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Separation and Characterization of All Configurational Isomers by Enzymatic Discrimination of Each Chiral Function[1]

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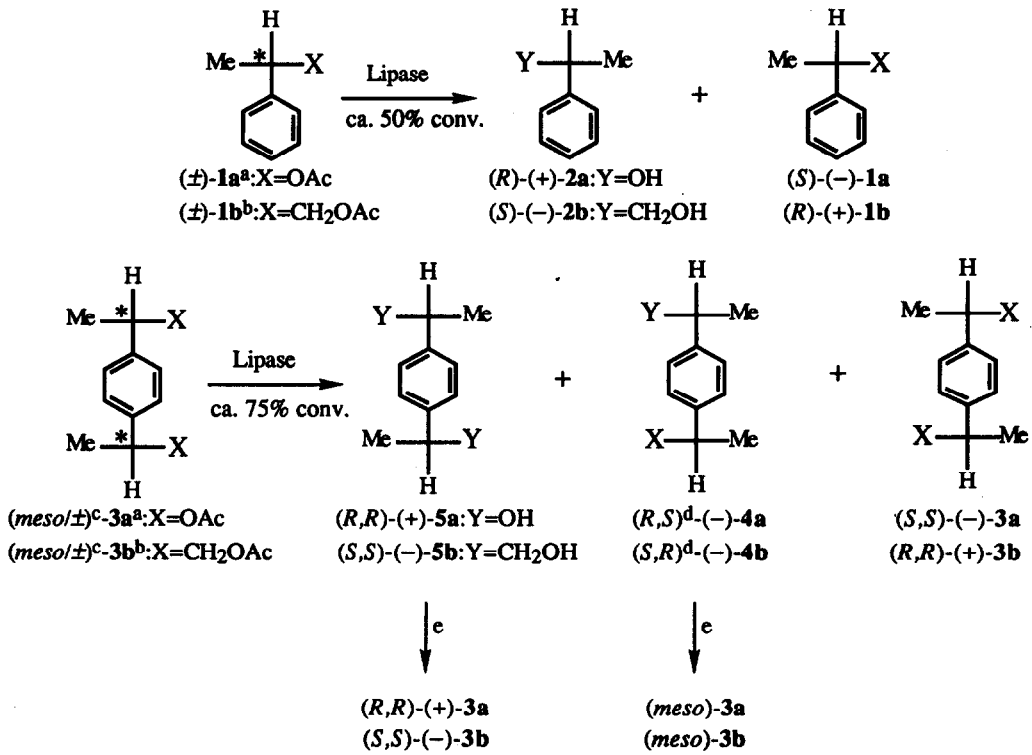
Abstract: An enzymatic method for simultaneous performance of separation and analysis of (meso/racemic)-diesters, i.e. 1,4-bis-(1-acetoxyethyl)benzene (3a) and 1,4-bis-(1-acetoxyisopropyl)benzene (3b), was demonstrated.

Enzyme actions can be conveniently used for organic synthetic purposes[2] without detail elucidation of their active sites. It is not known whether enzymes of high stereoselectivity on one chiral center recognize other remote chiral centers in the same molecule independently. We herein demonstrate a simple enzymatic strategy for otherwise difficult[3] preparative scale separation and characterization of all configurational isomers in mixtures of *meso* and *racemic* diesters **3a** and **3b**.[4] The strategy was as follows: 1) selection of an enzyme which exhibits high stereoselectivity toward racemic monoesters **1a** and **1b** having the same chiral center as that of **3a** and **3b**; 2) enzymatic hydrolysis of **3a** and **3b** using the enzyme; and 3) chromatographic separation of the resulting products.

The lipases, SAM-II and Steapsin, were first selected for **1a**,**1b**, respectively. The reactions were terminated after ca. 50% of the ester had been hydrolyzed. The absolute configurations of (−)-**1a**, (+)-**2a**,[5a] (+)-**1b**, and (−)-**2b**[5b] isolated from the reaction mixtures as shown in Scheme 1 were then assigned on the basis of their signs of optical rotations. Lipase SAM-II hydrolyzed preferentially the (*R*)-chiral center of the secondary alcoholic ester **1a** with high stereo selectivity as shown in Table 1. On the other hand, Steapsin under similar reaction conditions preferred the (*S*)-chiral center of the primary alcoholic ester **1b** with lower stereo selectivity compared to the former enzyme/substrate pair. The target diesters (*meso*/±)-**3a**,**b** were then exposed to the same enzyme-catalyzed hydrolysis conditions as those for the monoesters, except that the reactions were terminated at ca. 75% conversion stage. In a typical experiment, diester (*meso*/±)-**3b** (2.05 g) was stirred in 0.2 M sodium phosphate buffer (100 ml, pH 8, 25 °C) and enzymatically hydrolyzed in the presence of Steapsin (Tokyo Kasei Kogyo Co., Ltd., 150 mg). The reaction was monitored by GLC and terminated at 77% conversion. In the same way, (*meso*/±)-**3a** (375 mg) was hydrolyzed in the buffer (pH 7, 6 ml) with lipase SAM-II (Amano Pharmaceutical Co., Ltd., 60 mg) until 76% conversion. The reactions proceeded in a similar manner to give diols **5a**,[6]**b**, half esters **4a**,**b** and recovered **3a**,**b** after chromatographic separation.[7] The diols (*R*,*R*)-(+)-**5a**[8] and (*S*,*S*)-(−)-**5b**, as well as half esters (*R*,*S*)-(−)-**4a** and (*S*,*R*)-(−)-**4b**,[9] were acetylated to the corresponding



Scheme 1. [a] Lipase SAM-II. [b] Steapsin. [c] Italicized ± denotes *racemic*, emphasizing the configuration. [d] In the order of upper and lower chiral centers. [e] Acetylation.

