Tetrahedron Vol. 49, No. 16, pp. 3281-3290, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Biocatalytic Resolution of DL-Propranolol. A Successful Example of Computer-Aided Substrate Design[1]

Ching-Shih Chen,* Da-Ming Gou, Woan-Ru Shieh, and Yeuk-Chuen Liu

Department of Pharmacognosy and Environmental Health Sciences
College of Pharmacy, University of Rhode Island, Kingston, RI 02881, U.S.A

(*Received in USA 24 February* 1993)

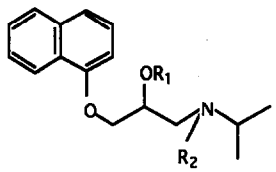
ABSTRACT: An approach entailing computer-aided substrate design was taken to develop biocatalytic resolution of racemic propranolol. This strategy provided useful insight into potential steric factors within the substrate, which might be crucial to the catalytic turnover and enantiomeric selection.

The past decade has witnessed a growing impact of biocatalysis in the field of asymmetric synthesis.[2] Enzymes are increasingly recognized as viable alternatives to transition-metal catalysts, and have been used for the preparation of a wide spectrum of optically active compounds. However, as the structure-activity relationships of most enzymatic reactions remain unclear, predictions of catalytic efficiency and stereochemical preference for unnatural substrates prove to be difficult.[3] Although researchers are now able to scrutinize the biocatalytic domains with the aid of protein crystallography and computer graphics,[4] the validity of applying the lock-and-key principle to examining enzyme-substrate interactions remains dubious.[5] Consequently, substrate modification to optimize chemical and/or optical yields has been conducted on a trial and error basis. In this paper, we report a new strategy entailing computer modeling to aid substrate design for biocatalytic reactions. This approach provides useful insight into the energy-minimized conformation of substrate, and thus allows the identification of potential steric factors crucial to catalytic turnover and enantiomeric differentiation. Based on this methodology, an efficient enzymatic method for preparing optically active propranolol **1** was developed. Although a number of approaches have been documented for the preparation of *S*-**1**, including asymmetric synthesis,[6] chemo-enzymatic synthesis,[7] and microbial resolution[8], to date, direct enzymatic resolution of **1** has not been successful.

RESULTS AND DISCUSSION

In the initial experiment, various commercial hydrolytic enzymes were examined for the enantioselective hydrolysis of the *N,O*-diacetyl derivative of **1** (*rac*-**2**). However, none of the enzymes tested,[9] including

lipases, protease and esterases, were capable of effecting the deacylation of **2**. This finding is in line with the results previously reported by other groups.[10] Presumably, the resistance to enzymatic hydrolysis stems from intrinsic steric hindrance about the cleavage site of **2**. Bearing this notion in mind, we subjected **2** to computer analysis using the *Chem3D plus*^{TM} program to generate the energy-minimized conformation through molecular mechanics calculation, which is shown in Figure 1A.



1: $R_1 = R_2 = H$
2: $R_1 = R_2 = C(O)CH_3$
3: $R_1 = R_2 = C(O)H$
4: $R_1 = R_2 = C(O)OCH_3$
5: $R_1 = R_2 = C(O)CH_2Cl$
6: $R_1 = R_2 = C(O)CCl_3$
7: $R_1 = R_2 = C(O)OC_2H_5$
8: $R_1 = C(O)OCH_3$ $R_2 = C(O)C_3H_7$
9: $R_1 = C(O)OCH_3$ $R_2 = C(O)$ 
10: $R_1 = C(O)OCH_3$ $R_2 = C(O)C_7H_{15}$
11: $R_1 = C(O)OCH_3$ $R_2 = C(O)C_{11}H_{23}$

Based on this computer-generated structure, a theory was thus proposed to account for the resistance of **2** toward enzymatic hydrolysis: Due to the bulkiness of the naphthyl ring and the tertiary acetamide group, the molecule may be restrained from free rotation in the enzyme pocket. Thus, the methyl moiety of the *O*-acetate function may impose hindrance to the nucleophilic attack at the carbonyl function (Figure 1A). In principle, this problem can be circumvented either by reducing the size of the acyl moiety or by moving the methyl group away from the nucleophile. Accordingly, two potential candidates were proposed for examination: *N,O*-diformyl-propranolol **3** and *N,O*-bis(methoxycarbonyl)-propranolol **4** whose energy-minimized conformations are presented in Fig. 1B and C, respectively. As shown, in these two molecules, especially the diformyl derivative **3**, the carbonyl function is more accessible to the nucleophile than in **2**.

