Tetrahedron Letters,Vol.27,No.16,pp 1763-1766,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain ©1986 Pergamon Press Ltd.

A FACILE ENZYMATIC RESOLUTION PROCESS FOR THE PREPARATION OF (+)-S-2-(6-METHOXY-2-NAPHTHYL)PROPIONIC ACID (NAPROXEN).

Qu-Ming Gu, Ching-Shih Chen and C. J. Sih*
School of Pharmacy, University of Wisconsin, Madison, WI 53706 U.S.A.

Summary: (+)-S-2-(6-Methoxy-2-naphthyl)propionic acid (1) has been prepared via enzymatic enantiospecific hydrolysis of (±)-chloroethyl-2-(6-methoxy-2-naphthyl)propionate (3), catalyzed by the lipase of Candida cylindracea.

2-Arylpropionic acids are an important class of non-steroidal antiinflammatory drugs. Two of the most commonly prescribed members of this family are p-isobutylhydratropic acid (Motrin) and (+)-S-2-(6-methoxy-2-naphthyl)propionic acid (Naproxen) (1). They are widely used to control the symptoms of arthritis and related connective tissue diseases.[1] While the antiinflammatory activity of the individual S(+) and R(-) isomers of p-isobutylhydratropic acid are essentially equivalent, because of the in vivo conversion of the R(-) to the S(+) enantiomer[2]; in contrast, the S(+) enantiomer of 2-(6-methoxy-2-naphthyl)propionic acid is 28 times more active than its R(-) enantiomer[3]. As the chemical synthesis of 2-(6-methoxy-2-naphthyl)propionic acid leads to a racemic mixture[4], chemical resolution methods are employed to obtain the (S)-enantiomer, Naproxen. Generally, these methods entail the selective stoichiometric crystallization of a diastereomeric salt by the use of an expensive amine such as cinchonidine[4], dehydrobiethylamine acetate[5a], or the use of a water soluble amine such as glucamine[5b], which is cumbersome to recover. Naproxen (1) was also prepared by chemical resolution of a precursor using the less accessible l-10-camphorsulfonic acid[6].

Our interest in the development of an enzymatic method for the preparation of 1 led us to first examine the enantiospecific transformation of (±)-methyl-2-(6-methoxy-2-naphthyl)propionate (2) by microorganisms. Although we had observed that Penicillium vinaceum and Streptomyces cavourensis catalyzed this enantiospecific hydrolysis to yield 1 of high optical purity[7] (ee = >0.98), unfortunately, the rate of conversion was slow and the microorganisms have low substrate concentration tolerance (<5 g/l). Hence, this process is impractical for large scale applications. On the other hand, we have recently discovered that the extracellular fungal lipases derived from the genera Rhizopus, Mucor and Candida are uniquely enantiospecific in catalyzing the hydrolysis of the ester grouping of (±)2. Moreover, they exhibit good stability and are able to tolerate high concentrations of substrate (>1 M). However, most of these lipases preferentially cleaved the R-enantiomer; only the lipase from Candida cylindracea possessed the desired S-stereochemical

preference (Table 1).

TABLE 1. Enantiospecific hydrolysis of (±)-methyl-2-(6-methoxy-2-naphthyl)propionate (2) by microbial lipases.

Lipase Source[1]	Stereochemical Preference	Extent of Conversion (%)	Enantiomeric Excess (%) Ester	Enantiomeric Excess (%) Acid	E
Candida cylindracea[1a]	S	39	63	>98	>100
Mucor meihei[1b]	R	18	21	95	51
Rhizopus arrhizus[1c]	R	11	13	97	78
Rhizopus sp.[1d]	R	19	21	92	27
Rhizopus oryzae[1e]	R	11	10	76	8

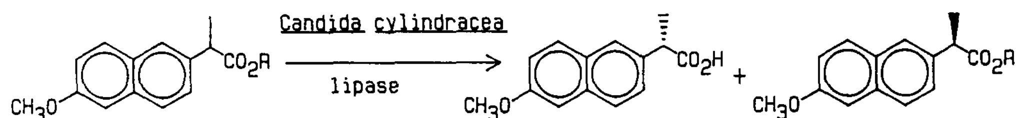
[1]To one ml of 0.2 M potassium phosphate buffer, pH 8.0, was added 244 mg (1 mmol) of (±)2 and varying amounts of different enzyme preparations. The contents were incubated at 22°C for 120-216 h under gentle stirring. [a]1 mg of pure enzyme[9] isolated from the Sigma type VII preparation, 216 h; [b]200 mg of Amano MAP10 powder, 120 h; [c]10 mg of enzyme of Boehringer-Mannheim, 120 h; [d]150 mg of powder from Serva, 120 h; [e]200 mg of Amano FAP powder, 120 h.

