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SEPARATION OF *CIS/TRANS*-CYCLOHEXANECARBOXYLATES BY ENZYMATIC HYDROLYSIS: PREFERENCE FOR DIEQUATORIAL ISOMERS

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Abstract: 4-Substituted *cis/trans*-cyclohexanecarboxylates have been separated into the isomers by enzymatic hydrolysis with lipase from *Candida rugosa* with very good selectivity. The enzyme preferentially recognizes diequatorial conformations. Copyright © 1996 Elsevier Science Ltd

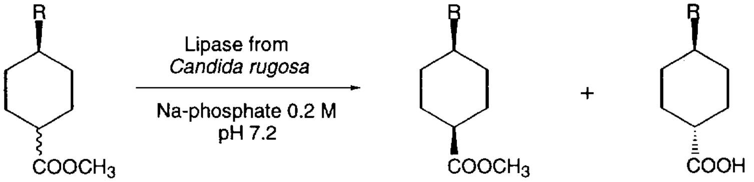
Cis- and *trans*-4-substituted cyclohexanecarboxylic acids are important building blocks for liquid crystal manufacture[1] and for the synthesis of various bioactive compounds including hypoglycemic drugs.[2] Traditionally, access to these compounds included of catalytic hydrogenation of the corresponding aromatic acids, yielding predominantly the *cis*–isomer, followed by thermal isomerization and separation via crystallization, where applicable.[3,4] In this communication we present a method for the separation of 4-substituted *cis*– and *trans*–cyclohexanecarboxylates by hydrolysis of their esters with lipase from *Candida rugosa.*

Lipases (usually triacylglycerol hydrolases) have been generally perceived as most suitable for the hydrolysis of substrates with stereochemistry in the alcohol part, although there are examples where chiral acids have been resolved using lipase-catalyzed hydrolysis of their esters.[5,6] To date, very little is known about the selectivity of enzymatic hydrolysis of 4-substituted cyclohexanecarboxylates, which are achiral due to a plane of symmetry. The only report to our knowledge is by Jones[7] on the hydrolysis of *cis*- and *trans*-4-*t*-butylcyclohexanecarboxylates with pig liver esterase. Preferential hydrolysis of an equatorially oriented carboxylate was observed, albeit with low selectivity (S = 3).

On initial screening of a number of enzymes for the selective hydrolysis of substrate **1a**, we obtained the corresponding acid **2a** in various *cis/trans* ratios from 2:3 to 5:1.[8] Only lipase from *Candida rugosa* (LCR) showed excellent selectivity for the hydrolysis of the *trans* ester. It is noteworthy to point out that LCR has previously been used for the synthesis of macrocyclic lactones from *trans*-1,4-cyclohexanedicarboxylic acid and 1,16-hexadecanediol.[9] In the present study we consequently performed preparative-scale hydrolysis reactions of substrates **1a**–**1f** (Table 1) with LCR using standard methodology (phosphate buffer, pH 7.2, rt).

Selectivity coefficients S (for isomeric selectivity) have been obtained analogous to the calculation of enantioselectivity coefficients E, as outlined by Chen *et al*,[10] by substituting conversion and *cis/trans* ratios into Chen's equation 6. Conversions were obtained mathematically from accurately determined *cis/trans* ratios of starting materials and products.

Table 1:



1a-f **1a-f** **2a-f**

	Substrate 1			Remaining Substrate 1		Product 2			
Entry	R	*cis/trans*	Time	Yield (%)	*cis/trans*	Yield (%)	*cis/trans*	conv.(%)[a]	S[b]
1a	$CH(CH_3)_2$	25.5/74.5	6.5 h	28.0	78.5/21.5	63.1	1.1/98.9	68	80
1b	$C(CH_3)_3$	43.8/56.2	24 h	40.4	91.1/8.9	51.9	2.5/97.5	53	84
1b	$C(CH_3)_3$	43.8/56.2	96 h	33.1	99.3/0.7	58.3	4.0/96.0	58	100
1c	CH_2CH_3	72.6/27.4	4.8 h	61.4	96.0/4.0	23.2	7.5/92.5	26	78
1d	CH_3	72.8/27.2	6.7 h	42.4	94.4/5.6	18.5	5.8/94.2	24	95
1e	OCH_3	56.5/43.5	11.7 h	54.0	76.9/23.1	31.4	33.6/66.4	47	4
1f	$COOCH_3$	70.6/29.4	14 h	70.0	98.9/1.1	23.2	3.1/96.9	29	270
1f	$COOCH_3$	70.6/29.4	24 h	66.6	99.93/0.07	23.6	7.0/93.0	32	240

a) Calculated from *cis/trans* ratios of product **2** and remaining substrate **1**.

b) Calculated using equation 6 of Chen *et al.*[10] A correct value for S is obtained for compounds **1c** - **1f**, if the isomeric excess of the starting material i.e. = (Trans-Cis)/(Cis+Trans) is used with the correct negative sign.

