

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

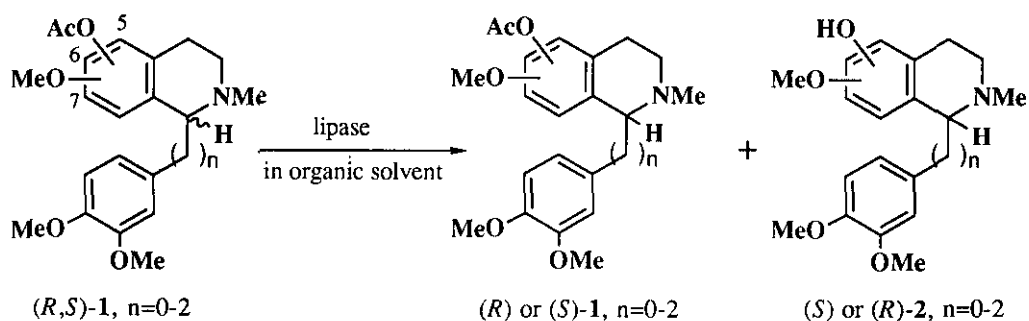
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Abstract--- Enzymatic resolution of (\pm)-1-acetoxy-2,9,10-trimethoxyaporphine (*O*-acetylthaliporphine)(3), (\pm)-2-acetoxy-1,9,10-trimethoxyaporphine (*O*-acetylpedicentrine)(4), and (\pm)-3-acetoxy-2,9,10-trimethoxyaporphines (5) by use of immobilized 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 (\pm)-1-acetoxy-2,10,11-trimethoxyhomoaporphine (6) did not take place, whereas that of (\pm)-2-acetoxy-1,10,11-trimethoxy- and (\pm)-3-acetoxy-2,10,11-trimethoxyhomoaporphines (7 and 8) produced optical active 7, 8 and hydroxyhomoaporphines (13, 14).

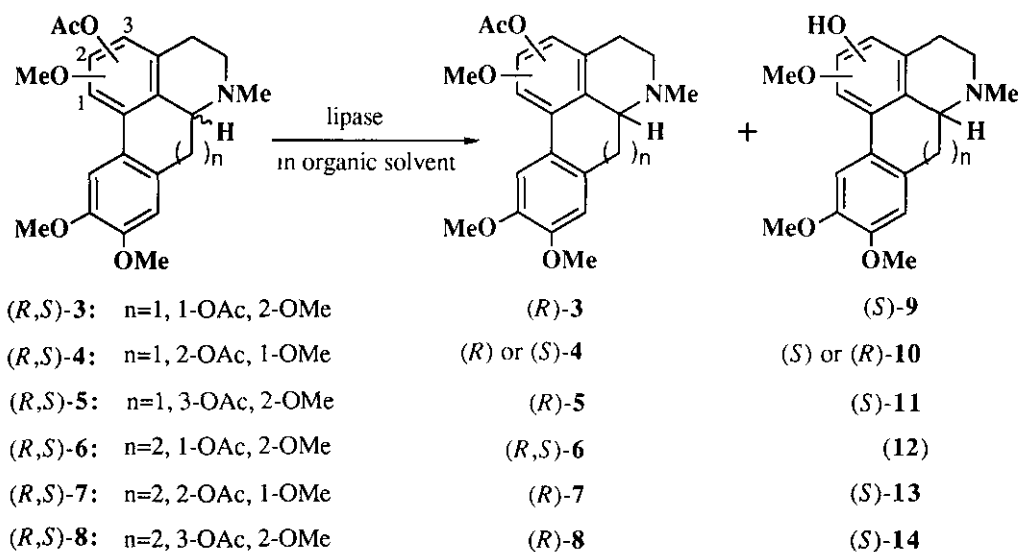
Kinetic resolution of prochiral acylates and esters using biocatalysts¹ is well known to be one of useful methods for preparation of chiral building blocks. Recently,² we have reported that enzymatic resolution of acetates of prochiral phenolic 1-substituted 1,2,3,4-tetrahydro-2-methylisoquinolines (1) 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 (\pm)-aporphines (9-11) and (\pm)-homoaporphines (12-14) under conditions similar to those reported previ-

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



ously² was investigated. The present paper deals with lipase-catalyzed resolution of acetates of phenolic (\pm)-aporphines and (\pm)-homoaporphines in organic solvent.

