

Enantioselective hydrolytic reactions of rice bran lipase (RBL): a first report [†]

N. W. Fadnavis* and Vasudev Jadhav

Biotransformation Laboratory, Indian Institute of Chemical Technology, Hyderabad 500 007, India

Abstract: Enantioselectivity has been observed in the hydrolysis of racemic N-acetyl amino acid esters with rice bran lipase (RBL). The enzyme shows selectivity towards the (S)-enantiomer. Products with high enantiomeric excess (e.e.>99%) are obtained depending upon the hydrophobicity of the amino acid as well as that of the leaving group. © 1997 Elsevier Science Ltd

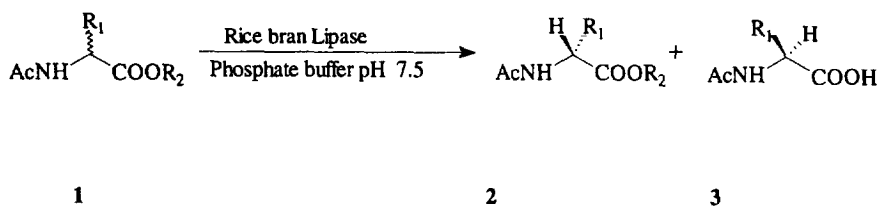
Lipases of different origins are widely used in the resolution of racemic mixtures of chiral molecules¹. Most frequently used lipases are from yeast (*Candida rugosa*), mold (*Mucor* sp.), bacteria (*Pseudomonas* sp.) and from animal sources such as pig pancreas. Due to their wide substrate specificity, high enantioselectivity, good stability, low cost and easy availability, literature reports on the applications of these lipases in asymmetric synthesis run in hundreds but surprisingly very little work has been reported on lipases from plant origin² and this is confined mainly to the chemistry of oils and fats³. As a part of our programme on the application of enzymes from plant sources in organic synthesis, we have explored the possibility of using lipase from rice bran in enantioselective hydrolysis of esters. In India, rice bran is produced in a very large tonnage and used as animal feed after extraction of rice bran oil. Apart from oil, rice bran is also an excellent source of lipase which has been isolated and characterized in early 70s⁴. Paradoxically, a lot of research work is being done to destroy the lipase present in rice bran instead of attempting to use it⁵. A practical industrial use of rice bran lipase (RBL), especially in the production of optically active intermediates would make the lipase an attractive value added product. Here we report for the first time, the RBL catalyzed enantioselective hydrolysis of esters of α -amino acids, products of great industrial use.

Generally, enzymatic resolution of racemic α -amino acids is carried out by an amidase such as acylase which stereospecifically hydrolyzes L-amino acid amides⁶ or by an esterase or other proteolytic enzyme which hydrolyzes amino acid esters with very high enantioselectivity⁷. Enzymes like trypsin, α -chymotrypsin, papain and even whole cells of baker's yeast⁸ have been used to carry out these resolutions.

Earlier, lipases from *Aspergillus niger*⁹, *Pseudomonas* sp. and porcine pancreas¹⁰ have been used in resolution of α -amino acids with excellent enantioselectivities. However, the possible presence of proteases in the enzyme preparations has been suspected to be responsible for the high enantioselectivities observed in these reactions¹¹. To rule out this possibility our lipase preparation from rice bran was checked for protease activity using standard assay methods¹² using large quantities of enzyme for assay. No observable protease activity was found in the enzyme preparation (20 mg/ml, contact time 24 h for casein). A range of amino acids from alanine to tyrosine were chosen, their corresponding N-acetyl derivatives were then esterified with alcohols of varying chain length from C₁ to C₈ and subjected to RBL catalyzed enantioselective hydrolysis in aqueous buffer at pH 7.5, 30°C (Scheme 1). The results are summarized in Table 1.

[†] IICT Communication No. 3820.

* Corresponding author. Email: root@csiict.ren.nic.in



Scheme 1.