Thus, racemic **3** and **4** were synthesized and subjected to enzymatic hydrolysis to test the validity of the theory. In accordance with our prediction, both compounds were readily digested by a number of enzymes with different degrees of enantiomeric selectivity (Table 1).

As shown, compound **3** was hydrolyzed at faster rates by a broader range of enzymes than **4**. Nearly all the enzymes examined were capable of hydrolyzing the *O*-formyl group at varying rates; whereas the ones cleaving the *O*-methoxycarbonyl group were limited to the esterases. This result agrees with the notion that a hydrogen atom poses less steric hindrance than a methoxy function. However, the fast deacylation of **3** was coupled to poor enantiomeric selectivity. This finding is borne out by the E values[11] that ranged from 1 to 3. Additionally, slow spontaneous hydrolysis of the formate ester **3** in buffer solutions took place, rendering this process unsuitable for preparative purposes. On the other hand, a partially purified esterase preparation from the acetone powder of porcine pancreas displayed moderate specificity (E = 19) toward *rac*-**4**. This enzyme (PPE) appeared to be different from cholesterol esterase (CE) whose E value was 5 in hydrolyzing compound **4**.

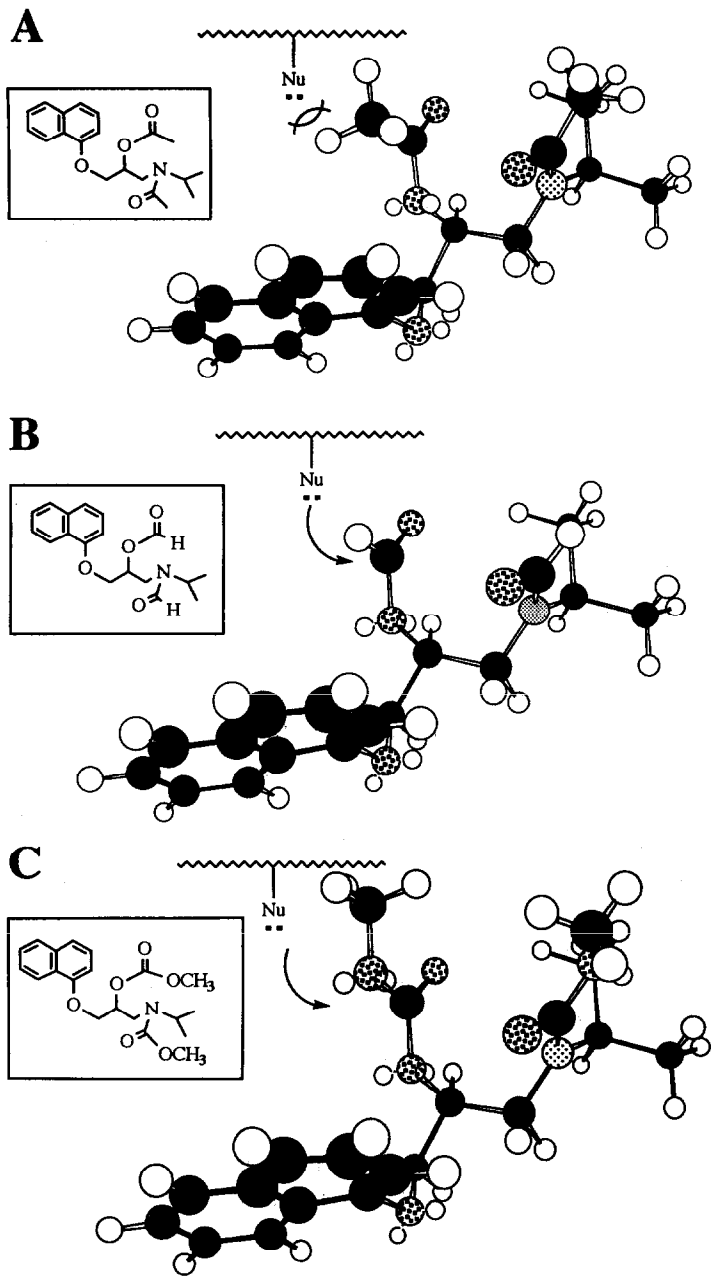
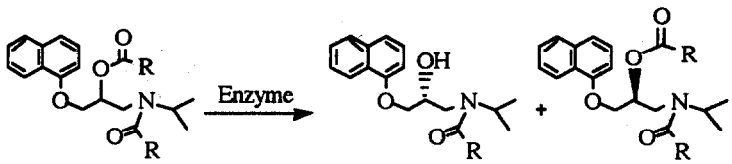


