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A lipase catalyzed condensation reaction with a tricyclic diketone: yet another example of biocatalytic promiscuity

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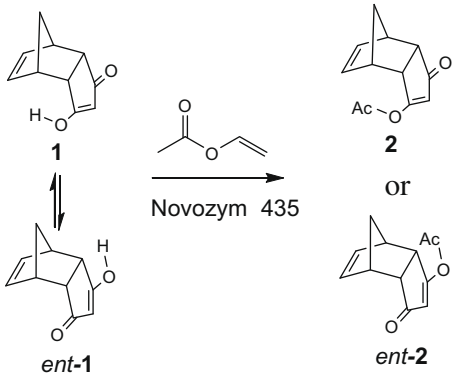
ABSTRACT

Novozym 435 (a commercially available immobilized form of *Candida antarctica* lipase B) was found to catalyze a condensation reaction of 5-hydroxy-endo-tricyclo[5.2.1.$0^{2,6}$]deca-4,8-dien-3-one with acetaldehyde (enzymatically produced from vinyl acetate in situ) under low water conditions, in presence of 10% organic co-solvent (*N*,*N*-dimethyl formamide or pyridine), to form a bis-adduct. Even though the condensation reaction occurred with pyridine (acting as a base catalyst) in the presence of acetaldehyde and in the absence of enzyme, the reaction was very slow as compared to the enzymatic process. Thus, while the non-enzymatic process took 4 days to achieve 100% conversion; in presence of enzyme it was possible within 4 h.

1. Introduction

In 1997, Sheldon stated that the fine chemical industry, in contrast to bulk chemicals manufacturing, is reluctant to apply catalytic technologies.[1] In the last decade, there have been vigorous efforts to leverage biotechnological toolbox to evolve green chemical approaches.[2] As Jaeger had put it more picturesquely, enzyme-catalyzed processes are slowly replacing 'fire and sword' chemistry.[3] The key to strengthen this approach is to use usual enzymes in different ways by exploiting their biological potential. The use of nearly anhydrous media created an opportunity to use hydrolases for organic synthesis.[4] Among such applications, lipases top the list. One of the reasons (perhaps the most important one) is that lipases show tremendous versatility vis-à-vis the range of substrates and biotransformations they catalyze. Acylation and esterification have been carried out by lipases with numerous substrates.[5] Vinyl esters are often preferred as acyl donors as the resulting vinyl alcohol is rapidly converted to acetaldehyde thereby making the reaction faster and irreversible. Weber et al.[6] and Hogberg et al.[7] have shown that in organic solvents, vinyl acetate can produce acetaldehyde and can result in hemiacetal esters. In the present work, we describe for the first time a condensation reaction of acetaldehyde with a tricyclic diketone catalyzed by a frequently used commercial lipase (Novozym 435) under low water conditions.

5-Hydroxy-*endo*-tricyclo[5.2.1.$0^{2,6}$]deca-4,8-dien-3-one (**1** or *ent*-**1**, Scheme 1) is an interesting tricyclic 1,3-diketone which exists in the enol form exhibiting pseudo meso character and a very important synthon for the synthesis of large variety of naturally occurring cyclopentanoids,[8] cubane-type polycyclic compounds,[9] and other pharmaceutically important compounds.[10] The fast tautomerization of the cyclic diketone such as **1**, makes it exist as a racemic mixture of the two enol forms **1** and *ent*-**1** (Scheme 1) and thus opens up the possibility of a dynamic kinetic resolution.



Scheme 1. The fast enantiomerization of the cyclic diketone **1** (enol forms **1** and *ent*-**1**) and its aimed desymmetrization via enzymatic transacetylation.

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Toward that aim, Novozym 435 and vinyl acetate were chosen to enantioselectively transacetylate **1** (Scheme 1). Instead, we observed the novel condensation product between the diketone and acetaldehyde.

2. Results and discussion

An excess of vinyl ester with a co-solvent, DMF or pyridine, in minimum amount required to dissolve **1** in vinyl ester was employed to maintain a nearly solvent-free condition and to accelerate desymmetrization as compared to racemization which is a key requirement to achieve an efficient dynamic kinetic resolution.[11]

The progress of the reaction was initially monitored by thin layer chromatography (TLC) which indicated a gradual disappearance of the starting material and the appearance of a new spot. The reaction reached completion in 4 h, which was further confirmed by HPLC analysis (Fig. 1). A control without enzyme failed to achieve this reaction even after 5 days (Table 1, entries 2 and 4).

