LIPASE-CATALYZED RESOLUTION OF ACETATES OF RACEMIC PHENOLIC APORPHINES AND HOMOAPORPHINES IN ORGANIC SOLVENT#

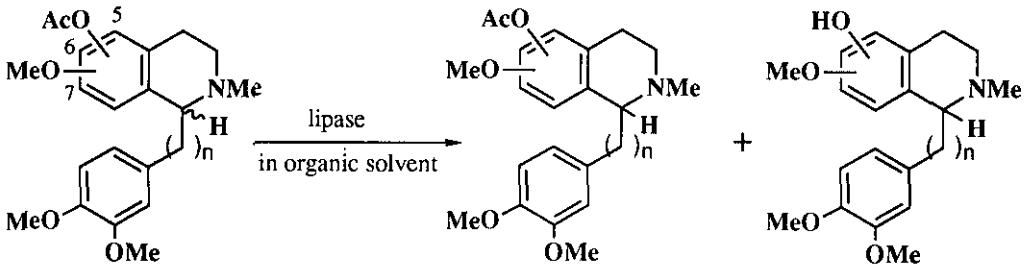
Osamu Hoshino*, Hiroyuki Fuchino, and Masafumi Kikuchi

Faculty of Pharmaceutical Sciences, Science University of Tokyo,
12, Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan

Abstract--- Enzymatic resolution of (±)-1-acetoxy-2,9,10-trimethoxyaporphine (*O*-acetylthaliporphine)(**3**), (±)-2-acetoxy-1,9,10-trimethoxyaporphine (*O*-acetylpredicentrine)(**4**), and (±)-3-acetoxy-2,9,10-trimethoxyaporphines (**5**) by use of immobilized lipase in organic solvent gave resolved **3-5** and the corresponding hydroxyaporphines (**9-11**) in fair to good chemical and optical yields. Analogous reaction of (±)-1-acetoxy-2,10,11-trimethoxyhomoaporphine (**6**) did not take place, whereas that of (±)-2-acetoxy-1,10,11-trimethoxy- and (±)-3-acetoxy-2,10,11-trimethoxyhomoaporphines (**7** and **8**) produced optical active **7**, **8** and hydroxyhomoaporphines (**13**, **14**).

Kinetic resolution of prochiral acylates and esters using biocatalysts[1] is well known to be one of useful methods for preparation of chiral building blocks. Recently,[2] we have reported that enzymatic resolution of acetates of prochiral phenolic 1-substituted 1,2,3,4-tetrahydro-2-methylisoquinolines (**1**) by using immobilized lipase in organic solvent affords resolved **1** and the corresponding phenols (**2**) in moderate chemical and optical yields. In order to examine the limitation and scope of this methodology, the reaction of acetates (**3-5** and **6-8**) of phenolic (±)-aporphines (**9-11**) and (±)-homoaporphines (**12-14**) under conditions similar to those reported previ-

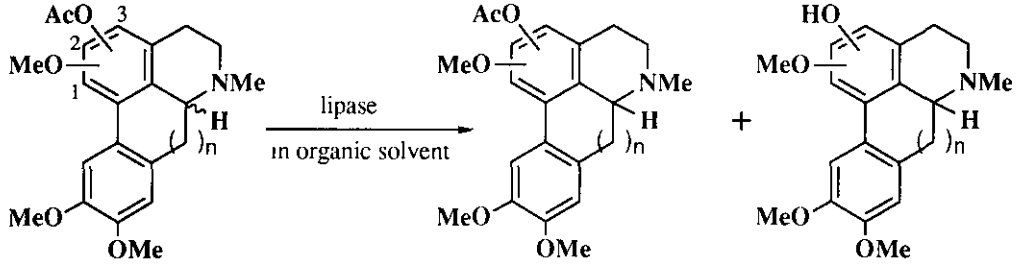
Dedicated to Dr. Arnold Brossi on the occasion of his 70th birthday.



(*R*,*S*)-**1**, n=0-2 (*R*) or (*S*)-**1**, n=0-2 (*S*) or (*R*)-**2**, n=0-2

ously[2] was investigated. The present paper deals with lipase-catalyzed resolution of acetates of phenolic (±)-aporphines and (±)-homoaporphines in organic solvent.

