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Kinetic Resolution of Racemic Benzaldehyde Cyanohydrin via Stereoselective Acetylation Catalyzed by Lipase in Organic Solvent

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Cyanohydrins are important key intermediates for the syntheses of drugs or biologically active compounds because they can be easily converted into variety of valuable chiral synthons such as β-aminoalcohols,[1] α-hydroxyacids,[2] and α-hydoxy-ketones.[3]   Several methods to prepare the chiral cyanohydrins by enzymatic[4] or microbial[5] hydrolysis of racemic esters thereof have been attempted in aqueous medium.   In these methods, the unreacted esters were recovered in high optical and chemical yields, but cyanohydrins were obtained in low yields or not isolated because they are susceptible to decomposition or racemization in aqueous medium.

We are therefore studying a kinetic resolution of cyanohydrins in organic solvent in order to overcome the disadvantages occurred in the aqueous systems.   Recently we demonstrated[6] that enol esters acylated various types of alcohol in the presence of lipase in diisopropyl ether.   As an extended application of these reactions, we here report the kinetic resolution of benzaldehyde cyanohydrin (mandelonitrile) via lipase-catalyzed stereoselective acetylation using isopropenyl acetate as an acylating reagent†
(Scheme 1).

Scheme 1.

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† Very recentry, Wong *et al.*[9] reported the resuolution of the cyanohydrins to provide the starting materials of β-blocker by lipase-catalyzed transesterification using enol esters as an acylating reagent.

Table 1. The screening of the commercial lipase preparations for stereoselective acetylation of mandelonitrile (**1**)[a]

origin of lipase	supplier	react. time (h)	conversion[b] (%)	e.e.[c] (S)-2 (%)	E
Pseudomonas sp.[d]	Nagase Biochemicals	22	32	96	77
Cromobacterium viscosum[e]	Toyo Jozo	38	38	93	49
Pseudomonas sp.[e]	Toyobo	38	28	92	34
Pseudomonas sp.[e]	Kurita	20	29	88	22
Pseudomonas fluorescens[d]	Amano Pharm.	25	57	64	12
Pseudomonas sp.[e]	Toyo Jozo	38	66	47	8

a) Conditions: (±)-mandelonitrile (300 mg, s.s 2.25 mmol), isopropenyl acetate (11.27 mmol), lipase preparations (about 30 mg), dry diisopropyl ether (11.3 ml), 40°C.
b) Determined by GC using ethylbenzene as an internal standard.
c) Determined by an HPLC analysis (CHIRAL CEL OB, hexane (9): iPrOH(1), 0.5 ml/min, 254 nm) of the reaction mixture.
d) A commercial lipase preparation was used as such.
e) A received lyophlized enzyme powder was dissolved in 5 mM potassium phosphate buffer and adsorbed on celite diatomite (Hyflosuper-cel).

As a preliminary experiment, six commercially available preparations of lipase were tested for the acetylation of mandelonitrile (±)-(**1**). The results are summarized in Table 1. Conversion of the reactions was determined by GC using ethylbenzene as an internal standard. Enantiomeric excess (e.e.) of 1-cyano-1-phenylmethyl acetate (mandelonitrile acetate) (**2**) was calculated from HPLC analysis of the reaction mixtures on optically active column (see experimental). From HPLC chromatogram, all enzymatically produced **2** was proved to have S configuration. Among the lipase preparations tested, a preparation from *Pseudomonas fluorescens* (Amano Pharm. Co., Ltd.) was higher in chemical conversion. A preparation from *Pseudomonas* sp. (Nagase Biochemicals Ltd.) showed high stereoselectivity based on the E values, enantiomeric ratio[7]; lipases from *Pseudomonas* sp. (Toyobo Co., Ltd.) and from *Chromobacterium viscosum* (Toyo Jozo Co., Ltd.) were in moderate range of stereoselectivity. Three preparations from *P. fluorescens* (Amano) and *Pseudomonas* sp. (Toyobo and Nagase) were therefore selected for the kinetic resolution of racemic mandelonitrile.