Fig. 1. Computer-generated diagrams of (A) *N,O* -diacetyl-propranolol **2**, (B) *N,O* -diformyl-propranolol **3**, and (C) *N,O* -bis(methoxycarbonyl)-propranolol **4**. Nu represents the nucleophilic residue in the catalytic site. Please note that lone pair electrons are also displayed.

Table 1. Enantioselective Hydrolysis of *N,O*-Diformyl-propranolol **3** and *N,O*-Bis(methoxycarbonyl)-propranolol *4* by Esterases. PLE: Pig Liver Esterase; CE: Cholesterol Esterase; PPE: Porcine Pancreatic Esterase.



R	Enzyme	Stereo-chemical preference	Conversion (%)	Enantiomeric Excess Product	Substrate	E
-H	PLE	*R*	64	0.03	0.19	1.03
(**3**)	CE	*R*	53	0.26	0.29	2.2
	PPE	*R*	87	0.29	0.52	2.9
-OCH3	PLE	*R*	35	0.11	0.06	1.3
(**4**)	CE	*R*	38	0.56	0.35	5.0
	PPE	*R*	40	0.83	0.55	19

However, the nature of these substituents (formyl and methoxycarbonyl) raised a plausible argument that the rate enhancement be attributed to the electronic factors rather than steric effect of these functional groups. In the literature, activated esters[10a,12] or formate esters[13] have been widely utilized to accelerate enzymatic hydrolysis and transesterification reactions. A better understanding of this reaction came when two activated esters, *N,O*-bis(chloroacetyl)- and *N,O*-bis(trichloroacetyl)- derivatives (**5** and **6**, respectively) were prepared and subjected to enzymatic hydrolysis. Computer analysis showed that the extent of steric hindrance imposed by the chloromethyl groups was similar to that of the methyl counterpart in **2**. It turned out that neither could be hydrolyzed by the enzymes even after prolonged exposure. This clearly lends support to the conclusion from computer modeling studies that the *O*-acyl function assumed a crucial role in the catalytic step. Moreover, it is worthy to note that *N,O*-bis(ethoxycarbonyl)-propranolol (**7**) was not susceptible to hydrolysis by any of these enzymes, implying lack of fit around the vicinity of the nucleophile. This finding apparently results from tight enzyme-substrate interactions coupled to restricted rotation at the biocatalytic site.

This moderate success observed in the above reaction prompted the search for additional parameters to improve the enantioselective process. Having established the necessity of the *O*-methoxy function in biocatalytic turnover, modifications at the *N*-acyl function were then examined. Computer graphics of various *N*-acyl-*O*-methoxycarbonyl derivatives **8** - **11** revealed the structural similarity of these compounds to **4** except in the *N*-acyl region. A representative comparison of the conformations of **8** and **4** is shown in Figure 2.

The close spatial configurations of compounds **8** - **11** to **4** at the hydrolytic site led to the assumption that their influence on the biocatalytic process is mainly in the enzyme-substrate (Enz-S) complexation but not in the nucleophilic attacking step. Based on the following simplified model, kinetic simulations indicate that

