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Enzymic resolution of an α-methylene-γ-lactonic ester

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Abstract

The possibility of using enzymes for the kinetic resolution of an α-methylene-γ-lactone was explored. The probe molecule, ethyl 2-methyl-4-methylene-tetrahydro-5-oxo-2-furancarboxylate was successfully resolved with Porcine pancreatic lipase and *Candida rugosa* lipase, in the presence of a cosolvent, leading to both enantiomers of the corresponding acid with high enantiomeric excesses.

The biological activity of natural products containing the α-methylene-γ-lactone ring as immunomodulators, antibiotics, antitumorals, antifungals, plant growth inhibitors and agents for vasorelaxing activity is related to the presence of the enone system, whose β-carbon atom is prone to undergo attack by a biological nucleophile belonging to an interacting enzyme.[1–9] Many syntheses of these types of compounds have been described.[10] However, the use of enzymes for their kinetic resolution is, to our knowledge, so far unexplored. Therefore it seemed interesting to verify the possibility of operating the resolution of chiral racemic γ-lactones containing the conjugated α-methylene function as well as an alkoxycarbonyl group as the site of reaction by hydrolytic enzymes. The substrate chosen for initial studies was the ethyl ester of 2-methyl-4-methylene-tetrahydro-5-oxo-2-furancarboxylic acid **3**,[11] (Scheme 1) in which the methylene group is sterically unaffected by the other substituents. The substrate was easily prepared by a Reformatsky-type reaction between 2-(bromomethyl)acrylic acid **1** and pyruvic acid ethyl ester, promoted by indium.[12–15] The initially formed hydroxy hemiester (±)-**2** was isolated and cyclised into the desired butanolide (±)-**3** under acidic conditions.

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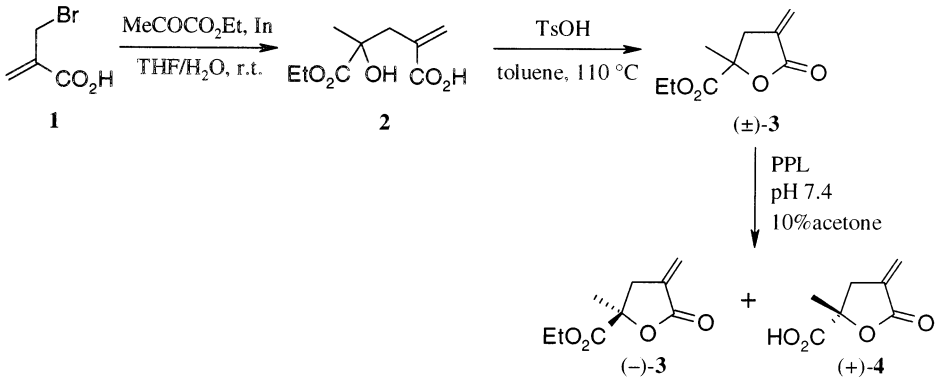


Scheme 1.

The enzymic hydrolyses of (±)-**3** were carried out in aqueous solution and in a 1:9 mixture of acetone:water,[16,17] both at pH 7.4, using a series of commercially available hydrolases. The most relevant results are summarized in Table 1.

Addition of a small amount (10%) of acetone resulted in a remarkable increase of the E[18,19] value and hence in a higher enantioselectivity. In fact, using Porcine pancreatic lipase, the corresponding lactonic acid (+)-**4** was isolated with 89% e.e., whereas the use of *Candida rugosa* lipase furnished the laevorotatory enantiomer (−)-**4** with 82% e.e. A second enzymic resolution carried out on these mixtures improved the e.e. of (+)-**4**[†] to 97% and that of (−)-**4**[‡] to 99% e.e. The e.e's of the acids were determined by chiral HRGC of their respective ethyl esters (+)-**3** and (−)-**3**, obtained by esterification of the acids with EDC and HOBT. α-Chymotrypsin also proved efficient in hydrolysing the ester group, furnishing the acid (+)-**4** with 77% e.e., while *Aspergillus niger* lipase, *Pseudomonas* sp. lipase and pig liver esterase were not enantioselective and *Pseudomonas fluorescens* lipase furnished (+)-**4** with only 29% e.e.

