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Original enzyme-catalyzed synthesis of chalcones: Utilization of hydrolase promiscuity

YAVOR N. MITREV[1], ALEKSANDER Y. MEHANDZHIYSKI[1], DANIELA I. BATOVSKA[1]*, ANDREAS LIESE[2] and BORIS GALUNSKY[2]**

[1]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 9, Sofia 1113, Bulgaria and* [2]*Institute of Technical Biocataysis, Hamburg University of Technology, Denickestr. 15, Hamburg 21073, Germany*

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Abstract: An *E*-chalcone was obtained with very high stereoselectivity for the first time by an enzyme-catalyzed Claisen–Schmidt condensation between benzaldehyde and acetophenone. From a set of lipases, only that from hog pancreas demonstrated promiscuity, catalyzing the reaction in the presence of imidazole as a promoter. Another enzyme, acylase from *Aspergillus melleu*s (EC 3.5.1.14) also proved to be active in the synthesis of *E*-chalcone under the same reaction conditions. This acylase along with the recombinant D-amino-acylase (EC 3.5.1.81) also catalyzed the reaction between acetophenone and *p*-nitrobenzaldehyde. Such a "green" approach to the synthesis of chalcones is of great interest because of the important applications of chalcones as formula ingredients in the pharmaceutical, food and cosmetic industries.

Keywords: Claisen–Schmidt condensation; enzyme promiscuity; lipase-catalyzed synthesis.

INTRODUCTION

Chalcones (1,3-diaryl-2-propen-1-ones) are open-chain flavonoids able to interact efficiently with various biological targets. Long-standing research,[1,2] revealed that these compounds can provide significant medicinal or other benefits. Hitherto, several chalcones, either from natural origin or developed by QSAR analyses, have been used for the treatment of viral diseases, cardiovascular disorders, parasitic infections, pain, gastritis, and stomach cancer, as well as food additives, non-azo dyes, cosmetic formulation ingredients and useful synthons.[1a,1d,1i,3] Development of chalcone-containing preparations is very topical, particularly in terms of further utilization of their pharmacological potential.[1]

* Corresponding authors. E-mail: (*)danibat@orgchm.bas.bg; (**)galunsky@tuhh.de
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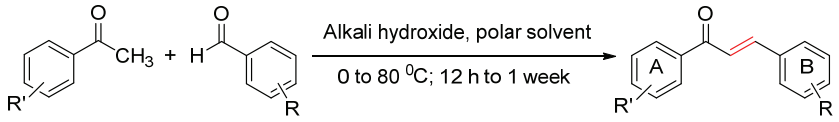
Thus, the demand for appropriate methods for the industrial synthesis of chalcones is increasing. Such methodologies should be simple, environmentally friendly, cost-efficient and provide pure products in high yields. In this regard, the Claisen–Schmidt condensation between aryl methyl ketones and aromatic aldehydes (Scheme 1)[4] is the most convenient synthetic route to chalcones. Herein, a modification of this method is reported that appears to be the first example of an enzyme-catalyzed synthesis of chalcones.



Scheme 1. Conventional synthesis of chalcones by the Claisen–Schmidt condensation

The Claisen–Schmidt condensation is generally performed between equimolar quantities of the reactants under basic or acid catalysis, by following either a homogeneous or a heterogeneous approach. When conducted under basic conditions, the reaction is restricted by side reactions while the acid catalyzed reaction often suffers from the drawbacks of lower yields and harsh, environmentally detrimental reaction conditions. During the past decade numerous methods, including the use of recyclable solid acids and bases,[5] often combined with solvent-free,[6] ionic liquid,[7] ultrasound[8] or microwave-assisted conditions,[6,9,10] have been developed in order to overcome the limitations of the conventional techniques. Although being more advantageous, these techniques suffer from poor selectivity to chalcones, the need for a large excess of reagents, and, occasionally, use of a toxic solvent.[9,11]

In this respect, and in line with the growing demand for "green" synthetic methods for fine chemicals, attention was focused on enzymes as a possible source of catalysts.[12] Particularly, lipases (EC 3.1.1.3) are widely used for industrial biotransformations because of their unusually broad substrate specificity, high enantio- and regioselectivity, and stability in organic solvents.[13] Some lipases display catalytic promiscuity, meaning the ability to catalyze unnatural reactions, including aldol and other valuable C–C bond forming reactions.[14] To the best of our knowledge, lipases have not hitherto been described as catalysts in the synthesis of chalcones.

