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Enzymatic Kinetic Resolution of (±)-4-Acetoxy[2.2]paracyclophane by *Candida cylindracea* Lipase. An Efficient Route for the Preparation of (+)-R-4-Hydroxy- and (+)-S-4-Acetoxy[2.2]paracyclophane

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Abstract: The enzymatic kinetic resolution of (±)-4-acetoxy[2.2]paracyclophane by *Candida cylindracea* lipase was investigated in water and in a two-phase aqueous organic system. The (+)-(R)-4-hydroxy- and (+)-(S)-4-acetoxy[2,2]-paracyclophanes were isolated in excellent yields and high enantiomeric excesses. The resolution was carried out on multi-gram scale in hexane-water at 40° C.
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[2.2]Paracyclophanes, cyclopentadiene-transition metal complexes such as ferrocenes and arenechromium complexes, are compounds with planar chirality that have received much attention[1] for their role in enantioselective syntheses. Efficient procedures for preparing the optically active forms of these compounds are therefore required.

As part of a project which uses [2.2]paracyclophanes as chiral auxiliaries and chiral catalysts, we recently reported[2] the preparation of some new (R)-4-substituted [2.2]paracyclophanes by using classical chemical procedures, showing a correlation between group polarizability and optical rotation.

Optically active 4-hydroxy[2.2]paracyclophane is a key compound for preparing suitable chiral auxiliaries with planar chirality and we resolved to investigate its preparation by enzymatic hydrolysis of the acetoxy derivative exploiting our previous experience in the field.[3]

In the last decade hydrolytic enzymes have received considerable attention as synthetic chiral catalysts for preparing optically active compounds[4] and the extracellular microbial lipases have been used most frequently because they have broad substrate specificities, do not require coenzymes, are stable either in water or in organic solvent and most of them are inexpensive.

The ability of lipases to provide optically active compounds has been mainly achieved in water and organic solvents,[5] in the resolution of racemic esters, alcohols and acids by selective hydrolysis or

transesterification. Most of the studies to date concern compounds with central chirality, compounds with planar chirality and compounds having both central and planar chirality have rarely been investigated.[6]

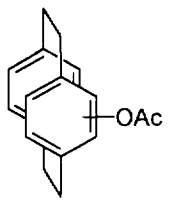
This paper reports the results of the enzymatic hydrolysis of (±)-4-acetoxy[2.2]paracyclophane **1**, a compound with planar chirality, in water and organic solvents by using *Candida cylindracea* lipase (CCL).

To our knowledge the only enzymatic resolution of [2.2]paracyclophane derivatives by lipases, concerns the esterification in organic solvents of (±)-4-hydroxymethyl[2.2]paracyclophane and hydrolysis of (±)-4-acetoxymethyl[2.2]paracyclophane with pig liver esterase and porcine pancreatic lipase.[6c] The procedure is not suitable for preparing optically active 4-substituted[2.2]paracyclophanes useful for enantioselective syntheses because the reaction yields and enantiomeric excesses are too low.

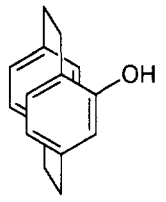
Results and Discussion

The hydrolysis of **1** was carried out in water at 22°C, 40°C and 50°C in a phosphate buffered heterogeneous medium in the presence of catalytic amounts of CCL. The pH was kept at 7.2 with the aid of an autoburette and the progress of the reaction was controlled by the consumption of NaOH 0.2 M. In the control experiment without enzyme, no hydrolysis was observed under the same conditions after a prolonged time.

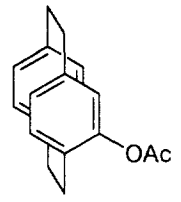
The reaction products **2** and **3** were isolated and the *ee* determined by the specific optical rotations[2] and ^{1}H-NMR chiral shift experiments. The results are reported in Table 1.



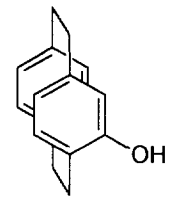
(±)-1



(+)-(*R*)-2



(+)-(*S*)-3



(-)-(*S*)-4

Table 1. Enzymatic hydrolysis of (±)-4-Acetoxy[2.2]paracyclophane **1** in water.[a]

				2		**3**	
Entry	T (°C)	t (h)	Conv.(%)[b]	Yield (%)[c]	ee (%)	Yield (%)[c]	ee (%)
1	22	193	50	92	≥98	85	≥98
2	40	96	50	92	≥98	85	≥98
3	50	72	50	88	92	80	95
4[c,d]	40	32	54	90	97	89	82
5[c,e]	40	18	46	91	74	96	82

[a] In phosphate buffer at pH 7.2. [b] Conversion determined by GC after consumption of proper amount of NaOH 0.2M. [c] Isolated product. d. In the presence of sodium taurocholate ($8\cdot10^{-3}$M). [e] In the presence of sodium taurocholate ($2\cdot10^{-3}$M).