As substrates for this resolution, (±)-1-, (±)-2-, and (±)-3-acetoxyaporphines (**3**,[3] **4**,[4] **5**) and (±)-1-, (±)-2-, and (±)-3-acetoxyhomoaporphines (**6**,[5] **7**,[6] **8**) were synthesized according to a lead tetraacetate oxidation method.[7]



(*R*,*S*)-**3**:	n=1, 1-OAc, 2-OMe	(*R*)-**3**	(*S*)-**9**
(*R*,*S*)-**4**:	n=1, 2-OAc, 1-OMe	(*R*) or (*S*)-**4**	(*S*) or (*R*)-**10**
(*R*,*S*)-**5**:	n=1, 3-OAc, 2-OMe	(*R*)-**5**	(*S*)-**11**
(*R*,*S*)-**6**:	n=2, 1-OAc, 2-OMe	(*R*,*S*)-**6**	(**12**)
(*R*,*S*)-**7**:	n=2, 2-OAc, 1-OMe	(*R*)-**7**	(*S*)-**13**
(*R*,*S*)-**8**:	n=2, 3-OAc, 2-OMe	(*R*)-**8**	(*S*)-**14**

At first, screening of all substrates (**3-8**) using thirteen kinds of lipases [Amano A-6 (*Asperigillus niger*), Amano P (*Pseudomonas fluorescens*), Amano F-AP-15 (*Rhizopus japonicus*), Amano M-10 (*Mucol japonicus*), Lipase F (*Rhizopus niveus*), Pancreatin F (*Porcine Pancreas*), Lipase No. L-3126 (*Porcine Pancreas*), Lipase

No. L-3001 (*Wheat Germ*), Amano GC-20 (*Geotrichum candidum*), Lipase No. L-1754 (*Candida cylindracea*), OF-360 (*Candida cylindracea*), MY-30 (*Candida cylindracea*), and Amano AY-30 (*Candida cylindracea*)] in benzene-isooctane saturated with water was performed by monitoring formation of phenol on tlc. Consequently, it was observed that although all the lipases did not hydrolyze (±)-1-acetoxyhomoaporphine (**6**) [(±)-**3** was found to be hydrolyzed in a prolonged reaction time], Amano A-6 and lipases from *Candida cylindracea* did (±)-2- and (±)-3-acetoxyaporphines (**4**, **5**) and (±)-2- and (±)-3-acetoxyhomoaporphines (**7**, **8**). It would be due to a steric hindrance of acetoxyl group at the 1-position that hydrolysis of (±)-**6** did not take place.

Table 1. Enzymatic Resolution of (±)-Acetoxyaporphines (**4**, **5**) and Homoaporphines (**7**, **8**) with Lipase in Organic Solvent[a)]

Entry	Substrate	Lipase[b)]	Reaction Time (h)	Yield (Optical Yield)		Abs. Config. of Phenol
				Acetate % (%ee)	Phenol % (%ee)	
1		A	0.5	45 (70)	46 (56)	*R*
2		A	1.0	20 (86)	66 (28)	*R*
3	**4**	B	0.6	**4** 59 (56)	**10** 39 (97)	*S*
4		C	0.6	51 (80)	45 (79)	*S*
5		D	0.6	57 (55)	35 (92)	*S*
6		A	1.3	41 (91)	52 (48)	*S*
7	**5**	B	24.0	**5** 28 (59)	**11** 9 (87)	*S*
8		E	24.0	59 (44)	25 (71)	*S*
9		A	0.3	29 (36)	54 (19)	*S*
10	**7**	B	5.3	**7** 54 (54)	**13** 40 (71)	*S*
11		C	5.0	25 (72)	57 (36)	*S*
12		A	0.6	75 (27)	16 (78)	*S*
13		A	1.3	64 (32)	26 (61)	*S*
14	**8**	B	24.0	**8** 72 (30)	**14** 17 (90)	*S*
15		D	24.0	55 (75)	39 (86)	*S*
16		F	1.6	32 (83)	51 (53)	*S*

a) All reactions were carried out at 33 °C in benzene-isooctane (1 : 5) saturated with water.

b) A : Amano A-6 ; B : No. L-1754 ; C : OF-360 ; D : Amano AY-30 ; E : MY-30 ; F : No. L-3001.