EXPERIMENTAL

Instrumentation

The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Avance II+ 600 in solutions of $CDCl_3$. Identification of the chalcone synthesized by enzyme catalysis was also performed by EI-MS after direct injection of a DCM solution of the sample onto a Thermo ISQ LT EI, coupled with a Thermo Trace 1300. Conversions of all reactions were analyzed by HPLC with a separations module – Alliance HT Waters 2795 and dual λ absorbance detector –

Waters 2487. The column was LiChrospher® 100 RP-18 (5 μm). A mixture consisting of 50/50 volumes of MeOH and water (deionized water with 0.01 % H_2SO_4) was used as the eluent at a flow rate of 1 ml min^{-1} and at 40 °C.

Preparation of reference chalcones

The reference *E*-chalcone was synthesized by a conventional Claisen–Schmidt condensation as described elsewhere.[2a] A mixture of *E*- and *Z*-isomers in the ratio 2.5:1 was prepared by simple daylight irradiation of a chloroform solution of *E*-chalcone for 30 min. The ratio of the isomer reference compounds and their structures were confirmed by their ^{1}H-NMR spectra (Fig. 1).

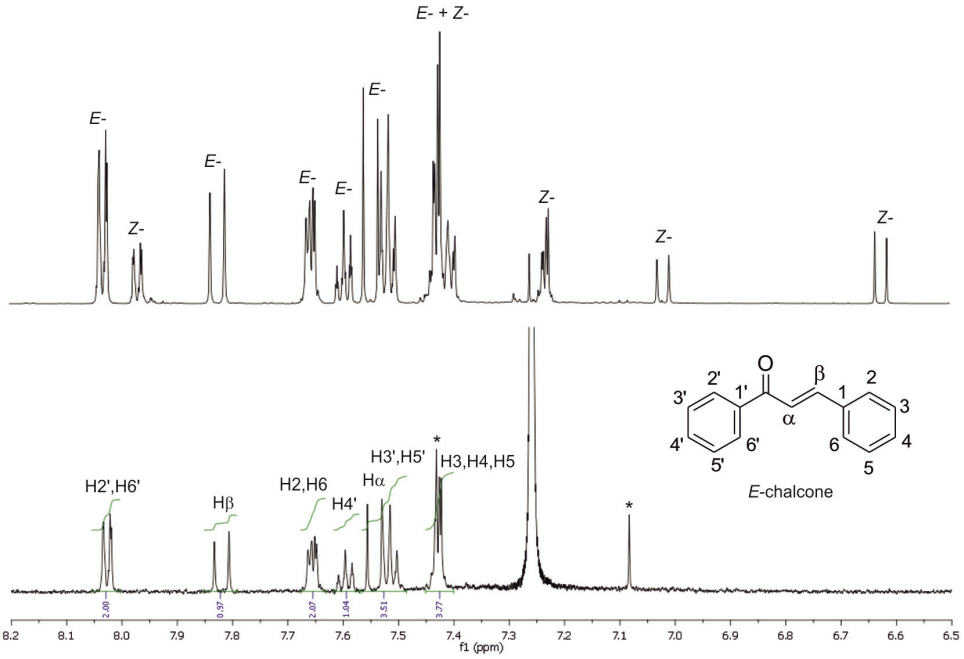


Fig. 1. ^{1}H-NMR spectra (600 MHz) of *E*-chalcone (bottom), isolated from the reaction mixture and reference mixture of *E*- and *Z*-chalcone (upper) in $CDCl_3$ at 293 K. Signals marked with an asterisk correspond to ^{13}C satellites of the solvent signal.