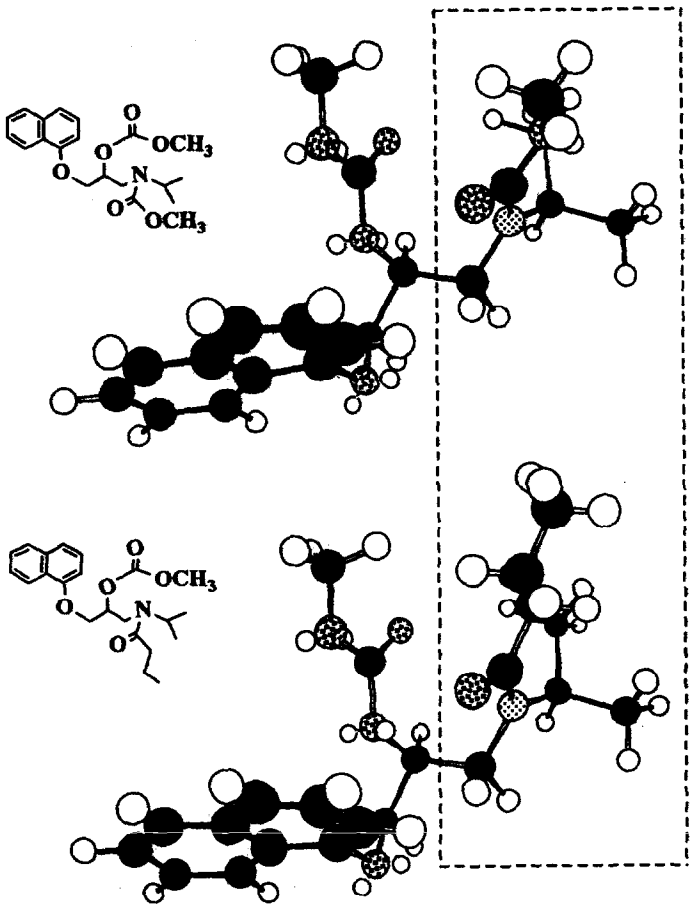


Fig. 2. Comparison of the energy-minimized conformations of *N,O* -bis-(methoxycarbonyl)-propranolol **4** and *N*-butyryl-*O*-methoxycarbonyl-propranolol **8**. Please note that lone pair electrons are also displayed.

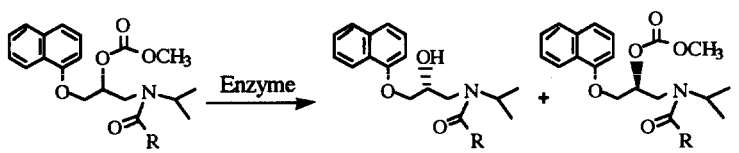
$$\text{Enz} + \text{A} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Enz-A} \xrightarrow{k_2} \text{Enz} + \text{P}$$

$$\text{Enz} + \text{B} \underset{k'_{-1}}{\overset{k'_1}{\rightleftharpoons}} \text{Enz-B} \xrightarrow{k'_2} \text{Enz} + \text{Q}$$

high enantiospecificity $[(k_{cat}/K_m)_A/(k_{cat}/K_m)_B]$ connotes rapid and reversible Enz-S complexation (i.e. $k_{-1} >> k_2$ and $k'_{-1} >> k'_2$).[14] Therefore, altering the *N*-acyl function might lead to better enantioselection by destabilizing the Enz-S complex. Should this be true, one could assess the steric effect of various substituents on enantioselectivity in a qualitative manner, allowing a better understanding of the conformation at the biocatalytic site.

To confirm the above assumption, a series of compounds (**8** - **11**) was prepared. *N*-Acyl-*O*-methoxycarbonyl derivatives were readily prepared by treating **1** with equivalent amounts of the corresponding acyl chloride, followed by dimethyl pyrocarbonate. When these compounds were subjected to PPE and crude pancreas acetone powder hydrolysis, they were found to be susceptible to enzymatic cleavage at rates comparable to that of **4**. The results are summarized in Table 2.

Table 2. Enantioselective Hydrolysis of *N*-acyl-*O*-methoxycarbonyl-propranolols (**8** - **11**) by PPE and Porcine Pancreas Acetone Powder (PPAP).



R	Enzyme	Stereo-chemical preference	Conversion (%)	Enantiomeric Excess Product	Enantiomeric Excess Substrate	E
$-C_3H_7$ (**8**)	PPE	*R*	29	0.96	0.41	76
	PPAP	*R*	47	0.91	0.82	54
 (**9**)	PPE	*R*	27	0.94	0.35	41
	PPAP	*R*	33	0.72	0.35	8.6
$-C_7H_{15}$ (**10**)	PPE	*R*	40	0.77	0.34	12
$-C_{11}H_{23}$ (**11**)	PPE	*R*	23	0.81	0.25	12

