Pergamon

Tetrahedron: Asymmetry, Vol. 7, No. 2, pp. 345-348, 1996

0957-4166/96 $15.00 + 0.00

S0957-4166(96)00002-X

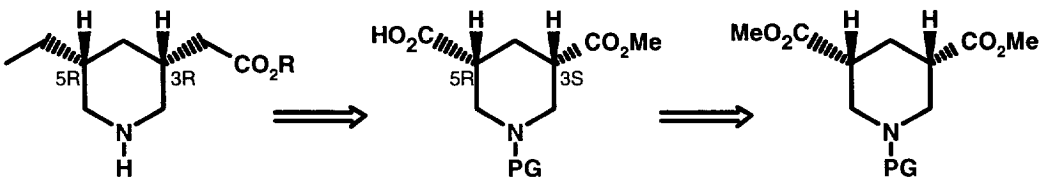
STEREOSELECTIVE ENZYMATIC HYDROLYSIS of DIMETHYL *meso*-PIPERIDINE-3,5-DICARBOXYLATES[1]

Bruno Danieli, Giordano Lesma,* Daniele Passarella, Alessandra Silvani

Dipartimento di Chimica Organica ed Industriale, Università degli Studi di Milano - Centro CNR di Studio sulle Sostanze Organiche Naturali - Via Venezian 21 - 20133 Milano (Italy).

Abstract: Desymmetrization of dimethyl *meso*-piperidine-3,5-dicarboxylates **3a-c** with pig liver esterase (PLE), lipase from *Candida cylindracea* (CCL) and porcine pancreatic lipase (PPL) is described. The enantioselectivities of the enzymatic transformations and the absolute configurations of the resulting half-esters **2a-c** were determined.

As part of an ongoing program aimed at the enantioselective total synthesis of indole alkaloids by chemo-enzymatic approach,[2] we envisioned the *cis*-(3*R*, 5*R*)-3,5-disubstituted piperidine **1** as a suitable precursor of the non-tryptamine portion of some *pseudo*-aspidospermidine and takamine alkaloids. This advanced intermediate may be derived from the C_1-symmetric hydrogen methyl-(3*S*, 5*R*)-piperidine-3,5-dicarboxylate **2** or its (3*R*, 5*S*) enantiomer **ent-2** (Scheme 1). In turn, access to these chiral half-esters can be achieved by desymmetrization, through enzyme-mediated hydrolysis, of σ-symmetrical dimethyl piperidine-3,5-dicarboxylates **3**. This methodology, which avoids the problem of loosing 50% of material which occurs in the kinetic resolution of racemates, has now become a very powerful tool in organic synthesis.[3]



1 **2** **3**

a) PG = Cbz b) PG = Ts c) PG = Bn

Scheme 1.

In the present work we wish to describe our preliminary results on the enzyme-catalyzed preparation of these heretofore unknown homochiral compounds **2**, and the determination of their absolute configuration.

The candidate substrates **3a-c**[4] employed in this study were obtained by a suitable *N*-protection of dimethyl *cis*-piperidine-3,5-dicarboxylate prepared from 3,5-dicarboxypyridine according to the procedure of *Stetter*.[5] In the early stage of the present study each of the *meso* substrates **3a-c** was submitted to the action of pig liver esterase (PLE, E.C. 3.1.1.1, 387 units/mmol substrate) at pH 7.5 in 25% DMSO/phosphate buffer. The pH value was adjusted and kept constant by continuous addition of 0.1 N NaOH and the progress of the hydrolysis reactions was followed by proton NMR (300 MHz).