As lipases useful for hydrolysis of acetoxyaporphines and homoaporphines except (±)-1-acetoxy congeners (**3**, **6**) were found by screening, we tried the reaction with the lipase selected under conditions similar to those reported previously.[2] After spots of phenol and starting material on tlc were observed as a ratio of approximately 1 : 1, the reaction mixture was filtered by suction. A residue obtained after usual workup was purified by Sephadex LH-20 column chromatography followed by preparative tlc. Optical yield of each product was estimated by hplc analysis using CHIRALCEL® of phenol or acetate or by ^{1}H nmr analysis of (*R*)-MTPA ester of the cor-responding phenol after alkaline hydrolysis for acetates (**4**-**5**, **7**) or acetylation for phenol (**14**). The results are listed in Table 1.

Among the lipases used, Amano A-6 was found to hydrolyze all substrates (Table 1, Entries 1, 2, 6, 9) faster than other lipases except (±)-**8** (Table 1, Entries 12, 13). It is noteworthy that the reaction (Table 1, Entries 3-5) of (±)-**4**, bearing an acetoxyl group at the farest distance from a prochiral center (the 6a-position), with lipase from *Candida cylindracea* gave the most satisfactory results.

As mentioned above, the lipases for hydrolysis of (±)-1-acetoxyaporphine (**3**) and homoaporphine (**6**) could not be searched by examination on tlc. However, with (±)-**3**, the prolonged reaction time[8] (9 days) and repeated addition of lipase resulted in formation of phenol (**9**). The results are shown in Table 2.

Table 2. Enzymatic Resolution of (±)-1-Acetoxyaporphines (**3**) with Lipase in Organic Solvent[a)]

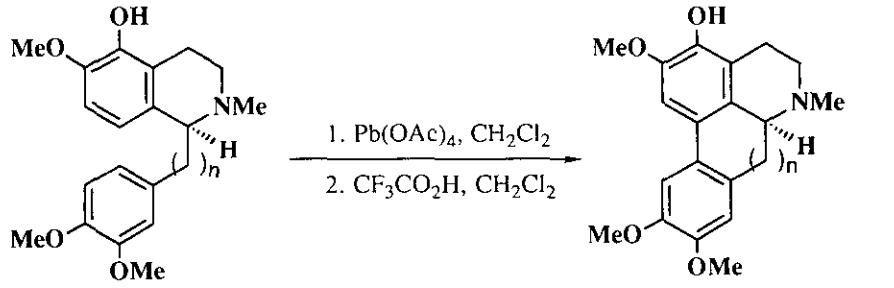
Entry	Lipase[b)]	Solvent[c)]	Yield (Optical Yield)		Abs. Config. of Phenol (**9**)
			Acetate (**3**) % (%ee)	Phenol (**9**) % (%ee)	
1	A	T / C	73 (23)	27 (77)	*S*
2	G	B / I	79 (11)	12 (69)	*S*
3	G	B / C	61 (21)	25 (57)	*S*
4	G	T / I	70 (14)	23 (61)	*S*
5	G	T / C	74 (12)	21 (58)	*S*

a) All reactions were carried out at 33 °C for 9 days. b) A : Amano A-6 ; G : No. L-3126.

c) T / C : toluene / cyclohexane (1 : 7) ; B / I . benzene / isooctane (1 : 7) ; B / I : benzene / isooctane (1 : 7) ; T / I : toluene / isooctane (1 : 7).

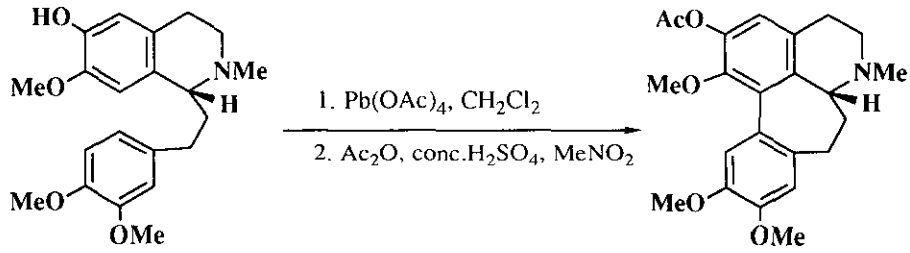
In these cases, Amano A-6 and lipase from *Porcine Pancreas* were found to be effective.

