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Studies on the Enzymatic Resolution of Chiral Tricarbonyl(benzaldehyde oxime)Chromium Complexes

Clara Baldoli*[a], Stefano Maiorana[a], Giacomo Carrea[b], Sergio Riva*[b]

[a]*CNR-Centro Studio Sintesi Stereochimica Speciali Sistemi Organici e Dipartimento di Chimica Organica e Industriale dell'Universita' - Via C. Golgi, 19 -20133 Milano.* [b]*CNR-Istituto di Chimica degli Ormoni- Via Mario Bianco,9 -20131 Milano*

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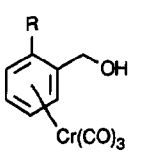
Abstract: Lipases from different sources have been used for the resolution of tricarbonyl(*ortho*-substituted benzaldehyde oxime)chromium complexes. The effect of the nature of the solvent on enantioselectivity has been investigated.

The high specificity of enzymes as catalysts is well known and many literature examples have also ascertained their compatibility with organometallic substrates [1]. For instance we and other authors[2] have reported the kinetic resolution of racemic tricarbonyl(benzaldehyde)chromium complexes using different dehydrogenases.

As a part of our research on the resolution of chiral tricarbonyl chromium complexes directed toward the obtainment of new optically pure organometallic synthons, we were interested in the preparation of optically active tricarbonyl(benzylamine)chromium complexes.

Recently, different authors have reported the resolution of the $Cr(CO)_3$ complexes of *ortho*- and *meta*-derivatives of benzyl alcohol **1** by asymmetric esterification with lipases from Pseudomonas species[3-5]. With our surprise, however, the acylation of the corresponding amines **2** under the same conditions was completely unspecific[6].

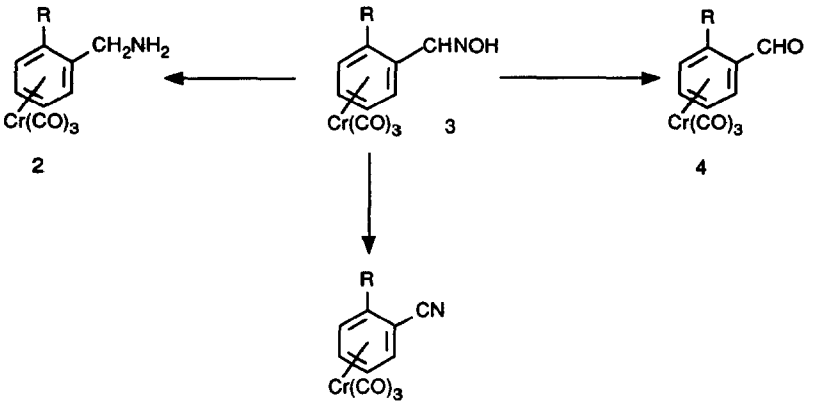


1

2

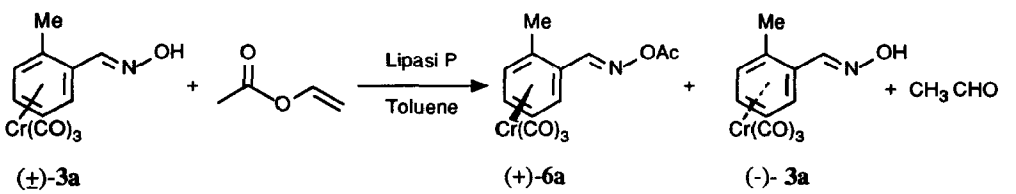
In order to overcome this unexpected result, we turned our attention to the oxime complexes (**3**), which, in principle can be chemically transformed into benzylamine and some other complexes (Scheme 1).

Scheme 1



As a model compound we chose the 2-methyl derivative **3a** which was subjected to the catalysis of lipase P (from *Pseudomonas cepacia*) in toluene, according to the Scheme 2 .

