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Enantioselective acetylation of racemic alcohols by *Manihot esculenta* and *Passiflora edulis* preparations

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ABSTRACT

Immobilized *Manihot esculenta* and *Passiflora edulis* juice preparations have been employed as stereoselective biocatalysts in the enzymatic acetylation of a set of racemic alcohols. Depending on the reaction conditions and the substrate structure, good to excellent enantioselectivities can be achieved in the preparation of the (*S*)-alcohols and (*R*)-esters, compounds presenting high interest in organic synthesis.

1. Introduction

In the last few years, the increasing interest for preparing organic compounds has expanded the development of new sustainable and environmentally friendly procedures. Among all these methods, biotransformations have emerged as efficient processes for the synthesis of high added value compounds [1–3]. Some of the advantages presented by the use of enzymes are their high efficiency and selectivity while working under mild and environmental friendly conditions, and an increasing applicability due to continuous advances in molecular biology for the development of new promising biocatalysts.

The large majority of enzymes used in biocatalytic processes are employed as crude preparations and are relatively inexpensive. The demanding task for the production of new and improved enzyme activities has allowed the discovery of very efficient biocatalysts in the last decade. For example, in recent years it has been described the use of a discard (*manipueira*) obtained from *Manihot esculenta* (cassava) as an important source of enzymes: a (*S*)-hydroxynitrile lyase has been cloned, overexpressed and applied in the preparation of (*S*)-cyanohydrins [4,5], and crude *manipueira* has been employed in the enzymatic bioreduction of different ketones and aldehydes, as well as in different hydrolytic biocatalyzed reactions [6]. Fruits' barks of *Passiflora edulis* (an industrial organic waste from the preparation of maracuja juice) have been also investigated as possible biocatalyst in the bioreduction of ketones and aldehydes in order to produce the corresponding secondary and primary alcohols respectively [7].

As a part of our ongoing project for the discovery of new enzymes from the Brazilian biodiversity, it has recently been developed the enzymatic acetylation of a set of racemic aromatic *sec*-alcohols catalyzed by an immobilized preparation of *M. esculenta* [8]. Now we have focused our efforts in the selective acetylation of other racemic alcohols using this promising biocatalyst as well as a novel enzymatic crude system obtained from the immobilization of *P. edulis* juice. The immobilization of a biocatalyst presents some advantages respecting its use without being supported, as an easier handling, possibility of enzyme recovery and reuse, an improvement in the enzymatic stability and a higher performance in organic solvents [9,10]. Enzymes can be immobilized onto different supports, being the entrapment of the biocatalyst on calcium-alginate hydrogels a well-studied methodology. This technique consists in the formation of a solidified water phase in an aqueous–organic two-phase system and provides an easy, fast, nontoxic, inexpensive and versatile immobilization process [11,12].

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2. Experimental

2.1. Materials and methods

Chemical reactions were monitored by analytical TLC, performed on Merck silica gel 60 F_{254} plates and visualized by UV irradiation. Flash chromatographies were carried out using silica gel 60 (230–240 mesh, Merck). IR spectra were recorded on a Perkin-Elmer 1720-X infrared Fourier transform spectrophotometer using NaCl plates. Optical rotations were measured using a Perkin-Elmer 241 polarimeter and are quoted in units of 10^{-1} ° cm^2 g^{-1}. ^{1}H NMR, ^{13}C NMR and DEPT spectra were recorded with TMS (tetramethylsilane) as internal standard using a Bruker AC-300-DPX (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometer. The chemical shift values (δ) are given in ppm and the coupling constants (J) in Hertz (Hz). APCI$^+$ using a Hewlett Packard 1100 chromatograph mass detector or EI$^+$ with a Hewlett Packard 5973 mass spectrometer was used to record mass spectra (MS). GC analyses were performed on a Hewlett Packard 6890 Series II chromatograph equipped with a CP-Chiralsil DEX CB column (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2) from Varian or a Restek RtβDEXse (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2). For all the analyses, the injector temperature was 225 °C and the FID temperature was 250 °C. HPLC analyses were developed using a Hewlett Packard 1100 LC liquid chromatograph equipped with a Chiralcel OD (0.46 cm × 25 cm) or Chiralcel OB-H (0.46 cm × 25 cm) chiral columns from Daicel.

