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STEREOSELECTIVE HYDROLYSIS of DIMETHYL AZIRIDIN-2,3-DICARBOXYLATES with PIG LIVER ESTERASE (PLE)

Peter Renold and Christoph Tamm*

Institut für Organische Chemie der Universität Basel,

St. Johanns-Ring 19, CH-4056 Basel, Switzerland

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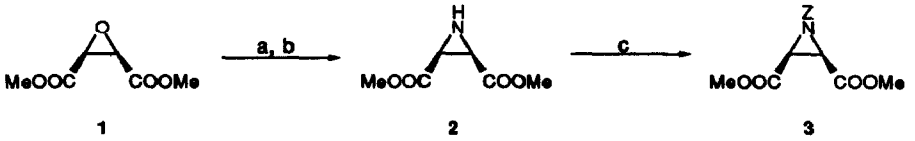
Abstract: The hydrolysis of the dimethyl *meso* aziridine-2,3-dicarboxylates **2** and **3** and the racemic dimethyl aziridine-2,3-dicarboxylates *rac*-**7** and *rac*-**8** with pig liver esterase (PLE) is described. The enantioselectivities of the enzymatic transformations and the absolute configurations of the resulting half esters **4** and **5** and diesters **7** and **8** were determined.

The transformation of achiral precursors into chiral synthons by enzymatic methods is a well established pathway in modern organic chemistry. The ability of pig liver esterase (PLE, E.C. 3.1.1.1) to hydrolyse stereoselectively *meso*-diesters or to resolve racemic mixtures of esters is well documented[1]. Stability, low costs, and broad substrate specificity are additional advantages of this enzyme which operates without the need of a coenzyme. The commercially available PLE preparations are mixtures of isoenzymes. It has been demonstrated that the stereospecificities of each isoenzyme are virtually identical, and for synthetic purposes PLE may be used as though it were a single enzyme[2]. Recently *Jones* and coworkers[3] proposed a cubic active site model for PLE that reflects the topology of the enzyme pocket. This model is generally used to predict the specificity of PLE toward methyl ester substrates.

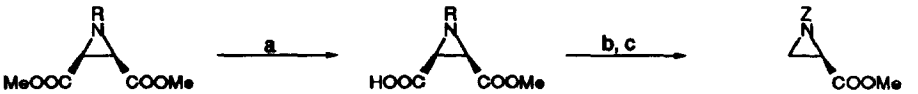
Some time ago we reported the hydrolyses of various cyclopropane-1,2-dicarboxylates with PLE[4]. In this paper we present the results of the enzymatic hydrolysis of a series of aziridines as another class of three membered ring substrates. Stereoselectively modified aziridine-carboxylates play an important role as chiral synthons for the synthesis of α- and β-aminoacids[5] and β-lactams[6].

The dimethyl oxirane-2,3-dicarboxylate **1** was prepared from fumaric acid according to a procedure published by *Payne* and *Williams*[7]. The cleavage of the oxirane ring to racemic dimethyl *syn* 3-azido-2-hydroxy-succinate was performed by *in situ* generated HN_3 from azidotrimethylsilane and methanol. The use of sodium azide for the ring opening reaction led to a mixture of *syn* and *anti* dimethyl 3-azido-2-hydroxy-succinates as decribed by *Zwanenburg et al.*[8]. After subsequent formation of the three membered ring with triphenylphosphine the *meso* dimethyl aziridine-2,3-dicarboxylate **2** could be isolated in 70% yield.



a: Me_3SiN_3, MeOH, DMF; b: PPh_3, DMF; c: benzyl chloroformate, CaH_2, THF.

A small amount of *trans*-aziridine **7** could easily be separated by column chromatography. When the mixture of *syn* and *anti* dimethyl 3-azido-2-hydroxy-succinates was treated with triphenylphosphine under the same conditions, *cis* -aziridine **2** and *trans*-aziridine *rac*-**7** were isolated in ratio of 5:4. The protection of the amino function of **2** and *rac*-**7** as N-benzyloxycarbonyl (*Z*) derivatives by standard methods[9] was unsuccessfull. Therefore we developed a method in THF and calcium hydride as base at 0° C. After addition of benzyl chloroformate and a reaction time from 1 to 17 hours the protected aziridines **3** and *rac*-**8** were isolated in a yield of 89 and 52%, respectively.



a: PLE, H_2O, pH 7; b: **4**→ **5**: benzyl chloroformate, NaOH/H_2O, pH 7; c: *Barton* decarb.[12]

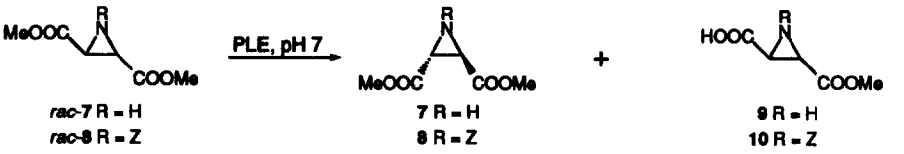
Meso-aziridines **2** and **3** were subjected to hydrolysis with PLE (35 units/mmol substrate) at pH 7.0 in water. The pH value was adjusted and kept constant by continuous addition of 0.1 N NaOH. After consumption of 1 equiv. of base the resulting half-esters were isolated by removal of the solvent by freeze drying as we have described earlier[10].

