Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 4, pp. 433-436, 1997
Copyright © 1997 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00028-0

Kinetic Resolution of Propranolol by a Lipase-Catalyzed *N*-Acetylation

Tzyy-Wen Chiou[1,2]*, Cheng-Chi Chang[2], Chung-Torr Lai[3] and Dar-Fu Tai[4]*

[1] Institute of Biotechnology, National Dong-Hwa University, Hualien, Taiwan, ROC.

[2] Institute of Chemistry, Academia Sinica, Taipei, Taiwan, R.O.C.

[3] Biochemical Engineering Center, Da-Yeh Institute of Technology, Chang Hwa, Taiwan, ROC.

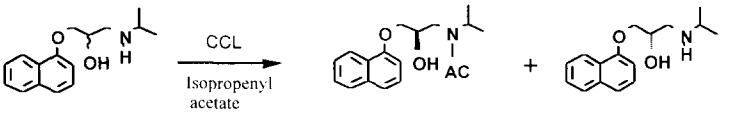
[4] Department of Chemistry, National Dong-Hwa University, Hualien, Taiwan, ROC.

Abstract: An enzymatic method for the direct resolution of propranolol is described. *Candida cylindracea* lipase enantioselectively catalyzed *N*-acetylation of *S*-propranolol with isopropenyl acetate in isopropyl ether. The ee values of the two enantiomers of *N*-acetylpropranolol were determined by an HPLC equipped with a chiral column. The effects of organic solvent nature and substrate concentration on enantioselectivity were also studied. © 1997, Elsevier Science Ltd. All rights reserved.

Homochiral aminol is a common building block for β-adrenergic receptor blocking agents(β-blockers). The β-blockers[1] are used clinically to control blood pressure, counteract depression, arrhythmia, and angina pectoris, to stimulate the appetite or to relieve asthma.[2] Generally, the *S*-enantiomers of β-blockers are more potent antagonists than the corresponding *R*-enantiomer. It has been demonstrated that *S*-(-)-propranolol possesses higher binding affinity toward both β_1 and β_2-receptors than *R*-isomer.[3] Asymmetric syntheses of *S*-propranolol by non-enzymatic[4] and enzymatic[5,6] methods were previously reported. In addition, HPLC methods were established for direct chiral separation and determination of β-blockers as well as their derivatives.[7] Lipase-catalyzed transesterification[5,8,9] is a good method for the kinetic resolution of secondary alcohol. Enzymatic resolutions of chiral amines by amidation in organic solvents were studied.[10] *Candida cylindracea* lipase (CCL) catalyzed aminolysis were reported previously.[11] However, direct enzymatic resolution of propranolol or a homochiral aminol by *N*-acetylation is still unknown.

It was our aim to use propranolol, which possesses a chiral secondary alcohol, as a substrate to undergo a lipase catalyzed transesterification. To our disappointment, for the three acetylating agents tested, we found that acetic anhydride underwent the chemical acetylation on O position predominately to give *O*-acetylpropranolol(OAP) with a yield above 70%. The variety of lipases and solvents tested had no influence on the enantioselectivity. On the other hand, using vinyl acetate or isopropenyl acetate as the reaction agent resulted in the production of *N*-acetyl-propranolol (Scheme I).

Scheme I



Vinyl acetate did not acetylate on O position, but the reaction mixture usually turned brown after 24 hours, and there were many unexpected side products that could be detected by HPLC. Among the three acetylating agents tested, isopropenyl acetate was shown to be a more suitable one. There were only four peaks (+P, -NAP, +NAP, -P) appeared on its HPLC chromatogram, the complete resolution of both *R* and *S* isomers of propranolol and *N*-acetylpropranolol was achieved in 50 min with Chiralcel OD column on HPLC. The retention period of *R*-propranolol(+P) was 17.85 min, *S*-*N*-acetylpropranolol(-NAP) 30.23 min, *R*-*N*-acetylpropranolol(+NAP) 35.85 min and *S*-propranolol(-P) 44.89 min. It is a direct and convenient method to quantify each enantiomer. The data obtained from these experiments were then used to optimize the resolution of the enzymatic *N*-acetylation.

Eight lipases (PS, MY, OF, L10, AP15, D20 and AY30 from Amano and CCL from Sigma) were tested for their selectivities in this reaction. The best result was obtained from *Candida cylindracea* lipase. The enantioselectivity was apparently influenced by the change of lipases and solvents. As indicated in Table 1, compared to the others, both the conversion rate and the enantioselectivity of CCL(OF, MY and CCL from Sigma) were among the highest. The optical rotation of *N*-acetyl-*S*-propranolol thus formed is $[\alpha]_D^{23} = -4.8$ (c= 2, EtOH).

