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# STRUCTURAL REQUIREMENTS IN THE ENZYMIC OPTICAL RESOLUTION OF BICYCLIC ESTERS USING PIG LIVER ESTERASE

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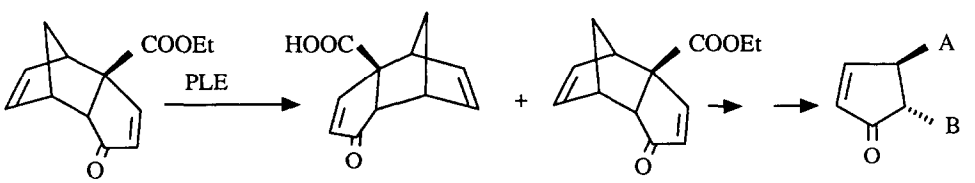
*Abstract: The PLE-catalyzed hydrolysis of bicyclic mono- and diesters **4-15** have been studied. Structural features such as bridge size and relative configuration of the ester functions have found to be essential for effective hydrolysis. A high degree of stereo- and enantioselectivity was observed for trans-bicyclo-[2.2.1]heptane-2,3-diesters **4, 5** and **6**.*

The use of biocatalysts for the synthesis of optically active compounds from both chiral and prochiral precursors is rapidly gaining recognition as a powerful synthetic methodology[1]. Recent work on enantioselective transformations has demonstrated that certain enzymes such as pig liver esterase (PLE) can accept a wide range of substrates.

In connection with our synthetic studies using polycyclic structures as synthons in natural product synthesis, we reported on the enzymic optical resolution of the unnatural substrate tricyclodecadienone ester **1**[2]. This rather bulky tricyclic ester appeared to be a surprisingly good substrate for PLE (Scheme). In a highly enantioselective

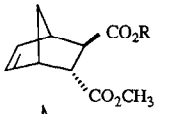
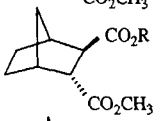
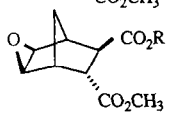
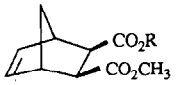
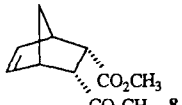
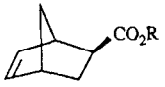
Scheme

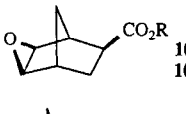
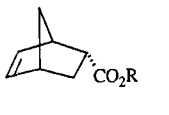
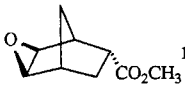
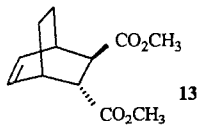
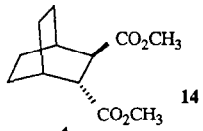
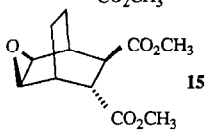



**1** **2** Y: 40% ee: 100% **3** Y: 48% ee: 80%

and rapid reaction, both the carboxylic acid **2** and the remaining ester **3** were obtained in excellent chemical yields and optical purities. This result and the fact that **1** is a conformationally completely defined structure offers an unique opportunity to establish the structural requirements which such an ester must meet for an effective PLE catalyzed hydrolysis. Based on studies of mono- and bicyclic *meso*-diesters[3,4,5], we hypothesized that in tricyclic ester **1**, the presence of a polar carbonyl function in the *trans*-position to the *exo*-ester function is essential for the observed effectiveness of PLE catalysis. To verify this hypothesis, we selected a series of

Table 1. PLE-catalyzed hydrolysis of bicyclic esters[a)]

| substrate | | reaction time | $[\alpha]_D$[b)] | %ee (%yield) | Absolute Configuration |
|---|---|---|---|---|---|
|  | **4a** | 3 hrs | +110.2 | 73 (45) | (+)-2S,3S[c)] |
| | **4b** | 3 hrs | -105.0 | 70 (45) | (-)-2R,3R[c)] |
|  | **5a** | 3.5 hrs | +31.7 | 82 (45) | (+)-2S,3S[c)] |
| | **5b** | 3.5 hrs | -33.2 | 90 (40) | (-)-2R,3R[c)] |
|  | **6a** | 6 hrs | -[d)] | 82 (45) | (+)-2S,3S[e)] |
| | **6b** | 6 hrs | -71.4 | 95 (40) | (-)-2R,3R[f)] |
|  | **7a** | | | <10[4)] | |
|  | **8** | no hydrolysis after 48 hrs[4)] | | | |
|  | **9a** | 1.75 hrs | +0.9 | <5 (45) | (+)-1R,2S,4R[g)] |
| | **9b** | 1.75 hrs | -1.0 | <5 (45) | (-) -1S,2R,4S[g)] |