The hydrolysis proceeds very slowly at room temperature but the reaction products **2** and **3** were isolated with high yields and high *ee*. At 50°C the reaction is ca. three times faster without an appreciable decrease in yield or enantioselectivity. The presence of catalytic amounts of sodium taurocholate (entries 4 and 5) greatly reduces the reaction time, but the *ee* of **2** depends on the concentration of bile salt.

Chromatographic separation and recrystallization of the products allows to obtain pure (+)-(R)-4-hydroxy[2.2]paracyclophane **2** and (+)-(S)-4-acetoxy[2.2]paracyclophane **3** which is then easily converted into (-)-(S)-4-hydroxy[2.2]paracyclophane **4**.

We also investigated the selectivity of the resolution of **1** in a 5:1 mixture of organic solvent and water because it is known that the organic medium affects both the enzymatic activity and selectivity of the reaction.

To date there is no satisfactory explanation of the organic solvent effect and only poor correlations have been observed between the enzymatic activity and selectivity and parameters that characterize the solvent.[7] Most of the effect has been related to the hydrophobicity of the solvent, which is a property of bulky solvent[8] but, it is probably more likely that the effect is related to the interaction of solvent molecule with active site of enzyme.[9]

We selected five organic solvents (Table 2) characterized by different log P values (from - 0.33 to 3.5).[10]

The hydrolysis of **1** was carried out at 40° C, save with diethyl ether, and the reaction was stopped when 0.5 equiv. of NaOH 0.1M were consumed. The conversion reaction was followed using GC analysis. The results are reported in Table 2.

Table 2. Enzymatic hydrolysis of 4-acetoxy[2.2]paracyclophane **1** in a two-phase aqueous-organic system.[a]

					2		**3**	
Entry	Medium	T(°C)	t(h)	Conv. (%)[b]	Yield (%)[c]	ee(%)	Yield (%)[c]	ee(%)
1	n-C_6H_{14}	40	18	52	88	89	89	92
2	Ph-H	40	65	40	95	70	94	52
3	t-BuOMe	40	18	54	96	78	94	86
4	Et_2O	25	216	47	85	84	86	75
5	MeCN[d]	40	34	55	87	80	92	≥98

[a] Organic solvent/phosphate buffer (pH 7.2) 5:1. [b,c] See footnotes of table 1. [d] 2% MeCN in phosphate buffer at pH 7.2 (Ref.11).

Except for diethyl ether the hydrolysis in aqueous organic solvent is faster than in phosphate buffer alone and the reaction yields are excellent as are those obtained in aqueous medium. By using n-hexane, t-butyl methyl ether and 2% acetonitrile[11] (entries 1,3,5) the compound **3** is obtained with the *ee* similar to that found when the resolution was carried out in water, while the *ee* of recovered hydroxy **2** was always at least 10% lower. In benzene and diethyl ether (entries 2 and 4), hydrolysis is slow and poorly enantioselective. The CCL is inactive in $CHCl_3$ dioxane and in aqueous MeCN higher than 2% v/v.[11]

As expected, the reaction medium (water or aqueous organic solvent) influences both the reactivity and

selectivity of the reaction. This can be justified by hypothesizing a remarkable conformational flexibility of CCL. The CCL can adopt a *closed* form,[9] which is inactive because the active site is shielded by a part of the polypeptide chain (the flap), or an *open* active form which has the active site accessible to the solvent and substrate. The movement of the flap determines the transition between the two conformations. We hypothesize that the flap position is determined by the specific characteristics of the reaction medium. As consequence of well known[13] conformational flexibility of enzymes in solution, the different positions of the flap influence not only the accessibility of the substrate to the active site but also the conformation of the active site itself and consequently, the reactivity and selectivity of the hydrolysis.

The resolution was then scaled-up. The reaction, carried out in aqueous medium on multi gram-scale required a longer reaction time and was less enantioselective. In contrast, 2 g of racemic acetoxy **1** in aqueous *n*-hexane at 40° C were converted into **2** and **3** in 18 h with the same *ee* as found in the micro-scale experiment. The products were easily and quantitatively separated by column chromatography and optically active pure form was obtained after one recrystallization.

