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Hemiacetals and their esters as side-products in lipase-catalysed transesterifications of vinyl esters with sterically hindered alcohols

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Abstract

Lipase-catalysed transesterifications of vinyl esters with various sterically hindered secondary alcohols sometimes give hemiacetals and hemiacetal esters as major side-products along with the expected esters, especially in the presence of aldehydes. The substrates, reaction conditions, and the lipases required for the formation of such hemiacetal products have been studied. Hemiacetals and their esters are very easily hydrolysed. Therefore, when conventional work-up procedures are used, the formation of such products in lipase-catalysed transesterification reactions may easily escape notice, leading to, e.g. an unexpectedly low enantiomeric purity of the isolated remaining substrate in a resolution reaction.

Keywords: Lipase; Sterically hindered alcohol; Hemiacetal esters

1. Introduction

Transesterification of esters catalysed by hydrolases in organic solvents constitutes a useful method for the resolution of chiral racemic secondary alcohols [1,2]. In order to obtain an irreversible acylation of the alcohol, vinyl esters are commonly used as acyl donors. During the reaction, vinyl alcohol is liberated and this is rapidly and practically irreversibly tautomerised into acetaldehyde (Scheme 1, step 1). Apart from its ability to deactivate some enzymes [3,4], the latter has been considered to be an inert product unable to interfere with the desired esterification reaction. However, in a recent communication we have, in collaboration with a Dutch group, shown that the aldehyde does indeed react with the alcohol in some cases [5]. Thus, when catalysed by a number of lipases in the presence of vinyl acetate, some sterically hindered secondary alcohols yield, beside the expected esters, hemiacetal esters. These undesired side-products are presumably formed by reaction of the alcohol with the acetaldehyde produced in the acylation reaction. Lipase-catalysed diastereoselective acylation of the resulting hemiacetal furnishes a hemiacetal ester [5], (Scheme 1).

Our previous results encouraged us to further study the formation of hemiacetals and their esters. Thus some sterically hindered alcohols were allowed to react with vinyl acetate using a number of enzymes as catalysts [PCL-L-6 (CHIRAZYME L-6), PCL-PS (Lipase PS "Amano") (*Pseudomonas* sp.) or CAL-B (NOVOZYM 435) (*Candida antarctica* sp.)]. In addition, two non-vinyl esters (trichlorethyl acetate and trifluoroethyl acetate) were tested as acyl donors.

2. Results and discussion

2.1. The general applicability of the reaction

To determine if the formation of hemiacetal esters is a general reaction for slow-reacting, sterically hindered alcohols other than borneol, **1**, and the tetra-, and octahy-

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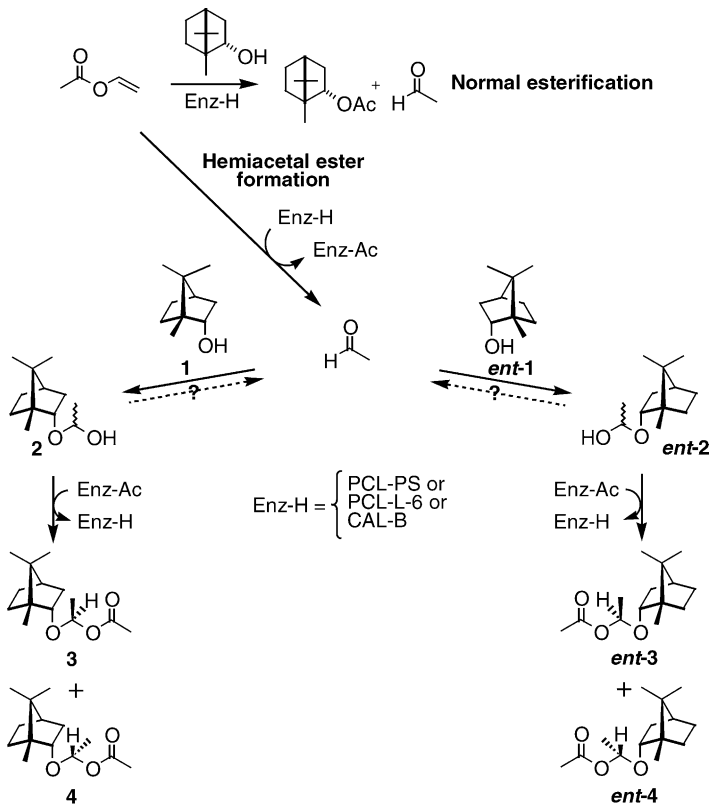