Absolute configuration of (+)-**9** was determined to be *S*-configuration by comparison of sign of its specific rotation with that reported in the literature,[9] while that of (+)-**10** was proved to be also *S*-configuration on the basis of the findings[9] on relationship between absolute configuration and sign of specific rotation. Absolute configuration of (-)-**14** was determined by transformation of (*S*)-(-)-**17**[2] into (*S*)-(-)-**14**. On the other hand, (*S*)-(+)-**11** and (*S*)-(+)-**7** were characterized by evidence that (*R*)-(-)-**15**[2] and (*R*)-(+)-**16**[2] were converted to (*R*)-(-)-**11** and (*R*)-(-)-**7**, which are enantiomers of acetates (**11** and **7**) obtained in the enzymatic reaction.



(*R*)-(-)-**15**, n=1
(*S*)-(-)-**17**, n=2

(*R*)-(-)-**11**, n=1
(*S*)-(-)-**14**, n=2



(*R*)-(+)-**16**

(*R*)-(-)-**7**

In conclusion, kinetic resolution of acetates of phenolic (±)-aporphines and (±)-homoaporphines except (±)-1-acetoxyhomoaporphine with Amano A-6 and lipase from *Candida cylindracea* was proved to take place in fair to good chemical and optical yields. Considering that all reactions excluding reaction (Table 1, Entries 1, 2) of (±)-**4** with Amano A-6 gave (*S*)-hydroxyaporphines and homoaporphines, the present reaction could be served as a method for determination of absolute configuration of certain hydroxyaporphines and homoaporphines.

ACKNOWLEDGEMENT

The authors are grateful to Professor T. Oishi, the President of Meiji College of Pharmacy, and Professor H. Akita, Faculty of Pharmaceutical Sciences, Toho University, for their valuable discussion. They are indebted to Amano Pharmaceutical Co., Ltd., and Meito Sangyo Co., Ltd., for kindly providing lipases. Thanks are also due to Miss N. Sawabe and Mrs. F. Hasegawa, this faculty, for ^{1}H nmr and mass spectral measurements and to Sankyo Co., Ltd., for elementary analysis.

EXPERIMENTAL

All melting points were measured on a Büchi melting point measuring apparatus and are uncorrected. ^{1}H Nmr spectra were taken on a JOEL FX-100 (100 MHz) or JOEL GSX-500 (500 MHz) spectrometer in $CDCl_3$ solution using TMS as internal standard. Ir spectra were run with a Hitachi 260-10 infrared spectrophotometer in $CHCl_3$ solution. Ms were taken with a Hitachi RMU-7M mass spectrometer. Specific rotation was run on a Nippon Bunko DIP-360 polarimeter in $CHCl_3$ solution. Hplc was carried out with a Senshu Kagaku instrument equipped with uv detector 3000A-II using CHIRALCEL® OD or OJ (Daicel Chemicals). Preparative tlc was run on Kieselgel F-250 (Merck) and column chromatography was performed using Sephadex LH-20 (Pharmacia Fine Chemicals), unless otherwise noted. Lipases (No. L-1754, No. L-3001, and No. L-3125) were purchased from Sigma Chemical Company.

(±)-3-Acetoxy-2,9,10-trimethoxyaporphine (5)---- Acetylation of (±)-**11**[10] in a usual manner gave (±)-**5**, mp 116-118 °C (ether-hexane) as pale yellow prisms. ^{1}H Nmr δ : 2.35 (3H, s, OAc), 2.56 (3H, s, NMe), 3.89, 3.92, 3.96 (each 3H, s, 3 x OMe), 6.76, 7.02, 7.12 (each 1H, s, 3 x arom. H) ; ir : 1755 cm^{-1}; HRms *m/z* calcd for $C_{22}H_{25}NO_5$ (M^+) · 383 1731. Found : 383 1729

(±)-3-Acetoxy-2,10,11-trimethoxyhomoaporphine (8)---- Acetylation of (±)-**14**[10] in a usual manner gave (±)-**8**, mp 165.5-167 °C (EtOH) as colorless prisms. ^{1}H Nmr δ · 2 35 (3H, s, OAc), 2.37 (3H, s, NMe), 3.84, 3.90, 3.92 (each 3H, s, 3 x OMe), 6.75, 6.81, 6.87 (each 1H, s, 3 x arom. H); ir ; 1760 cm^{-1}. Anal. Calcd for $C_{23}H_{27}NO_5$; C, 69.50 ; H, 6.85 ; N, 3.52. Found : C, 69.43 ; H, 7.02 ; N, 3.48.

