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Asymmetric transesterification of secondary alcohols catalyzed by feruloyl esterase from *Humicola insolens*

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Abstract

A new asymmetric transesterification of secondary alcohols catalyzed by feruloyl esterase from *Humicola insolens* has been found. Although alcohols are not the natural substrates for this enzyme, a high *R* enantioselectivity was observed. Stereochemical studies showed that variations in substrate structure lead to strong variations in enantioselectivity. The highest enantioselectivities are obtained when the β-carbon of the secondary alcohol is tertiary or quaternary.

Keywords: Ferulic acid; Feruloyl esterase; *Humicola insolens*; Transesterification; Enantioselectivity; Secondary alcohols

1. Introduction

Ferulic acid esterases (FAE) [1] are a subclass of the carboxylic ester hydrolases (EC 3.1.1.1) which hydrolyze the esters of hydroxycinnamic acids [2], such as *p*-coumaric acid, ferulic acid, and sinapic acid, with sugars. Hydroxycinnamates are

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important structural components of plant cell walls where they form cross-links with various polymers such as hemicellulose and pectin [3,4]. Feruloyl esterases are secreted by a number of bacterial [5,6] and fungal [7,8] organisms which exploit plants either to enter the plant cell or to utilize the cell wall material as nutritional resource [9]. The complete degradation of plant cell wall polymers requires multienzyme complex systems [10]. Most of the feruloyl esterases have been shown to act synergistically with xylanases, cellulases, and pectinases [11] to break down complex plant cell wall carbohydrates [12,13]. In addition to their fundamental biological importance, feruloyl esterases have many potential industrial and medicinal applications.

Most of the research done to date involves the isolation, purification, and characterization of feruloyl esterases derived from a wide range of microorganisms [5–8], as well as the enzymatic release of the products from cell-wall degradation [3,4]. In all cases, the activities of these enzymes have been determined by the release of free ferulic acid or other hydroxycinnamic acids. A few studies on substrate specificity and on the kinetics towards a range of methyl phenylalkanoates have been used to probe the active site of the enzyme [2,9].

Recently, the crystal structures of feruloyl esterase module of xylanase 10B [14] xylanase Z [15] from *Clostridium thermocellum* as well as ferulic acid esterase from *Aspergillus niger* were reported [16,17]. The primary structure analysis of FAEs has shown that they have an α/β hydrolase fold, with a Ser-His-Asp catalytic triad at their active site [10,14–17]. The overall fold of these proteins is very similar to that of the fungal lipases. Despite the fact that feruloyl esterases and lipases bear the same catalytic triad of residues in their active site, they do not show lipase activity. Recently, the synthesis of pentylferulate was achieved by using a water-in-oil microemulsion system containing a feruloyl esterase from *Aspergillus niger* [18]. However, the potential of feruloyl esterases for the transesterification of alcohols has never been reported.

We report here for the first time, experimental results of a new and high asymmetric transesterification of secondary alcohols catalyzed by a crude feruloyl esterase preparation from *Humicola insolens* [19]. The observed new stereoselectivity of this enzyme toward non natural substrates, such as secondary alcohols, was unexpected and may lead to a promising biocatalyst for the enantioselective synthesis of various alcohols and esters with interesting practical applications.

2. Results and discussion

Selective separation of one enantiomer from a racemic mixture by kinetic resolution techniques played a central role in the preparation of optically active compounds, despite the disadvantage that only a 50% maximum yield of one enantiomer can be obtained. In cases where the racemic substrates and the catalyst are readily available in large quantities, this method can be useful in organic synthesis.

In this work, a new and highly stereoselective transesterification of secondary alcohols catalyzed by a crude feruloyl esterase preparation from *H. insolens* has been

Table 1
Effect of temperature in the enzymatic resolution of 1-phenyl-1-ethanol (**1**)

Substrate	Temperature	Time (days)	Conversion (%)	ee (%)	*E*
1	25	3	12	94	36
1	45	3	23	92	32
1	65	3	30	80	12

studied. This is the first example of the feruloyl esterase reactivity towards substrates whose structural characteristics are completely different than those of the natural substrate of this enzyme.

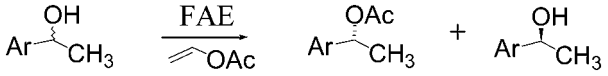
Initially, we investigated the effect of temperature in the enzyme's enantioselectivity and activity. Transesterification of 1-phenylethanol was used as a model reaction. In a typical run, 0.2 mmol of the substrate and a 30-fold excess of vinyl acetate were added in a vial to which 200 mg of the enzyme preparation was placed. Vinyl acetate was used as the activated acyl donor [20]. The resulting suspension was shaken at three different temperatures. Samples were collected periodically and were analyzed by gas chromatography. The reaction was performed in 25, 45, and 65 °C. The results obtained are summarized in Table 1.