The absolute configuration of (+)-**4** was determined to be *S* by means of CD spectroscopy. The ethyl ester (+)-**3** was reduced with sodium borohydride to a 1:1 mixture of the butanolides (+)-**7** and (+)-**8** through the intermediacy of the corresponding lactonic esters (−)-**5** and (+)-**6**, respectively (Scheme 2). Separation of the hydroxy lactones (+)-**7** and (+)-**8** afforded both diastereomers as pure compounds. A comparison was then made between the CD spectrum of (+)-**7**[§] and that of (3*S*,5*S*)-(+)-5-hydroxymethyl-3-methyl-4,5-dihydro-2(3*H*)-furanone **9**.[20] Both compounds exhibited a positive Cotton effect for the n→π* transition [(+)-**7**, $\Delta\varepsilon_{217}$ +0.2; UV, λ_{max} 210 nm, ε_{max} 200, EtOH; (+)-**9**, $\Delta\varepsilon_{220}$ +0.3, EtOH; UV, λ_{max} 206 nm, ε_{max} 200, EtOH]. Although the γ-carbon atom in (+)-**7** bears a methyl group in place of a hydrogen atom, the order of polarizability of the substituents is the same in both compounds, thus allowing a comparison between the curves to be made.[21,22] As a consequence, the absolute configuration of (+)-**7** is also (3*S*,5*S*) and that of (+)-**4** is *S*.

[†] (+)-**4**: $[\alpha]_D^{25}$ = +11.8 (*c* 0.64, MeOH); CD: $\Delta\varepsilon_{202}$ = −4.9, $\Delta\varepsilon_{226}$ = +3.2 (MeOH); UV: λ_{max} 224 nm, ε_{max} 2400 (MeOH); ^{1}H NMR (400 MHz) δ, ppm ($CDCl_3$): 9.5 (1H, bs, OH), 6.22 (1H, dt, *J* 2.9, 2.4 Hz, =CH), 5.66 (1H, dt, *J* 2.5 Hz, =CH), 3.27 (1H, dt, *J* 17.4, 2.4 Hz, H-3), 2.84 (1H, dt, *J* 17.4, 2.9 Hz, H-3), 1.65 (3H, s, Me); ^{13}C NMR δ, ppm ($CDCl_3$): 175.1 (s), 169.4 (s), 133.5 (s), 123.2 (t), 81.1 (s), 38.5 (t), 24.1 (q).

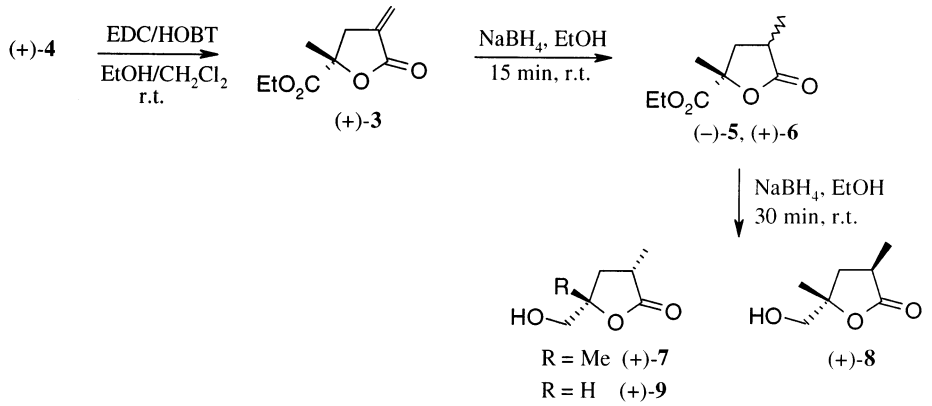
[‡] (−)-**4**: ($[\alpha]_D^{25}$ −12.3 (*c* 0.66, MeOH), CD: $\Delta\varepsilon_{201}$ = +6.6, $\Delta\varepsilon_{227}$ −3.9 (MeOH); UV: λ_{max} 224 nm, ε_{max} 2400 (MeOH).

[§] (+)-**7**: $[\alpha]_D^{25}$ +5.0 (*c* 0.90, EtOH); CD: $\Delta\varepsilon_{217}$ +0.2 (EtOH); UV: λ_{max} 210 nm, ε_{max} 200 (EtOH); ^{1}H NMR (400 MHz) δ, ppm ($CDCl_3$): 3.69 (1H, d, *J* 12.2 Hz, C*H*OH), 3.43 (1H, d, *J* 12.2 Hz, C*H*OH), 2.83 (2H, m and bs, H-3, OH), 2.08 (2H, 2 *pseudo*quartets of an AB system, 2 H-4), 1.31 (s, 3H, Me), 1.25 (d, 3H, Me); ^{13}C NMR δ, ppm ($CDCl_3$): 179.5 (s), 84.2 (s), 67.5 (t), 36.6 (t), 35.0 (d), 22.3 (q), 15.3 (q).