Scheme 2

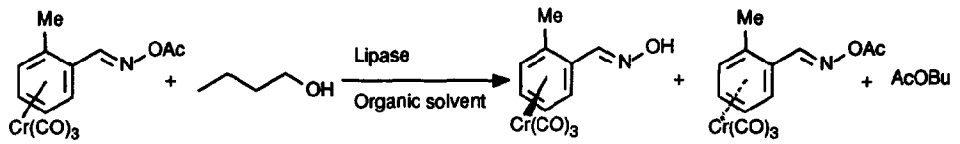


The reaction was followed by chiral HPLC[7], which allowed us to evaluate the enantiomeric ratio "E"[8]. The enantioselectivity was not very high (E= 9.8, average value of three HPLC runs with conversions (c) between 34 and 45%), but theoretically sufficient to give the unreacted residual substrate in enantiomerically pure form at c > 65%. On the contrary, we noticed that $e.e._s$ decreased at higher conversion: for instance $e.e._s$ was 74% at c= 52% and only 36% at c= 91%.

As oxime acetates have been used as activated donors[9], a possible explanation is that lipase P catalyses also the transesterification between the product (+)-**6a** and the residual substrate (-)-**3a**[10]. The influence of this side reaction on the overall equilibrium of the transformation increases at higher concentration of oxime acetate, thus causing the decrease of the optical purity of the remaining substrate (in accordance with the prediction of Chen et al.[11] for equilibrium reactions).

At this point, we considered the enzymatic transesterification depicted in Scheme 3, trying to optimize the reaction conditions. A screening of different lipases confirmed that Lipase P was the best catalyst (Table 1).

Scheme 3



(±) 6a (+) - 3a (-) - 6a

Table 1. Enantioselectivity of Different Lipases with (±) - 6a[a].

Lipase	%conv.[b]	%e.e. product[b]	E[c]
Pseudomonas cepacia[d]	33	76	10.6
Porcine pancreatic[e]	12	7	1.2
Chromobacterium viscosum[f]	22	40	2.6
Mucor miehei[g]	43	15	1.5
Humicola lanuginosa[d]	29	40[h]	2.7[h]
Candida cylindracea[e]	25	43	2.9

a) Conditions: to 1 ml of toluene containing 5 mg of racemic 6a and 15 μl (≅ 10 eq) of n-BuOH, 25 mg of Lipase supported on celite were added and the suspension was shaken at 250 rpm at 45 C

b) Determined by chiral HPLC: 10 μm Baker Bond Chiralcel OJ column; eluent hexane:2-propanol 5:1; flow rate 0.5 ml/min; UV detector 330 nm

c) E values were calculated from the degree of conversion and e.e. of the product according to Chen et al. d) Amano Pharmaceutical Co. e) Sigma Chemical Co

f) Finnsugar Biochemicals Inc. g) Biocatalyst h) Inversion of selectivity

Subsequently we studied the effect of the nature of the organic solvent on lipase activity and enantioselectivity[12]. As depicted in Table 2, enantioselectivity was quite similar in toluene, 3-pentanone and *t*-amyl alcohol. The last one was the solvent of choice, due to the much higher activity of lipase P in this" milieu".

Table 2. **Effect of Organic Solvents on the Enantioselectivity of *Pseudomonas Cepacia* Lipase with (±)-6a[a]**

Solvent[b]	Rel.rate[c]	E[d]
Dioxane	4	7.1
Acetonitrile	4	2.0
3-Pentanone	5	12.3
t-Amyl alcohol	100	11.5
Methylene chloride	1	2.7
Toluene	4	11.0
Dibuthyl-ether	20	9.0

a) Conditions: see footnote a) of Table 1 b) In order of increasing hydrophobicity c) At 30% of conversion

d) See Footnotes b) and c) of Table 1

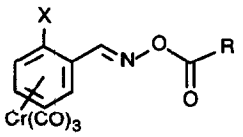
The reaction was scaled up (300 mg of racemic **6a**) and stopped at 62% of conversion. After usual work-up and flash-chromatography, purified residual (-)-**6a** was isolated in 92% e.e. (line 1 Table 3).Following the "homotopic double resolution" procedure[13], the enriched oxime (+)-**3a** was re-acylated according to Scheme 2. At 53% conversion the enantiomeric (+)-**6a** was isolated with 98% e.e.(line 4 of Table 3).