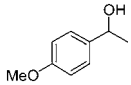
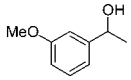
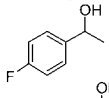
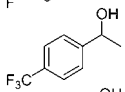
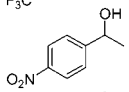
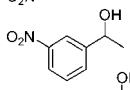
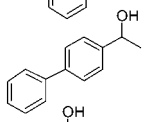
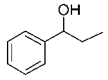
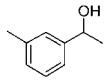
As seen from Table 1, the highest enantioselectivity is observed at 25 °C with a significant loss of activity (conversion = 12%), whereas the highest activity is at 65 °C with a significant loss of enantioselectivity, may be due to denaturation of the enzyme. After that, the optimum temperature for this reaction is 45 °C. All the rest reactions were performed at 45 °C.

The general protocol of the stereoselective transesterification of secondary alcohols is as follows: 0.2 mmol of the substrate and a 30-fold excess of vinyl acetate were added in a vial to which 200 mg of the enzyme preparation was placed. Vinyl acetate was used as the activated acyl donor [20]. The resulting suspension was shaken at 45 °C. Samples were collected periodically and were analyzed by gas chromatography. The enantioselectivity in each reaction was determined by analyzing the enantiomeric excess of the unreacted alcohol and the produced ester by using a 30 m chiral capillary column (HP-5 cross-linked 5% phenyl-methyl silicone), or by ^{1}H NMR spectroscopy by using chiral shift reagents or MTPA esters [21]. The results are summarized in Table 2.

As seen from Table 2, transesterification of a series of aryl alcohols **1**–**11** shows a high selectivity for the *R* enantiomer of the racemic mixture. In most cases, the *R* configuration was determined from the measured optical rotations. The high enantiopreference of $> 98\%$ with $E > 100$ is impressively illustrated by substrates **7**–**9**. Similarly para substituted aryl alcohols with either electron donating, **2** and **3**, or electron withdrawing groups, **4**–**7**, show high enantioselective transesterifications ranging from 88% to 95% ee. Apparently, the electronic properties of the phenyl substituent do not significantly affect enzyme's enantioselectivity. However, the substitution on the meta or para position alter the observed enantioselectivity. FAE shows higher enantioselectivity for meta than para substituted alcohols, as demonstrated by the two isomeric pairs **2**, **3**, and **6**, **7**. Furthermore, excellent enantioselectivity is demonstrated again in the 1-(4-pyridinyl)-1-ethanol, **9**, where more than 98% ee

Table 2



	Substrate[a]	Conversion (%)	ee (%)	Time (days)	Configuration	E[b]
1		23	92	3	*R*[c]	32
2		18	88	3	*R*[d]	19
3		28	98	3	–	>100
4		15	97	2	*R*	75
5		18	94	5	*R*	40
6		18	93	6	*R*	34
7		30	>99	7	–	>100
8		17	>98	2	–	>100
9		28	>98	0.5	*R*[e]	>224
10		25	93	3	*R*[f]	37
11		52	91	5	*R*[g]	>100

[a] Alcohols **1** and **9** were commercially available. Alcohol **6** was prepared by BF_3/EtSiH reduction [29] of the corresponding aryl ketone. Alcohol **10** was prepared by Grignard addition of EtMgI to the corresponding aldehyde. All the other alcohols were prepared by Grignard addition of CH_3MgI to the corresponding aldehydes.

[b] See [22].

[c] By GC analysis with a chiral capillary column of the remaining alcohol, compared with standard *S*-(−)-1-phenyl-1-ethanol.

[d] By the optical rotation of the remaining alcohol after 35% conversion. Measured $[\alpha]_D^{25}$ −15 (*c* 1, $CHCl_3$), ee % 30, reported $[\alpha]_D^{25}$ +52 (*c* 1, $CHCl_3$), ee % 87 (*R*)-alcohol, [23].

[e] The configuration of the ester was determined by the optical rotation of the isolated product after 50% conversion. Measured $[\alpha]_D^{25}$ +81 ± 1 (*c* 1, $CHCl_3$), reported $[\alpha]_D^{25}$ +81 (*c* 1.01, $CHCl_3$) [24].

[f] By the optical rotation of the isolated product after 50% conversion. Measured $[\alpha]_D^{25}$ +47 ± 1 (*c* 1, $CHCl_3$), reported $[\alpha]_D^{25}$ +49 (*c* 1, $CHCl_3$), [22].