| substrate | | reaction time | $[\alpha]_D$[b)] | %ee (%yield) | Absolute Configuration |
|---|---|---|---|---|---|
|  | **10a** | 0.5 hrs | | <2 (40) | |
| | **10b** | 0.5 hrs | | <2 (40) | |
|  | **11a** | 18 hrs | +44.5 | 30 (45) | (+)-1R,2R,4R[g)] |
| | **11b** | 18 hrs | -41.8 | 30 (45) | (-) -1S,2S,4S[g)] |
|  | **12** | no hydrolysis after 48 hrs | | | |
|  | **13** | no hydrolysis after 48 hrs | | | |
|  | **14** | no hydrolysis after 48 hrs | | | |
|  | **15** | no hydrolysis after 48 hrs | | | |

a) The optically active half esters are designated **a** (R=H) and the full esters **b** ($R=CH_3$). The racemic starting esters are notated by the number alone.

b) All optical rotations were measured in $CH_3OH$ (c=1) at roomtemperature.

c) The enantiomeric excess (ee) and absolute configurations of both the diesters **4b** and **5b**, and their respective half esters **4a** and **5a** have been established by their conversion into *trans*-bicyclo[2.2.1]heptane-2,3-dimethanol and comparison of their optical rotations with those reported for the latter compound[10]

d) Upon isolation half ester **6a** spontaneously hydrolyzed to the corresponding diacid which underwent an intramolecular lactonization to form 5-*endo*-6-*exo*-dihydroxy-bicyclo-[2.2.1]heptane-2-*exo*-3-*endo*-dicarboxylic acid-γ-lactone **16**[11] ($[\alpha]_D$[c)]= +25.1).

e) The enantiomeric excess and absolute configuration were determined by comparison with the optical rotation of the optically pure hydroxy-lactone **16**[11] obtained from optically pure **4a** by hydrolysis and peroxidation followed by lactonization.

f) The enantiomeric excess was established by converting **6b** into tricyclic hydroxy-lactone **16**[11].

g) The enantiomeric excess was determined by comparison with the optical data reported for this compound[12].

readily available bicyclic esters[6] and studied their hydrolysis by PLE.

The esters **4-15** were suspended in a 0.1. M phosphate buffer solution of pH 7.8 (5 mmol/ 50 ml) and incubated with PLE (240 µl/ 5 mmol, 360 units/ 5 mmol, purchased from SIGMA) at room temperature. The pH-value was maintained by continuous addition of 0.25 M NaOH using an auto-buret. At 50% conversion, the reaction was stopped by adding a $Na_2CO_3$-solution. Finally, acid-base extraction was used to seperate the carboxylic acid and the remaining ester. Table 1 summarizes the results.

Our experimental data clearly reveal that for norbornane diesters, the *trans*-relationship between the vicinal esters functions is essential for an effective enantioselective hydrolysis with PLE. All three *trans*-diesters studied, *viz.* **4**, **5** and **6** show a rapid, stereoselective hydrolysis with a high degree of enantioselectivity. The nature of the $C_5$-$C_6$ bridge has some but no critical effect on this enantioselectivity *viz.* reduction or epoxidation of the $C_5$-$C_6$ double bond slightly increases the enantioselectivity. Under identical conditions, the *endo-cis*-diester **8** does not show any hydrolysis at all, whereas the corresponding *exo-cis*-diester **7** is hydrolyzed with virtually no selectivity[7]. Apparently, in the norbornane 2,3-*trans*-diesters, the *endo*-ester function has just the right configuration to allow formation of a *rigid* enzyme-substrate complex in which the *exo*-ester function is favourably positioned at the active site of the enzyme. In the absence of such a vicinal *trans* ester function, the enzyme-substrate complex will be much more flexible and hardly any enantioselectivity is observed.