Table 3. **Preparative-Scale Resolution of 6a-d and 3a-d**

Substrate (mg)	Lipase	%c[a)]	%e.e.$_p$[a)](mg)	%e.e.$_s$[a)](mg)
(±) **6a** (300)	P	62	59 (145)	92 (100)
(±) **6b** (300)	P	63	46 (140)	74 (80)
(±) **6d** (200)	CE.5	69	32 (102)	72 (66)
(+) **3a** (140)[b)]	P	53	98 (86)	20 (75)
(+) **3b** (130)[b)]	P	51	76 (79)	19 (20)

a) Determined by HPLC (see experimental). b) Initial e.e.: **3a**= 59%; **3b**= 46%.

We applied the same reaction procedure to the other oxime acetates **6b-d**, obtaining the following E values (determined in toluene to overcome solubility problems):



	X	R	E
6a	Me	Ac	11.0
6b	OMe	Ac	7.9
6c	Cl	Ac	1.4
6d	F	Ac	1.8
6e	Cl	Pr	2.3

The E value for the *ortho*-methoxy derivatives **6b** was quite low, but sufficient to obtain a satisfactory resolution of the racemic mixture (lines 2 and 5 of Table 3). On the contrary, the E values for the *ortho*-halogens **6c** and **6d** were too low to allow a satisfactory resolution. A screening of different solvents in the case the chloro derivative **6e** (butanoate was used instead of acetate for analytical reasons) was unsuccessful. However we noticed an interesting inversion of the enantiopreference of lipase P on moving from *t*-amyl alcohol to methylene chloride (see table 4)[14].

Table 4 **Effect of Organic Solvent on the Enantioselectivity of *Pseudomonas Cepacia* Lipase with (±)-6e[a)]**

Solvent	%c	%e.e.$_p$	E[b)]	$\alpha_D$sign for **3c**
t-Amyl alchol	42	49	4.1	(-)
Dibutyl ether	29	47	3.4	(-)
Toluene	25	35	2.3	(-)
Dioxane	33	33	2.3	(-)
3-Pentanone	24	27	2.1	(-)
Acetonitrile	42	8	1.5	(+)
Methylenechloride	14	26	1.8	(+)

a) Conditions: see footnote a) of Table 1.b) Determined, according to ref.8 ,by chiral HPLC; see footnote b) of Table 1

With the haloderivatives better results were obtained with other lipases. For instance, the lipase from *Humicola lanuginosa* (CE-5) showed an E value of 5.9 in the transesterification of the *o*-fluoro oxime acetate **6d** according to Scheme 2. The reaction was scaled up and the results are reported in Table 3.

It is worthy of note that acetates **6a-d** are easily converted into the corresponding oximes, by treating them with 20% potassium hydroxide methanolic solution. Moreover, as complexed oximes **3** can be transformed to aldehydes by refluxing with 60% H_2SO_4 in benzene, the sequential application of these two hydrolytic processes to our enriched oxime acetate derivatives **6** would allow a new entry to both enantiomers of tricarbonyl(benzaldehyde)complexes **4**. Finally, we noticed that acetates **6a-d** were spontaneously transformed into nitriles **5** when stored at room temperature for long time.