The enzyme shows enantioselectivity towards the (S)-ester giving predominantly the (S)-acid **3**. It is also observed that the hydrophobicity and size of R_1 plays an important role in substrate recognition. In the case of amino acids with aliphatic groups the substrate recognition is not efficient; e.g. in case of N-acetyl alanine methyl ester ($R_1 = \text{Me}$), recovered ester after 50% hydrolysis has very low enantiomeric excess (e.e. 20%) while for valine ($R_1 = \text{iPr}$) it improves to 69%. Surprisingly, for isoleucine ($R_1 = \text{iBu}$), again the enantioselectivity is poor (e.e. 13%). Evidently, it is not just the hydrophobicity but also the size and shape of the alkyl residue that plays an important role in substrate recognition. As seen in several cases for typical lipase catalyzed reactions¹³ the enantioselectivity of the hydrolysis reaction is also found to be strongly dependent on the number of carbon atoms in the alcohol leaving group. Results in Table 1 highlight this effect very clearly. The enantioselectivity improves with increasing number of carbon atoms in the alcohol moiety. Almost invariably, n-butyl esters are the best substrates. In case of valine, the enantiomeric excess of the product can be improved from 69% to >99% by changing the ester group from methyl to n-butyl. Invariably, further increase in the chain length is detrimental and the enantioselectivity again drops.

n-Butyl esters of amino acids with aromatic groups such as phenylglycine, phenylalanine and tyrosine are also hydrolyzed with excellent enantioselectivity (e.e. >99%). Above results clearly indicate that the hydrolytic reaction is performed by the lipase and not by a contaminating protease.

In conclusion, the lipase from rice bran appears to have an excellent potential in biotransformations. This lipase is perhaps the most abundantly available in nature and also a very cheap enzyme which needs no fermentation for its production. Further work is in progress to determine the scope and limitations of RBL catalyzed reactions for use in asymmetric synthesis.

Experimental

Varian Gemini 200MHz spectrometer was used to obtain ¹H-NMR with CDCl₃ as a solvent (TMS as internal standard), Optical rotations were measured on JASCO DIP-270 digital polarimeter. HPLC grade solvents (Spectrochem, India) were used in all experiments. Racemic amino acids were obtained from Hi-Media, India. N-Acetyl amino acid esters were prepared according to standard procedure¹³ and their structures were confirmed by ¹H-NMR. Rice bran lipase was isolated by ammonium sulfate precipitation, dialysis and freeze drying as reported in literature⁴. Enzyme activity was determined by titration of butyric acid produced during hydrolysis of tributyrin and is expressed in International Units as the amount of enzyme that produces one μmole of butyric acid per minute. Typically, enzyme activity of 75 units/mg was obtained.

Resolution of racemic N-acetyl amino acid esters

In a typical experiment, rice bran lipase (200 mg) was dissolved in phosphate buffer (20 ml, 0.01 M, pH 7.5) and sodium chloride solution (20 ml, 0.01 M). Racemic N-acetyl amino acid ester (1 mmol) was added and the contents were magnetically stirred in a double walled glass reactor equipped with a pH electrode. Reaction temperature was maintained at 30°C by Julabo F-10 constant temperature circulating water bath. pH of the reaction was maintained at 7.5 by addition of 0.5 N sodium hydroxide. The reaction was stopped at 50% conversion (on the basis of NaOH consumed). The unreacted ester in the reaction mixture was extracted with ethyl acetate and recovered. The aqueous

Table 1. Enantioselective hydrolysis of various N-protected amino acid esters **1** by RB lipase[†]

S.No	R ₁	Ester	%ee of ester 2 (R)	%ee of acid 3 (S)
1a	Methyl (Ala)	Me	20.7	34.5
1b		Et	35.6	37.9
1c		Pr ⁿ	47.1	39.6
1d		Bu ⁿ	47.2	51.2
1e		Hex ⁿ	26.5	29.2
1f		Oct ⁿ	19.7	18.4
1g	Isopropyl (Val)	Me	69.4	67.0
1h		Et	74.1	70.9
1i		Pr ⁿ	71.2	68.4
1j		Bu ⁿ	>99	87.3
1k		Hex ⁿ	59.5	61.0
1l		Oct ⁿ	52.4	44.0
1m	Isobutyl (Ileu)	Me	13.1	17.0
1n		Et	25.3	24.0
1o		Pr ⁿ	45.3	51.0
1p		Bu ⁿ	68.8	61.9
1q		Hex ⁿ	56.7	46.0
1r		Oct ⁿ	33.8	41.3
1s	Phenyl (Phenylglycine)	Bu ⁿ	>99	87.5
1t	Benzyl (Phe)	Bu ⁿ	>99	93.7
1u	4-Hydroxy- benzyl (Tyr)	Bu ⁿ	>99	96.3
1v	4-Methoxy- benzyl	Bu ⁿ	>99	98.0

[†] Reaction stopped at 50% conversion

phase was acidified to pH 1 with 10% HCl, lyophilized and the residue was extracted with acetone to obtain the corresponding N-acetyl amino acid. Absolute configuration of the products were based on measurement of sign of specific rotation and comparison with that in literature¹⁴. Enantiomeric purity was determined by hydrolyzing the crude product with 6N HCl to free amino acid and analysing it directly by Chiral HPLC using Crownpack CR⁺ column (4 mm × 150 mm, Diacel, Japan) as per manufacturer's instructions. The absolute configurations were reconfirmed on the basis of elution pattern of the amino acid; the D-enantiomer elutes first.