Surprisingly, this enzymatic product appeared more polar (R_f = 0.6 in ethyl acetate, hexane, and methanol = 4:2:1) than the expected acetyl derivative **2** (or *ent*-**2**), Scheme 1, (R_f >0.9 in the same solvent) of the alcohol which, for this purpose, was synthesized using standard procedure.[10] Interestingly, when vinyl propionate was used instead of vinyl acetate (Table 1, entries 5 and 6) the reaction produced the same product exhibiting identical R_f value in TLC and the same retention time in HPLC. However, the only difference was that the reaction with vinyl propionate was found to proceed three times slower than that with vinyl acetate and under the same conditions, took 12 h for the completion (Table 1, entries 1 and 5). This indicated that, it was the vinyl moiety and not the acyl group that is participating in the reaction.

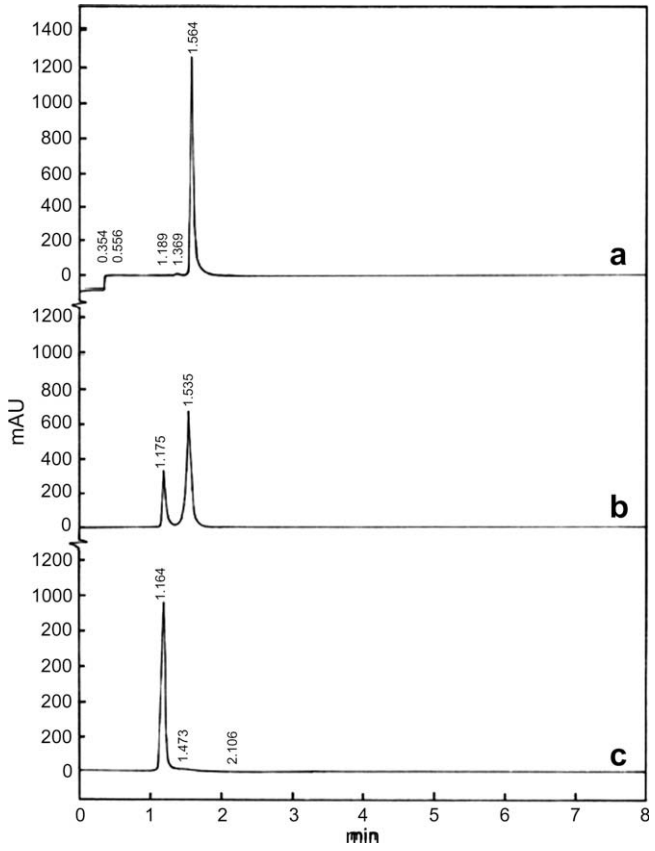
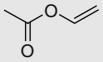
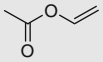
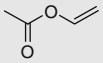
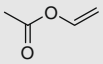
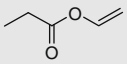
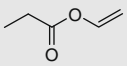
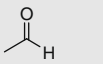
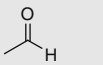


Figure 1. HPLC analysis of the enzymatic reaction: (a) at the start of the reaction, time t = 0 h showing only the starting material, the enol **1** (Scheme 2); (b) at time t = 0.5 h showing 30% conversion; (c) at t = 4 h showing complete disappearance of the enol **1** to the product **3** (Scheme 2).