While the stereochemical preference was maintained despite great variations in the size of the acyl

groups, the degree of enantiomeric discrimination differed considerably. It was found that the replacement of the carbamate function in **4** with butyryl and cyclohexylcarbonyl groups resulted in a marked improvement in enantiomeric selection, which was not observed with long chain amides. With PPE, the observed E values were 19, 76, and 41 for compounds **4**, **8**, and **9**, respectively; whereas the E values for the C_8 and C_{12} acyl derivatives decreased and were lower than 19. As expected, PPE appeared to be more enantiospecific than the crude acetone powder due to the removal of competing enzymes. Nonetheless, the crude enzyme reaction still represents a highly efficient process for the preparation of optically active **1**. For instance, its incubation with racemic **8** , after 47% conversion, gave (*R*)-*N*-butyryl propranolol and (*S*)-**8** with optical purity in 91% and 82% e.e., respectively. Removal of the protecting groups in both compounds by alkaline hydrolysis, followed by recrystallization, afforded (*R*)- and (*S*)-**1** with enantiomeric excess of 95 and 92%, respectively.

In conclusion, the present study clearly illustrates the potential use of computer modeling in improving the enantioselectivity and chemical yield of a biocatalytic resolution. When information concerning an enzyme's substrate preference is lacking, the energy-minimized conformations of substrate molecules can be used to identify steric factors crucial to the catalytic and enantio-discriminative steps, which may lead to designing a better substrate. Such a strategy proved to be successful in propranolol resolution, and its further applications are currently undergoing in this laboratory.

EXPERIMENTAL

General.

^{1}H NMR spectroscopy was carried out on a Bruker AM-300 spectrometer for solutions in deuteriochloroform with tetramethylsilane as the internal standard. Optical rotations were determined with a Rudolph Autopol III polarimeter for solutions in the indicated solvent. High-pressure liquid chromatography was performed using a Model 501 pump (Waters Associates) equipped with a Rheodyne injector and a Model 481 UV/Vis detector (Waters Associates). Cholesterol esterase, pig liver esterase and porcine pancreas acetone powder were purchased from Sigma Chemical Co., and the enzyme units were defined accordingly. Other enzymes mentioned in the paper were obtained from either Amano Enzyme Co. or Sigma Chemical Co. All other chemicals and solvents of the highest quality grade available were purchased from Aldrich Chemical Co. or Sigma Chemical Co.

Molecular Modeling.

The computer analysis was carried out using the *Chem3D plus*^{TM} molecular modeling program (Cambridge Scientific Computing, Inc., Cambridge, MA) and a Macintosh IIcx personal computer. For any putative substrate, a computer graphic display of the possible conformation was drawn and subjected to energy minimization through molecular mechanics calculation. The minimum RMS gradient was set at 0.001, and the energy-minimized conformation could normally be obtained after 200 iterations.

Propranolol Derivatives

N,O-Diacyl propranolols (**2**,**5**,**6**): The corresponding acyl chloride (22.8 mmol) was added dropwise to ice-cooled CH_2Cl_2 (20 ml) containing *DL*-propranolol (**1**) (2 g, 7.6 mmol) and triethylamine (2.3 g, 22.8 mmol). The resulting mixture was stirred at room temperature for 1 h, and was then washed successively with equal volumes of saturated aq. sodium bicarbonate, and brine. The organic layer was dried over sodium sulfate, and evaporated under reduced pressure. Purification of the crude residue over a silica gel column

(hexane-ethyl acetate, 5:1 to 1:1) afforded the product with yields ranging from 80 to 90%.

N,O-Diformyl propranolol (**3**): *DL*-**1** (2 g, 7.6 mmol) was refluxed with formic acid (30 ml) for 12 h. The remaining formic acid was removed under reduced pressure. Purification of the crude residue over a silica gel column (hexane-ethyl acetate, 5:1 to 2:1) yielded 400 mg (16%) of the diformyl derivative **3**. ^{1}H NMR δ_H 1.18-1.35 (6 H, m), 3.34-3.50 (1 H, m), 3.54-3.84 (4 H, m), 4.22-4.36 (1 H, m), 6.80-8.20 (8 H, m), 8.27 (1 H, s). Anal. Calcd. for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.72; H, 6.58; N, 4.27.