Table 1. Comparison of the lipase reactivities in isopropyl ether.[a]

Lipase	days	% of Conversion	e.e.[b] (Conf.)		E^{12}
			Aminol	Amide	
CCL(Sigma)	4	54.4	64 (R)	72 (S)	16.6
OF	3	57	28 (R)	73 (S)	21
OF	1	41.6	19 (R)	77 (S)	13
MY	3	57.1	56 (R)	71 (S)	20.7
MY	1	40.9	33 (R)	74 (S)	11
AY30	3	37.2	32 (R)	69 (S)	8
AY30	1	15.8	11 (R)	71 (S)	6.7
FAP15	3	2	0	20 (S)	---
L10	3	1.1	0	10 (R)	---
D20	3	0	---	---	---
PS	3	0	---	---	---

[a] Propranolol (0.05 mM), isopropenyl acetate (0.05 mM), isopropyl ether(1 mL) and lipase (20 mg) was shaken at 37°C with a speed of 200 rpm.

[b] Determined by HPLC analysis on chiralcel OD column with 10% isopropanol and 0.001% diethylamine in hexane as solvent system.

The stereoselectivity of an enzyme is strongly affected by hydrophobicity, functionality and structure of the reaction medium.[13] Therefore, the choice of solvents is the most critical part in the optimization of reaction conditions to achieve a better enantioselectivity. The catalytic activities of CCL toward propranolol with isopropenyl acetate in different organic solvents (toluene, chloroform, *t*-butyl methyl ether, *t*-amyl alcohol, isopropyl ether, tetrahydrofuran, and acetonitrile) were examined. As shown in Table 2, isopropyl ether and *t*-butyl methyl ether, which had higher hydrophobicities[14] (log P index) and solubility, were highly rated on both reactivity and enantioselectivity. It is worthy to note that *R*-propranolol has a uncommon higher rate of acetylation than its counterpart in *t*-amyl alcohol. The use of isopropenyl acetate as the solvent increased the rate of nonenzymatic reaction as to decrease the ee value of the product.

Table 2. Effect of organic solvent on CCL catalyzed acylation of racemic 1.[a]

Solvent	Log p	days	% of	e.e.[b] (Conf.)		E[12]
			Conversion	Aminol	Amide	
Isopropenyl acetate		1	58	0 (R)	0 (S)	---
Toluene	2.5	1	43	35 (R)	58 (S)	1.2
Chloroform	2.0	2	1	0 (R)	12 (S)	---
Isopropyl ether	1.9	4	61	40 (R)	76 (S)	16.6
t-Butyl methyl ether	1.35	6	51	32 (R)	54 (S)	7.9
t-Amyl alcohol	1.3	2	6	0 (S)	32 (R)	---
Tetrahydrofuran	0.49	4	6	6 (R)	80 (S)	1.5
Acetonitrile	- 0.33	2	0	---	---	---

[a] Propranolol (0.05 mM), isopropenyl acetate (0.05 mM), organic solvent (1 mL) and CCL (20 mg) was shaken at 37°C with a speed of 200 rpm.

[b] Determined by HPLC analysis on chiralcel OD column with 10% isopropanol and 0.001% diethylamine in hexane as solvent system.

The enantioselectivity was also influenced by the concentration of substrate. As shown in Table 3, the ee values and the E values of the transacetylation product-amide increased as the concentration of the propranolol decreased when the percentage of conversion are approximately the same. It was also found that the enantioselectivity of this enzymatic reaction reached the plateau at a certain concentration of substrate, which was varied upon the type of organic solvent we used. Variation of the temperature had affected the rate of the reaction but not the E values.

Table. 3 Effect of the concentration of propranolol on the E values of amides.

Solvent	Concentratio	days	% of	e.e.[b] (Conf.)		E[12]
	(M)		Conversion	Aminol	Amide	
t-Butyl methyl ether	0.2	6	60	30.6 (R)	37.8 (S)	3.8
t-Butyl methyl ether	0.1	3	58.3	57 (R)	51.6 (S)	6.5
t-Butyl methyl ether	0.05	3	57.8	37.6 (R)	56 (S)	7.9
Isopropyl ether	0.2	6	59.5	60.6 (R)	57 (S)	9.2
Isopropyl ether	0.05	2	54.4	63.6 (S)	72 (R)	16.6
Isopropyl ether	0.025	2	55.7	67 (R)	73.4 (S)	21

[a] Substrate (0.05 mM), isopropenyl acetate (0.05 mM), organic solvent (1 mL) and CCL (20 mg) at 37 °C.

[b] Determined by HPLC analysis on chiralcel OD column with 10% isopropanol and 0.001% diethylamine in hexane as solvent system.