The results of the kinetic resolution on preparative scale were summarized in Table 2. When the proper conversion of mandelonitrile to acetate was attained, lipase powder was filtered off. The filtrate was concentrated *in vacuo* and chromatographed on silica gel to separate mandelonitrile and acetate. The e.e. values of (+)-**1** were determined by comparing the observed specific rotations with the reported[8] value or by ¹H-NMR analyis of the corresponding MTPA-esters. The absolute configuration of (+)-**1** was proved to be R by the (+) sign of the rotation. On the other hand, the optical purities of the acetate (−)-**2** were determined by ¹H-NMR analysis in the presence of chiral shift reagent (see experimental). From the HPLC analysis using optically active column, the absolute configuration of (−)-**2** was revealed to be S by comparing with an authentic sample of (R)-isomer. Lipase from *Pseudomonas* sp. (Toyobo Co., Ltd.) attained 48% conversion for 53 h at 40°C

Table 2. Kinetic resolutions of $(\pm)-1$ via lipase-catalyzed stereoselective acetylation[a]

entry	origin of lipase	supplier	react. time (h)	conver-[b] sion (%)	(S)-(−)-acetate 2			(R)-(+)-cyanohydrin 1		
					yield[c] (%)	$[\alpha]_D$ in CHCl$_3$	e.e.[d] (%)	yield[c] (%)	$[\alpha]_D$ in CHCl$_3$	e.e. (%)
1	Pseudomonas sp.	Toyobo	53	48	46	−5.9[e]	83	49	+36.4°[f]	78[g]
2	Pseudomonas sp.	Nagase	79	22	23	−6.8[h]	94	75	+12.8°[i]	27[g]
3[j]	P. fluorescens	Amano	65	41	47	−5.8°[k]	80	35	+34.6°[l]	72[g][m]

a) Conditions: $(\pm)-1$ (1.00–5.33 g, 7.51–40.0 mmol), isopropenyl acetate (30–80 mmol), lipase preparations (0.76–4.0 g), diisopropyl ether (38–200 ml), 40°C or 23°C.
b) Determined by GC using ethylbenzene as an internal standard.
c) Isolated yield based on $(\pm)-1$.
d) Determined by a ^{1}H-NMR analysis in the presence of chiral shift reagent, Eu(hfc)$_3$.
e) At 25°C, c 5.03.
f) At 22°C, c 5.00.
g) Determined by comparing the rotation value with the reported.[9]
h) At 21°C, c 5.29.
i) At 21°C, c 5.01.
j) The mixture of cyclohexane (4): diisopropyl ether (1) was used as reaction solvent.
k) At 24°C, c 5.02.
l) At 24°C, c 5.06.
m) Determined by a ^{1}H-NMR analysis of the corresponding MTPA-ester.

to afford (S)-(−)-2 with 83% e.e. in 46% isolated yield; the (R)-(+)-1 with 78% e.e. was recovered in 49% isolated yield. With lipase from Pseudomonas sp. (Nagase Biochemicals Ltd.), the reaction proceeded slowly, but the stereoselectivity was high enough to give (−)-2 in 94% e.e.

In conclusion, a kinetic resolution of mandelonitrile was accomplished through lipase-catalyzed transesterification with isorpopenyl acetate in organic solvent. (S)-Mandelonitrile acetate with 80–94% optical purity was obtained in high chemical yields. The unreacted (R)-mandelonitrile was also recovered in high chemical yields.

EXPERIMENTAL

The optical rotations were measured with Perkin-Elmer 241 polarimeter. The conversion of the reactions was determined by GC analysis on Shimadzu GC-14A equipped with capillary column DB-5 (0.25 μm thick, 0.25 mm × 30 m). The e.e.'s of acetate 2 were analyzed by HPLC on optically active column (CHIRAL CEL OB, 4.6 × 250 mm, Daicel Co. Ltd., hexane(9):iPrOH(1), 0.5 ml/min, 254 nm). The retention times of the enantiomers were 32 min for (S)-2 and 40 min for (R)-2. Diisopropyl ether was distilled over CaH$_2$ and stored over 4Å molecular sieves. Isopropenyl acetate was distilled before use; its purity was ascertained by GC and ^{1}H-NMR.