[g] By comparison of GC analysis of the produced ester and the enzymatic hydrolysis of racemic ester of alcohol **7** with PFL, [25].

transesterification product was obtained with $E > 100$. In this case and in substrate **10**, when the conversion was extended to 60%, the unreacted alcohol was practically enantiopure (> 99% ee) having the *S* configuration. Also, substitution of an ethyl (substrate **10**) for the methyl group (substrate **1**) does not alter the high degree of stereoselectivity. At this point, it is difficult to rationalize the factors (electronic, steric) that contribute to this remarkable enantioselectivity with *R* preference. However, when the ''small'' substituent was isopropyl or *tert*-butyl group no selectivity was observed (data not shown). It must be pointed out, that all these results are consistent with the Kazlauskas model, [26] which predicts the enantiopreference of lipase towards secondary alcohols. This model is based on the size difference of the substituents at the stereogenic center and suggests that lipases distinguish between enantiomeric secondary alcohols according to the size of the two substituents.

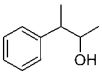
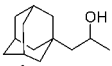
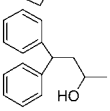
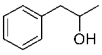
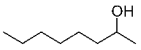
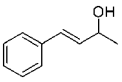
To examine further the stereochemical factors governing FAEs enantioselectivity towards secondary alcohols, we studied the secondary alcohols **12**–**22**, where the small substituent *S* (methyl) at the stereogenic center is the same through the series of the examined substrates while the large substituent L is varied. The results are summarized in Table 3.

As seen from Table 3, FAE exhibits high to excellent enantioselectivity in the transesterification of alcohols **12**–**15**. The common feature among these substrates is the existence of a tertiary or quaternary moiety next to the carbon which bears the –OH group. Alcohol **12**, with an adamantyl group (a quaternary carbon) next to the stereogenic alcohol carbon, impressively illustrates this point. However, a significant loss of enantioselectivity is observed when the large group L has an additional methylene group that is directly bonded to the stereogenic center(enantioselectivities ranging between 35% and 75% ee). Substrates **16**–**20** illustrate this point. For example, *E* factor is > 100 for substrate **12**, compared to *E* factor **9** for alcohol **16**. Again, the major product of these transesterifications is the *R* enantiomer.

3. Conclusion

In summary, these results show for the first time that feruloyl esterase from *H. insolens* can successfully catalyze the stereoselective transesterification of secondary alcohols that bear no structural similarity to the natural substrates of this enzyme. These reactions proceed with good to excellent enantioselectivity that is analogous to that of lipases [23–25]. Our results also demonstrate that the enantioselectivity of FAE in the transesterification of secondary alcohols depends significantly on the substitution of the carbon next to the stereogenic center which bears the hydroxyl group. Excellent enantiodiscrimination is achieved when the β-carbon is tertiary or quaternary, whereas moderate enantioselectivity is obtained when this carbon is secondary. Further increase in the substitution of the β-carbon results in improvement of the observed enantioselectivity. Additionally, the substitution of the phenyl ring of secondary benzylic alcohols affects the observed enantioselectivity of this enzyme. Higher enantioselectivity is observed for meta-substituted compared to para-substituted substrates.

Table 3
Asymmetric enzymatic transesterification of alcohols **12**–**22** with FAE

	Substrate[a]	Conversion (%)	ee (%)	Time (days)	Configuration	*E*
12		30	>95	15	*R*	>100
13		10	>95	3	*R*[b]	>100
14		22	92	24	–	31
15		23	85	9	–	16
16		22	75	18	–	9
17		22	60	16	–	6
18		21	40	30	*R*[c]	3
19		20	66	9	*R*[d]	6
20		21	34	3	–	2
21		25	62	4	*R*[e]	5
22		19	46	7	–	3

[a] Alcohols **12**, **14**, **15**, **16**, **17**, **18**, **21**, and **22** were prepared by MeLi addition to the corresponding carboxylic acids followed by $LiAlH_4$ reduction of the produced ketones. Alcohols **19** and **20** were prepared by reduction of the corresponding ketones with $LiAlH_4$. Synthesis of alcohol **13** was accomplished by Grignard addition of EtMgI to the corresponding aldehyde.

[b] See [27].

[c] The configuration of the alcohol was determined by the optical rotation of the isolated product after 35% conversion. Measured $[\alpha]_D^{25}$ +10.1 (*c* 1, $CHCl_3$) 38% ee, reported $[\alpha]_D^{25}$ +12.6 (*c* 1.01, $CHCl_3$) [23].

[d] By chemical esterification of commercially available *S*-alcohol to the *S*-ester and comparison of elution time in GC analysis.

[e] See [28].