Work is in progress to extend this procedure to the resolution of other tricarbonyl(*ortho* and *meta*-substituted benzaldehyde oxime)chromium complexes as well as of the corresponding keto-derivatives. The dramatic effect of small structural changes on the enantioselectivity of Lipase P (i.e the complete loss of selectivity on moving from **1** to **2** via **3**, or in the series **3a-3d**)can not be explained on the basis of the semplified active-site models proposed so far[15]. As the steric hindrance was quite similar in our substates, it is likely that a rationale based on electronic interactions must be found. Hopefully this goal will be achievable as soon as the tridimentional structure of the enzyme active site will be elucidated.

Experimental

General procedure for the synthesis of complexed oximes **3a-d**.

Complexed Benzaldehyde **4a-d**[2a] (1 mmol.) in methanol (10 ml) was added to a stirred solution of $NH_2OH.HCl$ (1.2 mmol.) and KOH (1.2 mmol.) in 5 ml H_2O. The reaction mixture was stirred at room temp. till the disappearance of starting complex (tlc: diethylether:light petroleum,1:1). After a standard work up, complexed oximes **3a-d** were recoverd as orange solid in 90% yield[6].

General procedure for the synthesis of complexed acetates **6a-d**.

Fresh distilled acetyl chloride (1.1 mmol.) in CH_2Cl_2 (2 ml) was added at 0°C to a stirred solution of **3a-d** (1 mmol.) and TEA (1.1 mmol.) in CH_2Cl_2 (10 ml). After a standard work-up, acetates **6a-d** were isolated by flash-chromatography. The butanoate **6e** was obtained similarly.

Lipase-catalysed acetylation of tricarbonyl(benzaldeide oxime)chromium complexes

The following procedure is representative: 140 mg of enantiomerically enriched oxime **3a** (59% e.e.) were dissolved in 14 ml of *t*-amyl alcohol containing 0.42 (- 10 eq) of vinyl acetate. Lipase P on celite[11] (200 mg) was added and the suspension shaken for 10 hours at 45°C (53% of conversion). Usual work up and flash-chromatography purification gave 86 mg of (+)-**6a** (98% e.e.) and 75 mg of residual oxime **3a** (20% e.e.).

Lipase-catalysed transesterification between tricarbonyl(benzaldehyde oxime acetate)chromium complexes and *n*-BuOH.

The following procedure is representative: 300mg of racemic **6a** were dissolved in 50 ml of *t*-amyl alcohol containing 0.9 ml (- 10 eq) of *n*-BuOH. Lipase P (750 mg) adsorbed over celite was added and the

suspension was shaken for 10 hours at 45°C. After this time (~62% of conversion as judged by chiral HPLC), the enzime was filtered off, the solvent evaporated and the crude residue purified by flash-chromatography (eluent: hexane:diethylether,1:1) to give 145 mg of oxime **3a** (59% e.e) and 100 mg of residual acetate (-)-**6a** (92%).

HPLC analyses.

Analyses were performed on a JASCO HPLC instrument (model 880-PU pump, model 870-UV detector) reading at 330 nm.

a) Transesterification between (±)-**6a** and n-BuOH: c, $e.e._p$ and $e.e._s$ were determined with a 10 μm Baker Bond Chiralcel OJ column; eluent: hexane:2-propanol, 5:1; flow rate 0.5 ml/min:

b) Transesterification between (±)-**6b** and n-BuOH: c was determined with a 10 μm Partisil 10 Whatman col.; eluent: hexane:AcOEt.,1:1; flow rate 0.5 ml/min.; $e.e._p$ was measured by a 10 μm Baker Bond Chiracel OD col.; eluent: hexane:2-propanol,4:1; flow rate 0.5 ml/min.; $e.e._s$ was obtained in the same condition after hydrolysis of the acetate with KOH in methanol

c) Transesterification between (±)-**6d** an n-BuOH: c and $e.e._s$ were determined with a 10 μm Baker Bond Chiracel OD col.; eluent: hexane:ethanol,4:1 flow rate 0.5 ml/min; $e.e._p$ was determined with a 10 μm Baker Bond chiracel OD col.; eluent: hexane:2-propanol,8:1, flow rate 0.6 ml/min.

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