Scheme 1. Formation of acetylated hemiacetal during lipase-catalysed transesterification of vinyl acetate with the sterically hindered alcohol borneol.

dronaphtols **7**–**9** [5], various alcohols (**6** and **10**–**20,** Fig. 1) were used as substrates in transesterifications of vinyl acetate using mainly PCL-L-6 as the catalyst (sometimes CAL-B and PCL-PS were used). The tertiary alcohols, α-terpineol

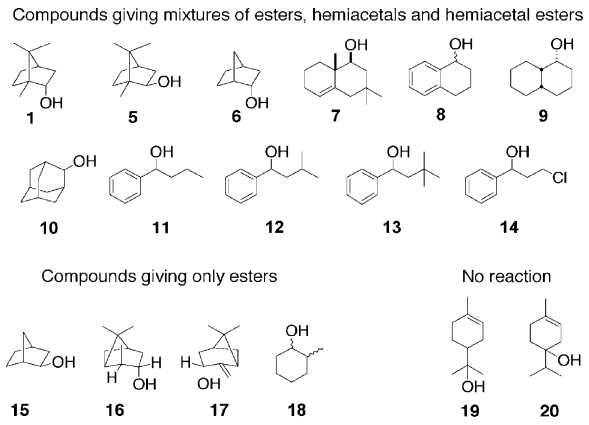

Fig. 1. Alcohols used as substrates in transesterification of vinyl acetate: (+)-borneol, (**1**); isoborneol, (**5**); *endo*-norborneol, (**6**); 3,3,8a-trimethyl-2,3,4,6,7,8,8a-octahydronaphtalene-1-ol, (**7**); 1,2,3,4-tetrahydronapht-1-ol, (**8**); (4,4,0)-bicyclodecan-1-ol, (**9**); 2-adamantol, (**10**); 1-phenyl-1-butanol, (**11**); 3-methyl-1-phenyl-1-butanol, (**12**); 3,3-dimethyl-1-phenyl-1-butanol, (**13**); 3-chloro-1-phenyl-1-propanol, (**14**); *exo*-norborneol, (**15**); (+)-isopinochampheol, (**16**); (−)-*trans*-pinocarveol, (**17**); 2-methylcyclohexanol, (**18**); α-terpineol, (**19**); terpinene-4-ol, (**20**). The lipases used in the reactions were PCL-L-6, PCL-PS (from *Pseudomonas* sp.) and CAL-B (from *Candida antarctica*).

(**19**) and (+)-terpinene-4-ol (**20**), and the secondary alcohols, isoborneol (**5**), *endo*-norborneol (**6**), *exo*-norborneol (**15**), (−)-isopinochampheol (**16**), (+)-isopinochampheol (***ent*-16**) and (−)-*trans*-pinocarveol (**17**) were allowed to react with vinyl acetate in TBME at room temperature (22 °C) using PCL-L-6 as catalyst. For (−)-*trans*-pinocarveol (**17**), PCL-PS (Lipase PS) was also tested. The 1-phenyl-1-butanols **11**–**13**, 3-chloro-1-phenyl-1-propanol (**14**), and 2-adamantol (**10**), were used under the same conditions employing both PCL-L-6 and CAL-B as catalysts. All the products were identified by GC-MS.

