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Lipase-Catalyzed Condensation Reaction of 4-Nitrobenzaldehyde with Acetyl Acetone in Aqueous-Organic Cosolvent Mixtures and in Nearly Anhydrous Media

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LIPASE-CATALYZED CONDENSATION REACTION OF 4-NITROBENZALDEHYDE WITH ACETYL ACETONE IN AQUEOUS-ORGANIC COSOLVENT MIXTURES AND IN NEARLY ANHYDROUS MEDIA

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GRAPHICAL ABSTRACT



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Abstract The nature of the product(s) in lipase-catalyzed reaction of acetyl acetone with 4-nitrobenzaldehyde was found to depend upon the source of lipase and the reaction medium. Mucor javanicus lipase was found to give 70% aldol with 80% enantiomeric excess in anhydrous t-amyl alcohol. A 2:2 adduct was formed by the dimerization of the aldol along with an unsaturated cyclic ether as the side products in varying proportions depending upon the reaction medium and the lipase used.

[Supplementary materials are available for this article. Go to the publisher's online edition of Synthetic Communications[®] for the following free supplemental resource(s): Full experimental and spectral details.]

Keywords Aldol condensation; asymmetric synthesis; biocatalytic promiscuity; 1,3-diketones; lipases; regioselectivity

INTRODUCTION

Replacement of chemical catalysts with biocatalysts is an important area in biotechnology.^[1–3] The recent results on catalytic promiscuity of enzymes have con-siderably enhanced the versatility of biocatalysts.^[4–7] So far, such observations in this area have been mostly limited to showing that some specific reactions, not expected from the known biological activity of the enzymes in vivo condition, can take place in *in vitro*. In the present work, we show that depending upon the source of the enzyme and the reaction medium, such promiscuous reaction can give a larger variety of products. This opens up the possibility of creating larger chemical libraries using enzymes. The utility of such approach in the synthesis of novel molecules of pharmaceutical importance is obvious. Carbon-carbon bond formation reactions are very important in organic synthesis.^[6,7] Of special interest are the C-C bond-formation reactions involving 1,3-diketones, which have considerable synthetic utility.^[8,9] The aldol reaction with 1,3-diketones with biocatalysts has been especially challenging. Both catalytic antibodies 33F12 and 38C2 raised with a 1,3-diketone as haptens (by reactive immunization) failed to carry out this reaction as ɛ-amino group of the active site lysine reacted with the diketone.^[10,11] More recently, a designer aldolase grafted from phase-display libraries also failed to catalyze aldol reaction with 1,3-diketones for a similar reason.^[11] Even by chemical methods, it requires a very long time to obtain the aldol of acetylacetone (the simplest 1,3-diketone) and substituted benzaldehydes.^[8,9] In this work we show that biocatalytic promiscuity of lipases can be well utilized for the aldol condensation of 4-nitrobenzaldehyde with acetylacetone, which acts as a donor, and also for the preparation of an acyclic and eight-membered unsaturated cyclic ether. It can be added that while the condensed products involving more than one free carbonyl functional groups are important synthons, the synthesis of cyclic ethers has been a matter of great interest to the organic chemists because the latter finds importance in the synthesis of allylic and homoallylic alcohols.^[12] The present work shows an enzymatic approach to prepare these three types of compounds.

RESULTS AND DISCUSSION

The aldol condensation reaction of 4-nitrobenzaldehyde (1, Scheme 1, 100 mM) and acetyl acetone (2, Scheme 1, 100 mM) was tried with lipases in phosphate buffer (50 mM, pH 7.0) containing 30% v/v water miscible organic cosolvent.



Scheme 1. Proposed lipase-catalyzed aldol condensation.

Three different organic solvents [viz., N,N'-dimethylfomamide (DMF), dioxan, and dimethylsulfoxide (DMSO)] were tried and 30% v/v was found to be the minimum concentration of any of these organic cosolvents that was necessary to dissolve the substrates at these concentrations. Of these three cosolvents (at this concentration) DMSO gave the best initial rate as well as conversion (details given as Fig. S1, supplementary information). It was found that different commercially available lipases (some free and some in immobilized forms), in aqueous-organic cosolvent mixture, did not give the expected aldol (3, Scheme 1) but yielded two different products (detected by TLC of $R_F 0.25$ and 0.4 in hexane-ethylacetate = 3:1) in varying proportions (quantified by HPLC, Table 1). The products were purified by column chromatography and characterized by ¹H NMR analysis, ¹³C NMR (including DEPT spectra), and high-resolution mass spectral analysis (given as Figs. S2-S6, supplementary information). The structure of one of the two products was found to be 4 (Scheme 2). In case of the second product (R_F 0.4), it was found that the ¹H NMR spectra exactly matched with the spectra recorded for deuterated 4 [i.e., the ¹H NMR spectra of **4** when taken after a deuterium exchange (Fig. S7, supplementary information)]. This confirmed that the two protons of the two enolic OH groups of 4 are no longer present in it and the structure of this product was anticipated as 5 (Scheme 2). This was further confirmed by the fact that when purified 4 was taken as the starting material, by Novozym 435, 5 was formed exclusively. The two enolic protons of 4 were removed during the ring closure to form 5 (Scheme 3). Compounds 4 and 5 were formed by an abstraction of a water molecule.