N,O-Dimethoxycarbonyl propranolol (**4**): *DL*-**1** (2 g, 7.6 mmol) was stirred with dimethyl pyrocarbonate (10.9 g, 76 mmol) in the presence of catalytic amounts of *N,N*-dimethylaminopyridine (DMAP) at 60 °C for 12 h. The solution was diluted with 20 ml of ethyl acetate, and the mixture was washed successively with equal volumes of saturated aq. sodium bicarbonate, 1 M HCl, and brine. The organic layer was dried over sodium sulfate, and evaporated under reduced pressure. Purification of the crude residue over a silica gel column (hexane-ethyl acetate, 5:1 to 2:1) afforded 2 g (75%) of **4**. ^{1}H NMR δ_H 1.0-1.8 (6 H, m), 3.2-3.6 (1 H, m), 3.63 (3 H, s), 3.77 (3 H, s), 3.8-4.6 (4 H, m), 5.3-5.6 (1 H, m), 6.68-7.0 (1 H, m), 7.1-7.9 (5 H, m), 8.1-8.3 (1 H, m). Anal. Calcd. for $C_{20}H_{25}NO_6$: C, 63.99; H, 6.71; N, 3.73. Found: C, 64.14; H, 6.55; N, 3.54.

N-Acyl-O-methoxycarbonyl propranolols (**8** - **11**). The corresponding acyl chloride (7.6 mmol) was added dropwise to ice-cooled CH_2Cl_2 (20 ml) containing **1** (2 g, 7.6 mmol) and triethylamine (0.93 g, 9.12 mmol). The resulting mixture was stirred at room temperature for 30 min, and was washed successively with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, and evaporated under reduced pressure. The oily residue was mixed with dimethyl pyrocarbonate (5 ml), and to the residue was added catalytic amounts of DMAP. The mixture was stirred at 50 °C for 12 h. The workup procedure was basically the same as described above. Purification of the crude residue over a silica gel column (hexane-ethyl acetate, 10:1 to 2:1 depending on the compound applied) afforded products with yields ranging from 75 to 85%. ^{1}H NMR δ_H **8**: 0.88-1.00 (3 H, m), 1.19-1.31 (6 H, m), 1.62-1.75 (2 H, m), 2.28-2.53 (2 H, m), 3.32-3.35 (1 H, m), 3.78 (3 H, s), 3.80-3.89 (1 H, m), 4.02-4.19 (1 H, m), 4.27-4.39 (2 H, m), 5.45-5.52 (1 H, m), 6.72 -6.81 (1 H, m), 7.32-7.51 (4 H, m), 7.75-7.81 (1 H, m), 8.21-8.25 (1 H, m). Anal. Calcd. for $C_{22}H_{29}NO_5$: C, 68.20; H, 7.54; N, 3.61. Found: C, 68.46, H, 7.41; N, 3.43. **9**: 1.18-1.37 (9 H, m), 1.45-1.81 (7 H, m), 2.47-2.57 (1 H, m), 3.36-3.51 (1 H, m), 3.77 (3 H, s), 3.79-3.88 (1 H, m), 4.13-4.22 (1 H, m), 4.26-4.37 (2 H, m), 5.44-5.51 (1 H, m), 6.78 (1 H, d, J = 7.4 Hz), 7.32 (4 H, m), 7.76-7.82 (1 H, m), 8.21 - 8.25 (1 H, m). Anal. Calcd. for $C_{25}H_{33}NO_5$: C, 70.23; H, 7.78; N, 3.28. Found: C, 70.49, H, 7.65; N, 3.13. **10**: 0.5-1.9 (19 H, m), 2.2-2.5 (2 H, m), 3.4-3.7 (2 H, m), 3.8-4.3 (3 H, m), 3.7 (3 H, s), 5.2-5.5 (1 H, m), 6.7-6.9 (1 H, m), 7.1-7.5 (4 H, m), 7.5-8.0 (1 H, m), 8.0-8.3 (1 H, m). Anal. Calcd. for $C_{26}H_{37}NO_5$: C, 70.40; H, 8.41, N, 3.16. Found: C, 70.61; H, 8.28, N, 3.03. **11**: 0.5-2.1 (27 H, m), 2.2-2.5 (2 H, m), 3.2-3.5 (2 H, m), 3.7 (3 H, s), 3.8-4.5 (3 H, m), 5.2-5.5 (1 H, m), 6.6-6.8 (1 H, m), 7.1-7.5 (4 H, m), 7.6-7.8 (1 H, m), 7.9-8.3 (1 H, m). Anal. Calcd. for $C_{30}H_{45}NO_5$: C, 72.11; H, 9.08; N, 2.80. Found: C, 72. 33; H, 8.97; N, 2.65.