Table 1. Asymmetric enzymatic hydrolysis of dimethyl *cis*-piperidine-3,5-dicarboxylates **3a-c.**

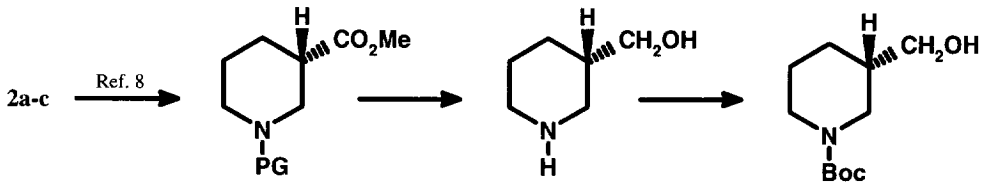
Entry	Substrate	Enzyme	Reaction time (h)	Product, %	% e.e.
1	**3a**	PLE[a]	1	**2a**, 28	44
2			6	96	27
3		CCL[b]	6	20	78[e]
4			20	58	57
5		PPL[c]	12	5	-
6	**3b**	PLE[a]	1	**2b**, 31	14
7			6	88	11
8	**3c**	PLE[a]	1	**2c**, 16	55
9			4	33	34
10		CCL[b]	6	25	80[f]
11			20	61	47
12		PPL[c]	1	14	-
13			7	28	12

[a] Substrate 46 mg/mL in 25% DMSO/phosphate buffer (pH 7.5), PLE (Sigma) 387 units/mmol substrate. [b] Substrate 16.6 mg/mL in *t*-butyl-methyl ether/H_2O (1:2, v/v), CCL (Sigma, Type VII) 10 units/mmol substrate. [c] Substrate 8.3 mg/mL in *n*-heptane/phosphate buffer (pH 7.2) (1:1, v/v), PPL(Sigma, Type II) 3350 units/mmol substrate. [d] A (3*S*, 5*R*) absolute configuration was determined in all cases for the major hydrolysis product (see text for more details). [e] $[\alpha]_{365}$ +29.5 (1% in MeOH). [f] $[\alpha]_{365}$ +46.0 (1% in Chloroform).

As can be seen from Table 1, all the reactions proceeded with good degree of conversion giving moderate stereoselectivity for substrates **3a** and **3c** (entries 1, 2, 8 and 9). On the other hand, PLE did not show significant stereoselectivity for **3b** (entries 6 and 7). Attempts to improve the stereoselectivity of the reactions by using different organic solvents (acetone, MeOH or MeCN) were unsuccessful.

While conditions for separating enantiomers of **2b** and **2c** have not yet been found, the e.e. of **2a** was determined by chiral HPLC using an LKB Enantiopak column, eluting with a buffered solution at pH 6.0 (10 mM NaH_2PO_4, 0.1 M NaCl, 8% propan-2-ol) and also confirmed by ^1H NMR (300 MHz, $CDCl_3$) spectroscopy in the presence of 0.9 mol eq. of (+)-ephedrine[6]. In order to determine its absolute configuration, the half-ester **2a**[7] (from entry 1) was converted into **5a**, in 78% yield, *via* a radical induced decarboxylation according to the *Barton* protocol[8] (Scheme 2). Selective reduction of the methoxycarbonyl group of **5a** with $LiBH_4$ in THF followed by removal of the Cbz protecting group (H_2, Pd/C, dioxane), afforded the piperidine-methanol intermediate **6** in 84% yield. Protection of the secondary amino group by reaction with $(Boc)_2O$ in THF-aq. NaOH gave **4** in quantitative yield.

The comparison of the specific rotation of **4** {$[\alpha]_{365}$ +27.6 (1% in EtOH)} with literature data showed that we have generated the (3*S*) enantiomer of the known *t*-butyl (3*R*)-3-(hydroxymethyl)-1-piperidinecarboxylate {$[\alpha]_{365}$ -60.7 (1% in EtOH)} documented by *Wirz*.[9] This result established a (3*S*, 5*R*) absolute configuration for the major hydrolysis product as indicated in structure **2a**. Moreover, the marginal preference of PLE toward the (*pro-R*) ester group in **3a** was confirmed by the separation of the enantiomers of **4** by GC analysis on a chiral column[10] in comparison with authentic samples of **4** prepared according to the literature[9] in both enantiomeric forms.



a) PG = Cbz b) PG = Ts c) PG = Bn

Scheme 2.

The same reaction sequence as described for **2a** was used to gain access to **4** from **2c**[7] while, in the case of **2b**, [7] hydrolytic cleavage was required to remove the *N*-tosyl protecting group. GC analysis[10] of crude **4** thus produced, allowed the determination of the e.e. and absolute configuration (3*S*, 5*R* as in **2a**) of the major enantiomer of the recovered half-esters as indicated in formulae **2b** and **2c**.