General Procedure for Enzymatic Resolution of (±)-Acetoxyaporphines (3-5) and Homoaporphines (7, 8)----The reaction of substrates (**3-5** and **7, 8**)(100 mg, 0.28 and 0.25 mmol) with lipase immobilized with celite [lipase (100 mg), H_2O (0.12 ml) and celite 535 (300 mg)] in organc solvent (30 ml for **4, 5, 7, 8** ; 35 ml for **3**) was carried out according to the procedure reported previously.[2] A residue obtained was

purified by column chromatography (benzene : MeOH = 5 : 2) followed by preparative tlc (developing solvent : benzene . AcOEt : MeOH = 10 : 10 : 3) to give acetates (**3-5**, **7**, **8**) and phenols (**9-11**, **13**, **14**), respectively. For the purpose of determining enantiomeric excess (ee), acetates (**3-5**, **7**) except **8** were hydrolyzed with 5% KOH-MeOH at room temperature to lead to phenols (**9-11**, **13**). Ee of each product was determined by calculation of each peak area obtained on hplc analysis (OD for **9**; i-PrOH : hexane = 9 : 1; flow rate : 0.5 ml / min; OJ for **11**; EtOH : hexane = 9 : 1; for **13** and **14** ; EtOH : hexane = 1 : 1; flow rate 0.5 ml / min), while that of (+)- and (-)-**10** was done by calculation of each peak (δ 3.33 and 3.45) due to methoxyl groups in (*R*)-MTPA esters of the corresponding phenols on the basis of 500 MHz ^{1}H nmr spectra. The results listed in Tables 1 and 2 are as follows.

With (±)-**4** : (1) (Table 1, Entry 1): Acetate (**4**)(45 mg, 45%, 70%ee) and phenol (**10**)(41 mg, 46%, 56%ee) were obtained. **10**: $[\alpha]_D$ -60.4° (c=1.4).

(2)(Table 1, Entry 3): Acetate (**4**)(59 mg, 59%, 56%ee) and phenol (**10**)(34 mg, 39%, 97%ee) were obtained. **10**: $[\alpha]_D$ +110.1° (c=0.9).

With (±)-**5** (Table 1, Entry 6)· Acetate (**5**)(41 mg, 41%, 91%ee) and phenol (**11**)(46.6 mg, 52%, 48%ee) were obtained. Hplc : Rt 12.0 min for (+)-**11**, Rt 17.8 min for (-)-**11**. **11**: $[\alpha]_D$ +26.1° (c=1.2).

With (±)-**7** (Table 1, Entry 9) : Acetate (**7**)(29 mg, 29%, 36%ee) and phenol (**13**)(47 mg, 54%, 19% ee) were obtained. Hplc : Rt 7.6 min for (+)-**13**; Rt 10.2 min for (-)-**13**. **13**:$[\alpha]_D$ +4.1° (c=0.3).

With (±)-**8** (Table 1, Entry 12) : Acetate (**8**)(75 mg, 75%, 27%ee) and phenol (**14**)(14.5 mg, 16%, 78%ee) were obtained. Hplc : Rt 10.6 min for (-)-**14**; Rt 12.8 min for (+)-**14** **14**: $[\alpha]_D$ -19.7° (c=0.7).

With (±)-**3** (Table 2, Entry 1): A mixture of (±)-**3** and lipase with celite in toluene-cyclohexane (1 : 7) was incubated. Furthermore, the same amounts of lipase were added to the mixture at intervals of 2, 3, and 2 days and incubation was continued for additional 2 days. After workup as noted above gave acetate (**3**)(73 mg, 73%, 23%ee) and phenol (**9**)(24 mg, 27%, 77%ee), respectively. Hplc Rt 8.6 min for (-)-**9**; Rt 13.4 min for (+)-**9**. **9**: $[\alpha]_D$ +36.4° (c=0.6).

(*R*)-(-)-3-Hydroxy-2,9,10-trimethoxyaporphine (11)---- The reaction of (*R*)-(-)-**15**[2] (41%ee, 150.5 mg, 0.44 mmol) according to the procedure reported previously[9] gave (*R*)-(-)-**11**, mp 210-213 °C (MeOH) [after purification by silica gel column chromatography ($CHCl_3$; MeOH = 100 : 1) followed by preparative tlc (developing solvent : benzene : AcOEt · MeOH = 10 : 10 : 3)]. $[\alpha]_D$ -11.7° (c=0.88) , ^{1}H nmr δ : 2.55 (3H, s, NMe), 3.90 (3H, s, OMe), 3.96 (6H, s, 2 x OMe), 6.77, 6.98, 7.08 (each 1H, s, 3 x arom. H); ir : 3550 cm^{-1}.