Whereas the sterically hindered tertiary alcohols α-terpineol (**19**) and terpinene-4-ol (**20**) did not react at all, the formation of acetylated hemiacetals was observed from isoborneol (**5**), *endo*-norborneol (**6**) (cf. Högberg et al. [5]), 2-adamantol (**10**, Table 1, entries 1–4), the phenylbutanols (Table 1, entries 5–16) and 3-chloro-1-phenyl-1-propanol (Table 1, entries 17–20). Thus, the conversion of isoborneol was 41% after 14 days and the product consisted of the acetate (23%) and the acetylated hemiacetal (18%). The mass spectrum of the isobornyl hemiacetal ester is almost identical to that of the bornyl acetal ester (Table 2). The norborneols **6** and **15** were totally converted into products in 4 days. Whereas 8% of the product formed from *endo*-norborneol consisted of the acetate of the hemiacetal, no such product was detected from *exo*-norborneol. Neither isopinochampheol [(+)- or (−)-**16**] nor (−)-*trans*-pinocarveol (**17**) gave any hemiacetal prod-

Table 1
Relative percentage of the constituents in the reaction mixture after 14 days

Entry	Substrate	Lipase	Acetaldehyde	Alcohol[a] (%)	Ester[a] (%)	Hemiacetal[a] (%)	Hemiacetal ester[a] (%)
1	**10**	PCL-L-6	No	50–57	18–23	26–34	0–0.5
2	**10**	PCL-L-6	Yes	25–30	4–5	53–56	7–8
3	**10**	CAL-B	No	72	5	19	2.5
4	**10**	CAL-B	Yes	55	3	36	6
5	**11**	PCL-L-6	No	38–43	57–62	–	–
6	**11**	PCL-L-6	Yes	68–82	17–31	–	0–0.5
7	**11**	CAL-B	No	53–56	38-40	6–7	–
8	**11**	CAL-B	Yes	44–48	23–31	23–28	1
9	**12**	PCL-L-6	No	96	2–3	0–0.2	0–0.2
10	**12**	PCL-L-6	Yes	98	1	–	0–0.4
11	**12**	CAL-B	No	83–85	0.5	6–7	8–9
12	**12**	CAL-B	Yes	80	0.3	8–9	7–11
13	**13**	PCL-L-6	No	96–97	2–3	0.3–0.6	0.4
14	**13**	PCL-L-6	Yes	97	1	0.6–0.9	1
15	**13**	CAL-B	No	77–81	–	8–10	10–12
16	**13**	CAL-B	Yes	65–67	0–2	14	19
17	**14**	PCL-L-6	No	34–37	63–65	–	0.2–0.4
18	**14**	PCL-L-6	Yes	34–36	60–61	1	1
19	**14**	CAL-B	No	50–52	38–40	7	2
20	**14**	CAL-B	Yes	46–48	24–33	22–24	4–5

[a] Sum of enantiomers and/or diastereomers.

Table 2
Major peaks in the Mass spectra of some hemiacetals and acetylated hemiacetals

1-HA	**1**-HAE	**5**-HAE	**6**-HAE	**10**-HA	**10**-HAE	**11**-HA	**11**-HAE	**12**-HA	**12**-HAE	**13**-HA	**13**-HAE	**14**-HA	**14**-HAE
180(1)	180(2)	180(4)	138(10)	178(3)	178(6)	176(1)	176(5)	148(4)	190(15)	161(24)	204(14)	155(8)	197(3)
137(58)	137(45)	137(66)	95(100)	136(11)	150(37)	133(25)	133(24)	147(28)	149(38)	105(27)	161(23)	153(26)	155(11)
121(3)	121(27)	121(47)	79(18)	135(100)	136(11)	117(7)	117(16)	131(5)	147(30)	91(26)	149(61)	117(19)	153(28)
95(43)	95(87)	95(70)	67(52)	134(12)	135(100)	92(8)	107(16)	105(30)	131(15)	57(100)	145(21)	115(13)	117(24)
93(14)	93(41)	93(75)	66(21)	93(24)	134(60)	91(100)	105(10)	104(7)	107(51)	41(12)	107(70)	92(8)	115(15)
81(100)	81(100)	81(100)	55(18)	91(10)	93(33)	77(8)	92(12)	92(9)	105(33)		105(34)	91(100)	91(100)
79(10)	79(18)	79(21)	43(22)	81(12)	92(45)		91(100)	91(100)	104(11)		104(17)	77(10)	43(22)
69(27)	69(38)	69(29)	41(27)	79(22)	91(18)		77(10)	77(7)	92(12)		103(10)		
67(20)	67(22)	67(43)	40(11)	67(23)	81(12)		43(18)	43(10)	91(100)		91(31)		
57(14)	57(7)	57(13)	32(12)	41(9)	79(33)				77(11)		77(12)		
55(16)	55(22)	53(21)			77(12)				43(58)		57(100)		
43(19)	43(45)	55(24)			67(23)						43(62)		
41(31)	41(49)	43(50)			43(39)						41(17)		
		41(56)			41(12)								