N-Acetyl-DL-alanine methyl ester (**1a**). $^1\text{H-NMR}$ (CDCl_3) δ 1.4 (d, 3H, $\alpha\text{-CH}_3$), 2 (s, 3H, COCH_3), 3.8 (s, 3H, COOCH_3), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.2 (br.d, 1H, NH).

N-Acetyl-DL-alanine ethyl ester (**1b**). $^1\text{H-NMR}$ (CDCl_3) δ 1.2 (t, 3H, CH_2CH_3), 1.4 (d, 3H, $\alpha\text{-CH}_3$), 2.0 (s, 3H, COCH_3), 4.2 (q, 2H, OCH_2), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.2 (br.d, 1H, NH).

N-Acetyl-DL-alanine propyl ester (**1c**). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (t, 3H, CH_2CH_3), 1.4 (d, 3H, $\alpha\text{-CH}_3$), 1.7 (m, 2H, CH_2), 2 (s, 3H, COCH_3), 4.2 (t, 2H, OCH_2), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.2 (br.d, 1H, NH).

N-Acetyl-D-alanine butyl ester (**2d**). $^1\text{H-NMR}$ (CDCl_3) δ 1.0 (t, 3H, CH_2CH_3), 1.4 (d, 5H, $\alpha\text{-CH}_3$, CH_2), 1.7 (m, 2H, CH_2), 2.0 (s, 3H, COCH_3), 4.2 (t, 2H, OCH_2), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.2 (br.d, 1H, NH). $[\alpha]_{\text{D}}^{25} = +30.1$ (c=1, methanol), e.e. 47.2%.

N-Acetyl-L-alanine (**3d**). $[\alpha]_{\text{D}}^{25} = -33.9$ (c=1, water), mp=124°C; (lit.¹⁴ $[\alpha]_{\text{D}}^{25} = -66.2$ (c=2, water), mp=125°C), e.e. 51.2%.

N-Acetyl-DL-alanine hexyl ester (**1e**). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (t, 3H, CH_2CH_3), 1.3 (m, 4H), 1.4 (d, 3H, $\alpha\text{-CH}_3$), 1.5–1.7 (m, 4H), 2.0 (s, 3H, COCH_3), 4.1 (t, 2H, OCH_2), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.1 (br.d, 1H, NH).

N-Acetyl-DL-alanine octyl ester (**1f**). $^1\text{H-NMR}$ (CDCl_3) δ 1.0 (t, 3H, CH_2CH_3), 1.3 (m, 4H), 1.4 (d, 3H, $\alpha\text{-CH}_3$), 1.5–1.7 (m, 6H), 2.0 (s, 3H, COCH_3), 4.1 (t, 2H, OCH_2), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.2 (br.d, 1H, NH).

N-Acetyl-DL-valine methyl ester (**1g**). $^1\text{H-NMR}$ (CDCl_3) δ 1.0 (t, 6H, $(\text{CH}_3)_2\text{C}$), 2.0 (s, 3H, COCH_3), 2.2 (m, 1H, $(\text{CH}_3)_2\text{CH}$), 3.9 (s, 3H, COOCH_3), 4.3 (m, 1H, $\alpha\text{-CH}$), 6.0 (br.d, 1H, NH).

N-Acetyl-DL-valine ethyl ester (**1h**). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (t, 6H, $(\text{CH}_3)_2\text{C}$), 1.3 (t, CH_2CH_3), 2.0 (s, 3H, COCH_3), 2.2 (m, 1H, $(\text{CH}_3)_2\text{CH}$), 4.4 (q, 3H, OCH_2CH_3), 4.5 (m, 1H, $\alpha\text{-CH}$), 6.0 (br.d, 1H, NH).