Partial purification of porcine pancreas acetone powder.

Porcine pancreas acetone powder (Sigma, 10 g) was suspended in 50 ml of 10 mM potassium phosphate

buffer (pH 7.0) (buffer A), and subjected to homogenization. The tissue debris was removed by centrifugation at 12,000 x g for 20 min, and the supernatant was dialyzed against 3 L of buffer A for 12 h. The solution was then applied to a DEAE-Sepharose CL-6B column (5 x 10 cm) equilibrated with buffer A. The column was washed with 200 ml of buffer A, and eluted with a linear gradient (600 ml) consisting of 0.01 to 0.5 M NaCl in buffer A. Fractions of 6 ml were collected. The esterase activity was assayed using *p*-nitrophenyl acetate as the substrate. Fractions 110 to 132, which contained the esterase, were collected, and the pooled solution was used for the enzyme reaction. One unit of PPE is that amount of enzyme catalyzing the hydrolysis of 1 μmol of *p*-nitrophenyl acetate per min at 25 °C.

Enzyme Incubations.

The substrate (200 mg), dissolved in DMF (0.5 ml), was introduced to 0.1 M potassium phosphate buffer (20 ml; pH 7.0) containing 0.5% Tween 80. To the mixture was added 200, 20, 20 units of PLE, CE, and PPE, respectively. The vigorously stirred mixture was incubated at 25 °C and the progress of the reaction was monitored by silica gel TLC analysis (hexane-ethyl acetate, 3:1 to 1:1, depending on the compound). After 2 - 120 h, the reaction was quenched by extracting the mixture with an equal volume of ethyl acetate three times. The combined extracts were dried over sodium sulfate, and concentrated to dryness. The residue was chromatographed over a silica gel column (hexane-ethyl acetate) to afford the *N*-acyl propranolol and the remaining substrate for optical purity determination.

Enantiomeric Purity Determination.

The *N*-acyl propranolols obtained from the biotransformation, with the exception of *N*-formyl propranolol, were treated with (*S*)-(-)-2-methoxy-2-(trifluoromethyl)-2-phenylacetyl (MTPA) chloride to form the corresponding (-)-MTPA esters. The MTPA derivatives were analyzed by HPLC using a silica gel column (4.6 mm x 25 cm) and hexane-ether, 3:1, as the mobile phase. The flow rates for individual MTPA esters, and the corresponding retention times were, respectively: ***N*-methoxycarbonyl**: 1.2 ml/min; (*S*): 40 min, (*R*): 43.6 min; ***N*-butyryl**: 1.2 ml/min; (*S*): 25.6 min, (*R*): 27 min; ***N*-cyclohexanecarbonyl**: 2 ml/min; (*S*): 12.5 min, (*R*): 13.5 min; ***N*-octanoyl**: 1.0 ml/min; (*S*): 25.5 min, (*R*): 29.7 min; ***N*-dodecanoyl**: 0.5 ml/min; (*S*): 45.5 min, (*R*): 53 min. To determine the optical purity of the N-formyl derivative, the compound was treated with alkaline, followed by (-)MTPA chloride to form the *N,O*-di-MTPA derivative. The retention times were 33.3 min and 31.3 min for the (*S*) and (*R*) isomers, respectively, at a flow rate of 1.5 ml/min. With regard to the remaining substrate fraction, the compound was treated with 1 N KOH/CH_3OH at room temperature for 30 min to yield the corresponding *N*-acyl propranolol. The optical purity could thus be determined according to the aforementioned procedure.

REFERENCES AND NOTES

(1) For a preliminary account of this study, see Shieh, W.-R.; Gou, D.-M.; Chen, C.-S. *J. Chem. Soc. Chem. Commun.* **1991**, p. 651.

(2) For recent reviews: (a) Sih, C. J.; Wu, S.-H. *Top. Stereochem.* **1989**, *19*, 63. (b) Wong, C.-H. *Science* **1989**, *244*, 1145. (c) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114.

(3) (a) Sih, C. J.; Gu, Q.-M.; Reddy, D. R. in *Trends in Medicinal Chemistry*, Mutschler E., Winterfieldt, E. Eds.; VCH, New York, 1987; pp. 181. (b) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A.

