigated via the enantioselective hydrolysis of the corresponding acetate. *Pseudomonas fluorescens* (Fluka) was found to give the highest enantiomeric ratios of the 11 lipases screened. At 51% conversion, the ee value (ee_p) for the product was found to be 89%, giving an enantiomeric ratio (E_p) of 58, while the ee value (ee_s) for the substrate was 89%, giving an enantiomeric ratio (E_p) of 38.

1. Introduction

We recently required enantiomerically pure 1-(3-furyl)-3-buten-1-ol, (*S*)- or (*R*)-**2**, for the synthesis of simplified analogues of salvinorin A (Fig. 1).

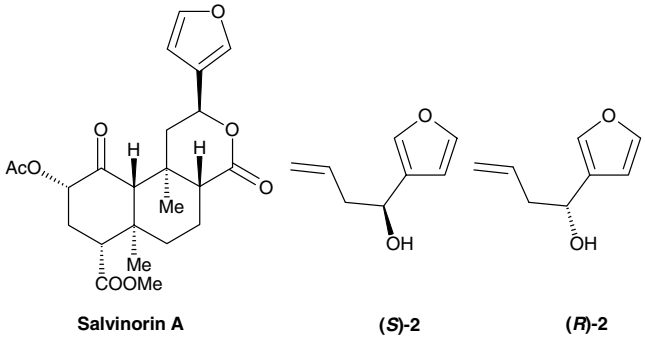

Figure 1. Salvinorin A together with (*S*)-**2** and (*R*)-**2**.

The enantiomers of **2** have previously served as important building blocks in the total synthesis of several natural products[1–4] and have been synthesized either via Brown allylation of 3-furaldehyde **1**[1,2,4] or by enzymatic kinetic resolution of **2**.[5] The Brown procedure has two disadvantages: (a) the reported ee of the procedure is 93–96% with no chance of further improvement. For useful structure–activity relationship (SAR) purposes an ee of >98% is normally needed[6] and (b) the tedious laboratory protocol, which requires a reaction temperature of −100 °C and filtering under inert conditions. Multigram quantities of resolved **2** were needed for our studies on the simplified analogues of salvinorin A, hence, the enzymatic kinetic resolution approach seemed much better suited.

Another attractive feature of enzymatic kinetic resolutions is the possibility of calculating the enantiomeric ratios E_s and E_p. E_s relates to the extent of conversion of the starting material to product, c, to the enantiomeric excess of the remaining starting material, ee_s while E_p relates c to the enantiomeric excess of the product ee_p. The E value facilitates the rapid evaluation of an enzyme's enantioselective property in a screening procedure. The enantiomeric ratios for the substrate E_s and for the product E_p were calculated using Sih and coworkers method,[7] Eqs. 1 and 2, assuming irreversibility.

$$E_s = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]} \quad (1)$$

$$E_p = \frac{\ln[1-c(1+ee_p)]}{\ln[1-c(1-ee_p)]} \quad (2)$$

It is important to distinguish between the enantiomeric ratio of the substrate (E_s) and that of the product (E_p). For the substrate, a high enantiomeric purity (ee_s >98%) can be obtained even with an E_s value as low as 5. However, the course of the kinetic resolution is different for the product. Initially, ee_p equals $(E-1)/(E+1)$, then at $c \sim 50\%$, the ee_p value rapidly diminishes, ending up as the racemic product at $c = 100\%$. Only systems with an $E_p > 100$ can be used

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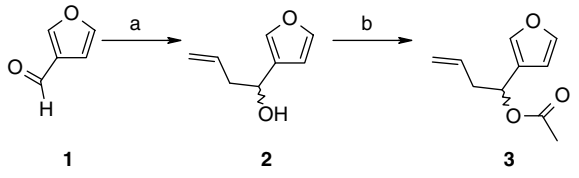
to obtain ee_p >98%. This kind of enantioselectivity is often not obtainable[6] and other strategies, such as product recycling,[7] coupled enantioselective action[8] and sequential biocatalytical resolution[9] have to be considered in order to obtain the desired ee_p.[10]

Since the enzyme preparation previously reported for this resolution is no longer commercially available,[5] we have reassessed the screening procedure using new sets of lipases obtained from Fluka (Lipase basic kit) and from Europa Bioproducts Ltd (*Alcaligines* spp. 12 and *Alcaligines* spp. 20).