Alcohols (±)-**3a**, (±)-**4a**, (±)-**5a**, (±)-**8a**, (±)-**11a**, (±)-**13a**, (±)-**15a**, (±)-**16a**, (±)-**17a** and ester (±)-**16b** were purchased from Sigma–Aldrich–Fluka. Compounds (±)-**12a**, (±)-**4b** and (±)-**17b** were available at Acros Organics. Racemic alcohol (±)-**7a** was obtained from Alfa Aesar. Starting alcohols (±)-**1a**, (±)-**2a**, (±)-**9a**, (±)-**10a** and (±)-**14a** were prepared from commercial ketones by treatment with $NaBH_4$ in dry methanol (yields higher than 80%). Compound (±)-**6a** was synthesised with high yield (90%) according to the literature, by treating a solution of benzothioazol in THF with *n*-BuLi in heptane and subsequent treatment with acetaldehyde [13]. Racemic esters (±)-**1**–**15b** were obtained by the chemical acetylation of the corresponding alcohols employing acetic anhydride and pyridine in dry CH_2Cl_2. In all cases, yields higher than 85% were achieved. Dry solvents were distilled over an appropriate desiccant under nitrogen. The synthesized compounds (±)-**1-2a** [14], (±)-**6a** [13], (±)-**10a** [15], (±)-**14a** [16], (±)-**1-3b** [14], (±)-**5b** [17], (±)-**6b** [13], (±)-**7b** [18], (±)-**11b** [19], (±)-**12b** [20], (±)-**13b** [21] and (±)-**15b** [18] exhibit physical and spectral properties in accordance with those reported. All other reagents and solvents were of the highest quality grade available, purchased from Sigma–Aldrich–Fluka.

Absolute configurations of alcohols (±)-**15**–**17a** were established by comparison of their GC chromatograms with samples obtained from the commercial chiral alcohols (*S*)-**15**–**17a**. For compounds **1**–**3a** [14], **4a** [22], **5a** [22], **6a** [13], **7a** [23], **8a** [24], **10a** [15], **11a** [25], **12a** [26] and **13a** [21] the absolute configurations were established by comparison of the specific rotation measured with ones previously reported. Absolute configuration of alcohol **9a** was determined by comparison of the GC chromatogram with the pattern described in previous experiments for the known configuration [27].

2.2. Preparation of immobilized *M. esculenta* and *P. edulis* biocatalysts

Immobilized *M. esculenta* biocatalyst was obtained as previously described [8]. Fresh fruits of *P. edulis* (maracuja) were obtained from a specimen located at Campus do Pici-UFC, Fortaleza-Ceara-Brazil and identified by Prof. E.P. Nunes. Voucher specimen (#12.811) was deposited at the Herbarium Prisco Bezerra (EAC) from Departamento de Biologia, Universidade Federal do Ceará, Brazil. Fruits were rinsed with 5% aqueous sodium hypochlorite solution. After this, 5.0 kg of fruits were separated in: juice (700 mL), seeds and fruits' barks. Seeds and barks were discarded and the juice was immobilized in sodium alginate using the following adapted technique: to a solution of maracuja juice (300 mL), sodium alginate 1.5% (w/v) was added and the solution was shaken for 3 h at room temperature. After homogenization, this mixture was dropped into 400 mL of a 5.0% (w/v) solution of calcium chloride, using a syringe with a 1.0 mm internal diameter needle. Upon contact with the solution, the drops were gelled to form constant and defined-size spheres, which remained in the solution for 12 h, under mild agitation, to complete gel formation. After hardening, microspheres were washed several times with water and acetone, and dried at room temperature until constant weight (4.0 g). Determination of the protein content using the Hartree method [28] allowed the calculation of protein content not incorporated into the beads (1.8%). By varying the orifice used for dropping the enzyme–sodium alginate mixture, the dimension of the beads was varied. In this case, the spherical diameter of the particles was determined as 1.0 mm by measuring microscopically the dimensions of beads using Electronic Microscopy of Sweeping (MEV), with the aid of a Philips XL-30 microscope.

2.3. General procedure for the enzymatic acetylation of racemic alcohols (±)-***1*–*17a*** *employing M. esculenta or P. edulis preparations*

Unless otherwise stated, the immobilized preparation of *M. esculenta* (30 mg) or *P. edulis* (30 mg) and vinyl acetate (10 equiv.) were added to a solution of the corresponding racemic alcohol (±)-**1**–**17a** (30 mg, 1.0 equiv.) in the selected organic solvent (4.0 mL) at 45 °C under nitrogen atmosphere. The resulting mixture was shaken at 250 rpm on an orbital shaker. The progress of the reaction was monitored by TLC analysis. Once the reaction was finished, the enzyme was filtered off, washed with ethyl acetate and the solvent evaporated under reduce pressure. The crude residue was then purified by flash chromatography using *n*-hexane/ethyl acetate 8:2 (compounds **1**–**6a**, **11**–**13a** and **17a**) or 7:3 (compounds **7**–**10a** and **14**–**16a**) in order to obtain the optically active alcohols (*S*)-**1**–**17a** and the corresponding esters (*R*)-**1**–**17b**.