Lipase-catalyzed acetylation of mandelonitrile (1); typical procedure. Mandelonitrile (5.33 g, 40 mmol) and isopropenyl acetate (8.01 g, 80 mmol) were dissolved in the mixture of cyclohexane (160 ml) and diisopropyl ether (40 ml) in the presence of ethylbenzene (1.06 g, 10 mmol) as an internal standard for GC analysis. Lipase from Pseudomonas fluorescens (Amano Pharm. Co., Ltd.) (4.0 g) was suspended in the

solution. The suspension was stirred for 27 h at 40°C and then for 38 h at 23°C. The reaction mixture was filtered and the lipase powder was washed twice with diisopropyl ether. The combined filtrate was concentrated *in vacuo* and chromatographed on silica gel (hexane:AcOEt=12:1–10:1) to afford (*S*)-(−)-**2** as clear oil (3.27 g, 47% yield) and (*R*)-(+)-**1** (1.85 g, 35% yield).

(*R*)-(+)-*Mandelonitrile.* $[\alpha]_D^{24} = +34.6°$ (*c* 5.06, CHCl$_3$). (Lit[8] $[\alpha]_D^{25} = +43.5°$ (*c* 5, CHCl$_3$) for the *R* isomer having 92.5% e.e.) ^{1}H-NMR (200 MHz) $\delta = 3.42$ (1H, d, *J*=6.8 Hz, OH), 5.51 (1H, d, *J*=6.8 Hz, CH), and 7.38–7.55 (5H, m, aromatic H). The e.e. value was determined as 72% by ^{1}H-NMR analysis of the corresponding MTPA-ester. The multiplet signals of methoxy proton were base line separated ($\delta = 3.46$ for the (*R*)-**1** containing diastereomer; $\delta = 3.58$ for the (*S*)-**1**).

(*S*)-(−)-*Mandelonitrile acetate.* $[\alpha]_D^{24} = -5.8°$ (*c* 5.02, CHCl$_3$). ^{1}H-NMR (200 MHz) $\delta = 2.17$ (3H, s, OAc), 6.41 (1H, s, CH), and 7.42–7.56 (5H, m, aromatic H). The e.e. value was determined as 80% by the ^{1}H-NMR analysis in the presence of chiral shift reagent, Eu(hfc)$_3$. A pair of the signals of acetyl group were base line separated ($\delta = 2.88$ for the (*S*)-**2** and $\delta = 3.00$ for the (*R*)-**2**).

REFERENCES

(1) W. Becker, H. Freund, and E. Pfeil, *Angew. Chem., Int. Ed. Engl.*, **4**, 1079 (1965).
(2) B.B. Corson, R.A. Dodge, S.A. Harris, and J.S. Yeaw, *Org. Synth., coll. vol.* I, p 336.
(3) J. Brussee, E.C. Roos, and A. van der Gen, *Tetrahedron Lett.*, **29**, 4485 (1988).
(4) N. Matsuo and N. Ohno, *Tetrahedron Lett.*, **26**, 5533 (1985).
(5) H. Ohta, S. Hiraga, K. Miyamoto and the late G. Tsuchihashi, *Agric. Biol. Chem.*, **52**, 3023 (1988); H. Ohta, Y. Miyamae, and the late G. Tsuchihashi, *Agric. Biol. Chem.*, **53**, 215 (1989); H. Ohta, Y. Miyamae, and the late G. Tsuchihashi, *Agric. Biol. Chem.*, **53**, 281 (1989).
(6) J. Hiratake, M. Inagaki, T. Nishioka, and J. Oda, *J. Org. Chem.*, **53**, 6130 (1988); M. Inagaki, J. Hiratake, T. Nishioka, and J. Oda, *Agric. Biol. Chem.*, **53**, 1879 (1989).
(7) C.-S. Chen, Y. Fujimoto, G. Girdaukas, and C.J. Sih, *J. Am. Chem. Soc.*, **104**, 7294 (1982).
(8) J.D. Elliott, V.M.F. Choi, and W.S. Johnson, *J. Org. Chem.*, **48**, 2294 (1983).
(9) Y.-F. Wang, S.-T. Chen, K.K.-C. Liu, and C.-H. Wong, *Tetrahedron Lett.*, **30**, 1917 (1989).