N-Acetyl-DL-valine propyl ester (**1i**). $^1\text{H-NMR}$ (CDCl_3) δ 1.0 (m, 9H, $(\text{CH}_3)_2\text{C}$, CH_3), 1.7 (m, 2H, CH_2), 2.0 (s, 3H, COCH_3), 2.2 (m, 1H, $(\text{CH}_3)_2\text{CH}$), 4.1 (t, 3H, OCH_2CH_3), 4.5 (m, 1H, $\alpha\text{-CH}$), 6.1 (br.d, 1H, NH).

N-Acetyl-D-valinebutylester (**2j**). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (m, 9H, $(\text{CH}_3)_2\text{C}$, CH_3), 1.4 (m, 2H, H_2), 6 (m, 2H, CH_2), 2.0 (s, 3H, COCH_3), 2.2 (m, 1H, $(\text{CH}_3)_2\text{CH}$), 4.2 (t, 3H, OCH_2CH_3), 4.5 (m, 1H, $\alpha\text{-CH}$), 6.2 (br.d, 1H, NH). $[\alpha]_{\text{D}}^{25} = +9.1$ (c=1, methanol); e.e.>99%.

N-Acetyl-L-valine (**3j**). $[\alpha]_{\text{D}}^{25} = -16.6$ (c=1, water), mp=168°C; (lit.¹⁵ $[\alpha]_{\text{D}}^{25} = -19.9$ (c=1.27, water), mp=168°C); e.e. 87.3%.

N-Acetyl-DL-valine hexyl ester (**1k**). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (m, 9H, $(\text{CH}_3)_2\text{C}$, CH_3), 1.4 (m, 6H, CH_2), 1.7 (m, 2H, CH_2), 2.0 (s, 3H, COCH_3), 2.2 (m, 1H, $(\text{CH}_3)_2\text{CH}$), 4.2 (t, 3H, OCH_2CH_3), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.1 (br.d, 1H, NH).

N-Acetyl-*DL*-valine octyl ester (**1l**). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (m, 9H, $(\text{CH}_3)_2\text{C}$, CH_3), 1.4 (m, 10H, CH_2), 1.7 (m, 2H, CH_2), 2.0 (s, 3H, COCH_3), 2.2 (m, 1H, $(\text{CH}_3)_2\text{CH}$), 4.2 (t, 3H, OCH_2CH_3), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.2 (br.d, 1H, NH).

N-Acetyl-*DL*-isoleucine methyl ester (**1m**). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (m, 6H, $(\text{CH}_3\text{CH}_2)\text{CH}_3$), 1.2–1.5 (m, 2H, CH_2), 1.9 (m, 1H, $(\text{CH}_3\text{CH}_2)\text{CHCH}_3$), 2.0 (s, 3H, COCH_3), 3.8 (s, 3H, OCH_3), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.0 (br.d, 1H, NH).

N-Acetyl-*DL*-isoleucine ethyl ester (**1n**). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (m, 6H, $(\text{CH}_3\text{CH}_2)\text{CH}_3$), 1.3–1.5 (m, 5H, CH_3 , CH_2), 1.9 (m, 1H, $(\text{CH}_3\text{CH}_2)\text{CHCH}_3$), 2.0 (s, 3H, COCH_3), 4.2 (q, 2H, OCH_2CH_3), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.1 (br.d, 1H, NH).

N-Acetyl-*DL*-isoleucine propyl ester (**1o**). $^1\text{H-NMR}$ (CDCl_3) δ 1.0 (m, 9H, $(\text{CH}_3\text{CH}_2)\text{CH}_3$, CH_3), 1.3–1.7 (m, 4H, CH_2), 1.9 (m, 1H, $(\text{CH}_3\text{CH}_2)\text{CHCH}_3$), 2.1 (s, 3H, COCH_3), 4.2 (t, 2H, OCH_2), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.1 (br.d, 1H, NH).

N-Acetyl-*D*-isoleucine butyl ester (**2p**). $^1\text{H-NMR}$ (CDCl_3) δ 0.8 (m, 9H, $(\text{CH}_3\text{CH}_2)\text{CH}_3$, CH_3), 1.3–1.7 (m, 6H, CH_2 , CH_2), 1.8 (m, 1H, $(\text{CH}_3\text{CH}_2)\text{CHCH}_3$), 2.0 (s, 3H, COCH_3), 4.0 (t, 2H, OCH_2), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.0 (br.d, 1H, NH). $[\alpha]_{\text{D}}^{25} = -9.4$ ($c=1$, methanol), e.e. 68.8%.

