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Enzymatic Alcoholysis of Alkoxymethyl Alkanoates: a Possible Approach for the Kinetic Resolution of Tertiary Alcohols

Maurice C.R. Franssen*, Earl L.V. Goetheer, Hugo Jongejan and Aede de Groot*

Department of Organic Chemistry, Wageningen Agricultural University, Dreijenplein 8, 6703 HB Wageningen, The Netherlands

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Abstract

The pivaloyloxymethyl and butanoyloxymethyl derivatives of *tert*-butanol and linalool (**1**, **4**) are readily accepted by hydrolases. Linalool derivative **4b** is alcoholysed stereoselectively by *Candida rugosa* lipase (E=9.7).

Keywords: lipase; esterase; tertiary alcohols; kinetic resolution

The use of hydrolytic enzymes like esterases, lipases, proteases and peptidases in organic synthesis has become common practice in recent years, both in academia and in industry. Their advantages are fourfold: i) they are cheap, ii) they do not need cofactors, iii) they are highly active and stable both in water and in organic solvents, and iv) they are able to convert a broad range of substrates with high stereoselectivity [1]. Despite their numerous applications, commercially available hydrolases rarely catalyse reactions involving sterically hindered compounds like tertiary alcohols or esters thereof [2]. *tert*-Butanol has even been used as an inert solvent in protease- and lipase-catalysed reactions [3]. Screening programmes afforded enzymes which accept more hindered alcohols, but these display low reaction rates [4] and/or low stereoselectivity [2c,4] and are still limited in their substrate range [4,5].

Presumably commercially available hydrolases are not reactive towards tertiary alcohols or esters thereof because either the substrate is too bulky to penetrate into the enzyme active site, or because correct positioning of the substrate inside the active site is impossible due to steric restrictions of the binding pocket. We reasoned that the alcohol derivative might be acceptable to commercially available hydrolases if a spacer would be introduced between the tertiary carbon atom and the reaction centre. This spacer should be easy to attach, easy to remove and should be connected to a group which is well accepted by hydrolases. The spacer should not be too long since there is evidence that the stereoselectivity of enzymes decreases when the distance between the chiral centre and the reaction centre increases [6]. We chose the alkanoyloxymethyl group because this is a mixed acetal/ester of formaldehyde. After hydrolysis, the formed hemiacetal of formaldehyde would split off formaldehyde (presumably spontaneously), thus liberating the free tertiary alcohol [7,8].

In order to test this hypothesis, the pivaloyloxymethyl derivatives of *t*-butanol (**1a**) and linalool (**1b**) [9] were prepared. Screening of 20 commercially available lipases, esterases and proteases revealed that only *Rhizomucor miehei* lipase [10a], *Pseudomonas fluorescens* lipase [10a], lipase G [10a] (*Penicillium sp.*) and cholesterol esterase [10b] showed acceptable activity in the alcoholysis of **1** with *n*-butanol in octane (see scheme

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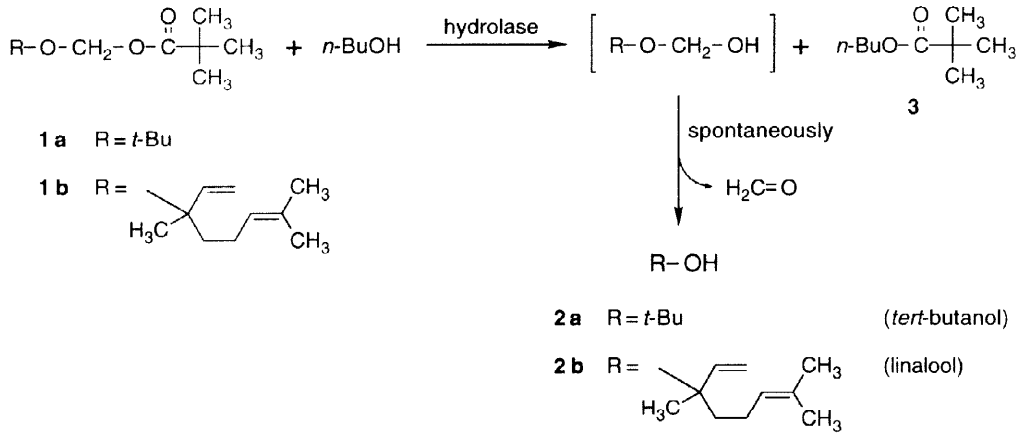
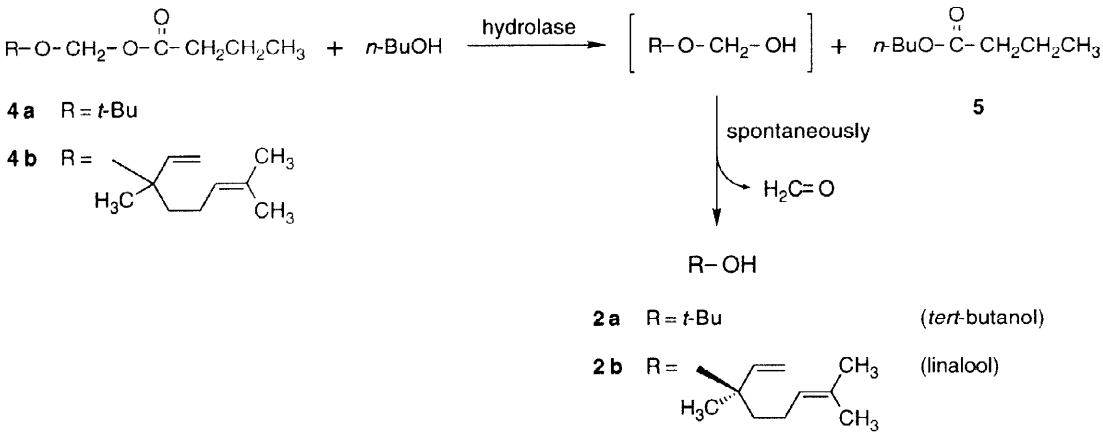
1). The conversion rate was 6-30% in 80 hours for the first three enzymes, whereas cholesterol esterase gave 100% **2a** and 46% **2b** in 80 h. Inspection of the stereoselectivity of these four enzymes revealed that linalool (**2b**) was formed with very low e.e. (**E** = 1.00 - 1.15).