[2]E is the ratio of the specificity constants (k_{cat}/K_m) of the two enantiomers.[10]

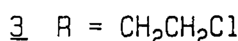
The enantiospecificity of C. cylindracea lipase was not affected by changes in the ester substituent, R, for the enantiomeric ratio, E, remained unperturbed for esters of varying chain length (C_1 ---> n-C_8). This observation led us to the use of activated esters as a means of enhancing the reaction rate. It was gratifying to find that the cyanomethyl (4), chloroethyl (3) and 2'-nitropropyl (5) esters were all hydrolyzed at a considerably faster rate than the methyl ester (2), which suggests that acyl enzyme formation may be the rate-limiting segment of the overall reaction. At the same time, the sense of chirality and enantiospecificity were not markedly influenced (Table 2).

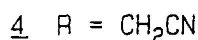
TABLE 2. Relative rates of enzymatic hydrolysis.

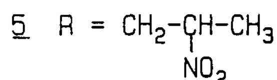
Compound	Relative rate	Enantiomeric ratio (E)
2	1	>100
3	15	>100
4	6	>100
5	3	81



1







Hence, the catalytic efficiency of the enzyme is greatly amplified and an illustration of the practicality of this simple catalytic process is given below.

To 50 mg of crude Candida cylindracea lipase (Sigma L1754 Type VII, 600 µg of protein) in 1 ml of 0.2 M phosphate buffer, pH 8.0, were added 292 mg of (±)2-(6-methoxy-2-naphthyl)propionic chloroethyl ester (3) (1 mmol) as a fine powder, 1 µmol of mercaptoethanol and 10 mg of polyvinyl alcohol. The resulting suspension was gently stirred with a magnetic stirrer for 42 hours at 22°C. The reaction mixture was then centrifuged for 5 min at 1000 x g and the precipitate was washed with 0.2 M phosphate buffer, pH 8.0, and again centrifuged to collect the water insoluble (-)-R-2-(6-methoxy-2-naphthyl)propionic chloroethyl ester (140 mg), $[\alpha]_D^{23}$ = -20.5° (c, 4.96, $CHCl_3$; ee = 0.70). The supernatant and the washing were combined and acidified to pH 2.0 with 3 N HCl and the precipitate was collected by filtration to afford 92 mg of 1, $[\alpha]_D^{23}$ = +64.2° (c, 3.49, $CHCl_3$; ee = >0.98).

The enantiospecificity and productivity number of various commercial C. cylindracea lipase preparations, as well as the kinetics of lipase action in this heterogeneous system are currently under investigation.

Acknowledgment. This investigation was supported in part by a grant from the University of Wisconsin Graduate School.

References and Notes

1. T. Y. Shen, Angew. Chem. Int. Ed., 11, 460 (1972).
2. W. J. Wechter, D. G. Loughhead, R. J. Reischer, G. J. VanGiessen and D. G. Kaiser, Biochem. Biophys. Res. Commun., 61, 833 (1974).
3. A. P. Roszkowski, W. H. Rooks II, A. J. Tomolonis and L. M. Miller, J. Pharmacol. Exp. Ther., 179, 114 (1971).
4. I. T. Harrison, B. Lewis, P. Nelson, W. Rooks, A. Roszkowski, A. Tomolonis and J. H. Fried, J. Med. Chem., 13, 203 (1970).
5. a) P. Newman, "Optical resolution procedures for organic compounds," vol. 2, part II, Manhattan College, New York, 1981, p. 653. b) E. Felder, D. Pitré and H. Zutter, U.K. Pat. Appl. GB2025968A (1980).
6. G. Tsuchihashi, Tetrahedron Lett., 23, 5427 (1982).
7. C. S. Chen and C. J. Sih, unpublished data. S. Iriuchiuma and A. Keiyu, Agr. Biol. Chem., 45, 1389 (1981) reported that Aspergillus sojae IAM 2703 preferentially hydrolyzed the R-enantiomer of (±)2 in low yields.
8. The enantiomeric excess of R-(-)-2 and S-(+)-1 were determined by comparison of their optical rotation with known standards and by PMR measurements of the methyl ester using $Eu(hfc)_3$.
9. N. Tomizuka, Y. Ota and K. Yamada, Agr. Biol. Chem., 30, 576 (1966).
10. The enantiomeric ratio (E value) is calculated from:

$$E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]} \qquad \text{where } c = ee_s/(ee_s + ee_p).$$

For a comprehensive treatment of the principles involved in biochemical kinetic resolutions, see: C. S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, J. Am. Chem. Soc., 104, 7294 (1982).

(Received in USA 4 February 1986)