2.3.1. (*S*)-1-(Pyridin-2-yl)ethanol, (*S*)-***1a***

Determination of the *ee* by HPLC analysis: Chiralcel OB-H, 20 °C, *n*-hexane/2-propanol (95:5), 0.8 mL/min; t_R (*S*) 14.9 min; t_R (*R*) 19.0 min.

2.3.2. (*R*)-1-(Pyridin-2-yl)ethyl acetate, (*R*)-***1b***

Determination of the *ee* by HPLC analysis: Chiralcel OB-H, 20 °C, *n*-hexane/2-propanol (95:5), 0.8 mL/min; t_R (*S*) 11.4 min; t_R (*R*) 12.8 min.

2.3.3. (*S*)-1-(Pyridin-3-yl)ethanol, (*S*)-***2a***

Determination of the *ee* by HPLC analysis: Chiralcel OB-H, 20 °C, *n*-hexane/2-propanol (95:5), 0.8 mL/min; t_R (*S*) 20.8 min; t_R (*R*) 37.0 min.

2.3.4. (*R*)-1-(Pyridin-3-yl)ethyl acetate, (*R*)-***2b***

Determination of the *ee* by GC analysis: CP-Chiralsil DEX CB, 70 °C (5 min), 3 °C/min, 200 °C; t_R (*S*) 23.2 min; t_R (*R*) 24.5 min.

2.3.5. (*S*)-1-(Pyridin-4-yl)ethanol, (*S*)-***3a***

Determination of the *ee* by HPLC analysis: Chiralcel OB-H, 20 °C, *n*-hexane/2-propanol (95:5), 0.8 mL/min; t_R (*S*) 29.9 min; t_R (*R*) 42.9 min.

*2.3.6. (R)-1-(Pyridin-4-yl)ethyl acetate, (R)-**3b***

Determination of the *ee* by GC analysis: CP-Chiralsil DEX CB, 70 °C (5 min), 3 °C/min, 200 °C; t_R (*S*) 23.1 min; t_R (*R*) 24.3 min.

*2.3.7. (S)-1-(Furan-2-yl)ethanol, (S)-**4a***

Determination of the *ee* by HPLC analysis: Chiralcel OB-H, 20 °C, *n*-hexane/2-propanol (97:3), 0.8 mL/min; t_R (*S*) 15.7 min; t_R (*R*) 18.7 min.

*2.3.8. (R)-1-(Furan-2-yl)ethyl acetate, (R)-**4b***

Determination of the *ee* by GC analysis: CP-Chiralsil DEX CB, 70 °C (5 min), 3 °C/min, 200 °C; t_R (*S*) 10.9 min; t_R (*R*) 13.1 min.

*2.3.9. (S)-1-(Tiophen-2-yl)ethanol, (S)-**5a***

Determination of the *ee* by HPLC analysis: Chiralcel OB-H, 20 °C, *n*-hexane/2-propanol (95:5), 0.8 mL/min; t_R (*S*) 14.4 min; t_R (*R*) 17.6 min.

*2.3.10. (S)-1-(Tiophen-2-yl)ethyl acetate, (S)-**5b***

Determination of the *ee* by GC analysis: CP-Chiralsil DEX CB, 70 °C (5 min), 3 °C/min, 200 °C; t_R (*S*) 18.3 min; t_R (*R*) 19.8 min.

*2.3.11. (S)-1-(1,3-Benzo[d]thiazol-2-yl)ethanol, (S)-**6a***

Determination of the *ee* by HPLC analysis after acetylation.

*2.3.12. (R)-1-(1,3-Benzo[d]thiazol-2-yl)ethyl acetate, (R)-**6b***

Determination of the *ee* by HPLC analysis: Chiralcel OD, 30 °C, *n*-hexane/2-propanol (97:3), 0.8 mL/min; t_R (*S*) 6.2 min; t_R (*R*) 6.9 min.

*2.3.13. (S)-1-Cyclohexylethanol, (S)-**7a***

Determination of the *ee* by GC analysis after acetylation.

*2.3.14. (R)-1-Cyclohexylethyl acetate, (R)-**7b***

Determination of the *ee* by GC analysis: CP-Chiralsil DEX CB, 70 °C (5 min), 3 °C/min, 200 °C; t_R (*S*) 18.0 min; t_R (*R*) 19.7 min.

*2.3.15. (S)-1-Cyclopropylethanol, (S)-**8a***

Determination of the *ee* by GC analysis: 60 °C (10 min), 2 °C/min, 120 °C; t_R (*S*) 7.3 min; t_R (*R*) 6.9 min.