Table 1
Enzymic resolution of (±)-**3**[a]

	H_2O					H_2O/acetone 9:1				
	E	React. time (min)	Conv. (%)	Ester (% e.e.)[b]	Acid (% e.e.)[c]	*E*	React. time (min)	Conv. (%)	Ester (% e.e.)[b]	Acid (% e.e.)[c]
Candida rugosa lipase	4	16	15	(+)-**3** (10)	(−)-**4** (58)	12	110	21	(+)-**3** (22)	(−)-**4** (82)
Porcine pancreatic lipase	7	45	37	(−)-**3** (38)	(+)-**4** (66)	24	30	28	(−)-**3** (35)	(+)-**4** (89)
α-Chymotrypsin	2	24	11	(−)-**3** (5)	(+)-**4** (41)	9	24	19	(−)-**3** (18)	(+)-**4** (77)

[a] Reaction conditions: 100 mg of substrate, 100 mg of enzyme, 0.1 M phosphate buffer at pH 7.4 (13 mL), acetone (1.3 mL), rt. The experiments were monitored with a PHM290 Meterlab™ pH-stat-controller.
[b] Determined by HRGC, capillary column Chiraldex™ type G-TA, trifluoroacetyl γ-cyclodextrin.
[c] Determined by HRGC on the ethyl ester derivative.



Scheme 2.

The chemical yields of the enzymic kinetic resolutions were accurately evaluated. It is important to emphasise that no significant loss of material was found. In fact, the enantiomerically pure acid was isolated in yields varying from 93 to 97% and the unreacted ester was recovered in yields ranging from 90 to 95% (both yields were calculated on the basis of the expected value at each conversion value).

These results would suggest that no irreversible Michael-type reaction occurred between the acceptor enone function of the substrate and some biological nucleophiles present in the enzymatic systems used.

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References

1. Yuuya, S.; Hagiwara, H.; Suzuki, T.; Ando, M.; Yamada, A.; Suda, K.; Kataoka, T.; Nagai, K. *J. Nat. Prod.* **1999**, *62*, 22–30.
2. Das, B.; Venkataiah, B.; Kashinatam, A. *Tetrahedron* **1999**, *55*, 6585–6594.
3. Chen, Y.-L.; Wang, T.-C.; Tzeng, C.-C. *Helv. Chim. Acta* **1999**, *82*, 191–197.
4. Chen, Y.-L.; Wang, T.-C.; Chang, N.-C.; Chang, Y.-L.; Teng, C.-M.; Tzeng, C.-C. *Chem. Pharm. Bull.* **1998**, *46*, 962–965.
5. Ghotak, A.; Sarkar, S.; Ghosh, S. *Tetrahedron* **1997**, *53*, 17335–17342.
6. Rodriguez, A. D.; Piña, I. C.; Barnes, C. L. *J. Org. Chem.* **1995**, *60*, 8096–8100.
7. Hoffmann, H. M. R.; Rabe, J. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 94–110.
8. Lee, K.-H.; Hall, I. H.; Mar, E.-C.; Starnes, C. O.; El Gebali, S. A.; Waddell, T. G.; Hadgraft, R. I.; Ruffner, C. G.; Weidner, I. *Science* **1977**, *196*, 533–536.
9. Kupchan, S. M; Thomas, A. M.; Eakin, M. A. *J. Med. Chem.* **1971**, *14*, 1147–1152.
10. Sidduri, A. R.; Knochel, P. *J. Am. Chem. Soc.* **1992**, *114*, 7579–7581 and references cited therein.
11. Fouquet, E.; Gabriel, A.; Maillard, B.; Pereyre, M. *Tetrahedron Lett.* **1993**, *34*, 7749–7752.
12. Li, C. J.; Chan, T. H. *Tetrahedron Lett.* **1991**, *32*, 7017–7020.

13. Bryan, V. J.; Chan, T.-H. *Tetrahedron Lett*. **1996**, *37*, 5341–5342.
14. Choudhury, P. K.; Foubelo, F.; Yus, M. *Tetrahedron Lett*. **1998**, *39*, 3581–3584.
15. Choudhury, P. K.; Foubelo, F.; Yus, M. *Tetrahedron* **1999**, *55*, 10779–10788.
16. Nagao, Y.; Kume, M.; Wakabayashi, R. C.; Nakamura, T.; Ochiai, M. *Chem. Lett*. **1989**, 239–242.
17. Ha, H.-J.; Yoon, K.-N.; Lee, S.-Y.; Park, Y.-S.; Lim, M.-S.; Yim, Y.-G. *J. Org. Chem*. **1998**, *63*, 8062–8066.
18. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc*. **1982**, *104*, 7294–7299.
19. Sih, C. J., Wu, S.-H. In *Topics in Stereochemistry*; Eliel, E. L.; Wilen, S. H., Eds.; John Wiley: New York, 1989; pp. 63–125.
20. Drioli, S.; Forzato, C.; Nitti, P.; Pitacco, G.; Valentin, E. *Tetrahedron*: *Asymmetry* **2000**, *11*, 1353–1366.
21. Uchida, I.; Kuriyama, K. *Tetrahedron Lett*. **1974**, *43*, 3761–3764.
22. Cain, R. B.; Kelly, S. M.; Kirby, G. W.; McLenaghan, H. J. S.; Marshall, A. J.; Price, N. C.; Rao, G. V.; Schmidt, S. *J. Chem. Res*. (*S*) **1996**, 526–527.