Scheme 1. Alcoholysis of pivaloyloxymethyl derivatives of *tert*-butanol (**1a**) and linalool (**1b**).

In order to make the substrates acceptable for a wider range of enzymes, derivatives with a less bulky acyl group were constructed. Reaction of chloromethyl butanoate [11] with the sodium salt of *t*-butanol or linalool in THF afforded butanoyloxymethyl derivatives **4** [12]. The alcoholysis of **4a** (see Scheme 2) with *n*-butanol in octane was catalysed by many hydrolases with a much higher rate compared to **1** (see Table 1).



Scheme 2. Alcoholysis of butanoyloxymethyl derivatives of *tert*-butanol (**4a**) and linalool (**4b**).

The lipases from *Candida rugosa*, *Pseudomonas fluorescens* and *Rhizomucor miehei* as well as lipoprotein lipase and cholesterol esterase displayed the fastest conversion of **4a**. These enzymes, together with two other lipases (*Candida antarctica* B and *Humicola lanuginosa*) were taken for the determination of the enantioselectivity of the alcoholysis of **4b** in octane. The results are displayed in Table 2. It is clear that all seven enzymes are able to react with **4b**, although the reaction rates differ to a large extent. Alcoholysis of **4b** was enantioselective, although the E-values were generally low. All enzymes preferably produced (R)-**2** [13], except *Pseudomonas fluorescens* lipase.

Table 1

Enzymatic alcoholysis of *t*-butyloxymethyl butanoate (**4a**) [14].

Enzyme (L=lipase, E=esterase)	supplier	mg enzyme added	time (h)	conversion (%)
Candida rugosa L	Sigma	100	110	100
Porcine pancreatic L	Sigma	100	110	6
Chromobacterium viscosum L	[10a]	5	110	0
Lipoprotein L	[10b]	5	86	75
Lipase AP6	[10b]	100	86	10
Lipase PS	[10b]	100	86	1
Lipase N conc	[10b]	100	86	12
Lipase R10	[10b]	100	86	3
Lipase G	[10b]	100	110	6
Aspergillus niger L	[10a]	20	110	2
Geotrichum candidum L	[10a]	20	86	9
Rhizomucor miehei L	[10a]	20	110	96
Pseudomonas fluorescens L	[10a]	20	86	72
Cholesterol E	[10b]	5	110	100
Naproxen E	[10b]	5	110	2
subtilisin (protease)	Sigma	5	110	8

Table 2

Enzymatic alcoholysis of linaloxymethyl butanoate (**4b**) [14].

Enzyme	time (h)	conversion (%)	E-value
Candida rugosa L	4.25	100	2.5
Lipoprotein L	49	35	1.9
Pseudomonas fluorescens L	48	50	2.3*
Cholesterol E	2.5	100	1.6
Rhizomucor miehei L	48	8	1.5
Candida antarctica B L [10d]	7	100	2.0
Humicola lanuginosa L [10d]	24	100	1.9

* Preference for the (S)-enantiomer.