Table 1. Results of the Enzymatic Hydrolysis of **1a,b** and **3a,b**.

Substrate	Time (h)	Product	Isolated Yield(%)	$[\alpha]^{25}_D$ ($CHCl_3$)	Stereoisomeric ratio (%) (*S,S*)(or *S*):*meso*:(*R,R*)(or *R*)		
(±)-**1a**	23	**2a**	37	+53	0.5	--	99.5
		1a	36	−102	97	--	3
(±)-**1b**	72	**2b**	63	−6	61	--	39
		1b	25	+2	4	--	96
(*meso*/±)-**3a**	24	**5a**	16	+69[a] (+178)[b]	0	0	100
		4a	44	−52 (+12)[b]	0	93	7
		3a	24	−169	100	0	0
(*meso*/±)-**3b**	84	**5b**	26	−25 (−10)[b]	86	14	0
		4b	43	−3 (+0.1)[b]	1	97	2
		3b	23	+12	1	2	97

[a] In MeOH. [b] After conversion to the corresponding diacetate.

diesters for the determination of stereoisomeric ratio by HPLC analysis using a chiral column.[10] The results are summarized in Table 1, showing the high stereochemical purities of the products. From a preparative point of view the present method provides a facile route to *(R,R)*-, *meso*- and *(S,S)*-isomers of **3a,b** and **5a,b** as well as optically active **4a,b** with high stereoisomeric purities. The compositions of the three products obtained by the enzymatic reaction of each mixture of *(meso/±)*-**3a,b** agreed well with those expected for the chemical preparation[4] of isomeric diols **5a,b** (i.e. *(R,R)* : *meso* : *(S,S)* = 1:2:1). The absolute configuration of (–)-**5b**[5b] were assigned from the literature data and those of (–)-**4a** and (–)-**5a** (from (–)-**3a**) by Mosher's rule after conversion into the MTPA esters.[11] The configurations of the chiral centers of the enzyme-derived disubstituted benzenes (**3a,b** - **5a,b**) coincide with those of the corresponding monosubstituted benzenes (**1a,b** and **2a,b**). It is thus apparent that the present lipases recognize the chiral center of the same configuration irrespective of the whole structure of the substrate, indicating that the ligands around the asymmetric carbon of both mono and disubstituted benzenes fit into the enzyme binding sites in the same manner.

Further application of the strategy to other enzyme/substrate systems are being studied.

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3. No different chemical shift due to diastereoisomerism was observed for **3a,b** - **5a,b** in their ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra. Their diastereomer pairs were also difficult to separate chromatographically under usual conditions (TLC Rf values and GLC retention times were identical). Furthermore the configurations of the stereo isomers could not be elucidated by the HPLC analysis using a chiral column as shown in Ref 10 data. The enzymatic strategy would be the only simultaneous method for separation and characterization of this kind of stereo isomers in preparative scale at the present stage.
4. Diol **5a** was prepared by Grignard reaction of terephthalaldehyde and **5b** via hydroboration/oxidation of p-diisopropenylbenzene. The alcohols **4a,b** and **5a,b** were acetylated by using either acetic anhydride under reflux or acetyl chloride-pyridine in $CHCl_3$ at room temperature. Acetates **3a** and **4a** were converted into diol **5a** with K_2CO_3 in MeOH without racemization. All new compounds gave satisfactory spectra and elemental analytical data.

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7. Chromatography on silica gel (hexane/ethyl acetate) of the resulting mixture followed by Kugelrohr distillation afforded the following results: diol (*S,S*)-(−)-**5b** (374 mg, bp 143 °C/2 Torr), half ester (*S,R*)-(−)-**4b** (747 mg, bp 178 °C/4 Torr) and recovered diester (*R,R*)-(+)-**3b** (470 mg, bp 164 °C/3 Torr); diol (*R,R*)-(+)-**5a** (39 mg, mp 119-120 °C (90-91 °C or 114-115 °C for the racemic form));[6] half ester (*R,S*)-(−)-**4a** (136 mg, bp 158 °C/3 Torr) and recovered diester (*S,S*)-(−)-**3a** (90 mg, bp 150 °C/3 Torr).
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9. The half esters **4a,b** were transformed to the corresponding *meso* compounds; the optical rotations of diesters **3a,b** (Table 1) and diol **5a** ($[\alpha]^{25}_{D}$ = +7.2° (c 0.44, $CHCl_3$)) derived from the half esters were less than 11% of the maximum rotations of the corresponding optically active stereoisomers.
10. Analytical conditions: column, Daicel Chiralcel OB 4.6 mm x 100 cm; solvent, hexane/2-propanol 9/1 for **3a** (flow rate 0.5 mL/min, retention times, (*R,R*) 95, (*S,S*) 114, and *meso* 137 min) or 50/1 for **3b** (flow rate 1.0 mL/min, retention times, (*S,S*) 99, *meso* 122, and (*R,R*) 164 min). Thus there seems to be no correlation between the elution orders of these stereo isomers in the HPLC and their configurations.
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