Entry	Lipase	Products formed	Initial rates for overall conversion $(\mu M mg^{-1} h^{-1})^a$	Time (h)	Yield $4(\%)^b$	Yield $5(\%)^b$
1	B. cepacia	4, 5	280	72	82	12
2	C. antarctica	4, 5	1500	24	70	25
3	C. antarctica (immobilized)	4, 5	170	72	15	50
4	M. javanicus	4	230	72	77	
5	M. miehei	4, 5	280	72	80	5
6	<i>M. miehei</i> (immobilized)	4, 5	300	72	83	7

 Table 1. Products formed in aqueous-organic cosolvent mixture by different lipases: Screening based upon higher product selectivity

^{*a*}4-Nitrobenzaldehyde (100 mM), acetylacetone (100 mM), 20 mg lipase formulations were taken in 1 mL of phosphate buffer (50 mM, pH 7.0) containing 30% DMSO and were shaken at 200 rpm at 30 °C. Aliquots were taken out at different time intervals and were analyzed by HPLC.

^bAfter purification by silica-gel column.



Scheme 2. Lipase-catalysed promiscuous reaction: formation of acyclic and cyclic 2:2 adducts.

A lipase from *Burkholderia cepacia* was found to catalyze the formation of **4** as a major product, resulting in an 82% yield within 72 h (entry 1, Table 1). Compound **5** was formed as the minor product. On the other hand, the use of immobilized formulation of *Candida antarctica* lipase B, Novozym 435, led to the formation of **5** as a predominant product (entry 3, Table 1). The free form of *Candida antarctica* lipase B catalyzed the formation of **4** in 70% yield within 24 h (entry 2, Table 1). Immobilization is known to influence the selectivity of the biocatalyst.^[13] However, no such effect was observed in the case of *Mucor miehei* lipase: Both the free and the immobilized forms catalyzed the formation of the product **4** as the major product (entry 5,6 Table 1). Interestingly *Mucor javanicus* lipase was found to give only



Scheme 3. Probable mechanism for the formation of cyclized product.

one product, **4** (entry 4, Table 1). Presumably with *Mucor javanicus* lipase, the reaction stops at the dimerization stage and hence only one product was obtained exclusively. While medium engineering is an established approach in use of enzymes for organic synthesis in low water media,^[14] this aspect has so far not been explored in the context of promiscuous reactions. It was decided to also explore nearly anhydrous conditions as the reaction medium. Enzyme reactions in such media have been extensively studied. However, promiscous reactions have been more often studied just in water.^[5,6] In an earlier work with a cyclic and more complex diketone we had used nearly anhydrous media for the lipase-catalyzed promiscuous reactions.^[4]

The reaction in different dry organic solvents was investigated in the presence of *M. javanicus* lipase (Table 2, detailed conversion vs time plots have been given as Fig. S8, supplementary information). Interestingly product **5** was not formed in these dry solvents. In addition to product **4**, another product was also formed (observed with an R_F of 0.53 on thin-layer chromatography, TLC) in all the organic solvents that were tried, but in varying proportions (as found by high-performance liquid chromatography, HPLC). The products were purified by column chromatography for characterization. The structure of this product was confirmed as **3** (Scheme 1), the expected aldol, by ¹H NMR spectra only as it showed a similar pattern as described in the literature.^[15] When the reaction was carried out in solvent-free medium (i.e., when the acetylacetone itself was used as the solvent), almost equal percentages of **3** and **4** were formed (entry 2, Table 2).

