Pig liver esterase catalyzed hydrolysis: substrate specificity and stereoselectivity

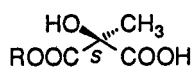Christoph Tamm

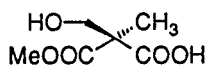Institut für Organische Chemie der Universität St. Johanns-Ring 19, CH-4056 Basel, Switzerland

Abstract: In order to gain a more detailed insight into the relationship between substrate structure and the stereoselectivity of the enzyme pig liver esterase (E.C. 3.1.1.1.) a large series of mainly *meso* and prochiral diesters with an open chain or a cyclic structure has been studied and evaluated. Results obtained with 3-substituted cyclopropane-1,2-dicarboxylates are incompatible with the three-dimensional (cubic) active-site model of PLE proposed by *J.B. Jones et al.* Kinetic resolution of *meso*- and racemic diesters as well as of racemic monoesters has been observed. Their synthetic potential as versatile enantiomerically pure synthons for the construction of complex natural products is demonstrated by the synthesis of the pheromone *endo*-1,3-dimethyl-2,9-dioxabicyclo[3.3.1]nonane, C(19)-to-C(27)-Segment of rifamycin S and the C(1)- to C(7)-segment of 14-membered macrolide antibiotics.

The efficient synthesis of biologically active compounds, either of natural or unnatural origin, frequently requires chiral synthons. Enzymes as chiral catalysts are now widely used for their preparation (ref. 1-11), because it is often rather difficult to introduce centres of chirality or perform regiospecific transformations by the application of purely "chemical methods". Especially esterases, such as pig liver esterase (PLE, E.C. 3.1.1.1.), a serine hydrolase, have been studied extensively in recent years (ref. 7, 12-15). Stability, low costs, and the ability of hydrolyse a wide range of substrates with high stereoselectivity represent additional advantages of this enzyme which operates without the need for co-enzymes. Until now, more than one hundred different esters, mainly *meso*- and *prochiral*-diesters have been subjected to the treatment with PLE (ref. 7). To be able to fully exploit the potential of this enzyme, it is indispensable to understand the factors which are responsible for the specificity. Accordingly, we as well as other research groups have initiated investigations for securing a large number of data on hydrolyses, allowing to gain more insight into the realtionship between substrate structure and enzymic activity of PLE. Commercially available PLE preparations are mixtures of at least six isoenzymes which, however were found to exhibit essentially the same stereospecificity (ref. 16). These findings justify the attempts to rationalize the results of the hydrolyses by an active-site model which is mainly based on the measured ee values and the absolute configuration of the hydrolysis products.
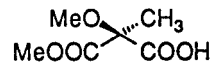
The following selected examples of results which were obtained by us and various other authors from *meso*-diesters in the malonate series appear to be very inconsistent and unpredictable with respect to the ee values and absolute configurations at first sight:
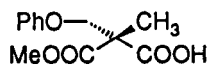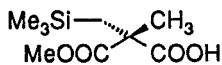
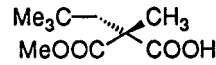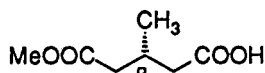|		
HO⸚CH₃ ROOC—ₛ—COOH	HO—CH₃ MeOOC—COOH	MeO⸚CH₃ MeOOC—COOH
R = Me: 46 %ee R = Et : 42 %ee	6 %ee	21 %ee
PhO—CH₃ MeOOC—COOH	Me₃Si—CH₃ MeOOC—COOH	Me₃C—CH₃ MeOOC—COOH
67 %ee	95 %ee	96 %ee