*2.3.16. (R)-1-Cyclopropoylethyl acetate, (R)-**8b***

IR (KBr): ν 2945 and 1745 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.37–0.45 (m, 2H), 0.71–0.79 (m, 2H), 1.03–1.07 (m, 1H), 1.48 (d, 3H, $^3J_{HH}$ 6.5 Hz), 2.17 (s, 3H) and 4.13 (q, 1H, $^3J_{HH}$ 6.1 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.6 (CH), 22.7 (CH_3), 23.5 (CH_3), 81.4 (CH) and 170.2 (C=O). MS (EI, *m/z*): 86 (M^+, 10%), 70 (100), 41 (50). Determination of the *ee* by GC analysis: CP-Chiralsil DEX CB, 60 °C (10 min), 2 °C/min, 120 °C; t_R (*S*) 8.2 min; t_R (*R*) 10.4 min.

*2.3.17. (S)-1-Cyclohexylpropan-2-ol, (S)-**9a***

IR (KBr): ν 3380 and 2920 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.17 (d, 3H, $^3J_{HH}$ 6.1 Hz), 1.22–1.38 (m, 5H), 1.41–1.51 (m, 3H), 1.63–1.73 (m, 5H) and 3.93 (q, 1H, $^3J_{HH}$ 6.1 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 22.3 (CH_3), 25.6 (CH_2), 25.7 (CH_2), 26.7 (CH_2), 31.4 (CH_2), 31.6 (CH_2), 33.4 (CH), 47.9 (CH_2) and 70.2 (CHOH). MS (EI, *m/z*): 142 (M^+, 5%), 124 (20), 82 (100), 55 (60). Determination of the *ee* by GC analysis by acetylation.

*2.3.18. (R)-1-Cyclohexylethyl acetate, (R)-**9b***

IR (KBr): ν 2950 and 1742 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.27–1.32 (m, 3H), 1.41 (d, 1H, $^3J_{HH}$ 6.5 Hz), 1.48–1.71 (m, 12H), 2.19 (s, 3H) and 4.87 (q, 1H, $^3J_{HH}$ 6.5 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 20.1 (CH_3), 22.7 (CH_3), 25.6 (CH_2), 25.7 (CH_2), 26.0 (CH_2), 30.8 (CH_2), 31.0 (CH_2), 31.4 (CH_2), 41.6 (CH), 70.8 (CH) and 173.7 (C=O). MS (EI, *m/z*): 184 (M^+, 15%), 142 (37), 82 (100) 55 (40). Determination of the *ee* by GC analysis: CP-Chiralsil DEX CB, 70 °C (5 min), 3 °C/min, 200 °C; t_R (*S*) 18.0 min; t_R (*R*) 19.7 min.

*2.3.19. (S)-Cyclohexylphenylmethanol, (S)-**10a***

Determination of the *ee* by GC analysis after acetylation.

*2.3.20. (R)-Cyclohexylphenylmethyl acetate, (R)-**10b***

IR (KBr): ν 2930 and 1740 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.98–1.23 (m, 5H), 1.53–1.78 (m, 2H), 2,12 (s, 3H), 2.45–2.51 (m, 1H), 5.53 (d, 1H, $^3J_{HH}$ 7.3 Hz) and 7.33–7.48 (m, 5H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.0 (CH_3), 25.4 (2C, CH_2), 25.9 (CH_2), 26.1 (CH_2), 28.8 (CH_2), 43.4 (CH), 79.1 (CH), 126.5 (CH_{ar}), 127.1 (CH_{ar}), 127.3 (CH_{ar}), 128.1 (2C, CH_{ar}), 143.9 (C_{ar}) and 170.6 (C=O). MS (EI, *m/z*): 232 (M^+, 8%), 190 (30), 172 (47), 107 (100). Determination of the *ee* by GC analysis: CP-Chiralsil DEX CB, 130 °C (5 min), 3 °C/min, 190 °C; t_R (*S*) 25.1 min; t_R (*R*) 26.9 min.

*2.3.21. (S)-1-Tetralol, (S)-**11a***

Determination of the ee by GC analysis after acetylation.

*2.3.22. (R)-1-Tetralyl acetate, (R)-**11b***

Determination of the *ee* by GC analysis: Restek RtβDEXse, 70 °C (5 min), 1 °C/min, 120 °C, 10 °C/min, 200 °C; t_R (*S*) 56.7 min; t_R (*R*) 57.1 min.

*2.3.23. (S)-2-Tetralol, (S)-**12a***

Determination of the ee by GC analysis after acetylation.