Our results show a clear preference of lipase from *Candida rugosa* for the hydrolysis of *trans*-4-substituted cyclohexane carboxylates over *cis*-compounds. The *tert*-butyl substituent is oriented almost exclusively in an equatorial conformation (substrate **1b**),[11] LCR therefore discriminates between an equatorial and an axial methyl carboxylate, selectively hydrolyzing the former. In the case of substrate **1f**, assuming only chair conformations, at least one of the two methyl carboxylates is in an equatorial position for both *cis* and *trans* isomers; the high selectivity of the hydrolysis lies, therefore, in a discrimination of the orientation of the remote substituent, diequatorial being favored over axial/equatorial. A combination of similar arguments applies to the cases where the free enthalpy difference between the axial and equatorial geometries of the remote residue and the methyl carboxylate (Table 2)[12] allows the *cis*-compounds to exist in an axial/equatorial or equatorial/axial conformation (substrates **1a**,**1c**,**1d**): diequatorial (*trans*) is always selected over equatorial/axial (*cis*). ^{1}H-NMR of the remaining highly enriched *cis*-esters **1a**, **1c**, and **1d** shows a slightly broadened quintet signal for the carboxylate methine indicative of an equatorial methine exhibiting four similar coupling constants (approx. 5 Hz). For this reason we assume that the methylcarboxylate exists preferentially in an axial conformation. The hydrolyzed *trans*-acids **2a-d** on the other hand exhibit the expected sharp triplet of triplets (12 and 3.5 Hz) expected for an axial methine. The low selectivity of hydrolysis of substrate **1e** compared to the almost isosteric **1c** is possibly rooted in additional electronic interactions introduced through the presence of the methoxy group, or significant contributions of other conformations to the set of possible geometries, hinted at by a complex ^{1}H-NMR-spectra which do not show the coupling patterns found for the other compounds.

Table 2:

entry	**substituent**	ΔG°(kcal/mol)
a	$CH(CH_3)_2$	2.15
b	$C(CH_3)_3$	4.90
c	C_2H_5	1.75
d	CH_3	1.70
e	OCH_3	0.75
f	$COOCH_3$	1.30

In conclusion, a mild and unique method has been developed to separate *cis/trans*-isomers of 4-substituted cyclohexanecarboxylates with high selectivity. Lipase from *Candida rugosa* was shown to hydrolyze preferentially substrates existing in diequatorial conformations. This method may be especially interesting for the preparation of the *cis*-isomers of these compounds, which currently cannot be obtained in a general and efficient manner.

Experimental.

Esters **1a–1e** were prepared with cesium carbonate and methyl iodide in acetone[13] from commercial acids or from the corresponding aromatic acids after catalytic hydrogenation using standard procedures. Enzymatic reactions were monitored by GC rather than an autotitrator, since base consumption did not reflect the actual conversion due to precipitation of the acid that is formed.

General procedure for the enzymatic hydrolysis of **1a–1f**: Lipase from *Candida rugosa* (Sigma, Lot 43F-0043, 595 units/mg, 300 mg) was suspended in sodium phosphate buffer (0.2 M, pH 7.2, 35 mL) using magnetic stirring. The ester (600 mg) was added after 10 min. At the appropriate time, the mixture was acidified to pH 3-4 with H_3PO_4, saturated with NaCl, and extracted with *tert*-BuOMe (5 x 40 mL). Drying over $MgSO_4$, concentration and chromatography (20 g of silica gel) with suitable mixtures of hexane and ethyl acetate (for the ester) and pure ethyl acetate (for the acid) afforded the ester, which was further purified by a bulb-to-bulb distillation at 1 mm, and the acid, which was dried *in vacuo*.

Cis/trans ratios were determined by gas chromatography using a 30 m x 0.25 mm HP-1 column (esters **1a-e**), or a 30 m x 0.32 mm Cyclodex-B column (ester **1f**). *Cis/trans* ratios of acids **2a–2d** were determined by ^{1}H-NMR. Samples of **2e** and **2f** were transformed into methyl esters with trimethylsilyldiazomethane[14] and analyzed by GC as indicated above.

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