Hydrolases used for the screening

Lipases from *Aspergillus niger* (Fluka), *Aspergillus oryzae* (Gentech), *Burkholderia cepacia* (Amano), *Candida antarctica* (C-lecta), *Candida cylindraceae* (Amano), *Mucor miehei* (Fluka), *Penicillium camembertii* (Amano), *Penicillium roquefortii* (Amano), *Pseudomonas alcallgines* (Gist-brocades), *Pseudomonas fluorescens* (Amano), *Rhizopus arrhizus* (Sigma), *Rhizopus niveus* (Sigma), *Rhizopus oryzae* (Biocatalysts), *Thermomyces lanuginosis* (Novozymes), mixed fungal Lipomod 768P (Biocatalysts) and hog pancreas (Fluka); acylase from *Aspergillus melleus* (Sigma); and recombinant D-aminoacylase from *Escherichia coli* (Sigma) were used.

Heterogeneous hydrolase-catalyzed synthesis of chalcone

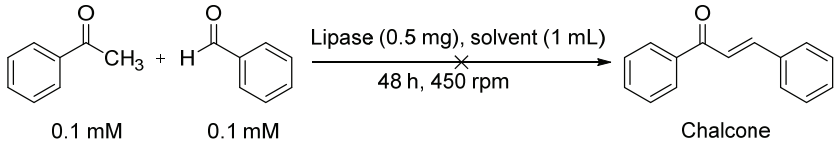
A suspension containing acetophenone (238 μL, 2.04 mmol), benzaldehyde (10 μL, 0.09 mmol), an enzyme (10.0 mg), imidazole (5.0 mg), *n*-octane (1 mL) was stirred at 50 °C for 48 h. The HPLC monitoring of enzyme-catalyzed reaction in presence of imidazole was performed as described above.

Preparative isolation of the chalcone produced by the enzyme

A suspension containing acetophenone (164 μL, 1.4 mmol), benzaldehyde (143 μL, 1.4 mmol), an enzyme (151.0 mg), imidazole (72.0 mg), *n*-octane (15 mL) was stirred at 50 °C. After 48 h, the reaction mixture was diluted with water to remove the suspended solid enzyme and imidazole. The organic layer was separated, washed with water and carefully evaporated under nitrogen until a crystal compound remained (16.6 mg, 0.08 mmol, 5 % isolated preparative yield).

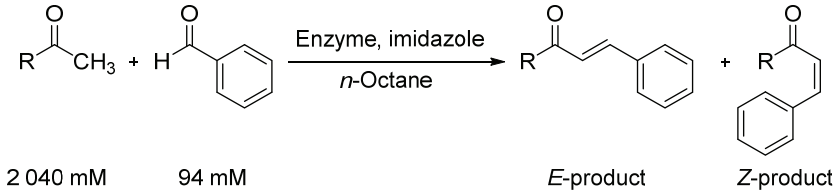
RESULTS AND DISCUSSION

A set of lipases were screened for their ability to catalyze the Claisen–Schmidt condensation between acetophenone and benzaldehyde (Scheme 2). At first, the conditions of the conventional homogeneous Claisen–Schmidt condensation were mimicked. Water–alcohol mixtures were chosen as the reaction media, but other solvents were also tested (Scheme 2). However, no product formation was observed in the thus-conducted reactions.



Scheme 2. Enzymatic mimic of a Claisen–Schmidt condensation. Reaction conditions: a) potassium phosphate buffer (0.1 M, pH 7.0) or H_2O:MeOH (55:45), 30 °C; b) H_2O:DMSO (90:10; 55:45) or H_2O:EtOH (90:10; 55:45; 10:90), 35 °C.

In a search for the best reaction conditions, a strategy for the implementation of a heterogeneous enzyme-catalyzed aldol condensation was discovered. This reaction was performed between acetone (R = CH_3) and aromatic aldehydes (Scheme 3). The products, arylbut-3-en-2-ones, were obtained in high yields (74 %) and with high *E*-selectivity (245:1) in the presence of recombinant D-aminoacylase (EC 3.5.1.81) and imidazole.[15]



Scheme 3. Enzyme-catalyzed aldol condensation in the presence of imidazole.