*2.3.24. (R)-2-Tetralyl acetate, (R)-**12b***

Determination of the *ee* by GC analysis: Restek RtβDEXse, 70 °C (5 min), 1 °C/min, 120 °C, 10 °C/min, 200 °C; t_R (*S*) 53.4 min; t_R (*R*) 55.1 min.

*2.3.25. (S)-1-Indanol, (S)-**13a***

Determination of the *ee* by GC analysis after acetylation.

*2.3.26. (R)-1-Indanyl acetate, (R)-**13b***

Determination of the *ee* by GC analysis: Restek RtβDEXse, 70 °C (5 min), 1 °C/min, 120 °C, 10 °C/min, 200 °C; t_R (*S*) 46.5 min; t_R (*R*) 47.6 min.

*2.3.27. (S)-2-Octanol, (S)-**15a***

Determination of the ee by GC analysis after acetylation.

*2.3.28. (R)-2-Octyl acetate, (R)-**15b***

Determination of the *ee* by GC analysis: CP-Chiralsil DEX CB, 70 °C (5 min), 1 °C/min, 100 °C, 10 °C/min, 200 °C; t_R (*S*) 21.1 min; t_R (*R*) 23.3 min.

*2.3.29. (S)-2-Butanol, (S)-**16a***

Determination of the ee by GC analysis after acetylation.

*2.3.30. (R)-2-Butyl acetate, (R)-**16b***

Determination of the *ee* by GC analysis: CP-Chiralsil DEX CB, 40 °C, 4 °C/min, 120 °C, 10 °C/min, 200 °C; t_R (*S*) 3.5 min; t_R (*R*) 3.8 min.

*2.3.31. (S)-Citronellol, (S)-**17a***

Determination of the ee by GC analysis after derivatization by acetylation.

*2.3.32. (R)-Citronellyl acetate, (R)-**17b***

Determination of the *ee* by GC analysis: CP-Chiralsil DEX CB 70 °C (5 min), 3 °C/min, 200 °C; t_R (*S*) 16.7 min; t_R (*R*) 18.2 min.

Table 1
Enzymatic acetylation of heteroaryl secondary alcohols (±)-**1**–**6a** catalyzed by *M. esculenta* and *P. edulis* preparations in Et_2O and vinyl acetate.[a].

Entry	Alcohol	Enzyme	Time (h)	*ee* (%)		*c* (%)[c]	*E*[d]
				1–**6a**[b]	**1**–**6b**[b]		
1	(±)-**1a**	*Manihot*	96	41	92	32	35
2	(±)-**1a**	*Passiflora*	120	6	91	6	22
3	(±)-**2a**	*Manihot*	96	19	92	17	30
4	(±)-**2a**	*Passiflora*	120	5	87	6	11
5	(±)-**3a**	*Manihot*	116	20	83	19	13
6	(±)-**4a**	*Manihot*	116	34	83	29	14
7	(±)-**5a**	*Manihot*	116	38	87	30	20
8	(±)-**6a**	*Manihot*	120	18	93	16	32

[a] For reaction conditions see Section 2.
[b] Determined by HPLC or by GC.
[c] Conversion, $c = ee_s/(ee_s + ee_p)$.
[d] Enantiomeric ratio, $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$.

3. Results and discussion

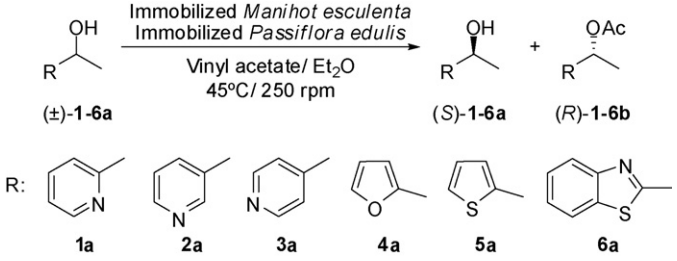
For all the compounds herein described, the *R* enantiomer of the starting material was acetylated, remaining the alcohol of (*S*)-configuration. As previously described for *M. esculenta* and *P. edulis* preparations both follow Kazlauskas' rule for the stereoselective acylation of secondary alcohols [29].