The same is true for glutarates:
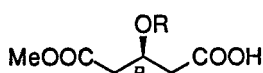
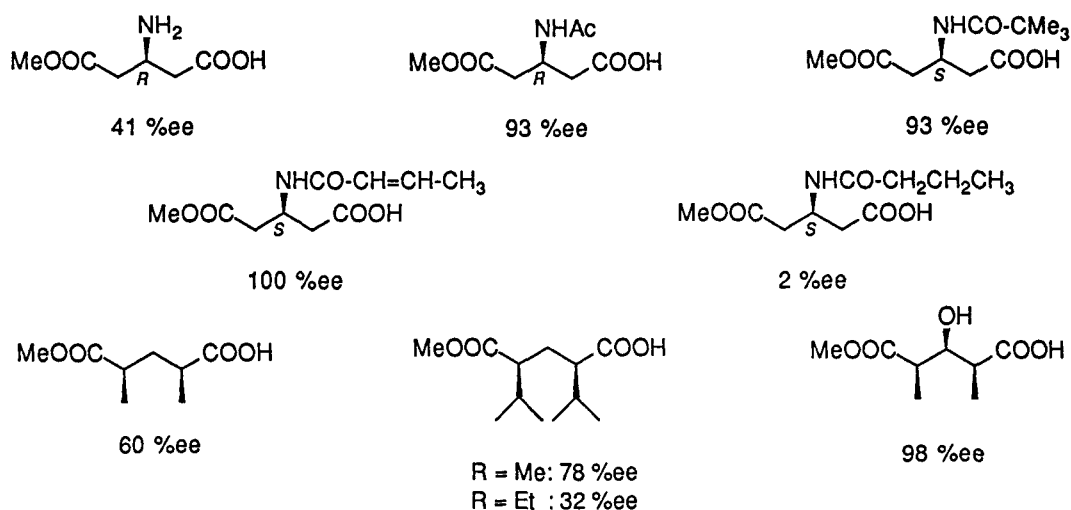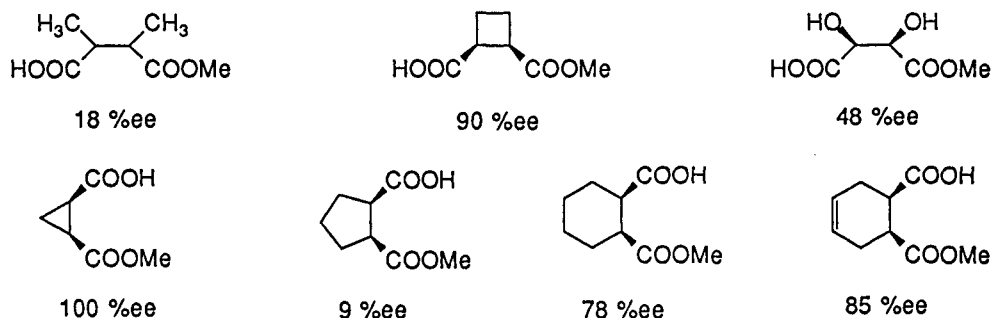|		
CH₃ MeOOC⌄⌃⌄COOH R	OR MeOOC⌄⌃⌄COOH R	HO CH₃ ROOC⌄⌃⌄COOH R
90 %ee	R = H : 12 %ee R = Ac : 51 %ee	R = Me: 98 %ee R = Et : 84 %ee

MeOOC—(NH₂)(R)—COOH
41 %ee

MeOOC—(NHAc)(R)—COOH
93 %ee

MeOOC—(NHCO-CMe₃)(S)—COOH
93 %ee

MeOOC—(NHCO-CH=CH-CH₃)(S)—COOH
100 %ee

MeOOC—(NHCO-CH₂CH₂CH₃)(S)—COOH
2 %ee

MeOOC—COOH
60 %ee

MeOOC—COOH
R = Me: 78 %ee
R = Et : 32 %ee
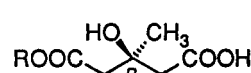
MeOOC—(OH)—COOH
98 %ee

Cyclic diesters again yielded different ee-values as well as changes of stereoselectivity from (S) to (R) were encountered. Cyclic structures exhibited higher stereoselectivity as compared to an acyclic analogue:

H₃C—CH₃
HOOC COOMe
18 %ee

HOOC COOMe
90 %ee

HO OH
HOOC COOMe
48 %ee

COOH
COOMe
100 %ee

COOH
COOMe
9 %ee

COOH
COOMe
78 %ee

COOH
COOMe
85 %ee

We concluded that high stereoselectivity is found only if the *prochiral* centre is in α- or β-position of the ester group. Rigid conformation is favorable. Substituents of different polarity and different size show opposite effects on the selectivity of enzyme hydrolysis. Whereas in one case the (*pro-S*)-group is hydrolysed, in the other case the (*pro-R*)-group is preferably attacked. In six-membered rings equatorial orientation of the ester group is required (ref. 17). The latter conclusion was demonstrated by the hydrolysis of dimethyl 4,5-epoxy-1,2-*cis*-cyclohexanedicarboxylate which finally led to a hydroxy acid with a γ-lactone group. This polyfunction-alized molecule is a versatile chiron (ref. 18).