HAE: hemiacetal ester from acetaldehyde and acetate; HA: hemiacetal from acetaldehyde.

ucts. Only the corresponding acetates were obtained after 14 days (100%, 87%, and 100% conversion, respectively).

In contrast to the terpenoid substrates **1**, **5** and **6**, which only occasionally yielded traces of hemiacetals, the substrates **10**–**14** often gave substantial amounts of hemiacetals. The occurrence of hemiacetals as side-products in lipase-catalysed reactions is a new but not unexpected observation. If the solvent was not dried over molecular sieves prior to use, the ratio hemiacetal/hemiacetal ester often increased.

2.2. Reactions in presence of aldehydes

Earlier we have shown, that when transesterification reactions of vinyl acetate and borneol are performed in the presence of acetaldehyde, not only does the yield of the hemiacetal ester increase but so does also the diastereoselectivity (cf. Högberg et al. [5] Fig. 2).

In parallel with the experiments using substrates **10**–**14** and vinyl acetate, additional experiments were performed with acetaldehyde present in the reaction mixtures (Table 1). Except for entries 5, 6, 9 and 10 (Table 1) the yields of both hemiacetals and hemiacetal esters increased. In the presence

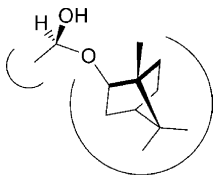

Fig. 2. Bornyl hemiacetal 2 docked into Kazlauskas' model pockets [6]. The diastereoselectivity increases in presence of extra acetaldehyde.

Table 3
Transesterification of (+)-, (−)-borneol, (**1** and ***ent*-1**) using propanal

Entry	Substrate[a]	Lipase (mg)	Additives (mg)	C (%)	Of product mixture after 14 days[b] (%)
1	***ent*-1**	PCL-L-6/4.8	Vinyl acetate 128–129, propanal 15.1–15.2	37–50	***ent*-1-Ac** 0%, HAE (acetaldehyde) 39%, HAE (propanal) 61%
2	**1**	PCL-L-6/4.3-7.1	Vinyl acetate 126–127, propanal 12.0–15.8	12–18	**1**-Ac 0%, HAE (acetaldehyde) 43–49%, HAE (propanal) 51–57%

[a] 0.65 ml solution of (+) or (−) borneol (0.1 M) with undecane (0.05M) as internal standard in TBME.
[b] **1**-Ac: bornyl acetate; HAE: hemiacetal ester.

of acetaldehyde the conversion of the alcohol substrate increased. However, in contrast to reactions with borneol the diastereoselectivety remained unchanged in reactions with the substrates **11**–**14**.