To improve the enantioselectivity of the enzymatic hydrolysis, further experiments were carried out on **3a** and **3c** with several commercially available enzyme preparations including Naproxen esterase, in phosphate buffer pH 8.0, lipases from *Candida cylindracea* (CCL), *Pseudomonas fluorescens* (Amano PS and SAM 2), *Mucor miehei* (Lipozyme) and porcine pancreatic lipase (PPL) in various solvent systems.

Of these enzymes, CCL gave the best results (Table 1, entries 3 and 10), while PPL was found only slightly active with **3c**. Among the solvent systems screened, *t*-butyl-methyl ether/H_2O[11] was the best, in terms of chemical yield and e.e., when CCL was used as catalyst, while *n*-heptane/ phosphate buffer (pH 7.2) was the most effective in the reaction catalyzed by PPL.

As can be seen from the results collected in Table 1, CCL, which is known to prefer bulky substrates,[11] was more selective than PLE. On the other hand, as for PLE, the enantiomeric excess of the half-esters **2a** and **2c** obtained with CCL is strongly dependent on the degree of conversion, with higher conversions leading to reduced e.e. values. Noteworthy is the fact that in all the substrates investigated with the enzymes PLE, CCL and PPL, the stereochemical course of the reaction is the same, giving the same enriched enantiomer **2a-c**.

Since a moderate ability of enzymes to discriminate between enantiotopic groups can be a consequence of the conformational mobility of the molecule,[3] we performed at this stage extensive molecular mechanics calculations[12] on **3a-c**. Accordingly, these analyses showed that **3a-c** adopt a large number of low energy

conformations belonging to chair and twist-boat families, the former being less energetic than the latter within a 2.20-3.03 Kcal/mol range.[13]

In conclusion, we have shown that enzymatic hydrolysis of *meso*-diesters **3a** and **3c** catalyzed by CCL can be used in preparation of hydrogen methyl piperidine-3,5-dicarboxylates **2a** and **2c** with acceptable enantiomeric excess, but with a fair degree of conversion. Because of the synthetic potential of optically active C_1-symmetric 3,5-disubstituted piperidines, we have undertaken an investigation on the enzymatic asymmetrization of *meso* piperidine-3,5-dimethanols. Details regarding this work will be presented in due course.

Acknowledgment: The financial support by MURST is gratefully acknowledged.

References and Notes:

1. Part of this work was presented at the Eight European Symposium on Organic Chemistry, Sitges, Barcelona, Spain, August 1993.
2. Danieli, B.; Lesma, G.; Mauro, M.; Palmisano, G.; Passarella, D. *J. Org. Chem.* **1995,** *60*, 2506-2513 and references cited therein.
3. Danieli, B.; Lesma, G.; Passarella, D.; Riva, S. Chiral Synthons via Enzyme-Mediated Asymmetrization of Meso-Compounds, in *Advances in the Use of Synthons in Organic Chemistry*; Dondoni, A.; Ed.; **1993**; Vol. 1, pp. 143-219.
4. Selected spectral data: **3a:** TLC, R_f 0.23 (Et_2O/hexane, 7:3); ^1H NMR (300 MHz, $CDCl_3$, 50°C) δ 5.12(2H, s), 4.41(2H, br, d, J = 12.0 Hz), 3.67(6H, s), 2.76(2H, t, J = 12.0 Hz), 2.46-2.40(3H, m), 1.68(1H, q, J = 13.2 Hz); IR($CHCl_3$) 1735, 1690 cm^{-1}; MS(FAB$^+$), *m/z* 336 (MH^+). **3b:** TLC, R_f 0.40 (Et_2O/hexane, 7:3); ^1H NMR (200 MHz, $CDCl_3$) δ 4.04(2H, dd, J = 11.6, 4.0 Hz), 3.65(6H, s), 2.64(2H, tt, J = 11.6, 4.0 Hz), 2.42(3H, s), 2.38(1H, dtt, J = 13.2, 4.0, 0.8 Hz), 2.21(2H, t, J = 11.6 Hz), 1.39(1H, q, J = 13.2 Hz); IR($CHCl_3$) 1728 cm^{-1}; MS(EI) *m/z* (%) 355(M^+, 4), 228(100). **3c:** TLC, R_f 0.44 (EtOAc/hexane, 7:3); ^1H NMR (300 MHz, $CDCl_3$) δ 3.65(6H, s), 3.55(2H, s), 3.10(2H, dd, J = 10.8, 4.2 Hz), 2.63(2H, tt, J = 12.0, 4.2 Hz), 2.33(1H, br, dt, J = 13.8, 4.2 Hz), 2.04(2H, t, J = 10.8 Hz), 1.54(1H, q, J = 13.8 Hz); IR($CHCl_3$) 1720 cm^{-1}; MS(EI) *m/z* (%) 291(M^+, 51), 200(100).
5. Stetter, H.; Hennig, H. *Chem. Ber.* **1955**, *88*, 789-795.
6. In these conditions **2a** (3*S*, 5*R*) showed at δ 3.69 for the methyl group of the methoxycarbonyl while, **ent-2a** (3*R*, 5*S*) showed at δ 3.67.
7. Selected spectral data: **2a:** TLC, R_f 0.27 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 5.56(1H, br, s), 5.12(2H, s), 4.45(2H, br, d, J = 11.9 Hz), 3.65(3H, s), 2.77(2H, br, t, J = 11.9 Hz), 2.62-2.40(3H, m), 1.72(1H, q, J = 13.0 Hz); MS(EI), *m/z* (%) 321(M^+, 21), 186(100). **2b:** TLC, R_f 0.44 ($CHCl_3$/MeOH, 9:1); ^1H NMR (200 MHz, $CDCl_3$) δ 4.13-3.95(2H, m), 3.66(3H, s), 2.74-2.51(3H, m), 2.39(3H, s), 2.21(2H, br, t, J = 11.4 Hz), 1.38(1H, q, J = 13.2 Hz); MS(EI) *m/z* (%) 342(M^++1, 6), 200(100). **2c:** TLC, R_f 0.26 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 3.63(3H, s), 3.60(2H, s), 3.12(2H, br, d, J = 12.0 Hz), 3.08(1H, br, d, J = 12.0 Hz), 2.62(1H, tt, J = 12.0, 3.9 Hz), 2.44(1H, br, tt, J = 12.0, 3.9 Hz), 2.28(1H, br, d, J = 13.6 Hz), 2.05(1H, t, J = 12.0 Hz), 2.03(1H, t, J = 12.0 Hz), 1.46(1H, q, J = 13.6 Hz); MS(EI) *m/z* (%) 277(M^+, 66), 186(100).
8. Barton, D.H.R.; Hervé, Y.; Potier, P.; Thierry, J. *Tetrahedron* **1988**, *44*, 5479-5486
9. Wirz, B.; Walther, W. *Tetrahedron: Asym.* **1992**, *3*, 1049-1054.
10. GC separation of the enantiomers of **4** was performed on permethylated 50 m β-cyclodextrine (CD-M) column (150-200°C with 1°C/min).
11. Wong, C.H.; Whitesides, G.M. Enzymes in Synthetic Organic Chemistry, in *Tetrahedron Organic Chemistry Series*; Baldwin, J.E.; Magnus, P.D.; Eds.; Pergamon, **1994**, Vol. 12, pp. 94-98.
12. All calculations were performed with HyperChem program (release 4.0, Autodesk, Inc.). Candidate conformations were generated by dihedral driving using constrained minimization. Once the alternative conformation was generated, constraints were removed and the structure was reminimized.
13. These results were consistent with a recent report on molecular mechanics study of acylpiperidine conformations, see: Schnur, D.M.; Yuh, Y.H.; Dalton, D.R. *J. Org. Chem.* **1989**, *54*, 3779-3785. In addition these calculations showed that for **2a** the chair conformations with the two methoxycarbonyl groups equatorial disposed, are more stable by about 0.40-0.58 Kcal/mol with respect to the others with the same groups axially oriented. In the case of compound **2c**, this difference ranges from 0.46 to 0.65 Kcal/mol in the opposite sense.

(*Received in UK* 10 *October* 1995; *accepted* 15 *December* 1995)