T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656.

(4) For example, the tertiary structures of pancreatic lipase, *Mucor miehei* lipase and *Geotrichum candidum* lipase have recently been elucidated. see: (a) Brady, L.; Brzozowski, A. M.; Derewenda, Z. S.; Dodson, E.; Dodson, G.; Tolley, S.; Turkenburg, J. P.; Christiansen, L.; Huge-Jensen, B.; Norskov, L.; Thim, L.; Menge, U. *Nature* **1990**, *343*, 767. (b) Winkler, F. K.; D'Arcy, A.; Hunziker, W. *ibid* **1990**, *343*,771. (c) Schrag, J. D.; Li, Y.; Wu, S.; Cygler, M. *ibid* **1991**, *351*, 761.

(5) Jorgensen, W.L. *Science*, **1991**, *254*, 954.

(6) (a) Backvall, J. E.; Bjorkman, E. E.; Bystrom, S. E. *Tetrahedron Lett.* **1982**, *23*, 943. (b) Katsuki, T. *Tetrahedron Lett.* **1984**, *25*, 2821. (c) Miyano, S.; Lu, L. D. L.; Viti, S. M.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 4350. (d) Klunder, J. M.; Soo, Y. K.; Sharpless, K. B. *ibid*, **1986**, *51*, 3710.

(7) Several preparations of (*S*)-propranolol which relied upon the elaboration of enzymatically prepared precursors have been reported. For example, see (a) Iriuchijima, S.; Keiyu, A.; Kojima, N. *Agri. Biol. Chem.* **1982**, *46*, 1593. (b) Marsuo, N.; Ohno, N. *Tetrahedron Lett.* **1985**, *26*, 5533. (c) Fuganti, C.; Grasselli, P.; Seneci, P. F.; Servi, S. *Tetraherdon Lett.* **1986**, *27*, 2061. (d) Terao, Y.; Murata, M.; Achiwa, K. *Tetrahedron Lett.* **1988**, *29*, 5173. (e) Wang, Y.-F.; Chen, S.-T.; Liu, K. K. C.; Wong, C.-H. *Tetrahedron Lett.* **1989**, *30*, 1917. (f) Bevinakatti, H. S.; Banerji, A. A. *J. Org. Chem.* **1991**, *56*, 5372. (g) Kaimal, T. N. B.; Prasad, R. B. N.; Chandrasekhara Rao, T. *Biotechnology Lett.* **1992**, *14*, 21.

(8) (a) Jpn. Kokai Tokyo Koho JP 62 151,196 (*Chem Abstr.* **1988**, *108*, 110832d). (b) Jpn. Kokai Tokyo Koho JP 63 94,992 (*Chem Abstr.* **1989**, *110*, 93558r).

(9) Proteases included: chymotrypsin, thermolysin and proteasès from *Aspergillus oryzae*, *A. sojae*, *A. satoi*, *Rhizopus* sp., and *Streptomyces caeapitosus*. Lipases included: crude lipase preparations from porcine pancreas, *Candida cylindracea*, *A. niger*, *Geotrichum candidum*, *Humicola lanuginosa*, *Mucor miehei*, *Pseudomonas* sp., *Rhizopus niveus*, and *R. oryzae*. Esterases included pig liver esterase, cholesterol esterase, and partially purified porcine pancreatic esterase.

(10) (a) Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7071. (b) Sih, C. J., personal communication.

(11) E (enantiomeric ratio) is defined as the ratio of the specificity constants of the two enantiomers, $(k_{cat}/K_m)_S/(k_{cat}/K_m)_R$, and the value is calculated from

$$E = \ln [1 - c(1 + ee(P))]/\ln [1 - c(1 - ee(P)],$$

where ee(P) is the ee value of product. See; Chen, C.-S., Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

(12) Gu, Q.-M.; Chen, C.-S.; Sih, C. J. *Tetrahedron Lett.* **1986**, *27*, 1763.

(13) Bevinakatti, H. S.; Newadkar, R. V. *Biotechnol. Lett.* **1989**, *11*, 785.

(14) (a) Sih, C. J.; Chen, C.-S. *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 570. (b) Wang, Y. F.; Chen, C.-S.; Girdaukas, G.; Sih, C. J. in *Enzymes in Organic Synthesis*, Battersby, A. R., ed.; Pitman, London, **1985**; pp.128.