Addition of propanal instead of acetaldehyde to a reaction mixture containing (−)-borneol, enzyme, and vinyl acetate furnished a mixture of acetate esters of two hemiacetals, a single diastereomer of the acetate of the acetaldehyde derived hemiacetal of borneol along with a single diastereomer of the ester arising from acetylation of the corresponding borneol hemiacetal with propanal. After 14 days (50% conversion) the ratio of the two hemiacetal esters was ≈2:3 (entry 1, Table 3). Apart from these esters, traces of aldol condensation products were also detected (cf. Branneby et al. [7]). Under similar conditions and when (+)-borneol was used as the substrate, the relative amount of the esters of the hemiacetals from acetaldehyde and propanal was 6:7 (18% conversion, entry 2, Table 3). In contrast to the single diastereomers obtained from (−)-borneol, its enantiomer furnished a mixture of two acetate esters of each of the hemiacetals, two from acetaldehyde (dr 17:2), along with two from propanal (dr 19:2).

2.3. Prerequisites for formation of hemiacetal derivatives

Earlier, we have shown that, in the absence of an enzyme, borneol and vinyl acetate do not form any products [5]. Neither were hemiacetal esters formed from a mixture of borneol, acetaldehyde and acetic acid nor from borneol, acetaldehyde, acetic acid and acetic anhydride. Only a minute amount of bornyl acetate was detected in some of the samples from these experiments.

A fairly sterically hindered alcohol which will not give diastereomeric hemiacetal esters is 2-adamantol (**10**). As mentioned above (entries 1–4, Table 1), when treated with enzyme and vinyl acetate, this alcohol furnished not only the expected hemiacetal ester but also substantial amounts of the hemiacetal, especially in the presence of added acetaldehyde (entries 2 and 4). The obvious explanation for this observation is that a spontaneous non-catalysed reaction occurs between acetaldehyde and 2-adamantol (**10**), which is then acylated in an enzyme catalysed transesterification of vinyl acetate. However, in the absence of vinyl acetate and with or without the addition of enzyme, 2-adamantol and acetaldehyde failed to produce any hemiacetal. In addition, in the absence of enzyme, 2-adamantol (**10**), vinyl acetate, and acetaldehyde did not yield any hemiacetal or hemiacetal ester.

In order to see, if the presence of a vinyl ester was a necessary requirement for hemiacetal formation in enzyme catalysed reactions of sterically hindered alcohols, we studied the alternative acyl donors 2,2,2-trifluoroethyl and 2,2,2-trichloroethyl acetate. Both the trihaloesters and (−)-borneol in the presence of Chirazyme L-6 yielded bornyl acetate and thus they were indeed acyl donors. When acetaldehyde was added prior to addition of the enzyme, small amounts of acetates of hemiacetals were also detected in the reaction mixture.

Thus, the presence of a lipase, an acyl donor, and an aldehyde is a prerequisite for the formation of both hemiacetals and hemiacetal esters. The yields of both hemiacetals and hemiacetal esters can be improved by addition of an aldehyde.

In order to establish that the formation of hemiacetal esters were truly lipase catalysed, and not mediated by impurities present in the crude enzyme preparation, a purified sample of CAL-B was used as catalyst. It did indeed catalyse the formation of hemiacetal esters. To confirm that the active site of CAL-B was the same in this reaction as that used by the enzyme for normal acylations, the active site of the same enzyme sample was inhibited by treatment with excess methyl *p*-nitrophenyl *n*-hexylphosphonate [8] resulting in a 99% reduction of lipase activity. The inhibited enzyme did neither promote hemiacetal nor hemiacetal ester formation to any appreciable degree not even when acetaldehyde was added to the reaction mixture.