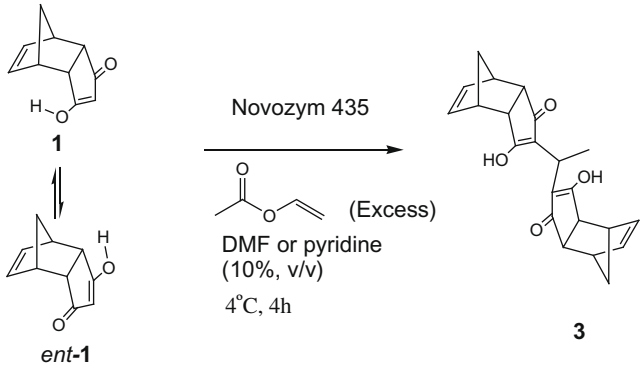
Table 1
Novozym 435 (Novo 435) catalyzed condensation reaction of 1 (or *ent*-1, Scheme 1) with vinyl esters and with acetaldehyde in nearly anhydrous conditions and in solvent-free media

Entry no.	Reactant **2** (excess)	Enzyme (10%, w/w)	Co-solvent (10%, v/v)	Time	*C* (%)*	Yield** (%)
1		Novo 435	DMF	4 h	100	95
2		—	DMF	5 days	0	—
3		Novo 435	Pyridine	4 h	100	97
4		—	Pyridine	5 days	0	—
5		Novo 435	Pyridine	12 h	100	98
6		—	Pyridine	5 days	0	—
7		Novo 435	Pyridine	4 h	100	94
8		—	Pyridine	4 days	100	95

* *C* corresponds to the conversion value obtained by HPLC.
** This corresponds to the product yield after work-up. The variations between three sets of experimental results were within 2%.

After the purification of the enzymatic product by column chromatography the ^{1}H NMR spectral data of the product revealed that it was not the expected acetyl derivative, **2** (or *ent*-**2**). Upon further analysis (with ^{13}C NMR, DEPT, and high resolution mass spectral data; details given as Supplementary data) the product was identified to be the bis-adduct of **1** or *ent*-**1** (**3**, Scheme 2).

Lipases have been reported to give abnormal reactions with vinyl acetate due to its hydrolysis to acetaldehyde.[6,7] To confirm that acetaldehyde produced was involved in the reaction, the reaction of 1 with an excess of acetaldehyde in presence of Novozym 435 and pyridine (10% v/v) was tried. This reaction led to the formation of the same product **3** (Table 1, entry 7). Evidently, here the



Scheme 2. The formation of an unusual bis-adduct **3**.

reaction did not follow the known abnormal pathway giving hemiacetal or acetylated hemiacetal derivative of the alcohol as earlier reported by Hogberg et al.[7] The formation of this bis-adduct can be explained as follows: enzyme-promoted nucleophilic addition of **1** or *ent*-**1** with acetaldehyde initially affording the mono-adduct which further undergoes second reaction with the loss of water molecule to afford the bis-adduct in 95–97% yield. A control experiment in the absence of enzyme was carried out with 1 (or *ent*-1), acetaldehyde, and pyridine. Even though the reaction afforded the bis-adduct **3**, it was much slower, requiring 4 days for the completion of the reaction (Table 1, entry 8). Despite the fact that pyridine, taken as a co-solvent, could catalyze the condensation reaction it was preferred due to the ease of work-up. On the other hand, the reaction in DMF, which unlike pyridine was not found to catalyze or assist the condensation, showed more clearly the role of enzyme catalysis. Thus it was clear that Novozym 435 was not only catalyzing the acyl-oxygen bond cleavage of vinyl acetate to form acetaldehyde but it also facilitated the formation of **3** from **1** with acetaldehyde.

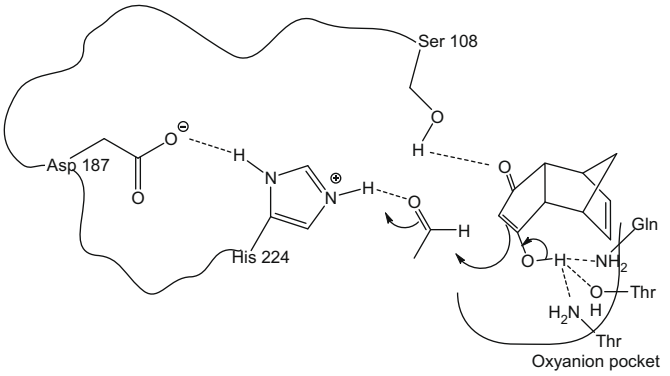
All these facts collectively point out to a reaction sequence (Scheme 3) which involves: enzymatic cleavage of vinyl esters to produce acetaldehyde even under anhydrous conditions (which has already been reported by Weber et al.[6]), condensation between 1 and acetaldehyde gives an initial product in situ followed by formation of the bis-adduct (diketo form), which was found to exist in its enolic form (confirmed by ^{1}H NMR spectra showing two enolic protons which could be exchanged by D_2O and also by FTIR spectra which did not show any isolated carbonyl frequency around 1700–1710 cm^{-1}). The enzyme (being an immobilized formulation) and the excess unreacted vinyl ester could be easily recovered and reused. The percentage of yield in all the cases was very high, 95–97%.