This hypothesis is convincingly substantiated by the behaviour of some norbornane mono-esters towards PLE. Although, both *exo*-esters **9** and **10** are rapidly hydrolyzed by PLE, hardly any enantioselectivity is observed. A somewhat better optical result is obtained for *endo*-ester **11**, which, however in a very slow reaction, afforded the corresponding acid in 30% ee. Strikingly, the *endo*-epoxy ester **12** is not hydrolyzed at all by PLE.

The high degree of stereoselectivity observed for PLE hydrolysis of *trans*-diesters **4**, **5** and **6**, and the observation that *exo*-esters **9** and **10** are much more rapidly hydrolyzed than *endo*-esters **11** and **12**, illustrate the discriminative power of PLE towards the norbornane *exo*- and *endo*-ester function[8]. This preference of PLE for the *exo*-ester function can be understood by assuming that the orientation of the bicyclic diester in the enzyme pocket is critically dependent on the steric size of the $C_5$-$C_6$ ethylene and $C_7$ methylene bridges. With the *exo*-ester function (*syn* to the methylene bridge) bonded at the active side of the enzyme, the space available for both the methylene bridge and ethylene bridge is apparently large enough to allow such an orientation. However, hydrolysis of the *endo*-ester function (*syn* to the ethylene bridge) would require the more bulky ethylene bridge to fit into the same area that accomodated the methylene bridge in the aforementioned case of *exo*-ester hydrolysis. The available space is apparently quite limited and as a consequence this orientation is unfavourable.

In accordance with this model, the enzymic hydrolysis of the corresponding bicyclo[2.2.2]octane *trans*-diesters **13**, **14** and **15** could not be accomplished. For the bicyclic mono-ester **11** having an *endo*-ester function, PLE hydrolysis is possible however at a very low rate.

In conclusion, our results on the PLE catalyzed hydrolysis of the norbornane esters clearly prove the crucial role of a carbonyl containing function *anti* and *vicinal* to the target ester group in this enzymic process. In addition, these results show that the difference in size of the methylene and ethylene bridge is largely responsible for the high degree of stereoselectivity in the norbornane esters. Finally, in contrast to earlier speculations[4] access to optically active norbornane derivatives by enzymic resolution is now quite feasible. As norbornane derivatives constitute the starting material in many synthetic routes, this enzymic resolution of norbornene diesters offers excellent prospects for enantioselective syntheses of important natural products[9].

## References and Notes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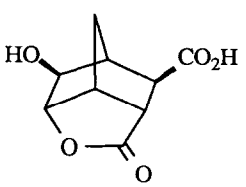
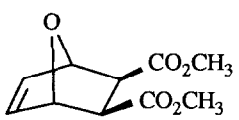
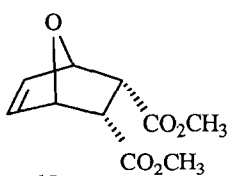
5. M. Ohno, Y. Ito, M. Arita, T. Shibata, K. Adachi and H. Sawai, Tetrahedron **40**, 145 (1984).
6. All the racemic esters studied, **4-15**, are methyl esters (R=$CH_3$) which were readily obtained in high yields following literature procedures.
7. Interestingly, Bloch[4] *et al.* showed that the corresponding *exo-* and *endo-cis-meso*-oxabicyclo[2.2.1]heptane diesters **17** and **18** are good substrates for PLE affording the corresponding half-esters in both high chemical and optical yields. Apparently, replacement of the lipophylic methylene bridge in **7** and **8** by the more polar oxa-bridge in **17** and **18** changes the nature of these molecules in such a way that now effective formation of an enzym-substrate complex is again possible.
8. Sicsic *et al.* who studied the PLE-hydrolysis of the ethylesters of the *exo-* and *endo*-7-oxabicyclo[2.2.1]heptene-2-carboxylic acids made a similar observation; S. Sicsic, J. Leroy, C. Wakselman, Synthesis **1987**, 155.
9. See also: Th. Oberhauser, M. Bodenteich, K. Faber and H. Griengl, Tetrahedron **43**, 3931 (1987).
10. D. Horton, T. Machinami and Y. Takagi, Carbohydr. Res. **121**, 135 (1983). The diol was purified by column chromatography (ethyl acetate/benzene 3:1).
11. 

**16**


**17**


**18**
12. J.A. Bernson, D.A. Ben-Efraïm, J. Amer. Chem. Soc., **81**, 4083 (1959); W. Kirmse, R. Siegfried, *Ibid.*, **105**, 950 (1983).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