2.4. Effects of having hemiacetal esters present among the products

When subjected to conventional work-up procedures, e.g. treatment with water or when chromatographic purification is attempted, the hemiacetal esters easily decompose into the alcohol, aldehyde, and acetic acid. Thus, in routine scanning for suitable conditions in lipase-catalysed irreversible transesterifications of vinyl acetate with sterically hindered secondary alcohols, the formation of hemiacetals and their esters might easily be overlooked. If, during a resolution, hemiacetal-esters are formed and escape notice, their presence can, after workup, lead to unexpectedly low ee-values for the remaining substrate. For example Liu et al. ([9] and [10]; cf. also Raju et al., [11]) have reported that CAL-B with

vinyl butanoate as the acyl donor can resolve 3-chloro-1-(2-thienyl)-1-propanol and 3-chloro-1-phenyl-1-propanol (**14**, Fig. 1) with exceptionally high *E*-values of 300 and 1000, respectively. If, however, the *E*-values are calculated based on the ee:s of the isolated resolved compounds, they are both close to 200, and the authors briefly comment on this. A possible explanation for this unexpected decrease is that small amounts of hemiacetal esters were formed and that these were decomposed during hydrolytic work-up, with the liberated racemic alcohol contaminating the product mixture. Another example of side-products from liberated acetaldehyde has been observed on attempted resolution of a 2-aminoalcohol by lipase-catalysed acylation with vinyl acetate, which yielded a cyclic aminal, (an oxazolidine) [12]. Other groups have reported that side reactions can occur when using vinyl esters as acyl donors, although they neither describe the nature of these reactions nor their products [13,14].

2.5. Conclusions

From the data available, it is not possible to establish a simple and general rule, which can predict the extent of formation of hemiacetal esters from the structure of the substrate, the lipase used, or the reaction conditions employed. However, it is more likely that hemiacetal esters will be formed during a lipase catalysed resolution of a sterically hindered alcohol with vinyl acetate or acyl donor and aldehyde present under dry conditions than under other circumstances.

3. Experimental

3.1. Solvents and chemicals

tert-Butyl methyl ether (TBME) (>99%) was purchased from Merck. The TBME was distilled before use. Vinyl acetate (>99%) was purchased from Merck-Schuchardt and was dried by addition of molecular sieves (4 Å). Acetic anhydride (97%), acetaldehyde, and propanal (>99%) were purchased from Kebo Lab (Merck-Schuchardt). Acetaldehyde was distilled before use. Acetic acid (>99%) was purchased from Tamro (J.T. Baker). The purity of the following compounds was determined by GC: (+)-Borneol (**1**), (98% ee, 98%) and (−)-borneol (***ent*-1**), (100% ee, 98%) were purchased from Aldrich Chem. Co. Racemic borneol was obtained by mixing the two enantiomers of borneol. 2-Adamantol (98%) (**10**), α-terpineol (65%) (**19**), (+)-terpinene-4-ol (97%) (**20**) and (−)-trans-pinocarveol (96%) (**17**) were purchased from Fluka. Isoborneol (97%) (**7**), *endo*-norborneol (97%) (**6**), *exo*-norborneol (99%) (**15**), (–)-isopinochampheol (95% ee, 97%) (**16**) and (+)-isopinochampheol (>99% ee, 99%) (***ent*-16**) were purchased from Aldrich Chem. Co.

3.2. Enzymes

Commercially available lipases (EC 3.1.1.3) were used without further purification unless otherwise stated. NOVOZYM 435, from *Candida antarctica B*, CAL-B were kindly supplied by Novo Nordisk. Two samples of *Candida antarctica* lipase, which were prepared according to two different procedures were kindly supplied by Anders Magnusson, Department of Biotechnology, The Royal Institute of Technology (KTH) in Stockholm. One of them was cultivated at KTH and then immobilised on powdered polypropylene and the other sample was from Novo Nordisk and was a pure *Candida antarctica* lipase, which was immobilised at KTH. CHIRAZYME L-6 from *Pseudomonas cepacia*, PCL (old name: *Pseudomonas fluoroscens*, PFL) was purchased from Boehringer Mannheim, and Lipase PS “Amano” from *Pseudomonas cepacia*, PCL was a gift from Amano International Enzyme Co.