O=⟨COOMe⟩—COOH (e) ⟶ O=⟨COOMe⟩—COOH ⟶⟶ ⟨lactone⟩—COOH, HO
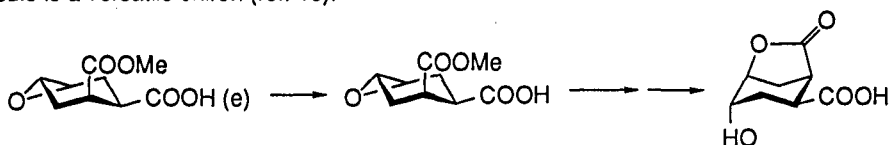
PLE can also be used for resolution of racemic esters (ref. 19) and for the preparation of a variety of useful synthons (ref. 12, 13, 19, 20) as demonstrated by the dimethyl *meso-* and *rac.* 3,4-epoxyadipates:

MeOOC—⟨O⟩—COOMe ⟶ MeOOC—⟨O⟩—COOMe
99 %ee

MeOOC—⟨O⟩—COOMe ⟶ MeOOC—⟨O⟩—COOH
MeOOC—⟨O⟩—COOMe
95 %ee
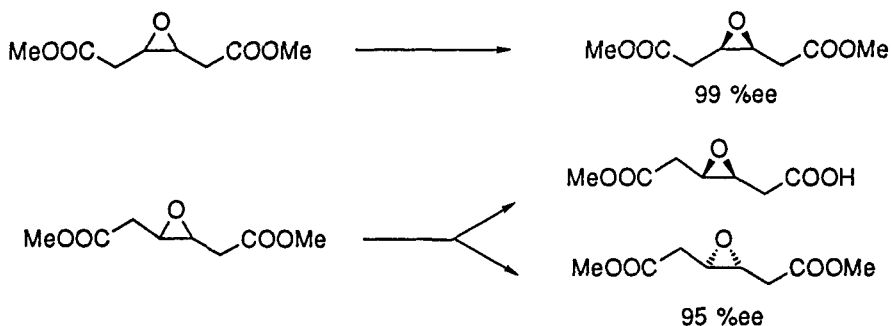
Kinetic resolution is also observed for racemic monoesters, e.g. methyl 3,4-epoxybutanoate:


An optically active 3,4-epoxybutanoate served as starting material for the presentation of functionalized *erythro*-1,3-diols, one of which was transformed readily to the pheromone (R=CH₃) *endo*-1,3-dimethyl-2,9-dioxabicyclic [3.3.1]nonane (ref. 21):
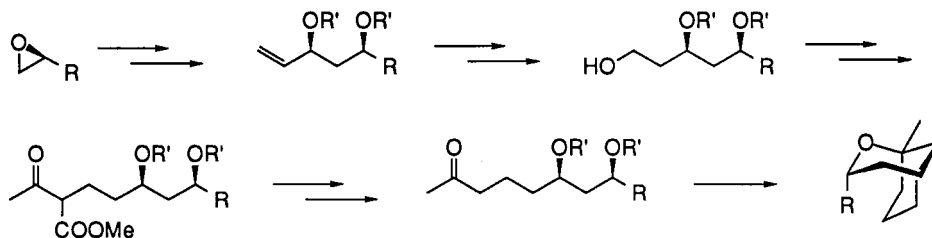