As substrates for this resolution, (\pm)-1-, (\pm)-2-, and (\pm)-3-acetoxyaporphines (3,³ 4,⁴ 5¹) and (\pm)-1-, (\pm)-2-, and (\pm)-3-acetoxyhomoaporphines (6,⁵ 7,⁶ 8) were synthesized according to a lead tetraacetate oxidation method.⁷



At first, screening of all substrates (3-8) using thirteen kinds of lipases [Amano A-6 (*Aspergillus niger*), Amano P (*Pseudomonas fluorescens*), Amano F-AP-15 (*Rhizopus japonicus*), Amano M-10 (*Mucol japonicus*), Lipase F (*Rhizopus niveus*), Pancreatn 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 (\pm)-1-acetoxyhomoaporphine (**6**) [(\pm)-**3** was found to be hydrolyzed in a prolonged reaction time], Amano A-6 and lipases from *Candida cylindracea* did (\pm)-2- and (\pm)-3-acetoxyaporphines (**4**, **5**) and (\pm)-2- and (\pm)-3-acetoxyhomoaporphines (**7**, **8**). It would be due to a steric hindrance of acetoxy group at the 1-position that hydrolysis of (\pm)-**6** did not take place.

Table 1. Enzymatic Resolution of (\pm)-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)	<i>R</i>
2		A	1.0	20 (86)	66 (28)	<i>R</i>
3	4	B	0.6	4 59 (56)	10 39 (97)	<i>S</i>
4		C	0.6	51 (80)	45 (79)	<i>S</i>
5		D	0.6	57 (55)	35 (92)	<i>S</i>
6		A	1.3	41 (91)	52 (48)	<i>S</i>
7	5	B	24.0	5 28 (59)	11 9 (87)	<i>S</i>
8		E	24.0	59 (44)	25 (71)	<i>S</i>
9		A	0.3	29 (36)	54 (19)	<i>S</i>
10	7	B	5.3	7 54 (54)	13 40 (71)	<i>S</i>
11		C	5.0	25 (72)	57 (36)	<i>S</i>
12		A	0.6	75 (27)	16 (78)	<i>S</i>
13		A	1.3	64 (32)	26 (61)	<i>S</i>
14	8	B	24.0	8 72 (30)	14 17 (90)	<i>S</i>
15		D	24.0	55 (75)	39 (86)	<i>S</i>
16		F	1.6	32 (83)	51 (53)	<i>S</i>

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 (\pm)-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 ¹H 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 (\pm)-**8** (Table 1, Entries 12, 13). It is noteworthy that the reaction (Table 1, Entries 3-5) of (\pm)-**4**, bearing an acetoxy group at the farthest 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 (\pm)-1-acetoxyaporphine (**3**) and homoaporphine (**6**) could not be searched by examination on tlc. However, with (\pm)-**3**, the prolonged reaction time⁸ (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 (\pm)-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