The enzyme active site involvement (based on the models as reported earlier)[12] is given in Scheme 4. The free CAL B active site contains a serine residue, Ser 108, (a part of the catalytic triad) which binds vinyl acetate and liberates acetaldehyde as it commonly does in the case of hydrolysis and then it is the imidazolium nucleus of the histidine residue, His 224, which facilitates the condensation reaction of acetaldehyde by making it a better electron acceptor through hydrogen-bonding interactions (Scheme 4).[13,14]

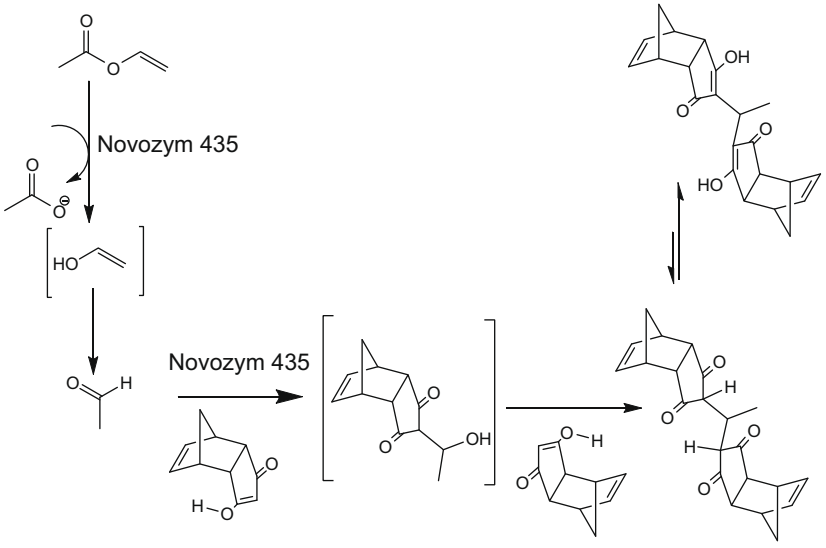
Svedendahl et al. reported that a mutant of *Candida antarctica* lipase B (Ser105Ala) could catalyze efficiently Michael type condensation.[13] More recently, Li et al. described the first lipase-catalyzed asymmetric aldol condensation (between acetone and aromatic aldehydes) in presence of water as an illustration of biocatalytic promiscuity by the enzyme.[14] The present case, to the best of our knowledge, is the first example ever reported where Novozym 435 exhibits the behavior of both lipase and aldolase in a single reaction under anhydrous conditions, and in nearly solvent-free media resulting in a bis-adduct. Interestingly, the temperature used in the present case (4 °C) is lower than that used by Li et al. or Svedendahl et al. but it was found to give a fast conversion.

3. Conclusion

Lipases are known to catalyze acylation of alcohols, a reaction frequently carried out by vinyl acetate.[7] In the present case, the cyclic diketone (exists as an enol) failed to get acylated. Instead, the cyclic diketone underwent an aldol-type condensation reaction with acetaldehyde (acetaldehyde was produced in situ from hydrolysis of vinyl acetate). In this respect, Novozyme 435 behaved more like an aldolase under nearly anhydrous media. This opens up a new synthetic opportunity using lipases.



Scheme 4. Condensation of acetaldehyde with **1** (based on the model as reported earlier by Li et al.).[14]



Scheme 3. An unusual reaction sequence which involves: enzymatic deacetylation of vinyl esters to produce acetaldehyde even under anhydrous conditions, condensation between **1** and acetaldehyde gives an initial product in situ followed by formation of the bis-adduct (diketo form), which tautomerizes in its enolic form.