The enantioselectivity of the enzymatic hydrolysis and the absolute configuration of the resulting major products were determined as follows. Half-ester **4** was treated with benzyl chloroformate in water at pH 7.0. In order to prevent isomerisation at C(2) and C(3) the hydrochloric acid, which was formed in the course of the reaction, was neutralized by constant addition of 0.1 N NaOH. The N-protected half-ester **5** was isolated as described above.

The decarboxylation of half-ester **4** according to a method published by *Barton et al.*[11] for amino acids led to the mono-ester **6**. The comparison of the value and sign of the optical rotation of **6** with literature data shows that we have generated the (2*R*) enatiomer ($[\alpha]_D^{23}$ = +34.0 (c = 0.364, MeOH)) of the known methyl (2*S*)-1-benzyloxy-carbonyl-2-aziridinecarboxylate ($[\alpha]_D^{23}$ = -34.7 (c = 0.950, MeOH)) documented by *Sato* and *Kozikowski*[12]. The enantioselectivity of the enzymatic hydrolysis was determined by the separation of the enantiomers in the crude product of the *Barton* decarboxylation by GC analysis on a chiral column[13].

Aziridine **3** was hydrolyzed and then analyzed in a analogous manner. The results of these reactions are summarized in the table.

The kinetic resolution of the aziridines *rac*-**7** and *rac*-**8** by enzymatic hydrolysis with PLE was performed in water at pH 7.0 under the same conditions as described above for substrates **2** and **3**. The transformation was stopped



after a conversion of 50% indicated by the consumption of 0.5 equiv. of base and the unhydrolyzed diesters **7** and **8** were extracted from the aequeous solution with EtOAc. No further investigations were carried out on the remaining *trans* half-esters **9** and **10**. The enantioselectivity of the enzymatic reaction was estimated from GC analyses of the crude **7** and **8** on a chiral column[13]. The absolute configuration of the major enantiomer of the recovered diesters was determined by comparison of the value and sign of the optical rotations with literature data[8] after its conversion into the corresponding diacids with lithium hydroxide.

Table : Results of the PLE-Catalyzed Hydrolysis of Diesters **2**, **3**, *rac*-**7** and *rac*-**8**

Substrate	Product	t 1/2 (h)	% e.e.	Abs. Config.
2	**4**	2	92	2*S*, 3*R*
3	**5**	4	38	2*S*, 3*R*
rac-**7**	**7**	1	27	2*R*, 3*R*
rac-**8**	**8**	1	28	2*R*, 3*R*

The whole series of the dimethyl aziridine-2,3-dicarboxylates **2**, **3**, *rac*-**7** and *rac*-**8** proved to be substrates for PLE. The enzymatic reaction in the case of *rac*-**7** and *rac*-**8** was performed in a short half life time (1 h) but with low enantiomeric excess. The N-benzyloxycarbonyl substituent exerts no obvious influence on the reaction time and the stereoselectivity. In our opinion, it probably would be more successful to perform the transformation with a lipase e.g. from *Candida cylindracea*, as demonstrated for similar substrates by *Bucciarelli et al.*[14]

Inspection of the data in the table for *meso*-aziridines **2** and **3** reveals that the benzyloxycarbonyl group at the nitrogen in substrate **3** led to an increase of the reaction time and to a decrease of the e.e. value (38% e.e.) as compared with substrate **2** (92% e.e.). In both substrates the (*pro-S*) ester groups are preferentially hydrolyzed. The preference of PLE toward (*pro-S*) ester groups fully agree with findings on *meso* dimethyl cyclopropane-1,2-dicarboxylates with various substituents at C(3)[4].

The three-dimensional (cubic) active site model of PLE proposed by *Jones et al.*[3] deals with four pockets surrounding the catalytically active serine. Whereas two pockets with different size exhibit a hydrophobic character and interact with aliphatic and aromatic substituents of the substrates, the other regions accept polar groups. In the case of *meso*-diesters they bind the hydrolyzed and the non-hydrolyzed ester groups of the substrate.

To analyse our findings the ester groups of *meso*-aziridine 2 and 3 were placed into the two hydrophobic pockets of the *Jones* model. In consequence we had to disregard the polar character of the aziridine ring and to position the basic structures of the substrates either in the smaller or larger hydrophobic domain. The unsubstituted aziridine 2 fits well into the small hydrophobic pocket. Therefore the prediction by the model is compatible with the observed stereoselectivity. In the case of aziridine 3 the bulky benzyloxycarbonyl group has to be placed into the larger hydrophobic pocket and therefore we would predict an attack on the (*pro-R*) ester group by the catalytically active serine. However it is the (*pro-S*) ester group which is preferentially hydrolyzed by PLE.
The *Jones* model seems to be suitable for the analysis of the results of enzymatic hydrolyses of a series of different cyclic and noncyclic esters. But for substrates bearing additional polar groups the PLE active site model should be modified and take into consideration supplementary H-bond interactions between polar substituents and the protein surface of the catalytic pocket of the enzyme.

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