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ENANTIOSELECTIVE HYDROLYSIS OF 2-(CHLOROPHENOXY)PROPIONIC ESTERS BY ESTERASES.

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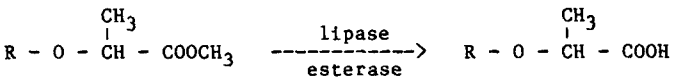
Abstract: Enzymatic hydrolysis of racemic 2-(mono-,di- and trichlorophenoxy)-propionic acids methyl esters by α-chymotrypsin, pig liver esterase and porcine pancreatic or Candida cylindracea lipases was found to be poorly to moderately enantioselective; in most cases, the R-ester was preferentially hydrolyzed.

Chlorophenoxypropionic acids **2b-f** are well-known herbicides which are currently used as racemates although only R-enantiomers are known to be biologically active [1]. Acid **2b** is also known as an hypocholesterolemic agent (R-enantiomer again biologically active) [2] and as an activator of microsomal stearoyl-Coa desaturase activity [3]. Several methods have been reported for the resolution of racemic phenoxypropionic acid derivatives: chemical methods involve kinetic resolution [4], crystallization of diastereoisomeric salts [5]; biological methods involve enantioselective microbial reduction by Gloeosporium olivarum [6] or Glomerella cingulata [7], enantioselective esterification of acid **2d** [8,9] and enantioselective hydrolysis or transesterification of ester **1d** [8,10] by Candida cylindracea lipase.



1a : R = Phenyl
b : R = 2'-Cl-phenyl
c : R = 3'-Cl-phenyl
d : R = 4'-Cl-phenyl
e : R = 2',4-diCl-phenyl
f : R = 2',4',5'-triCl-phenyl

2a-f

We have investigated the enantioselectivity of the hydrolysis of methyl esters **1a-f** by currently used esterases such as α-chymotrypsin, Candida cylindracea lipase, pig liver esterase and porcine pancreatic lipase.

EXPERIMENTAL: Esters **1a-f** were prepared from the corresponding acid chlorides. Enzymatic hydrolyses were carried out in conditions described in Table 1. One unit of α-chymotrypsin (type VII, Sigma) hydrolyzes 1.0 micromole of N-benzoyl-L-tyrosine ethyl ester per minute at pH 7.8 and 25°C. One unit of lipase from Candida cylindracea (type VII, Sigma) or lipase from porcine pancreas (type II, Sigma) liberates 1.0 microequivalent of fatty acid from olive oil in one hour at pH 7.7 and 37°C. One unit of pig liver esterase (Boehringer) hydrolyzes 1.0 micromole of ethyl butyrate per minute at pH 8 and 25°C. The course of the reaction was followed by reversed-phase HPLC on a 25x0.46 cm I.D. Nucleosil (5μ)-C8 column eluted with a pH 3, 0.1 M phosphate buffer containing 40 to 60 % methanol; the detection was done at the

following wavelength: **1a**, 269 nm; **1b**, 275 nm; **1c**, 274 nm; **1d**, 279 nm; **1e**, 284 nm; **1f**, 288 nm. The λ_{max} and corresponding ε_{max} were found to be identical for carboxylic acids and methyl esters. The extent of hydrolysis was calculated from the relative areas of the ester and acid peaks. After extraction of the remaining ester from the reaction mixture with hexane, the enantiomeric excess was directly determined by HPLC on a 25x0.46 cm I.D. covalently linked N-(3,5-dinitrobenzoyl)-R-phenylglycine column (J.T.Baker); the mobile phase was 0.25% 2-propanol in hexane [11]. Absolute configuration of the preferentially hydrolyzed enantiomer was determined by comparing the optical rotation of the acid obtained by extraction from the acidified hydrolysis mixture with data from the litterature [5,17,18].

Table I. Stereospecificity of the hydrolysis of esters **1a-f** by α-chymotrypsin (α-CT), lipase from Candida cylindracea (CcL), pig liver esterase (PLE), and porcine pancreatic lipase (PPL).

Ester	Enzyme[a] (U/ml)		Time (hours)	conversion (%)	ee[b] (%)	E[c] (absol. config. of the acid)
1a	α-CT	(50)	3	60	69.2	6.6 (R)
1b	"	(50)	3.5	51	43.2	3.9 (R)
1c	"	(50)	1.5	55	69.4	8.3 (R)
1d	"	(50)	2	52	26.3	2.0 (R)
1e	"	(50)	2.7	56	10.6	1.4 (R)
1f	"	(100)	5	44	4.2	1.2 (R)
1a	CcL	(800)	1.3	54	49.2	4.1 (R)
1b	"	(800)	1.1	55	59.8	5.0 (R)
1c	"	(400)	0.3	59	32.6	2.6 (R)
1d	"	(800)	0.7	52	69.2	10.5 (R)
1e	"	(800)	0.8	41	14.7	1.9 (R)
1f	"	(800)	2	52	1.4	1.0 -
1a	PLE	(0.04)	0,4	32	11.8	1.8 (R)
1b	"	(0.04)	0.5	60	14.2	1.3 (S)
1c	"	(0.02)	0.75	51	10.4	1.3 (R)
1d	"	(0.02)	0.5	41	32.4	3.4 (R)
1e	"	(0.02)	0.5	31	3.7	1.1 (R)
1f	"	(0.02)	3	27	1.0	1.0 -
1a	PPL	(560)	1	19	7.8	2.1 (R)
1b	"	(560)	2	32	12.6	1.9 (R)
1c	"	(560)	0.5	48	7.8	1.1 (S)
1d	"	(560)	1	50	20.2	1.9 (R)
1e	"	(560)	0.75	42	23.8	2.7 (R)