4. Experimental

4.1. Synthesis of the enol adduct (1 or *ent*-1)

5-Hydroxy-*endo*-tricyclo[5.2.1.0^{2,6}]deca-4,8-dien-3-one was synthesized by a Diels–Alder reaction between cyclopenten-1,4-dione(Aldrich, Germany) and cyclopentadiene (Fluka, USA) as reported earlier by Ramesh et al. (mp 170–172 °C , after recrystallization from dichloromethane and ethyl acetate) and De Puy et al.[10,15] ^{1}H NMR (300 MHz: $CDCl_3$, Me_4Si), δ (ppm): 1.67 (1H, d, J = 8.4 Hz), 1.78 (1H, d, J = 8.4 Hz), 3.1(4H, s), 4.9 (1H, s), 5.9 (2H, s), 8.9 (1H, br s).^{13}C NMR ($CDCl_3$) δ (ppm): 43.54, 48.98, 107.87, 132.82, 202.77 (given as Supplementary data).

4.2. Enzymatic synthesis of 3, the bis-adduct of 1

In a 1.5 ml reactor alcohol **1** (or *ent*-**1**, 100 mg, 0.6 mol) was dissolved in *N*,*N*-dimethylformamide, or pyridine (100 μL, 0.1% water by GC, Qualigens fine chemicals, India) and then an excess of vinyl acetate (Merck, Germany) or vinyl propionate (Aldrich, Germany) or acetaldehyde (anhydrous, Fluka, Switzerland) was added to make the volume 1 ml. Novozym 435 (100 mg, 10 mg enzyme protein)[16] was added so that its load was 10% (w/w, enol **1**). Reaction was set at 4 °C with an orbital shaking at 300 rpm. After different time intervals aliquots were taken and were analyzed by TLC and HPLC (see later). Each of the reaction set was run in triplicates and the variations between three sets of experimental results were within 2%. The liquid chemicals were distilled and dried over activated 3 Å molecular sieves (Merck, India) for overnight before use.

4.3. Purification and product characterization

The enzyme was easily separated from the medium by filtration and the crude product was recovered by decantation of the unreacted vinyl ester contaminated with DMF (or pyridine) followed by evaporation under strong vacuum. The crude product was purified by a 15 × 2 cm silica gel (100–200 mesh) column using an eluent consisting of ethyl acetate, methanol, and hexane (in 8:2:1 v/v ratio). The fractions were collected and evaporated in a rotor evaporator to get the pure bis-adduct **3** (104 mg, 95–97%) as a colorless solid which was further recrystallized from dichloromethane and ethyl acetate. (mp 243–245 °C). ν_{max} (neat)/cm^{-1} 2590 br, 1600, 1400, 1310, and 715; ^{1}H NMR (300 MHz: $CDCl_3$ with 2 drops of DMSO-d_6; Me_4Si) δ (ppm): 1.04 (3H, d, J = 7.5 Hz), 1.53 (2H, d, J = 8.4 Hz), 1.72 (2H, d, J = 8.5 Hz), 2.98 (4H, m), 3.58 (1H, q, J = 7.5 Hz), 5.82 (s, 4H, merged with a br s, 2H, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$ with 2 drops of DMSO-d_6) δ (ppm): 16.13, 18.3, 42.6, 42.65, 46.1, 46.56, 51.03, 122.36, 131.41, 131.72, 196.01, 196.63; *m/z* 351.1598 (M^+ $C_{20}H_{22}O_4$ requires 350.1518), 301.1417, 236.0731, 224.5758 (see Supplementary data).

4.4. HPLC analysis of the enzymatic reaction

The aliquots taken in different time intervals were diluted with hexane (10×) to precipitate the unreacted **1** (or *ent*-**1**) and the product **3**, centrifuged and the recovered solid mass was dissolved in the eluent (acetonitrile/water = 87:13) and was run in a Zorbax C-18 reverse phase column (fitted in Agilent 1100 series) at a flow rate of 1 ml/min and was monitored by DAD–UV at 245 nm. Alcohol and the product appeared at 1.5 min and 1.2 min, respectively. The positions were confirmed after running pure NMR grade samples.

Acknowledgments

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Supplementary data

^{1}H NMR, ^{13}C NMR, Mass spectral data are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.06.108.

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