When *t*-alcohols were used as the solvent, the formation of **4** was minimum and mostly aldol **3** was obtained (entries 6,7, Table 2). The greatest ratio of the aldol **3** to the 2:2 uncyclized product **4** (12:1, entry 7, Table 2) was obtained when *t*-amyl alcohol was used as the reaction medium. It may be added that *t*-alcohols are generally not the substrates of *Mucor javanicus* lipase.^[16]

Table 2 gives the initial rates for the enzyme catalyzed formation of **3** and **4** and also shows that in all the cases [except cosolvents cyclopentyl methyl ether (CPME)

Entry	Solvent	Initial rates for 3 $(\mu M mg^{-1} h^{-1})^a$	Initial rates for 4 $(\mu M mg^{-1} h^{-1})^a$	Time (h)	Yield (%) 3	Yield (%) 4	ee (+) (%), 3
1	Acetonitrile	10	72	72	10^{b}	48	$< 10^{c}$
2	Acetylacetone	180	390	48	45	52	24 ^a
3	CPME	5	32	72	6	35	0
4	DCM	5		72	3	0	0
5	DMSO	280	80	72	72	18	45
6	t-Butanol	128	14	72	64	8	68
7	<i>t</i> -Amyl alcohol	140	15	72	70	6	80

Table 2. Optimization for the synthesis of **3**: Effect of low-water media on initial rates and product nature as exhibited by *Mucor javanicus* lipase

^{*a*}Determined by analysis of the crude reaction mixture (by analytical HPLC using Zobax Sil C18 column) of hexane and isopropanol (4:1, v/v) at a flow rate of 1 mL/min and was monitored by UV-diode array detector at 254 nm.

^bAfter column purification.

^cDetermined by chiral HPLC using Chiralcel-ODRH column of the column purified aldol product, and polarimetric studies confirmed a dextrorotation in all the cases.



Figure 1. Chiral HPLC analysis of the column purified aldol product (**3**, Scheme 1): (a) the racemic mixture (obtained by using CPME), (b) at 50% conversion in *t*-amyl alcohol exhibiting 38% *ee* of *d* form (by polarimetric studies of the injected sample) after 24 h, and (c) at 70% conversion in *t*-amyl alcohol corresponding to 80% *ee* of the *d* form after 72 h.

and dichloromethane (DCM)], enantiopreference of the d form (confirmed by polarimetric analysis) of the aldol product 3 was observed. The racemic aldol (3) was produced as a result of enzymatic reaction using CPME (entry 3, Table 2) as a green solvent.^[17] Its resolution on the chiral column is shown in Fig. 1a [efforts to improve this resolution by changing the flow rate did not succeed]. Figure 1b shows the chromatogram after 24 h of the reaction in t-amyl alcohol. This corresponded to 50% conversion and 38% ee of d-form. Figure 1c gives the chromatogram after 72h and corresponds to 70% conversion and 80% ee of the d-form. The best enantiomeric excess, ee (80%) was obtained in the case of t-amyl alcohol (Fig. 1). The compound **3** has not been reported in the literature so far. Hence, determination of its absolute configuration by crystallographic analysis or NMR studies may be interesting but was not pursued in the present work. Products 4 and 5 were found to be optically inactive. This raised several possibilities. These could be both meso compounds. Alternatively, these could be racemic compounds. The efforts to resolve either of these on chiral column did not succeed and only a single peak was obtained in each case (data not shown). Hence, it looks more likely that these were meso forms (Scheme 2) but further work would be needed to settle this issue. Also, as these are new compounds the assignment of R and S configuration around chiral center cannot be done without detailed structural characterization.

CONCLUSION

To sum up, this first-ever study of lipase-catalyzed condensation of acyclic 1,3-diketone acetylacetone with 4-nitrobenzaldehyde showed that multiple products are formed, including an unsaturated cyclic ether. However, choice of the lipase and the solvent conditions dictate the nature of the products. It is interesting to note that in aqueous medium lipases were found to give the 2:2 adduct **4** as the major product

by a fast dimerization of **3** and **5** as a minor product. The dimerization of the aldol **3** was restricted in the low-water media. It is also of interest to note that there was a regioselectivity in this catalytic promiscuity because the reaction occurred only via C-3 atom of acetylacetone. Similar regioselectivity was observed with a cyclic 1,3-diketone as well.^[4]

EXPERIMENTAL

Lipases from *Mucor javanicus, Mucor miehei* (both free and immobilized on anion exchange resin), and *Candida antarctica* (B, both free and immobilized on acrylic resin) were obtained from Novozymes A/S, Bagsvaerd, Denmark. *Burkhol-deria cepacia* lipase (Lipase PS) was a gift from Amano Enzymes Inc., Nagoya, Japan. 4-Nitrobenzaldehyde (>99%) and acetylacetone (>99.5%, GC) were purchased from Spectrochem, Mumbai, India.

Condensation Reaction

4-Nitrobenzaldehyde (15 mg, 0.1 M), acetylacetone (10 mg, 0.1 M) were taken in phosphate buffer (50 mM, pH 7.0) containing 30% v/v organic cosolvent (up to 1 mL) with 20 mg lipase formulations and were shaken at 200 rpm at 30 °C. For the reactions in low-water media, 4-nitrobenzaldehyde (15 mg, 0.1 M), acetylacetone (10 mg, 0.1 M or in excess in case of solvent free conditions), and pH-tuned (in 50 mM phosphate buffer, pH 7) freeze-dried lipase formulations were taken in 1 mL of various organic solvents (distilled and dried by molecular sieves) and were shaken at 200 rpm at 30 °C.