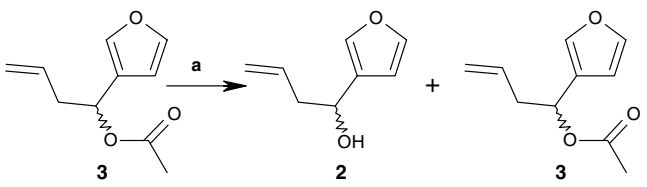
2. Results and discussion

The synthesis of the ester **3** (Scheme 1) is straightforward and was performed essentially as in the work of Bierstedt et al.[5] Aldehyde **1** was subjected to a Barbier reaction[11] with allylbromide and Zn powder in a saturated solution of ammonium chloride and THF to give the racemic homoallylic alcohol **2** in 99% yield. The alcohol was then acetylated with acetic anhydride to afford **3**, the starting material for the enzymatic kinetic resolution.



Scheme 1. Reagents: (a) allylbromide, Zn powder, THF–NH_4Cl (aq) (1:5) (99%); (b) acetic anhydride, Et_3N, CH_2Cl_2 (86%).

The screening procedure (Scheme 2) was performed with 11 different lipases in 10 mL of a 9:1 mixture of a phosphate buffer (pH 7) and DMSO, at 40 °C, using 100 mg of substrate for each lipase. A control experiment, without any enzyme, was performed to assure that the substrate was not subjected to any competing non-catalyzed background reactions. The hydrolysis conversion rate was followed by ^1H NMR spectroscopy, using 1,4-dimethoxybenzene as the internal standard. The ee_s values were determined by GC on a Supelco β-Dex 120 column and the ee_p values by HPLC on a Chiracel OD-H column. The results are presented in Table 1.



Scheme 2. Reagents and condition: (a) lipase, DMSO–H_2O (1:9), pH 7.

Lipases from *Candida antarctica*, *Mucor miehei*, *Rhizopus arrhizus*, *Rhizopus niveus* and *Hog pancreas* gave little or no conversion. *Candida cylindracea* gave a reasonably high E_s value of 22, but only showed a 29% conversion to the alcohol over five days. The E_s values for *Alcaligines* spp. 20 from Europa Bioproducts Ltd gave an E_s value of 24. This E_s value is comparable with the value obtained with the lipase from the same organism, but from Boehringer Mannheim, which was used for a previous resolution.[5] It would indeed be possible to change the commercial sources of the lipases from the *Alcaligines* species, whilst retaining the enantioselectivity for the substrate. The most efficient lipase identified in our screening was from *Pseudomonas fluorescens*, with $E_s = 38$ and $E_p = 58$. The conversion time was also reasonable, making it the most promising candidate for future scale-up experiments. To gain scale-up experience with these enzyme reactions, we chose to use *Pseudomonas cepacia* lipase. This lipase gave a good E_s value of 13 and is considerably cheaper compared with *P. fluorescens* lipase. The scale-up experiment was performed with 793 mg of substrate, affording a similar E_s value (13) and conversion (63%) as in the screening procedure (E_s = 12% and 66% conversion). The ee_s value of acetate **3** was determined indirectly on the corresponding alcohol, the hydrolysis product of **3**. Ester **3** was hydrolyzed to alcohol **2** using 2 M NaOH at room temperature. The alcohol was then subjected to analysis of the enantiomeric purity by chiral HPLC. The absolute configuration was determined by comparison of measured with published $[\alpha]_D$ values of (*R*)- and (*S*)-**2**. Interestingly Sih's Eq. 2 failed to calculate E_p for *P. cepacia* (entry 5) and *Alcaligines* spp. 20 (entry 11). In both cases the ee_p values were too high for Eq. 2 to handle. Both Eqs. 1 and 2 are sensitive to side reactions.[6] The chemical background reactions in this study were accounted for by the control experiment in entry 12. In addition, the conversions in the enzyme catalyzed hydrolysis were followed by ^1H NMR spectroscopy with no traces of any by-products detected. The use of an internal standard made it possible to calculate the yield before any work-up was performed and the yields obtained were in all cases quantitative. This excludes many of the stated side reactions known to occur.[6] A plausible explanation of the failure to use Sih's equations might be that not only is the reaction reversible but it is also enantioselective in the synthetic direction towards the ester. Further experiments are, however, needed to determine the mechanism behind the described phenomena.