No product chalcone was obtained in this reaction (Scheme 3) when acetone (R = CH_3) was replaced by acetophenone (R = phenyl). Probably the size and shape of acetophenone did not fit the active site of D-aminoacylase. Although several lipases were proved inactive towards the synthesis of arylbut-3-en-2--ones,[15] the reaction conditions used for the lipase screening were adopted. It was found that from the entire set of lipases, only that from hog pancreas (EC 3.1.1.3) catalyzed chalcone formation. According to HPLC monitoring of the reaction, a compound with a retention time of 16 min, identical with the retention time of the reference *E*-chalcone, was obtained within 3 h after reaction onset and its amount increased with increasing reaction time. In blank experiments under same reaction conditions, no reaction was observed without an enzyme, or without imidazole, as well as in the absence of both.

To obtain unambiguous proof for the identity of the chalcone obtained, the reaction was performed with equimolar quantities of the reactants in the presence of lipase from hog pancreas (EC 3.1.1.3) and imidazole. The structure of the product, obtained by preparative isolation from the reaction mixture, was shown to be *E*-chalcone by comparison of its ^{1}H-NMR spectrum with that of the reference *E*- and *Z*-chalcones (Fig. 1) and according to literature data.[16] This reaction showed very high stereo-selectivity, since no *Z*-isomer could be detected in the ^{1}H-NMR spectrum. It provided 16.6 mg (0.08 mmol, 5 % isolated yield) of pure *E*-chalcone, as determined by preparative isolation. The high purity of the product was also proved by its mass spectrum. Lack of by-products showed that under these reaction conditions, other possible reactions, such as Cannizzaro and Michael addition reactions, did not occur.[2c,11]

CONCLUSIONS

In summary, *E*-chalcone was obtained for the first time by an enzyme-catalyzed reaction with very high stereoselectivity. Such a “green” approach to the synthesis of these compounds is of great interest, because of their important applications as formula ingredients in pharmaceutical, food and cosmetic industries. From the studied set of lipases, only that from hog pancreas demonstrated promiscuity, catalyzing the Claisen–Schmidt condensation in the presence of imidazole as a promoter. Another enzyme, acylase from *Aspergillus melleu*s (EC 3.5.1.14) also proved to be active in the synthesis of *E*-chalcone under the same reaction conditions. This acylase along with the recombinant D-aminoacylase (EC 3.5.1.81) catalyzed also the reaction between acetophenone and *p*-nitrobenzaldehyde. Further investigation of this reaction will be performed with the aim of clarifying the *modus operandi* of imidazole. Applying molecular modeling, protein engineering, and process engineering tools should aid in the determination of the optimal conditions to attain high yields and selectivity in the biocatalytic synthesis of variously substituted chalcones.

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ИЗВОД

ОРИГИНАЛНА, ЕНЗИМСКИ КАТАЛИЗОВАНА СИНТЕЗА ХАЛКОНА: ПРИМЕНА ПРОМИСКУИТЕТНОГ ПОНАШАЊА ХИДРОЛАЗА

YAVOR N. MITREV[1], ALEKSANDER Y. MEHANDZHIYSKI[1], DANIELA I. BATOVSKA[1], ANDREAS LIESE[2] и BORIS GALUNSKY[2]

[1]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria и* [2]*Institute of Technical Biocataysis, Hamburg University of Technology, Hamburg 21073, Germany*

У ензимски катализованој Claisen–Schmidt реакцији кондензације између бензалдехида и ацетофенона добијен је *E*-халкон уз високу стереоспецифичност. Поредећи разне липазе, нађено је да је само липаза из панкреаса свиње испољила промискуитетно понашање, катализујући ову реакцију у присуству имидазола као иницијатора. Још један ензим, ацилаза из гљиве *Aspergillus melleus* (EC 3.5.1.14), се показао способним за синтезу *E*-халкона под истим реакционим условима. Ова ацилаза је, заједно са рекомбинантном D-аминоацилазом (EC 3.5.1.81), такође катализовала реакцију између ацетофенона и *p*-нитробензалдехида. "Зелени приступ" у синтези халкона је веома важан, због примене халкона као састојака фармацеутских, прехрамбених и козметичких производа.

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