Further studies are currently under way in our laboratories in order to gain a better insight on substrate specificity and to explore the usefulness of this enzyme as a potent catalyst for the enantioselective resolution of various alcohols and esters with interesting practical applications.

4. Experimental

4.1. General methods and materials

All commercially available reagents were used as received (Merck and Sigma-Aldrich) without any further purification. Solvents were distilled and dried using standard techniques.

The FAE preparation (Pentopan 500 BG) was kindly provided by Novo Nordisk as a commercial culture supernatant from a strain of *H. insolens*. Pseudomonas fluorescens lipase (PFL) was purchased from Fluka. Diethyl ether and THF were freshly distilled over sodium. Standard Schlenk techniques were employed by using argon as the inert atmosphere for all manipulations of air or moisture sensitive reactions.

The enzymatic reactions were incubated at 45 °C under shaking at 400 rpm. Reactions were quenched on completion by filtering off the enzyme. Solvents were removed under reduced pressure and the products were purified by flash chromatography using Merck silica gel (230–400 mesh). Control reactions without enzyme were carried out under the same conditions. All enzymatic reactions were performed two times.

The progress of the FAE catalyzed reactions and the enantiomeric excesses were determined by gas chromatography (HP5890II gas chromatograph equipped with an FID detector; column: 30 m chiral capillary column, HP-5 cross-linked 5% phenylmethyl silicone). The estimated error was <2%. In some cases, the enantioselectivity was determined by ^{1}H NMR analysis of the corresponding MTPA esters or by using optically active shift reagents [Eu(hfc)$_3$].

MS were taken on a GC–MS (Simatzu GCMS-QP5050 equipped with a SPB-5 column and CI mass detector). ^{1}H NMR and ^{13}C NMR spectra were recorded on a 300 or a 500 MHz Bruker spectrometers in $CDCl_3$ solutions, by using Me_4Si as internal standard. Chemical shifts are reported in parts per million downfield from Me_4Si. Yields refer to isolated and spectroscopically pure materials. Optical rotations were measured on a D,P 360 Jasco polarimeter with a Hg 360 lamp. Measurements were made in 1 cm, permanent-window cell at room temperature.

Feruloyl esterase (FAE) activity was calculated with destarched wheat bran as substrate. FAE's activity was assayed by the analysis of free ferulic acid (FA) released from destarched wheat bran. Ferulate release was analyzed by HPLC (Nucleosil C18, column). The assay was carried out in 100 mM MOPS buffer, pH 6, and 45 °C. One unit (U) of activity was defined as the amount of enzyme that catalyzes the release of 1 μmol FA/min. The FAE activity was found to be 0.435 U/gr.

4.2. Preparation of aryl alcohols

Alcohols **2**, **3**, **4**, **5**, **7**, **8**, **9**, and **10** were prepared by the addition of the proper Grignard reagent to the corresponding aldehyde as follows:

To a stirred mixture of 12 mmol of crushed Mg in 20 ml freshly distilled absolute Et_2O, under inert atmosphere, 10 mmol of alkyl iodide in 10 ml of absolute Et_2O was added dropwise, followed by 30 min reflux. In some cases the addition of an iodine crystal was required. After cooling the reaction mixture at 0 °C, 10 mmol of the appropriate carbonyl compound in 10 ml of absolute Et_2O was added dropwise. The reaction mixture was stirred under reflux conditions and after cooling at 0 °C, 0.36 mmol of water was added dropwise and the produced alcohol was isolated following standard Grignard work-up conditions.

Alcohol **6** was prepared by reduction of the corresponding *p*-nitro-acetophenone with Et_3SiH/BF_3 system [29].

Alcohol **11** was prepared from the corresponding ketone by reduction with $LiAlH_4$ [30].

1-(4-Methoxyphenyl)-1-ethanol, **2**: yield 1.32 g (90%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.32 (d, $J = 8.2$ Hz, 2H), 6.90 (d, $J = 8.2$ Hz, 2H), 4.88 (m, $J = 6.3$ Hz, 1H), 3.82 (s, 3H), 1.50 (d, $J = 6.3$ Hz, 3H). MS: *m*/*z* 152.

1-(3-Methoxyphenyl)-1-ethanol, **3**: yield 2.2 g (96%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.16–7.29 (t, $J = 7.8$ Hz, 1H), 6.95 (d, $J = 6.2$ Hz, 2H), 6.83 (d, $J = 6.2$ Hz, 1H) 4.87 (q, $J = 6.4$ Hz, 1H), 3.82 (s, 3H), 1.5 (d, $J = 6.4$ Hz, 3H). ^{13}C NMR ($CDCl_3$ 500 MHz, δ ppm) 159.74, 147.59, 129.49, 117.67, 112.84, 110.89, 70.27, 55.19, 25.10.