3.2.1. Preparation of 1-phenyl-1-butanols (*11*, *12*, *13*) and 3-chloro-1-phenyl-1-propanol (*14*)

The alcohols were prepared from the corresponding ketones by reduction with sodium borohydride in methanol or ethanol. Purification was made by recrystallisation or flash chromatography. The ketone precursors for **11** and **14** were commercially available and the ones for **12** and **13** were prepared by Friedel–Craft acylations with benzene and the appropriate acid chloride according to a standard procedure [15]. Isovaleric acid chloride was commercially available and 3,3-dimethylbutanoic acid chloride was prepared from the corresponding acid according to a standard procedure with $SOCl_2$ [15].

3.2.2. Preparation of 2,2,2-trichloroethyl acetate

The ester was prepared according to Boullais et al. [16]. Acetyl chloride (6.4 ml) was added dropwise to 2,2,2-trichloroethanol (3.8 g) and was left with stirring at room temperature over night. The mixture was then refluxed for 4 h and then poured out on 50 ml of ice water. White crystals precipitated. The mixture was extracted with 100 ml of ether. The organic phase was washed with 100 ml $NaHCO_3$ and 100 ml distilled water and then dried on $MgSO_4$. The solvent was removed on a rotary evaporator and the crude product was distilled at 53 °C (10 Torr) to give 3.2 g of ester.

3.2.3. Preparation of lipase CAL-B inhibited with methyl p-nitrophenyl n-hexylphosphonate and its reactions

The inhibitor was prepared as described by Rotticci et al. [8] except that acetonitrile was used as solvent instead of benzene. The inhibition of the lipase (in this case CAL-B) was achieved following the previously published procedure [8]. In order to see whether the inhibition was successful or not, the activities of the non-inhibited and the inhibited lipase samples were compared using octanol and vinyl acetate. After 20 min, non-inhibited CAL-B gave a 90% conversion of octanol into its acetate ester, whereas under the same conditions after 20 min, inhibited CAL-B had only converted 0.6% (after 18 h, only 26% conversion was observed). When the inhibited enzyme was reacted with vinyl acetate and borneol (−)-borneol for 14 days 0.5% of bornyl acetate and 0% of the

hemiacetal ester were formed. When enzyme that had been treated in exactly the same way, albeit without addition of the inhibitor, was used under the same conditions, the reaction yielded 5% bornyl acetate and 2% hemiacetal ester after 14 days.

3.3. Formation of acylated hemiacetals from sterically hindered alcohols, general procedure

A TBME solution (0.65 ml) containing the substrate alcohol (0.1 M) with undecane and/or pentadecane (0.05 or 0.1 M) as internal standards, were mixed with the relevant additives (vinyl acetate, acetaldehyde, propanal, acetic acid and/or acetic acid anhydride) in a 2 ml glass vessel (Not all of the additives were used every time). The vinyl esters were added as acyl donors. When lipases were used, the reactions were started by addition of PCL-L-6 (4–9 mg) or PCL-PS (41–46 mg) or CAL-B (17–46 mg). The glass vessel was sealed with a screw cap and left at room temperature (22 °C) on a roller mixer (model KEBO Lab, Assistant 348, RAM 5). Samples were withdrawn after 1, 3, 6 and 14 days (approximately 5 μl). All reactions were performed in duplicate or triplicate. The samples were analysed by GC or GC-MS.

3.4. Transformation of enantiomerically pure and racemic borneol

The reactions were performed using the general procedure described above. Internal standard: undecane (0.1 M) or pentadecane (0.1 M). Additive: vinyl acetate (130–175 mg). Addition of lipase PCL-L-6 (4.9–7.8 mg) or PCL-PS (41–46 mg) or CAL-B (40–46 mg) started the reaction. All reactions were performed in duplicate or triplicate. Samples were analysed by GC or GC-MS (Table 4, Methods 1–3).

3.5. Formation of acetylated bornyl hemiacetals in the presence of aldehyde

Experiments to confirm the importance of the presence of an aldehyde were performed using the general procedure described above. Substrate: (−)-borneol or (+)-borneol. Internal standard: undecane (0.05 M). Additives: acetaldehyde (11.44 mg 4 equiv.) or propanal (15.10 mg 4 equiv.) and vinyl acetate (130 mg). Addition of CHIRAZYME L-6 (5 mg) started the reaction. All reactions were performed in duplicate or triplicate. Samples were analysed by GC or GC-MS (Table 4, Method 1).