N-Acetyl-*L*-isoleucine (**3p**). $[\alpha]_{\text{D}}^{25} = +9.2$ ($c=1$, water), mp=149°C, (lit.¹⁴ $[\alpha]_{\text{D}}^{25} = +14.9$ ($c=2$, ethanol), mp=150°C), e.e. 61.9%.

N-Acetyl-*DL*-isoleucine hexyl ester (**1q**). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (m, 9H, $(\text{CH}_3\text{CH}_2)\text{CH}_3$, CH_3), 1.3–1.7 (m, 8H, CH_2 , CH_2), 1.8 (m, 1H, $(\text{CH}_3\text{CH}_2)\text{CHCH}_3$), 2.0 (s, 3H, COCH_3), 4.2 (t, 2H, OCH_2), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.0 (br.d, 1H, NH).

N-Acetyl-*DL*-isoleucine octyl ester (**1r**). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (m, 9H, $(\text{CH}_3\text{CH}_2)\text{CH}_3$, CH_3), 1.3–1.7 (m, 10H, CH_2 , CH_2), 1.8 (m, 1H, $(\text{CH}_3\text{CH}_2)\text{CHCH}_3$), 2.0 (s, 3H, COCH_3), 4.1 (t, 2H, OCH_2), 4.6 (m, 1H, $\alpha\text{-CH}$), 6.1 (br.d, 1H, NH).

D-*N*-acetyl-phenylglycine butyl ester (**2s**). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (t, 3H, CH_3), 1.4 (m, 2H, CH_2), 1.6 (m, 2H, CH_2), 2.0 (s, 3H, COCH_3), 4.2 (t, 2H, OCH_2), 5.6 (d, 1H, $\alpha\text{-CH}$), 6.6 (br.d, 1H, NH), 7.4 (s, 5H, H_{Ar}). $[\alpha]_{\text{D}}^{25} = -14.4$ ($c=1$, methanol); e.e. >99%.

L- α -(Acetylamino)benzeneacetic acid (**3s**). $[\alpha]_{\text{D}}^{25} = +165$ ($c=1$, 1 N NaOH), mp=193–194°C; (lit.¹⁶ $[\alpha]_{\text{D}}^{25} = +189$ ($c=1$, 1 N NaOH), mp=194–195°C); e.e. 87.5%.

N-Acetyl-*D*-phenylalanine butyl ester (**2t**). $^1\text{H-NMR}$ (CDCl_3) δ 1.0 (t, 3H, CH_3), 1.4 (m, 2H, CH_2), δ 1.7 (m, 2H, CH_2), 2.0 (s, 3H, COCH_3), 3.2 (d, 2H, CH_2Ph), 4.2 (t, 2H, OCH_2), 4.9 (m, 1H, $\alpha\text{-CH}$), 6.2 (br.d, 1H, NH), 7.3 (m, 5H, H_{Ar}). $[\alpha]_{\text{D}}^{25} = -7.4$ ($c=1$, methanol); e.e. >99%.

N-Acetyl-*L*-phenylalanine (**3t**). $[\alpha]_{\text{D}}^{25} = +47.2$ ($c=1$, methanol), mp=170°C; (lit.¹⁷ $[\alpha]_{\text{D}}^{25} = +50.4$ ($c=2$, methanol), mp=171–172°C); e.e. 93.7%.

N-Acetyl-D-tyrosine butyl ester (2u). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (t, 3H, CH_3), 1.4 (m, 2H, CH_2), 1.6 (m, 2H, CH_2), 2.0 (s, 3H, COCH_3), 3.0 (m, 2H, CH_2Ph), 4.2 (t, 2H, OCH_2), 4.8 (m, 1H, $\alpha\text{-CH}$), 6.2 (br.d, 1H, NH), 6.7–7.0 (dd, 4H, H_{Ar}). $[\alpha]_{\text{D}}^{25} = -19.1$ (c=1, methanol); e.e.>99%.

N-Acetyl-L-tyrosine (3u). $[\alpha]_{\text{D}}^{25} = +46.5$ (c=1, methanol), mp=152–153°C (lit.¹⁸ $[\alpha]_{\text{D}}^{25} = +48.3$ (c=2, water), mp=153–154°C); e.e. 96.3%.