Experimental

Compounds **1**, **2** and **3** were described previously.[2] The *ee* were calculated by measuring the specific optical rotations (in $CHCl_3$ solution on a JASCO-DIP 360 polarimeter) and by ^{1}H-NMR chiral shift experiments with Eu(hfc)$_3$ in $CDCl_3$ solutions on a Brucker AC 200 MHz spectrometer. GC analyses were performed on a Hewlett-Packard 5890 chromatograph with HP-5-fused silica capillary column (30 m, 0.25 internal diameter, 0.25 μm film thickness), an "on column" injector system", a FID detector and hydrogen as gas carrier.

Hydrolysis in aqueous medium

Candida cylindracea (120 mg) was suspended in phosphate buffer (NaH_2PO_4/ Na_2PO_4 12 ml, 0.1 M, pH 7.2) and stirred for 15 min. at 40°C. The acetoxy **1** (1.0 mmol) was added and the mixture maintained at pH 7.2 under stirring at 40°C for 96 h by automatic titration with NaOH 0.2 M using a Mettler DK pH-Stat. When were consumed 0.5 mmol of 0.2 NaOH the reaction was stopped by adding a saturated solution of NaCl (20 ml) and the mixture was cooled at room temperature and then extracted with diethyl ether (3x30 ml). The organic phases were dried (Na_2SO_4), evaporated under reduced pressure and the residue chromatographed on silica gel eluting with petroleum ether/diethyl ether 9:1.

(+)-R-4-hydroxy[2.2]paraciclophane **2** (103 mg) yield 85%, $[\alpha]_D^{20}$ 8.1° (c 1.20) *ee* 98%, mp 232-234°C (from EtOH-H_2O). After recrystallization $[\alpha]_D^{20}$ 8.4° [Lit.[2] m.p.232-234°C, $[\alpha]_D^{20}$ 8.4°]. ^{1}H-NMR and GC-MS in agreement with those previously reported.[2] (+)-S-4-acetoxy[2.2]paracyclophane **3** (113 mg), yield 92%, $[\alpha]_D^{20}$ 40.4° (c 1.06) *ee* 98%, mp 122-123°C (from hexane). After recrystallitation $[\alpha]_D^{20}$ 41.2° [Lit.[2] for the enantiomer : mp 122-123°C, $[\alpha]_D^{20}$ -41.2°]. ^{1}H-NMR and GC-MS in agreement with those reported for the enantiomer.

Hydrolysis in the presence of sodium taurocholate

Candida cylindracea (120 mg) was suspended and stirred at 40°C in phosphate buffer (0.1 M, 12 ml) at

pH 7.2 containing sodium taurocholate ($8x10^{-3}$ M). After stirring 15 min. acetoxy **1** was added and the reaction carried out as described when executed in sole aqueous medium. For reaction conditions and yields see Table 1.

Hydrolysis in aqueous organic solvent

Candida cylindracea (240 mg) was suspended and stirred in phosphate buffer (0.1 M, 4ml) at pH 7.2 and the pH adjusted. Organic solvent (20 ml) was added and the mixture stirred at 40°C (except when diethyl ether was used) for 10 min. The acetoxy **1** (2 mmol) was added and the stirring continued at 40°C for the time reported in Table 2. The reaction was stopped when 1 mmol of 0.2 M NaOH was consumed. The products **2** and **3** were isolated as described above.

Scale-up of the reaction

A solution of acetoxy **1** (2 g) in *n*-hexane (96 ml) heated at 40°C was added to a stirred suspension of *Candida cylindracea* (1.5 g) in phosphate buffer (16 ml, 0.1 M) at pH 7.2 and 40°C. The stirring was maintained for 18 h, the pH kept at 7.2 and the reaction stopped when 4 mmol of 0.2 M NaOH were consumed. The reaction was then worked up as described above **2** : 0.88 g, yield 89%, *ee* 89%; **3** : 0.80 g, yield 89%, *ee* 92%.

(-)-(S)-4-hydroxy [2.2]paracyclophane 4

A mixture of enantiomerically pure **3** (0.5 g, 1.9 mmol) and 10% NaOH (15 ml) was stirred for 3 h at 85°C. After cooling the mixture was acidified with dil. HCl and extracted with diethyl ether. After work-up and recrystallization of the crude product 0.4 g of **4** were isolated, mp 232-234°C from EtOH/ H_2O, $[\alpha]_D^{20}$ -8.3° (c 1.08). ^{1}H-NMR and GC-MS in agreement with those reported for its enantiomer.[2]

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