1-(4-Fluorophenyl)-1-ethanol, **4**: yield 1.25 g (90%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.38–7.41 (m, 2H), 7.06–7.1 (m, 3H), 4.83 (m, 1H), 1.39 (d, $J =$ 4.5 Hz, 3H).

1-(4-Trifluoromethylphenyl)-1-ethanol, **5**: yield 1.4 g (90%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.63 (d, $J = 7.7$ Hz, 2H), 7.51 (d, $J = 8.0$ Hz, 2H), 4.99 (m, $J = 6.4$ Hz, 1H), 1.53 (d, $J = 6.4$ Hz, 3H). MS: *m*/*z* 190.

1-(4-Nitrophenyl)-1-ethanol, **6**: yield 1.6 g (95%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 8.22 (d, $J = 7.7$ Hz, 2H), 7.56 (d, $J = 8.3$ Hz, 2H), 5.04 (m, $J =$ 6.4 Hz, 1H), 1.54 (d, $J = 6.4$ Hz, 3H). MS: *m*/*z* 152.

1-(3-Nitrophenyl)-1-ethanol, **7**: yield 0.84 g (50%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 8.25 (s, 1H), 8.12 (d, $J = 7.6$ Hz, 1H), 7.72 (d, $J = 6.7$ Hz, 1H), 7.53 (m, 1H), 5.03 (m, $J = 6.1$ Hz, 1H), 1.55 (d, $J = 6.1$ Hz, 3H).

1-(4-Phenyl-phenyl)-1-ethanol, **8**: yield 0.75 g (94%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.61 (d, $J = 8.0$ Hz, 4H), 7.47 (m, $J = 8.0$ Hz, 4H), 7.37 (m, 1H), 4.98 (q, $J = 6.4$ Hz, 1H), 1.57 (d, $J = 6.4$ Hz, 3H).

1-(4-Pyridinyl)-1-ethanol, **9**; yield 0.5 g (94%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 8.54 (d, $J = 6.6$ Hz, 2H), 7.31 (d, $J = 3.8$ Hz, 2H), 4.92 (m, $J = 3.8$ Hz, 1H), 1.59 (d, $J = 3.8$ Hz, 3H).

1-Phenyl-1-propanol, **10**: yield 1.3 g (96%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.37 (t, $J = 3.0$ Hz, 4H), 7.28 (m, $J = 2.5$ Hz, 1H), 4.62 (t, $J = 6.6$ Hz, 1H), 1.78 (dd, $J = 7.1$ Hz, 1H), 1.86 (dd, $J = 7.3$ Hz, 1H), 0.95 (t, $J = 7.4$ Hz, 2H).

^{13}C NMR ($CDCl_3$ 500 MHz, δ ppm) 144.59, 128.38, 125.96, 76.006, 31.86, 10.12.

1-(3-Methylphenyl)-1-ethanol, **11**: yield 1.17 g (98%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.26–7.29 (t, $J = 3.7$ Hz, 1H), 7.19 (d, $J = 7.5$ Hz, 2H), 7.12 (d, $J = 7.5$ Hz, 1H), 4.87 (q, $J = 6.4$ Hz, 1H), 2.4 (s, 3H), 1.51 (d, $J = 6.4$ Hz, 3H).

^{13}C NMR ($CDCl_3$ 500 MHz, δ ppm) 145.81, 138.10, 128.38, 128.17, 126.10, 122.42, 70.36, 25.09, 21.43.

4.3. Preparation of alcohols **12**, **14**, **16**, **17**, **18**, **21**, and **22**

The above alcohols were prepared from their corresponding ketones by reduction with $LiAlH_4$. These ketones were prepared from their carboxylic acids by addition of MeLi [31] as follows:

To a stirred solution of 5 mmol of carboxylic acid dissolved in 50 ml freshly distilled absolute Et_2O, under inert atmosphere, which was cooled to −78 °C, 11 mmol of MeLi was added dropwise. The reaction mixture was stirred at this temperature for 15 min and then it was left to reach room temperature. It was then stirred for 3 h. After completion of the reaction, the reaction mixture was cooled at 0 °C and 1 ml of water was added dropwise. The organic layer with washed with distilled water and saturated aqueous NaCl and then it was dried over $MgSO_4$. The corresponding ketone was isolated after removal of the ether under vacuum.

The overall yield of isolated alcohols was in the range of 50–60%.