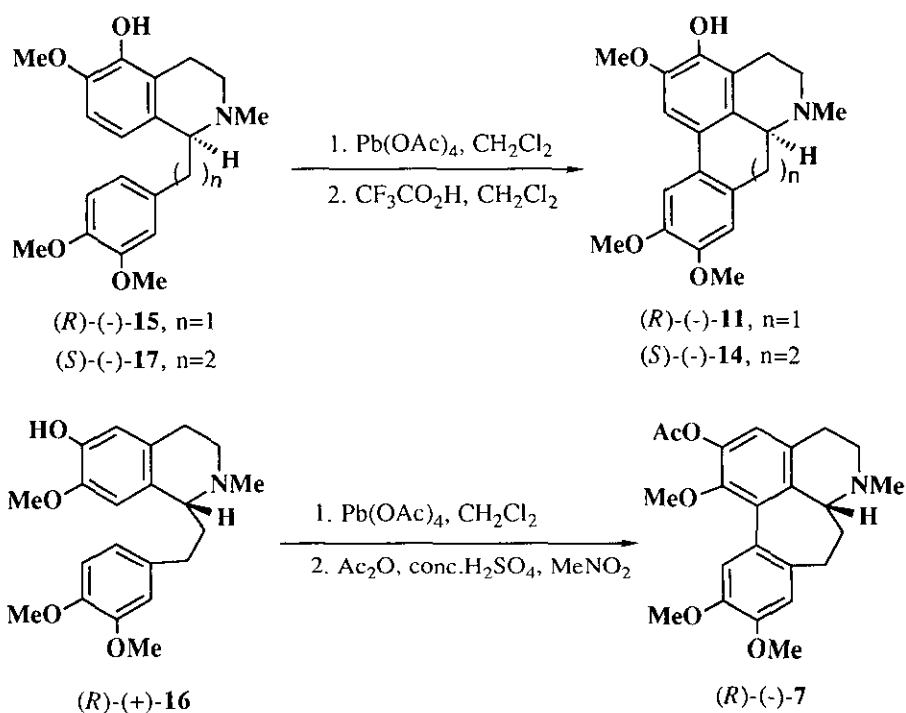
a) All reactions were carried out at 33 °C for 9 days.

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 findings⁹ 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.



In conclusion, kinetic resolution of acetates of phenolic (\pm)-aporphines and (\pm)-homoaporphines except (\pm)-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 (\pm)-**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.

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EXPERIMENTAL

All melting points were measured on a Büchi melting point measuring apparatus and are uncorrected. ^1H 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.

(\pm)-3-Acetoxy-2,9,10-trimethoxyaporphine (**5**)--- Acetylation of (\pm)-**11**¹⁰ in a usual manner gave (\pm)-**5**, mp 116-118 °C (ether-hexane) as pale yellow prisms. ^1H 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 $\text{C}_{22}\text{H}_{25}\text{NO}_5$ (M^+): 383 1731. Found: 383 1729

(\pm)-3-Acetoxy-2,10,11-trimethoxyhomoaporphine (**8**)--- Acetylation of (\pm)-**14**¹⁰ in a usual manner gave (\pm)-**8**, mp 165.5-167 °C (EtOH) as colorless prisms. ^1H 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 $\text{C}_{23}\text{H}_{27}\text{NO}_5$; 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 (\pm)-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 organic 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 ^1H nmr spectra. The results listed in Tables 1 and 2 are as follows.

With (\pm)-**4** : (1) (Table 1, Entry 1): Acetate (**4**)(45 mg, 45%, 70%ee) and phenol (**10**)(41 mg, 46%, 56%ee) were obtained. **10**: $[\alpha]_{\text{D}}$ -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**: $[\alpha]_{\text{D}}$ +110.1° (c=0.9).

With (\pm)-**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**: $[\alpha]_{\text{D}}$ +26.1° (c=1.2).

With (\pm)-**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**: $[\alpha]_{\text{D}}$ +4.1° (c=0.3).

With (\pm)-**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**: $[\alpha]_{\text{D}}$ -19.7° (c=0.7).

With (\pm)-**3** (Table 2, Entry 1): A mixture of (\pm)-**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**: $[\alpha]_{\text{D}}$ +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 previously⁹ gave (*R*)-(-)-**11**, mp 210-213 °C (MeOH) [after purification by silica gel column chromatography (CHCl₃ ; MeOH = 100 : 1) followed by preparative tlc (developing solvent : benzene : AcOEt · MeOH = 10 : 10 : 3)]. $[\alpha]_{\text{D}}$ -11.7° (c=0.88), ^1H 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 reaction of **(S)-(-)-17²** (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. $[\alpha]_D -9.0^\circ$ ($c=1.15$); 1H 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)-(+)-16²** (39%ee, 154.6 mg, 0.44 mmol) according to the procedure reported previously⁶ 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. $[\alpha]_D -5.6^\circ$ ($c=1.06$); 1H 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.

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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 (\pm)-**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.
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