HPLC Analysis

The aliquots were taken at different time intervals, diluted with acetonitrile (10 times to precipitate the enzyme, which was required especially in cases of the reactions in aqueous–organic media), and centrifuged, and the supernatant was analyzed on Zorbax SIL C-18 column (fitted in Agilent 1100 GC system) with an eluent consisting of a mixture of hexane and isopropanol (4:1, v/v) at a flow rate of 1 mL min⁻¹ and was monitored by a UV-diode array detector at 254 nm.

Workup Details and Analysis

The crude product was extracted from the aqueous solutions by DCM and was purified by column chromatography (column length 20 cm, 2.5 cm diameter) with hexane and ethyl acetetate (5:1) as eluent, and the isolated products were recrystallized from HPLC-grade hexane and ethyl acetate. The purity was cross-checked by TLC and confirmed by HPLC.

Chiral HPLC Analysis

The column-purified, NMR-tested product 3 was dissolved in isopropanol and analyzed on Chiralcel ODRH (fitted in Agilent 1100 GC system) with an eluent

composed of hexane and isopropanol (5: 2, v/v ratio) at a flow rate of 1 mLmin^{-1} and was monitored by a UV-diode array detector at 254 nm. The *l* and *d* enantiomers were detected at 5.5 and 5.9 min respectively. Enantiomeric excess were calculated from the corresponding peak areas.

Polarimetric Analysis

The column-purified characterized (by NMR) product was dissolved in ethanol (conc. 0.7-1.2%) and its optical rotation was measured by a digital polarimeter, Autopol V. A dextrorotation was observed for **3**, while **4** and **5** were found to be optically inactive.

Product Characterization

(Z)-4-Hydroxy-3-(hydroxy(4-nitrophenyl)methyl)pent-3-en-2-one (3). $C_{12}H_{13}NO_5$ yellow, semisolid mass, $R_F = 0.53$ in hexane–ethylacetate (3:1). IR (KBr), ν (cm⁻¹): 735, 1059, 1344, 1537, 1572, 1640, 1690, 3200 (b). ¹H NMR (CDCl₃, 300 MHz), δ (ppm): 1.85 (3H, s), 2.2 (3H, s), 5.12 (1H, bs, exchangeable with D₂O), 5.3 (1H, s), 8.08 (2H, d, J = 8.4 Hz), 8.40 (2H, d, J = 8.4 Hz), 10.16 (1H, exchangeable with D₂O).

(3Z,3'Z)-3,3'-(Oxybis((4-nitrophenyl)methylene))bis(4-hydroxypent-3-en-2-one) (4). C₂₄H₂₄N₂O₉, orange yellow solid, mp 72–73.5 °C. R_F = 0.25 in hexane– ethylacetate (3:1). IR (KBr), ν (cm⁻¹): 737, 1059, 1210, 1344,1537, 1572, 1640, 3300 (b). ¹H NMR (CDCl₃, 300 MHz), δ (ppm): 2.25 (6H, s), 2.46 (6H, s), 5.3 (2H, s; exchangeable with D₂O), 7.49 (2H, s), 7.55 (4H, d, J=8.4 Hz), 8.24 (4H, d, J=8.4 Hz). ¹³C NMR: 26.72, 31.78, 124.16, 130.25, 136.5, 139.24, 145.59, 148.48, 196.01, 204. 36. DEPT: 26.7, 31.79, 124.17, 130.26, 136.51; m/z 509.3036 (M⁺ NaC₂₄H₂₂ D₂ N₂O₉ requires 509.2027).

1,1'-((3*E***,6***E***)-4,6-Dimethyl-2,8-bis(4-nitrophenyl)-2,8-dihydro-1,5-dioxocine-3,7diyl)diethanone (5).** C₂₄H₂₂N₂O₉, orange-yellow solid (mp 60–62 °C), R_F = 0.4 in hexane–ethylacetate (3:1). IR (KBr), ν (cm⁻¹): 735, 1059, 1215, 1270, 1348, 1537, 1572, 1690. ¹H NMR (CDCl₃, 300 MHz), δ (ppm): 2.25 (6H, s), 2.46 (6H, s), 7.49 (2H, s), 7.55 (4H, d, J = 8.4 Hz), 8.24 (4H, d, J = 8.4 Hz).

SUPPORTING INFORMATION

Screening of organic cosolvents, NMR spectral data of new compounds, and mass spectra (HRMS) can be found via the Supplementary Content section of this article's Web page.

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