HRms *m/z* calcd for $C_{20}H_{23}NO_4$ (M^+) : 341.1625. Found : 341.1619. This material was identical in all respects except sign of specific rotation with a sample obtained in the enzymatic reaction.

(*S*)-(-)-3-Hydroxy-2,10,11-trimethoxyhomoaporphine (14)---- The reeaction of (*S*)-(-)-**17**[2] (38%ee, 38.7 mg, 0.11 mmol) in a way similar to that noted for (*R*)-(-)-**11** gave (*S*)-(-)-**14** (17.3 mg, 45%), mp 182-185 °C. $[\alpha]_D$ -9.0° (c=1.15) ; ^{1}H nmr δ : 2.40 (3H, s, NMe), 3.91 (9H, s, 3 x OMe), 6.74 (2H, s, 2 x arom. H), 6.81 (1H, s, arom. H); ir : 3530 cm^{-1}. HRms *m/z* calcd for $C_{21}H_{25}NO_4$ (M^+) : 355.1790. Found : 355.1786.

(*R*)-(-)-2-Acetoxy-1,10,11-trimethoxyhomoaporphine (7)---- Treatment of (*R*)-(+)-**16**[2] (39%ee, 154.6 mg, 0.44 mmol) according to the procedure reported previously[6] gave an oil, purification of which on silica gel column chromatography ($CHCl_3$; MeOH= 100 : 1 - 50 : 1) afforded (*R*)-(-)-**7** (58.2 mg, 42.3%). A part of it was purified by preparative tlc (developing solvent . benzene : AcOEt : MeOH = 10 : 10 : 3) to give an oil. $[\alpha]_D$ -5.6° (c=1.06) ; ^{1}H nmr δ : 2.34 (3H, s, OAc), 2.38 (3H, s, NMe), 3.29 (3H, s, 1-OMe), 3.84, 3.92 (each 3H, s, 2 x OMe), 6.76, 6.81, 7.04 (each 1H, s, 3 x arom. H); ir : 1760 cm^{-1}. HRms *m/z* calcd for $C_{23}H_{27}NO_5$ (M^+) : 397.1901. Found : 397.1894. This material was identical in all respects except sign of specific rotation with a sample obtained in the enzymatic reaction.

REFERENCES AND NOTES

1. Reviews: S. Servi, *Synthesis*, **1990**, 1 ; A. M. Klivanov, *Acc. Chem. Res.*, **1990**, *23*, 114 ; R. Csuk and B. I. Glänzer, *Chem. Rev.*, **1991**, *91*, 49 ; K. Faber and S Riva, *Synthesis*, **1992**, 895.
2. O. Hoshino, R. Tanahashi, M. Okada, H. Akita, and T. Oishi, *Tetrahedron : Asymm.*, **1993**, *4*, 933.
3. O. Hoshino, T. Toshioka, M. Ohtani, and B. Umezawa, *Chem Pharm. Bull* , **1974**, *22*, 1302.
4. O. Hoshino, M. Ohtani, and B. Umezawa, *Chem Pharm Bull.*, **1979**, *27*, 3101.
5. O. Hoshino, T. Toshioka, K. Ohyama, and B. Umezawa, *Chem. Pharm. Bull.*, **1974**, *22*, 1307.
6. O. Hoshino, K. Kikuchi, H. Ogose, B. Umezawa, and Y. Iitaka, *Chem Pharm. Bull.*, **1987**, *35*, 3666.
7. *Cf.* O. Hoshino and B. Umezawa, "The Alkaloids", Vol. 36, ed. by A. Brossi, Academic Press, Inc., 1989, Chapter 2.
8. Analogous reaction of (±)-**3** with Amano A-6 in toluene-cyclohexane (1 : 7) for 5 days gave (*R*)-**3** (84%, 13 %ee) and (*S*)-**9** (15%, 53%ee), respectively.
9. M. Shamma and M. J. Hillman, *Experimentia*, **1969**, *25*, 544.
10. H. Hara, H. Shinoki, T. Komatsu, O. Hoshino, and B. Umezawa, *Chem. Pharm. Bull.*, **1986**, *34*, 1924.

Received, 3rd February, 1994