3.1. Enzymatic acetylation of heteroaryl secondary alcohols

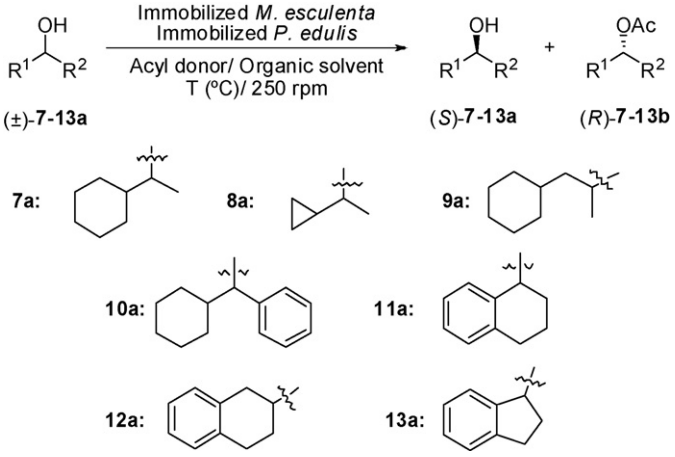
Our initial efforts were devoted to study the enzymatic acetylation of different heteroaryl secondary alcohols, as shown in Table 1. Immobilized *M. esculenta* and *P. edulis* were employed in the enzymatic acetylation of 1-pyridilethanols with vinyl acetate in Et_2O at 45 °C (Scheme 1). It was observed that the reaction was faster and more enantioselective using *M. esculenta* for racemic 1-(pyridin-2-yl)ethanol (±)-**1a**, being obtained (*S*)-**1a** and (*R*)-**1b** with a moderate *E* value [30] (*E* = 35) and a 32% conversion after 96 h, meanwhile *P. edulis* showed a slow reaction rate (entry 2). The processes reached a small conversion value when employing the 3-pyridil derivative (±)-**2a**, achieving a similar enantioselectivity to (±)-**1a** (entries 3 and 4), while there was an important loss in *Manihot* selectivity in the biocatalytic resolution of 1-(pyridin-4-yl)ethanol (±)-**3a** (entry 5). Next, the enzymatic kinetic resolutions of 1-(2-furyl)ethanol (±)-**4a** and (±)-1-(2-thienyl)ethanol (±)-**5a** were carried out using the best enzyme found for the pyridine derivatives (*M. esculenta*) observing low enantioselectivities and conversion values close to 30% after 116 h (entries 6 and 7). Finally, the benzofused heteroaryl racemic alcohol 1-(1,3-benzo[*d*]thiazol-2-yl)ethanol (±)-**6a** was acetylated in the presence of *M. esculenta* with a moderate enantioselectivity and 16% conversion after 120 h (entry 8).

3.2. Enzymatic acetylation of racemic cyclic and aliphatic secondary alcohols

Based on the excellent results described when employing immobilized *M. esculenta* as biocatalyst in the enzymatic kinetic resolution of *rac*-1-phenylethanol [8], we decided to study the non aromatic analogue, *rac*-1-cyclohexylethanol (±)-**7a**, as shown in Scheme 2. Table 2 summarized the results obtained. The enzymatic acetylation of this racemic compound with vinyl acetate in Et_2O at 45 °C allowed the preparation to (*S*)-**7a** and (*R*)-**7b** in a process with an excellent *E* value (*E* = 117, entry 1) and a 33% conversion after 120 h. The reaction was also performed with isopropenyl acetate as acylating reagent, as shown in entry 2, leading to a decrease in the enzymatic activity (*c* = 21% after 116 h), but maintaining a similar *E* value. *P. edulis* showed a moderate enantioselectivity, leading to lower conversions after longer reaction times (entry 3). In order to improve the enzymatic activity in the resolution of (±)-**7a**, the effect of different organic solvents was analyzed. Results are summarized in entries 4–13 of Table 2. The use of DIPE allowed to obtain an excellent *E* value (*E* > 100, entry 4) and the same conversion than when employing Et_2O (*c* = 33% after 120 h). Good results were achieved in the biocatalyzed acetylation of (±)-**7a** when using toluene, EtOAc or hexane (entries 5–7). Reactions performed in toluene or hexane led to low conversions (*c* = 11% after 120 h), while (*R*)-**7b** was produced in 22% conversion by employing EtOAc. Ethers as TBME or THF increased in a great extent the enzymatic activity (conversions close to 40% after 120 h for TBME and after 96 h for THF), while there was an important loss in the enantioselectivity. Nevertheless,



Scheme 1. Biocatalyzed acetylation of racemic heteroaryl alcohols.



Scheme 2. Acetylation of racemic *sec*-alcohols (±)-7-13a catalyzed by *Manihot esculenta* and *Passiflora edulis* preparations.

Table 2
Biocatalyzed acetylation of 1-cyclohexylethanol (±)-**7a** by *M. esculenta* and *P. edulis* preparations using 10 equiv. of acyl donor.[a].