The reaction of the most selective enzyme (*Candida rugosa* lipase) was optimised with respect to organic solvent and temperature, since it is known that these parameters may affect the stereoselectivity of enzymes [1a,15]. The results are listed in Table 3.

Table 3

Effect of organic solvent and temperature on the *Candida rugosa* lipase-mediated alcoholysis of linaloxymethyl butanoate (**4b**) [14].

Solvent	logP of solvent	Temperature (°C)	Initial rate (μmol.mg enzyme^{-1}.h^{-1})	E-value
acetonitrile	-0.33	45	0.1	1.4
tetrahydrofuran	0.49	45	very slow	1.0
di-*iso*propyl ether	1.9	45	0.9	2.1
benzene	2.0	45	1.05	5.4
toluene	2.5	45	1.20	5.5
octane	4.5	45	2.30	2.5
hexadecane	8.8	45	2.60	1.0
toluene	2.5	20	0.5	6.2
toluene	2.5	0	0.15	9.7
toluene	2.5	-10	0.08	9.9

The organic solvent indeed has a profound effect on the reaction rate and the stereoselectivity of the alcoholysis of **4b**. Toluene appeared to be the best of all the solvents tested. There is a linear relation between the initial rate of the enzymatic reaction and the logP of the solvent, but there is no relation between the E-value and the nature of the organic solvent [16]. The background of solvent effects on enzyme stereoselectivity remains enigmatic [1d]. Decreasing the reaction temperature lead to a higher E-value, which means that the stereoselectivity of the reaction is dominated by $\Delta\Delta H^{\ddagger}$ [15]. The most selective reaction was obtained at -10°C, but the alcoholysis at 0°C is more practical without losing too much selectivity [17].

It is clear from the results, depicted in Table 3, that *Candida rugosa* lipase is well suited for the kinetic resolution of linalool (E=9.7-9.9). It is for the first time that this tertiary alcohol is enzymatically resolved with appreciable selectivity. These results show that our strategy to turn tertiary alcohols into suitable substrates for commercially available hydrolases is fruitful. Investigations to determine the scope and limitations of this method are currently in progress.

References and notes

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[7] This approach has been used succesfully for the resolution of hindered carboxylic acids (Ebiike H, Terao Y, Achiwa K. Tetrahedron Lett. 1991;41:5805-5808) and barbiturates (Murata M, Achiwa K. Tetrahedron Lett. 1991;32:6763-6766).

[8] See for a similar approach using a stable spacer: Brackenridge I, Mc.Cague R, Roberts SM, Turner NJ. J. Chem. Soc. Perkin Trans. I 1993:1093-1094.

[9] **1a** Was prepared from potassium *tert*-butoxide and chloromethyl pivalate (Acros Organics) in THF, 47% yield [18]. **1b** Was prepared by reaction of linalool with NaH in THF followed by treatment with chloromethyl pivalate, 40% yield [18].

[10] The enzymes were gifts from a) Biocatalysts Ltd.; b) Amano; c) Gist brocades; d) Novo Nordisk.

[11] Obtained from butyryl chloride and paraformaldehyde using zinc chloride as a catalyst (63% after distillation [18]): Rasmussen M, Leonard NJ. J. Am. Chem. Soc. 1967;89:5439-5445.

[12] Yields are 71% for **4a** and 61% for **4b**, respectively [18].

[13] Established by retention time on chiral GC (Beta-DEX™ 120) through comparison to racemic linalool and oil of coriander, which contains (R)-linalool: Connolly JD, Hill RA. Dictionary of Terpenoids, Vol. I. London: Chapman & Hall, 1991:12.

[14] Standard procedure: 1 ml of a solution of 100 mM **4** in octane (unless indicated otherwise) containing 500 mM *n*-butanol and 45 mM hexadecane (internal standard) was mixed with enzyme and shaken at 45°C (unless indicated otherwise) and 250 rpm. The reaction was monitored by GC. Blanks showed no reaction.

[15] Phillips RS. TIBTECH 1996;14:13-16.

[16] The thermodynamic water activity (a_w, varied between 0.07 and 1.0) influenced the initial activity (V_{ini} is highest at a_w=1) but had no effect on the E-value.

[17] Typical procedure: 6 ml of a solution of 100 mM **4b** and 500 mM *n*-butanol in toluene was added to 100 mg *Candida rugosa* lipase. After 2 h of magnetic stirring at 0°C (conversion = 52%), the solution was filtered, concentrated and chromatographed to give 5.1 mg linalool [18] (e.e. = 68%, detd. by GC).

[18] Numbers refer to isolated yields and are not optimised. NMR and HRMS data of all substrates and products are in full accordance with the depicted structures.