In summary, propranolol is a homochiral aminol, the chemical environment of its chiral center on binding with a lipase is different from that of a general secondary alcohol. The amino group has a higher nucleophilicity to react with acetylating agent. Among the lipases tested, CCL was found to be the most reactive and enantioselective for catalyzing *N*-acetylation of propranolol. Except chloroform, solvents with higher hydrophobicities, such as toluene, isopropyl ether and *t*-butyl methyl ether, showed more potential in terms of reactivity and enantioselectivity.[8,14] As for the enantioselectivity, an ee value of 80 was achieved in this study. We can obtain high E value in different organic solvent by controlling the concentration of the substrate. Their ee values are obviously affected by the concentration of propranolol.

REFERENCES

1. Cruickshank, J. M., *Am. Heart J.*, **1980**, 100, 160.
2. Frishman, W., *N. Engl. J. Med.*, **1982**, 306, 1456.
3. Morris, T. M.; Kaumann, A. J. *Naunyn-Schmiedeberg's Arch.Pharmacol.*, **1984**, 327, 176.
4. Sasai, H.; Suzuki, T.; Itoh, N.; Shibasaki, M., *Appl. Organometal. Chem.*, **1995**, 9, 421; Takahashi, H.; Sakuraba, S.; Takeda, H.; Achiwa, K. *J. Am. Chem. Soc.*, **1990**, 112, 5876; Thompson, J. A; Klunder, J.M.; Ko, S.Y.; Sharpless, K.B. *J. Org. Chem.*, **1986**, 51, 3710.; Holtzman, J. L.; Tsuru, M.; Lerman, C.; Holtzman, J. L. *J. Chromatogr.*, **1982**, 238, 470.
5. Bevinakatti, H.S.; Banerji, A.A. *J. Org. Chem.*, **1991**, 56, 5372.
6. Kamal, A.; Rao, M.V *Tetrahedron: Asymmetry*, **1994**, 5, 1881; Shieh, W.-R.; Gao, D.-M.; Chen, C.-S. *J. Chem. Soc. Chem. Commun.*, **1991**, 651; Wang, Y. F.; Chen, S. T.; Liu, K. K. C.; Wong, C. H. *Tetrahedron Lett.*, **1989**, 30, 1917; Terao, Y.; Murata, M.; Achiwa, K.; Nishino, T.; Akamatsu, M.; Kamimura, M. *Tetrahedron Lett.*, **1988**, 29, 5173; Matsuo, M.; Ohno, N. *Tetrahedron Lett.*, **1985**, 26, 5533.
7. Haginaka, J.; Wakai, J.; Takahashi, K.; Yasuda, H.; Katagi, T. *Chromatographia*, **1990**, 29, 587; Okamoto, Y.; Aburatani, R.; Hatano, K.; Hatada, K. *J. Liq. Chromatogr.*, **1988**, 11, 2147; Straka, R.J.; Lalonde, R.L.; Wainer, I.W. *Pharmaceutical Res.*, **1988**, 5, 187; Hermansson, J. *J. Chromatogr.*, **1985**, 325, 379.
8. Nakamura, K.; Kitayama, T.; Takebe, Y.; Ohno A. *Tetrahedron Lett*, **1991**, 32, 4941.
9. Raju, S. B., Chiou, T. -W, Tai, D. -F.*Tetrahedron: Asymmetry*, **1995**, 6, 1519.
10. Puertas, S.; Rebolledo, F.; Gotor, V. *J. Org. Chem.*, **1996**, 61, 6024; Puertas, S.; Brieva, R.; Rebolledo, F.; Gotor, V. *Tetrahedron*, **1993**, 49, 4007; Kamal, A.; Rao, M.V. *Tetrahedron: Asymmetry*, **1991**, 2, 751; Kitaguchi, H.; Fitzpatrick, P. A.; Huber, J. E.; Klibnov, A.M. *J. Am. Chem. Soc.*, **1989**, 111, 3094.
11. Takaokazu, Y. ; Kajimoto, T.; Wong, C.-H. *J. Org. Chem.*, **1993**, 58, 4809; Gotor, V.; Brieva, R.; Gonzalez, C.; Robolledo, F. *Tetrahedron*, **1991**, 47, 9207; Gotor, V.; Garcia, M.J.; Robolledo, F. *Tetrahedron: Asymmetry*, **1990**, 1, 277.
12. Chen, C.-S., Fujimoto, Y., Girdaukas, G., Sih, C.J. *J. Am. Chem. Soc.*, **1982**, 104, 7294.
13. Klibanov A.M. *Acc. Chem. Res.*, **1990**, 23, 114.
14. Nakamura, K.; Kinoshita, M.; Ohno A. *Tetrahedron*, **1994**, 50, 4681.

(*Received in Japan* 14 *November* 1996; *accepted* 9 *January* 1997)