Entry	Solvent	Acetate	*T* (°C)	Time (h)	*ee* (%)		*c* (%)[c]	*E*[d]
					7a[b]	**7b**[b]		
1	Et_2O	Vinyl	45	116	47	97	33	117
2	Et_2O	Isopropenyl	45	116	26	98	21	134
3[e]	Et_2O	Vinyl	45	120	15	95	14	45
4	DIPE	Vinyl	45	120	48	97	33	105
5	Toluene	Vinyl	45	120	12	97	11	73
6	EtOAc	Vinyl	45	116	27	96	22	63
7	Hexane	Vinyl	45	120	17	97	12	77
8	TBME	Vinyl	45	120	57	89	39	30
9	Dioxane	Vinyl	45	96	64	81	44	18
10	Dioxane	Vinyl	30	120	31	90	26	25
11	THF	Vinyl	45	96	63	90	41	32
12	THF	Vinyl	30	120	27	94	22	42
13	MeCN	Vinyl	45	96	23	80	22	11

[a] For reaction conditions see Section 2.
[b] Determined by GC.
[c] Conversion, $c = ee_s/(ee_s + ee_p)$.
[d] Enantiomeric ratio, $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$.
[e] Reaction carried out with immobilized *Passiflora edulis* as biocatalyst.

Table 3
Biocatalyzed acetylation of cyclic secondary alcohols (±)-**8**–**13a** by immobilized *M. esculenta* and *P. edulis* preparations using 10 equiv. of acyl donor.[a].

Entry	Alcohol	Biocatalyst	Solvent	Time (h)	*ee* (%)		*c* (%)[c]	*E*[d]
					8–**13a**[b]	**8**–**13b**[b]		
1	(±)-**8a**	*Manihot*	Et_2O	120	26	90	22	24
2[e]	(±)-**8a**	*Manihot*	Et_2O	120	7	93	7	29
3	(±)-**8a**	*Manihot*	DIPE	120	42	82	34	15
4	(±)-**8a**	*Passiflora*	Et_2O	120	3	54	6	4
5	(±)-**9a**	*Manihot*	Et_2O	96	28	97	22	86
6[e]	(±)-**9a**	*Manihot*	Et_2O	96	16	97	14	76
7	(±)-**9a**	*Manihot*	DIPE	96	24	96	20	61
8	(±)-**9a**	*Manihot*	Dioxane	96	54	78	41	13
9	(±)-**9a**	*Passiflora*	Et_2O	96	10	93	10	30
10	(±)-**10a**	*Manihot*	Et_2O	120	22	90	20	24
11	(±)-**11a**	*Manihot*	Et_2O	120	37	89	27	24
12	(±)-**12a**	*Manihot*	Et_2O	120	16	83	16	12
13	(±)-**13a**	*Manihot*	Et_2O	120	13	87	13	16

[a] For reaction conditions see Section 2.
[b] Determined by GC.
[c] Conversion, $c = ee_s/(ee_s + ee_p)$.
[d] Enantiomeric ratio, $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$.
[e] Reaction performed using 10 equiv. of isopropenyl acetate instead of vinyl acetate.

(±)-**7a** was resolved with $E = 30$–32 (entries 8 and 11). We decided to study the resolutions performed with 1,4-dioxane and THF at a lower temperature as 30 °C, in order to obtain higher *E* values. For both solvents (entries 10 and 12), it was observed an important loss in the enzymatic activity with only a slight increase in the *E* values. Finally a more polar solvent as acetonitrile led to the lowest value of enantioselectivity (entry 13).

In view of the promising results obtained with low polar solvents, an alcohol presenting a smaller aliphatic ring as racemic 1-cyclopropylethanol (±)-**8a** was studied as substrate for the enzymatic processes. Unfortunately, this compound was acetylated with moderate *E* values and conversions when employing immobilized *M. esculenta* with vinyl acetate in Et_2O or DIPE (Table 3, entries 1 and 3). The same reaction performed with isopropenyl acetate was very slow (entry 2) and the use of *P. edulis* led to a resolution with very low activity and selectivity (entry 4).

1-Cyclohexylpropan-2-ol was also analyzed as substrate for these two biocatalyst preparations. A high *E* value could be obtained when acetylating (±)-**9a** with *M. esculenta* and vinyl acetate in Et_2O, as shown in entry 5 of Table 3. After 96 h, 22% of (*S*)-**9b** was achieved, a lower conversion when compared to 1-cyclohexylethanol. The reaction performed with isopropenyl acetate led to a similar enantioselectivity but a slower resolution process ($c = 14\%$, entry 6), while the use of other solvents as DIPE produced a negative effect in the biocatalytic properties of the enzyme (entry 7). This was especially clear when using a hydrophilic solvent as 1,4-dioxane, being observed an increase in the enzymatic activity but a dramatic decrease in the *E* value (entry 8). Acetylation catalyzed by *P. edulis* occurred with a moderate *E* value ($E = 30$) and a low conversion (only 10% of (±)-**9a** was converted in 96 h, entry 9).