1-Adamantyl-1-ethanol, **12**: yield 0.50 g (58%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 3.3 (m, 1H), 2.0 (s, 3H), 1.74 (d, 3H), 1.65 (m, 7H), 1.50 (d, 3H), 1.2 (d, $J = 6.4$ Hz, 3H). ^{13}C NMR ($CDCl_3$ 500 MHz, δ ppm) NMR ($CDCl_3$ 500 MHz, δ ppm) 75.75, 37.69, 37.23, 36.53, 28.31, 16.41.

3,3-Dimethyl-2-butanol, **14**: yield 0.50 g (58%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 3.49 (q, $J = 6.3$ Hz, 1H), 1.13 (d, $J = 6.3$ Hz, 3H), 0.91 (s, 9H).

3-Methyl-2-butanol, **15**: yield 0.42 g (57%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm), 3.49 (q, 1H, $J = 6.3$ Hz), 1.13 (d, 3H, $J = 6.3$ Hz), 0.91 (s, 9H).

1-Adamantyl-2-propanol, **16**: yield 0.66 g (60%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 4.05 (m, 1H), 1.96 (s, 3H), 1.67 (dd, 6H, $J_1 = 12.1$ Hz, $J_2 = 33.3$ Hz), 1.57 (s, 6H), 1.25 (m, 2H), 1.19 (d, $J = 6.2$ Hz, 3H). ^{13}C NMR ($CDCl_3$ 500 MHz, δ ppm) 64.35, 54.18, 42.98, 37.04, 32.21, 28.66, 26.04.

4,4-Diphenyl-2-butanol, **17**: yield 0.66 g (68.5%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.36 (m, 8H), 7.27 (m, 2H), 4.28 (m, 1H), 3.73 (m, 1H), 2.26 (t, $J = 7.1$ Hz, 2H), 1.28 (d, 6.1 Hz, 3H). ^{13}C NMR ($CDCl_3$ 500 MHz, δ ppm) 145.11, 144.44, 128.60, 128.58, 128.13, 127.82, 126.33, 126.25, 65.85, 47.74, 45.02, 24.06.

1-Phenyl-2-propanol, **18**: yield 0.63 g (72%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.34 (m, 2H), 7.26 (m, 3H), 4.04 (m, 1H), 1.27 (d, $J = 6.2$ Hz, 3H).

4-Phenyl-3-buten-2-ol, **21**: yield 0.91 g (63%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.40 (d, $J = 6.9$ Hz, 2H), 7.34 (d, $J = 7.3$ Hz, 2H), 7.27 (m, 1H), 6.59 (d, $J = 16.2$ Hz, 1H), 6.29 (dd, $J_1 = 6.2$ Hz, $J_2 = 15.8$ Hz, 1H), 4.51 (m, $J = 6.2$ Hz, 1H), 1.40 (t, $J = 6.6$ Hz, 3H).

4-Methyl-3-buten-2-ol, **22**: yield 0.50 g (60%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 5.22 (m, 1H), 4.56 (m, 1H), 1.73 (s, 3H), 1.71 (s, 3H), 1.23 (d, $J = 7.7$ Hz, 3H).

Alcohols **15**, **19**, and **20** were prepared from their corresponding ketones by reduction with $LiAlH_4$.

3-Methyl-2-butanol, **15**: yield 0.31 g (70%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 3.58 (m, 1H), 1.63 (m, 1H), 1.15 (d, $J = 6$ Hz, 3H), 0.93 (d, $J = 6.8$ Hz, 3H), 0.91 (d, , $J = 6.8$ Hz, 3H).

2-Octanol, **19**: yield 0.54 g (85%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm), 3.80 (m, 1H), 1.45 (m, 2H), 1.26–1.36 (m, 8H), 1.21 (d, $J = 6.2$ Hz, 3H), 0.90 (t, $J = 6.9$ Hz, 3H).

2-Butanol, **20**: yield 0.26 g (70%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm), 3.58 (m, 1H), 1.40 (m, 2H), 1.10 (d, $J = 6.2$ Hz, 3H), 0.89 (t, $J = 6.3$ Hz, 3H).

4.4. Acyl esterification of alcohols [32]

To a stirred mixture of 0.5 mmol of the appropriate alcohol in 30 ml of ethyl acetate were added 15 mmol of distilled acetic anhydride, 15 mmol of anhydrous K_2CO_3 and a catalytic amount of *N*,*N*-dimethyl-amino pyridine (DMAP).The reaction mixture was stirred at room temperature for 24 h. Following the addition of diethyl ether, the mixture was extracted with saturated $CuSO_4{\cdot}5H_2O$, saturated aqueous sodium bicarbonate $NaHCO_3$ and finally washed with brine. The organic layer was dried over $MgSO_4$ and the corresponding acetyl ester was isolated after removal of the solvent under vacuum in good yields.