3. Conclusion

In conclusion, *P. fluorescens* lipase (Fluka) was found to give the highest enantiomeric ratio of the 11 lipases screened. At 51% conversion, the ee value (ee_p) for the product was found to be 89%, giving an enantiomeric ratio (E_p) of 58, whilst the ee value (ee_s) for the substrate was 89%, giving an enantiomeric ratio (E_s) of 38. These values suggest the enzyme preparation from *P. fluorescens* as a suitable substitute to obtain the desired ee of >98%. Future experiments planned to obtain a desired ee_p in reasonable yields include large scale resolution followed by product recycling.

Table 1. Enzyme catalyzed hydrolysis of (±)-**3**

Entry	Enzyme	Time (h)	Conversion (%)[a]	ee_s (%)[b]	ee_p (%)[c]	E_s[d]	E_p[e]	$[\alpha]_{Ds}^{18}$ [f]	$[\alpha]_{Dp}^{18}$ [g]
1	*Aspergillus*[h]	96	81	84	19	3	3	—[i]	—
2	*Candida antarctica*[h]	120	<10	—	—	—	—	—	—
3	*Candida cylindracea*[h]	120	29	36	48	22	3	—	—
4	*Mucor miehei*[h]	144	<10	—	—	—	—	—	—
5	*Pseudomonas cepacia*[h]	72	67	98	72	12	n.c.[j]	—	—
6	*Pseudomonas fluorescens*[h]	72	51	89	89	38	58	—	—
7	*Rhizopus arrhizus*[h]	120	<10	—	—	—	—	—	—
8	*Rhizopus niveus*[h]	144	<10	—	—	—	—	—	—
9	*Hog pancreas*[h]	144	<10	—	—	—	—	—	—
10	*Alcaligines* spp. 12[k]	192	19	3	61	1	4	—	—
11	*Alcaligines* spp. 20[k]	72	57	96	85	24	n.c.	—	—
12	Control[l]	144	0	0	0	0	0	—	—
13	*Pseudomonas cepacia*[m]	72	63	96[n]	74	13	n.c.	−25 (*S*)[o]	20 (*R*)[o]

[a] Determined by ^{1}H NMR.
[b] ee_s = enantiomeric excess of substrate. Determined by GCon a Supelco β-Dex 120 column.
[c] ee_p = enantiomeric excess of product. Determined by HPLC on a Chiracel OD-H column.
[d] E_s = enantiomeric ratio of substrate, calculated by Eq. 1.
[e] E_p = enantiomeric ratio of product, calculated by Eq. 2.
[f] $[\alpha]_{Ds} = [\alpha]_D$ for the substrate, measured on the alcohol (*c* 1.0, CH_2Cl_2), see footnote m.
[g] $[\alpha]_{Dp} = [\alpha]_D$ for the product (*c* 1.0, CH_2Cl_2).
[h] Fluka lipase basic kit.
[i] — = not determined.
[j] n.c. = not possible to calculate with Eq. 2.
[k] Lipases from Europa Bioproducts Ltd.
[l] Same reaction conditions, but without enzyme.
[m] Scale-up experiment with 793 mg of acetate.
[n] The ester was hydrolyzed with 2 M NaOH, then the ee_s was determined for the alcohol by HPLC on a Chiracel OD-H column.
[o] Absolute configuration determined by measuring the $[\alpha]_D$ value and comparing with Ref. 5.

The scale-up experiment with *P. cepacia* lipase proved that it is possible to perform the resolution with these substrates on a larger scale with the enantioselectivity and conversion rate remaining intact.

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