Racemic cyclohexylphenylmethanol (±)-**10a** was acetylated by immobilized *M. esculenta* with moderate activity and selectivity ($c = 20\%$ and $E = 24$, entry 10), while no reaction was observed when using *P. edulis* after longer reaction times. We focused then in the enzymatic acetylation of different benzofused secondary alcohols, as shown in entries 11-13 of Table 3. 1-Tetralol was resolved by immobilized *M. esculenta* in Et_2O with a moderate enantioselectivity ($E = 24$), being obtained a 27% of (*R*)-**11b** after 120 h (entry 11). The acetylation of 2-tetralol occurred with a loss in both the enantioselectivity and the conversion, achieving (*S*)-**12a** and (*R*)-**12b** in a process with $E = 12$. Only a 13% of (*R*)-1-indanyl acetate **13b** was obtained after 120 h when acetylated with vinyl acetate in Et_2O at 45 °C, in a process presenting a poor selectivity (entry 13). Unfortunately, resolutions catalyzed by immobilized *P. edulis* did not lead to the formation of the corresponding esters even by modifying the reactions parameters (organic solvent and temperature). Finally, 4-phenylcyclohexanol (±)-**14a** was treated with vinyl acetate in the presence of both biocatalysts, but no reaction was observed when performed at 45 °C with vinyl acetate and different organic solvents.

Table 4
Immobilized *M. esculenta* and *P. edulis* biocatalyzed acetylation of secondary aliphatic racemic alcohols **15**–**16a** with 10 equiv. of vinyl acetate.[a].

Entry	Alcohol	Biocatalyst	Time (h)	*ee* (%)		*c* (%)[c]	*E*[d]
				15–**16a**[b]	**15**–**16b**[b]		
1	(±)-**15a**	*Manihot*	106	32	75	30	10
2	(±)-**15a**	*Passiflora*	106	20	81	19	13
3	(±)-**16a**	*Manihot*	72	19	29	41	2
4	(±)-**16a**	*Passiflora*	106	12	43	22	3

[a] For reaction conditions see Section 2.
[b] Determined by GC.
[c] Conversion, $c = ee_s/(ee_s + ee_p)$.
[d] Enantiomeric ratio, $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$.

The study was extended to the enzymatic acetylation of two racemic aliphatic secondary alcohols as 2-octanol (±)-**15a** and 2-butanol (±)-**16a**, as shown in Table 4. Reactions were catalyzed by *M. esculenta* and *P. edulis* with vinyl acetate in Et_2O and 45 °C and the corresponding (*R*)-alcohols and (*S*)-esters were obtained with low enantioselectivities. For both enzymes, resolution of 2-octanol was more selective than when starting from the shorter alkyl chain alcohol. Concerning the effect on the enzymatic activity, *M. esculenta* was able to acetylate much faster *rac*-2-butanol than *rac*-2-octanol, while for *P. edulis* (*R*)-**15b** and (*R*)-**16b** were obtained with similar conversions after 116 h (entries 2 and 4, respectively).

With the aim of obtaining a deeper knowledge on these novel enzymatic systems, an aliphatic primary racemic alcohol as *rac*-citronellol (±)-**17a** was studied. Previously, it has been described that *M. esculenta* was not a good biocatalyst for the resolution of 1-phenyl-2-propanol. Herein, we carried out the biocatalyzed acetylation of this highly interesting alcohol with *M. esculenta* and *P. edulis* employing Et_2O as solvent and vinyl acetate. For both biocatalysts, very low enantioselectivities ($E \approx 3$) and low conversions were achieved, being the reaction slightly faster with *M. esculenta* ($c = 17\%$ after 115 h) than with *P. edulis* [10% of (*R*)-**17b** in the same time].

4. Conclusions

Immobilized *M. esculenta* and *P. edulis* preparations have been used in the enzymatic acetylation of different heteroaromatic, cyclic and aliphatic alcohols. Both enzymatic preparations are able to catalyze the acetylation of secondary aliphatic alcohols; observing some grade of stereopreference in the processes favoring in all cases the formation of the (*R*)-acetates. Meanwhile in general *P. edulis* led to moderate results, *M. esculenta* preparation has demonstrated to be a suitable biocatalyst for the resolution of a set of heteroaryl and cyclic racemic secondary alcohols. While the resolution of pyridilethanols occurred with moderate to good *E* values, excellent enantioselectivities can be achieved for the resolution of 1-cyclohexylethanol with both vinyl or isopropenyl acetate and different organic solvents. 1-Cyclopropylethanol as well as 1-cyclohexylpropan-2-ol can be resolved with high selectivity while the use as substrates of other cyclic alcohols led to moderate *E* values depending on the substrate structure.

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