All the prepared substrates are known compounds and were identified by their NMR, and MS spectra.

Acetyl ester of alcohol **2**: yield 0.082 g (85%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.31 (d, $J = 8.5$ Hz, 2H), 6.90 (d, $J = 8.5$ Hz, 2H), 5.87 (m, $J = 6.5$ Hz, 1H), 3.82 (s, 3H), 2.06 (s, 3H), 1.54 (d, $J = 6.6$ Hz, 2H). MS: m/z 152.

Acetyl ester of alcohol **3**: yield 0.084 g (87%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.26–7.29 (t, $J = 7.8$ Hz, 1H), 6.95 (d, $J = 6.2$ Hz, 2H), 6.83 (d, $J = 6.2$ Hz, 1H), 5.77 (q, $J = 6.4$ Hz, 1H), 3.82 (s, 3H), 1.50 (d, $J = 6.4$ Hz, 3H).

Acetyl ester of alcohol **4**: yield 0.082 g (91%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.33–7.36 (m, 2H), 7.03–7.06 (m, 2H), 5.8 (q, $J = 6.5$ Hz, 1H), 2.08 (s, 3H), 1.5 (d, $J = 6.5$ Hz, 3H).

Acetyl ester of alcohol **5**: yield 0.096 g (81%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.63 (d, $J = 8.0$ Hz, 2H), 7.48 (d, $J = 8.0$ Hz, 2H), 5.92 (m, $J = 6.7$ Hz, 1H), 2.11 (s, 3H), 1.56 (d, $J = 6.7$ Hz, 3H).

Acetyl ester of alcohol **6**: yield 0.083 g (80%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.23 (d, $J = 8.6$ Hz, 2H), 7.53 (d, $J = 8.6$ Hz, 2H), 5.94 (m, $J = 6.7$ Hz, 1H), 2.13 (s, 3H), 1.57 (d, $J = 6.7$ Hz, 3H).

Acetyl ester of alcohol **7**: yield 0.077 g (75%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.61 (d, $J = 7.0$ Hz, 2H), 7.43 (m, 1H), 7.31 (m, 1H), 5. 72 (m, 1H), 2.1 (s, 3H), 1.57 (d, $J = 6.5$ Hz, 3H).

Acetyl ester of alcohol **8**: yield 0.101 g (90%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.60 (d, $J = 7.9$ Hz, 4H), 7.46 (t, $J = 7.0$ Hz, 4H), 7.37 (m, 1H), 5.96 (q, $J = 6.5$ Hz, 1H), 1.60 (d, $J = 6.4$ Hz, 3H).

Acetyl ester of alcohol **9**: yield 0.070 g (85%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 8.61 (d, $J = 6.0$ Hz, 2H), 7.26 (d, $J = 6.0$ Hz, 2H), 5.84 (m, $J = 6.5$ Hz, 1H), 2.1 (s, 3H), 1.54 (d, $J = 6.5$ Hz, 3H).

Acetyl ester of alcohol **10**: yield 0.087 g (94%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.35 (d, $J = 6.5$ Hz, 4H), 7.29 (m, $J = 3.4$ Hz, 1H), 5.69 (t, $J = 6.9$ Hz, 1H), 2.1 (s, 3H), 1.56 (m, $J = 7.4$ Hz, 1H), 1.85 (m, $J = 7.1$ Hz, 1H), 0.91 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR ($CDCl_3$ 500 MHz, δ ppm) 170.39, 140.54, 128.357, 127.79, 126.56, 29.27, 21.24, 9.88.

Acetyl ester of alcohol **11**: yield 0.080 g (97%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.27 (t, $J = 7.4$ Hz, 1H), 7.18 (d, $J = 7.4$ Hz, 2H), 7.13 (d, $J = 7.4$ Hz, 1H), 5.88 (q, $J = 6.6$ Hz, 1H), 2.4 (s, 3H), 2.1 (s, 3H), 1.55 (d, $J = 6.6$ Hz, 3H). ^{13}C NMR ($CDCl_3$ 500 MHz, δ ppm) 170.30, 141.60, 138.10, 128.60, 128.38, 126.81, 123.09, 72.33, 22.17, 21.41.

Acetyl ester of alcohol **12**: yield 0.10 g (91%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 4.52 (q, $J = 6.5$ Hz, 1H), 2.08 (s, 3H), 1.98 (s, 3H), 1.67 (dd, $J_1 = 12.1$ Hz, $J_2 = 38.63$ Hz, 6H), 1.54 (dd, $J_1 = 12.1$ Hz, $J_2 = 26.1$ Hz, 6H), 1.01 (d, $J = 6.5$ Hz, 3H). ^{13}C NMR ($CDCl_3$ 500 MHz, δ ppm) 170.86, 77.69, 37.82, 37.06, 35.67, 28.14, 21.16, 13.34.