The stereoselective synthesis of the C(19)- to C(27)-segment 2 of the the antibiotic rifamycin S (1) represents an instructive example for the usefulness of chirons made available by a PLE hydrolysis. Dimethyl 2,4-dimethyl-3-hydroxyglutarate (4) is converted to the desired monoester 5 with 98% ee. At first it was converted to the protected 23-epimer 6 of the C(19)- to C(27)-segment, because the epimerization at C(23) caused unexpected difficulties. They were overcome by the stereoselective reduction of an intermediate possessing a 23-keto group by zinc borohydride. The building block 3 was obtained in a satisfactory yield. After removal of the isopropylidene group the resulting diester did not exhibit any optical activity thus proving the correct stereochemistry (*meso* form). It was confirmed by X-ray diffraction (ref. 22).
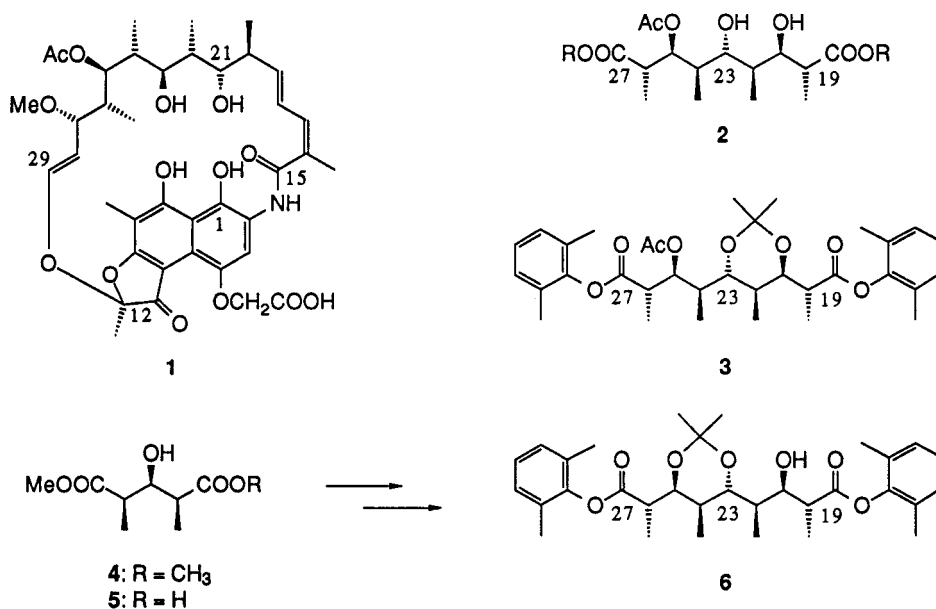
In a similar way a chiral synthon of the C(1)-to-C(7)-segment of 14-membered macrolide antibiotics of type 7 and 8, respectively, was synthesized using the same glutarate 4 as starting material (ref. 23, 24):
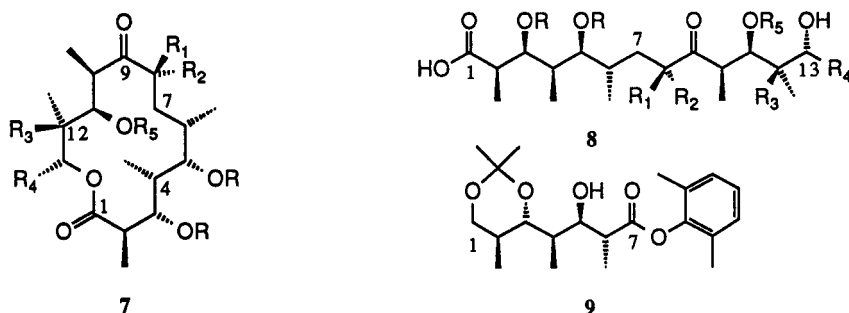
In order to rationalize the results of the PLE hydrolyses several approaches to an active-site model have been reported (ref. 16, 17, 26, 27). The most recent proposal made by *Jones* and coworkers which is based on cubic-space descriptors, is at present the most precise published model (ref. 28, 29). For testing its validity a study of the hydrolysis of a series of structurally related cyclopropane-1,2-dicarboxylates was carried out (ref. 30). These substrates were chosen for the following reasons: 1. The cyclopropane ring provides rigidity. Therefore only one preferred conformation has to be accounted for the analysis of the substrate/active site interaction. 2. Cyclopropane-dicarboxylates have been studied only rarely inspite of the fact that cyclopropane derivatives have become versatile building blocks for the synthesis of various natural products. 3. Cyclopropane rings also occur as structural elements of natural products. - The following ee-values were observed:

100 %ee

R = Me: 74 %ee
R = Et : 45 %ee
R = Pr : no hydrolysis

91 %ee

88 %ee

no hydrolysis
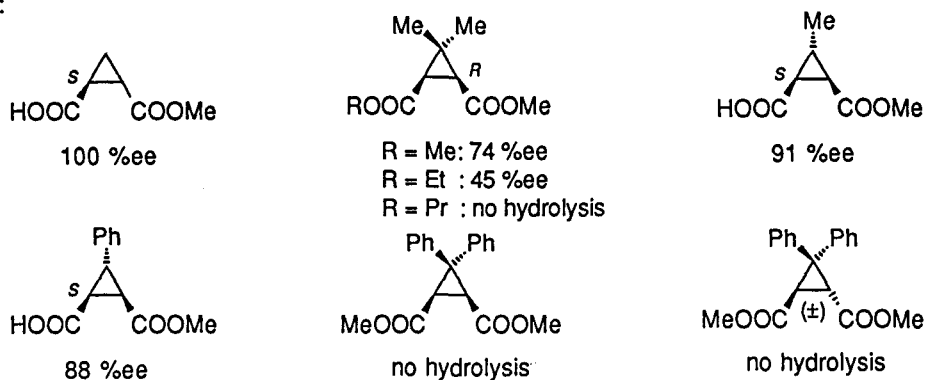
no hydrolysis

The absolute configuration of the hitherto unknown half-esters was proven by their conversion to the known monoesters and acids respectively by a *Barton* decarboxylation.

R = Me
R = H

The hydrolysis products of the unsymmetrical racemic 3-phenyl-1,2-*trans* dicarboxylates were obtained in yields of 90-99%, but remained nearly racemic. Thus the enzyme does not distinguish the racemic esters.

R = Me
R = Et

PLE

R = Me: 6 %ee
R = Et : 5 %ee
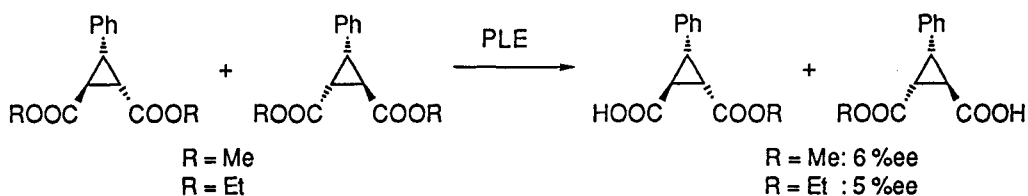
The results of the hydrolyses of the unsubstituted 1,2-dicarboxylate, the 3,3-dimethyl- and 3-Methyl-1,2-dicarboxylates as well as of both 3,3-diphenyl-1,2-dicarboxylates which did not undergoe hydrolysis due to the large steric hindrance by both phenyl groups at C(3), are compatible with the *Jones* active-site model. However regarding the configuration of the hydrolysis products of the 3-phenyl-1,2-dicarboxylate and the racemic diesters, the results are not in agreement with the model. In the case of the 3-methyl derivative it is the (*pro-S*)-ester group which undergoes hydrolysis, and not the (*pro-R*)-group as expected. Concerning the racemic 3-phenyl-1,2-dicarboxylates, the ester groups, *cis*-oriented with respect to the phenyl substituent, were expected to hydrolyse according to the model. However, the ester groups in *trans*-position were attacked as shown by the ^{1}H-NMR data. It is important to note that the enzyme does not distinguish between both enantiomers. Both are hydrolyzed. These results require the development of a modified active-site model for PLE which is compatible with the unexpected results mentioned. A proposal will be presented in due time. - Regarding the impact of larger alkyl rests in the ester groups on the stereoselectivity of PLE only the dimethyl ester of the 3,3-dimethyl-1,2-carboxylic acid is hydrolyzed with relatively high stereoselectivity. The homologous diethyl ester represents a worse substrate, and the dipropyl ester is not converted at all. These findings fully agree with the results of other substrates (ref. 31).

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