D-N-acetyl-4-methoxyphenylalanine butyl ester (2v). $^1\text{H-NMR}$ (CDCl_3) δ 0.9 (t, 3H, CH_3), 1.4 (m, 2H, CH_2), 1.6 (m, 2H, CH_2), 2.0 (s, 3H, COCH_3), 3.0 (d, 2H, CH_2Ph), 3.8 (s, 3H, OCH_3), 4.1 (t, 2H, OCH_2), 4.8 (m, 1H, $\alpha\text{-CH}$), 5.8 (br.d, 1H, NH), 6.8–7.0 (dd, 4H, H_{Ar}). $[\alpha]_{\text{D}}^{25} = -5.5$ (c=1, methanol); e.e.>99%.

L-N-acetyl-4-methoxyphenylalanine (3v). $[\alpha]_{\text{D}}^{25} = +32.2$ (c=1, methanol).

Acknowledgements

We are thankful to CSIR, New Delhi for financial support and grant of Senior Research Fellowship to Vasudev Jadhav.

References

1. E. Schoffers, A. Golebiowski and C. R. Johnson, *Tetrahedron*, **1996**, 52, 3769.; K. Drauz and H. Waldmann (Eds.), *Enzyme Catalysis in Organic Synthesis*, VCH Publishers Inc. New York, **1995**.
2. J. R. Green, *Proc. Roy. Soc. London*, **1890**, 48, 370; T. P. Singer, *J. Biol. Chem.*, **1948**, 11, 174.
3. R. L. Ory, A. J. St. Angelo and A. M. Attschul, *J. Lipid. Res.*, **1960**, 1, 20.
4. M. Funatsu, Y. Aizono, K. Hayashi, M. Watanabe and M. Eto., *Agri. Biol. Chem.*, **1971**, 35, 5734; Y. Aizono, M. Funatsu, M. Sugano, K. Hayashi and Y. Fujiki, *Agr. Biol. Chem.*, **1973**, 37, 2031.
5. J. V. Prabhakar and K. V. L. Venkatesh, *J. Am. Oil Chem. Soc.*, **1986**, 66, 644; Nasirullah, M. N. Krishnamurthy and K. V. Nagaraj, *J. Am. Oil Chem. Soc.*, **1989**, 66, 661.
6. J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids*, Wiley and Sons, New York, **1961**, p. 715; H. K. Chenault, J. Dahmer and G. M. Whitesides, *J. Am. Chem. Soc.*, **1989**, 111, 6354.
7. G. W. Schwert, H. Neurath, S. Kaufman and J. E. Snoke, *J. Biol. Chem.*, **1948**, 172, 221.
8. N. W. Fadnavis, N. P. Reddy and U. T. Bhalerao, *J. Org. Chem.*, **1989**, 54, 3218.
9. T. Miyazawa, T. Takitani, S. Ueji, T. Yamada, and S. Kuwata, *J. Chem. Soc. Chem. Commun.*, **1988**, 1214.
10. E. Guibe-Jampel, G. Rousseau and J. Sataun, *J. Chem. Soc. Chem. Commun.*, **1987**, 1080.
11. L. Poppe and L. Novak (Eds.), *Selective Biocatalysis*, VCH Publishers Inc. New York, **1992**, p. 91; K. Drauz and H. Waldmann (Eds.), *Enzyme Catalysis in Organic Synthesis*, VCH Publishers Inc. New York, **1995**, p. 201.
12. *Methods in Enzymol.* 2, 55.
13. N. W. Fadnavis and K. Koteswar, *Tetrahedron: Asymmetry*, **1997**, 8, 337; K. Faber, G. Ottolina and S. Riva, *Biocatalysis*, **1993**, 8, 91.; U. T. Bhalerao, L. Dasaradhi, P. Neelkathan and N. W. Fadnavis, *J. Chem. Soc. Chem. Commun.*, **1987**, 838.
14. S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **1952**, 194, 455.
15. T. W. Pattabiraman and W. B. Lawson, *Biochem. J.*, **1972**, 48, 695.
16. S. G. Cohen, R. M. Schultz and Y. S. Weinstein, *J. Am. Chem. Soc.*, **1966**, 88, 5315.
17. W. H. Schuller and C. Niemann, *J. Am. Chem. Soc.*, **1951**, 73, 1644.
18. J. H. Barnes, R. C. Cookson, G. T. Dickson, J. Elks and V. D. Poole, *J. Chem. Soc.*, **1953**, 1448.

(Received in UK 6 May 1997)