Acetyl ester of alcohol **14**: yield 0.64 g (89%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 4.70 (q, $J = 6.7$ Hz, 1H), 2.1 (s, 3H), 1.15 (d, $J = 6.7$ Hz, 3H), 0.92 (s, 9H).

Acetyl ester of alcohol **15**: yield 0.55 g (90%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 4.7 (m, $J = 6.3$ Hz, 1H), 2.04 (s, 3H), 1.77 (m, 1H), 1.16 (d, $J = 6.3$ Hz, 3H), 0.91 (d, $J = 6.7$ Hz, 6H).

Acetyl ester of alcohol **16**: yield 0.11 g (92%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 5.09 (m, 1H), 2.02 (s, 3H), 1.95 (s, 3H), 1.06 (dd, $J_1 = 11.7$ Hz, $J_2 = 37.5$, 6H), 1.52 (m, 9H), 1.20 (d, $J = 6.2$ Hz, 3H).

Acetyl ester of alcohol **17**: yield 0.91 g (88%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.30–7.35 (m, 6H), 7.20–7.25 (m, 4H), 4.84 (m, 1H), 4.08 (t, $J = 7.6$ Hz, 1H), 2.47 (m, 1H), 2.25 (m, 1H), 1.98 (s, 3H), 1.28 (d, $J = 6.2$ Hz, 3H). ^{13}C NMR ($CDCl_3$ 500 MHz, δ ppm) 170.53, 128.61, 128.51, 127.79, 127.75, 126.40, 126.29, 65.59, 47.86, 41.69, 21.17, 20.32.

Acetyl ester of alcohol **18**: yield 0.77 g (88%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.30 (m, 2H), 7.22 (m, 3H), 5.14 (m, 1H), 2.94 (dd, $J_1 = 6.5$ Hz, $J_2 = 13.5$ Hz, 1H), 2.77 (dd, $J_1 = 6.5$ Hz, $J_2 = 13.5$ Hz, 1H), 2.02 (s, 1H), 1.24 (d, $J = 6.5$ Hz, 3H).

Acetyl ester of alcohol **19**: yield 0.60 g (92%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 4.88 (m, 1H), 2.04 (s, 3H), 1.58 (m, 1H), 1.48 (m, 1H), 1.3 (m, 8H), 1.22 (d, $J = 6.2$ Hz, 3H), 0.90 (t, $J = 6.9$ Hz, 3H).

Acetyl ester of alcohol **20**: yield 0.40 g (82%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 4.35 (m, 1H), 2.01 (s, 3H), 1.68 (m, 1H), 1.55 (m, 1H), 1.23 (d, $J = 6.4$ Hz, 3H), 0.88 (t, $J = 7.3$ Hz, 3H).

Acetyl ester of alcohol **21**: yield 0.77 g (82%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 7.40 (d, $J = 7.3$ Hz, 2H), 7.34 (d, $J = 7.7$ Hz, 2H), 7.27 (m, 1H), 6.62 (d, $J = 15.8$ Hz, 1H), 6.22 (dd, $J_1 = 6.6$ Hz , $J_2 = 15.8$ Hz, 1H), 5.56 (m, $J = 6.6$ Hz, 1H), 2.10 (s, 3H), 1.42 (t, $J = 6.2$ Hz, 3H).

Acetyl ester of alcohol **22**: yield 0.62 g (88%). ^{1}H NMR ($CDCl_3$ 500 MHz, δ ppm) 5.6 (m, 1H), 5.18 (d, $J = 7.3$ Hz, 1H), 2.04 (s, 3H), 1.74 (d, $J = 5.5$ Hz, 6H), 1.27 (d, $J = 6.2$ Hz, 3H).

4.5. General procedure for the enzymatic transesterification of alcohols catalyzed by feruloyl esterase

All enzymatic transesterification reactions were performed as follows:

100 mg (0.0435 U) of the FAE preparation and 0.277 ml (3 mmol) of dry vinyl acetate were placed in a vial and 0.1 mmol of the (*R*,*S*)-alcohol was added. Molecular sieves 3 Å were also added to the reaction mixture. The resulting suspension was shaken at 400 rpm, 45 °C. Samples were collected periodically and were analyzed by gas chromatography. When the desired conversion was reached the mixture was filtered through a paper funnel and the mixture of the residual substrate and the resulting ester was washed three times with ethyl acetate. The solvent was removed under vacuum and the products were separated from the remaining substrate by flash column chromatography with 5–30% diethyl ether in hexane as eluent.

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