3.6. Formation of acetylated bornyl hemiacetals with alternative acyl donors and aldehyde

The experiments were performed using the general procedure with the following exceptions. Acyl donor: trifluoroethyl acetate (277 mg) or trichloroetyl acetate (370 mg). Substrate: 0,65 ml 0.1 M TBME solution of (−)-borneol. Additives: acetaldehyde (20 mg). Addition of CHIRAZYME L-6 (6–13 mg) started the reaction. All reactions were performed in duplicates. Samples were analysed by GC or GC-MS (Table 4, Method 1).

3.7. Transformation using α-terpineol, (+)-terpinene-4-ol, isoborneol, endo-norborneol, exo-norborneol, (−)-isopinochampheol, (+)-isopinochampheol and (−)-trans-pinocarveol

The reactions were performed using the general procedure described above. Internal standard: undecane (0.1 M) [for reaction with (−)-*trans*-pinocarveol: pentadecane (0.05 M)], additive: vinyl acetate (∼130 mg). Addition of lipase PCL-L6 (5–9 mg) [for (−)-*trans*-pinocarveol PCL-PS 40 mg was also tested] started the reaction. All reactions were performed in duplicate. The samples were analysed by GC-MS (Table 4, Method 1).

3.8. Transformation using 2-adamantol, 1-phenyl-1-butanol, 3-methyl-1-phenyl-1-butanol, 3,3-dimethyl-1-phenyl-1-butanol and 3-chloro-1-phenyl-1-propanol

The reactions were performed using the general procedure described above with the exception that no internal standard was used. Vinyl acetate (approximately 170 mg) was added to each reaction. Experiments in which acetaldehyde (15–20 mg) had been added were performed in parallel with the above-mentioned ones. The reactions were started by addition of lipase PCL-L6 (4–6 mg) or lipase CAL-B (17–25 mg). All reactions were performed in duplicate. The samples were analysed by GC-MS (Table 4, Method 4).

3.8.1. Mass spectra

Both EI and CI were used in the GC-MS analysis (Table 2). However, the molecular ion was neither observed from the acetates nor from the corresponding butyrates. One product, the hemiacetal, showing a similar MS as the acetylated hemiacetal, was sometimes detected.

Table 4
Methods used for GC and GC-MS analyses

Method	Column/detector	Split/splitless	GC-temperature programme
1	β-dex 120/FID or MS	1:80/1:40/1:5	70 °C, 15 min → 120 °C, 10 min (2 °C/min) → 180 °C (3 °C/min)
2	β-dex 120/FID	1:80	130 °C, 17 min → 180 °C (3 °C/min)
3	HP-5/MS	1:40/1:5	80 °C, 1 min → 160 °C (5 °C/min)
4	β-dex 120/MS	1:10	70 °C, 0 min → 180 °C, 20 min (3 °C/min) → 70 °C (5 °C/min)

3.8.2. Analysis by GC and GC-MS

The GC analyses were performed using a Varian 3300 gas chromatograph (FID-detector, detector temperature 225 °C, split ratio 1:80, injector temperature 200 °C). The GC-MS analyses were made using a Hewlett Packard GC-MS (GC: 6890, injector temperature 200 °C, split/splitless injector, MS: 5973). He (0.7–1.0 ml/min) was used as carrier gas. A HP-5 fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) and a chiral column β-dex 120 (30 m × 0.25 mm i.d., film thickness 0.25 μm) were used in the GC-analyses. The MS source temperature was 200 °C when using the β-dex 120 column and 230 °C when using the HP-5 column. If nothing else was stated the differences in response factors were neglected. The conversion of the various substrates was calculated from the GC integration area of the substrate peak related to that of the internal standard.

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