(a) Hydrolyses were carried out with 2.5 mM ester (excepted for **1a**/α-CT, 15 mM and **1a**/PPL or PLE, 5 mM) in the presence of 20 % DMSO, in 0.1 M, pH 7 phosphate buffer for α-CT (pH 7.5 for other enzymes) at 30°C. (b) enantiomeric excess of the residual ester. (c) values given were obtained by correlating C (% conversion) and ee% of residual ester (ee(S)) in equation 1 (see text). They are regression values for several experimental measurements at various % conversion and therefore may slightly differ from estimation of E obtained from isolated data given in this table.

RESULTS: Selected results are reported in Table 1. The most active enzyme was found to be PLE, as expected for a methyl ester substrate; however the enantioselectivity of the hydrolysis of most substituted substrates by PLE was negligible. α-CT was moderately active towards substrates **1a-f**; interestingly, we observed that to the higher reaction rate corresponded the higher degree of stereoselectivity, culminating with the meta-monosubstituted ester **1c** (E = 8.3). In all cases, the R-ester was preferentially hydrolyzed, as previously noted for other substituted 2-phenoxypropionic esters [13], in accordance with classical α-CT models [14]. Figure 1 illustrates the variation of stereoselectivity observed with α-CT acting on racemic substrates **1a-f**. Each curve was computer-generated with an enantioselectivity ratio E obtained by adjusting [15] all experimental values to equation 1, according to Sih et al. [12]:

$$E = \frac{\text{Log}\left[(1-C)(1-ee(S))\right]}{\text{Log}\left[(1-C)(1+ee(S))\right]} \qquad \text{(equation 1)}$$

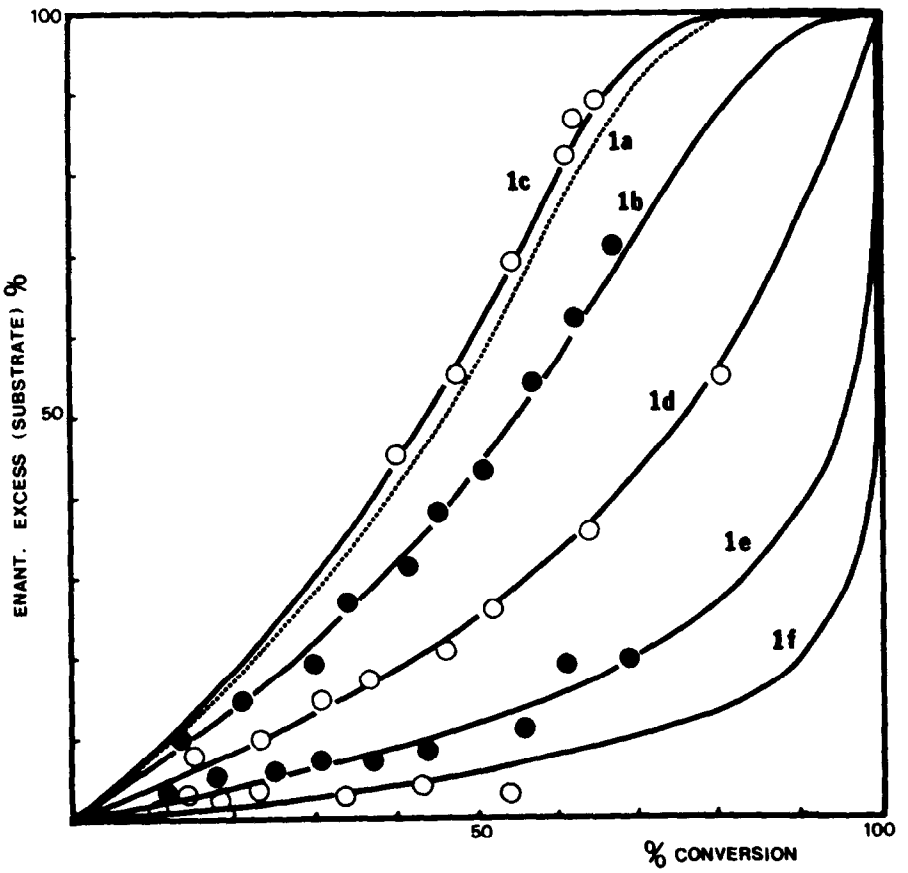


Figure 1: Evolution of the enantiomeric excess of the residual esters **1a-f** related to the progress of the hydrolysis by α-chymotrypsin (for the conditions, see table I); each curve was generated from equation 1 (see text) with the adjusted value of E calculated from all experimental data (-o-).

This formulation was previously found to apply correctly to α-CT hydrolysis of such esters by comparing the value of E obtained by equation 1 and the theoretical value ($E = Vm_R.Km_S/Vm_S.Km_R$)[12,13] deduced from the initial rate determination of the kinetic parameters of the hydrolysis of optically pure R- and S-ester **1a**. A similar conclusion can be inferred from the high correlation noted in fig.1 between experimental data at any conversion stage and regression curves.

While PPL exhibited a slightly higher hydrolytic activity compared to Candida cylindracea lipase, the later was more enantioselective, specially with the para-monosubstituted ester **1d** (E = 10.5) [16]. With all enzymes, the R-esters were again preferentially hydrolyzed, excepted for **1b** with PLE and **1c** with PPL, these two reversals been associated with very low enantioselectivity factors (1.3 and 1.1).

In conclusion, only moderately enantioselective hydrolysis methods could be found for the resolution of monosubstituted chlorophenoxypropionic esters **1b-d**, using α-CT or CcL; such methods, even by using product recycling [12,13], cannot compete with chemical methods [4,5,17]. Di- and trichlorophenoxyesters **1e-f** were generally more resistant to hydrolysis and poor enantioselectivity was found with classical commercial enzymes.

REFERENCES and NOTES:

1- C.R.Worthing, Ed., in "The Pesticide Manual", 6th ed. (BCPC Publication, London, 1979), p.329.
2- D.T.Witiak, T.C-L.Ho, R.E.Hackney and W.E.Connor, J.Med.Chem., **11**, 1086 (1968).
3- Y.Kawashima, N.Hanioka and H.Kozuka, J.Pharm.Dyn., **7**, 286 (1984).
4- U.Salz and C.Rüchardt, Chem.Ber., **117**, 3457 (1984).
5- E.Fourneau and G.Sandulesco, Bull.Soc.Chim.Fr., **31**, 988, 991 (1922); **33**, 459 (1923); A.Fredga and M.Matell, Arkiv Kemi, **4**, 325 (1952); M.S.Smith, R.L.Wain and F.Wightman, Ann.Appl.Biol., **39**, 295 (1952); M.Matell, Arkiv Kemi, **4**, 473 (1952); **7**, 437 (1954); A.Fredga, A.M.Weider and C.Grönwall, Arkiv Kemi, **17**, 265 (1961); B.Sjöberg and S.Sjöberg, Arkiv Kemi, **22**, 447 (1964); W.J.Gottstein and L.C.Cheney, J.Org.Chem., **30**, 2072 (1965).
6- Y.Tsuda, K.Kawai and S.Nakajima, Chem.Pharm.Bull., **33**, 1961 (1985).
7- Y.Tsuda, K.Kawai and S.Nakajima, Agric.Biol.Chem., **48**, 1373 (1984).
8- B.Cambou and A.Klibanov, Biotechnol.Bioeng., **26**, 1449 (1984)
9- G.Kirchner, M.P.Scollar and A.M.Klibanov, J.Am.Chem.Soc., **107**, 7072 (1985).
10- C.S Chen, S.H.Wu, G.Girkaudas and C.J.Sih, J.Am.Chem.Soc., **109**, 2812 (1987).
11- R.Dernoncour and R.Azerad, submitted to J.Chromatogr., 1987.
12- C.S Chen, Y.Fujimoto, G.Girkaudas and C.J.Sih, J.Am.Chem.Soc., **104**, 7294 (1982).
13- R.Dernoncour, S.Letellier and R.Azerad, unpublished results.
14- J.B.Jones and J.F.Beck, in "Applications of Biochemical systems in Organic Chemistry", J.B.Jones, C.J.Sih and D.Perlman ed., Techniques in Chemistry, vol.X, chap.4, Wiley, New York, 1976 .
15- R.G.Duggleby, Anal.Biochem., **110**, 9 (1981).
16- However such a measured enantioselectivity factor does not allow to explain precedently described results (ref.8), which asserted a nearly complete resolution of **2d** (96% ee. at 50% hydrolysis), in one hydrolytic operation.
17- J.Gabard and A.Collet, Nouveau J.Chim., **10**, 685 (1986).
18- M.Matell, Arkiv Kemi,**5**, 341 (1953); **6**, 251 (1953); E.Gamstedt, Arkiv Kemi, **32**, 151 (1970).

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