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Enzymatic synthesis of a chiral building block for perhydrofuro[2,3b]furans

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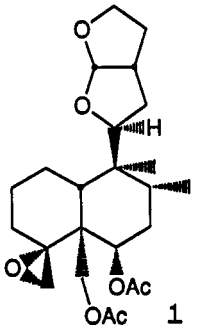
Abstract - 2-Methoxy-3-carbomethoxytetrahydrofuran (3) is a building block for perhydrofuro[2,3b]furans. Compound 3 was resolved by transesterification with butanol using the lipase of *Candida cylindracea* suspended in dry octane. The resulting mixture of methyl- and butylesters was separated by preparative gas chromatography. In this way 7-8 g of enantiomerically pure material (> 98% e.e) could be obtained in almost 100% yield.

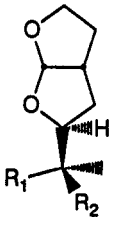
INTRODUCTION

The search for new crop-protective agents that are environmentally safe has led to increased interest in the exploration of indirect acting insecticides. These compounds interfere with specific processes in the insect life and are not harmful to other living creatures, in contrast to the direct acting insecticides which are broad-spectrum poisons. Within the group of indirect acting insecticides the antifeedants have received a lot of attention. Antifeedants inhibit the uptake of food by the insect and in this way can protect plants against insects (ref. 1). In our laboratory there is special interest in the total synthesis of sesqui- and diterpene antifeedants. Examples of compounds which have been synthesized by us are the sesquiterpenes polygodial, warburganal and muzigadial (ref. 2). More recently attempts have been made to synthesize the diterpene dihydroclerodin (1, see Scheme 1; ref. 3). This compound, which is found in the plant *Caryopteris divaricata* has strong antifeedant activity against caterpillars of *Spodoptera litura* (ref. 1). Dihydroclerodin is a representative of the clerodanes, a group of diterpenes which all possess a decalin skeleton and quite often also a perhydrofuro[2,3b]furan system (2) at the 9-position. For a total synthesis of dihydroclerodin an enantioselective synthesis of the furofuran system is a necessity, because coupling of a racemic furofuran to the decalin system would lead to four stereoisomers which will be difficult to separate. Besides, the effect of the stereochemistry in the furofuran system on the antifeedant activity has not been well documented yet and this induced us to the search for an enantioselective synthetic route to perhydrofuro-[2,3b]furans. We here report on the enzyme-catalyzed resolution of 2-methoxy-3-carbomethoxytetrahydrofuran (3) which is a building block for these systems (ref. 4).

Scheme 1



1



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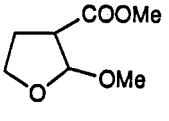
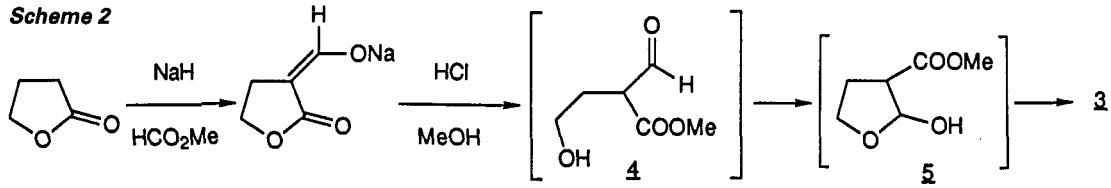


3

RESULTS AND DISCUSSION

The synthesis of racemic 3 is depicted in Scheme 2. γ-Butyrolactone is first formylated by sodium hydride and methyl formate. The resulting sodium salt is then treated with hydrochloric acid in methanol giving the ring-opened compound 4 which spontaneously closes to hemiacetal 5. Reaction with methanol then gives 3 in 50-55% yield (ref. 4).

Scheme 2



Synthesis of 2-methoxy-3-carbomethoxytetrahydrofuran (3)

Compound 3 has two chiral centers and the product obtained therefore consists of four stereo-isomers. Since the cis-trans mixtures can be easily separated by column chromatography in a later stage of the synthetic route to furofurans (ref. 4) we have confined ourselves to the resolution of the C-3-epimers.

Since 3 is an ester, and a lot of hydrolytic enzymes are well known for their good stereoselectivity (ref. 5), we have screened about ten commercially available esterases and lipases for their ability to react with 3. Although several enzymes hydrolyzed 3 rapidly, only in the case of the lipase from *Candida cylindracea* (CCL) the reaction rate strongly decreased around 50% conversion (see Fig. 1), indicating stereoselectivity. Indeed, the remaining ester isolated at the end of the reaction had an e.e. of 68%. This figure was not very impressive and there were some difficulties in the workup of the reaction as well. First, a large amount of emulsion was formed at the interface during the extraction of the products with ether. This is due to the fact that a crude lipase preparation was used, which is contaminated with membrane fragments, surfactants and structural proteins (ref. 6). Second, both products (3 and the carboxylic acid derived from it) appeared to be remarkably water-soluble, making their extraction quite inefficient. Third, the enzyme is partly inactivated during the extraction and cannot be reused. Although the first and third problem can be partly overcome by immobilization of the enzyme, the second point and the moderate e.e. remain and so another approach was chosen.

It has been recognized that biocatalysts, and especially membrane-associated enzymes like lipases are very well able to work in organic solvents, even when these solvents are almost completely water-free (ref. 7). This system has some profound advantages. The biocatalyst does not dissolve and can therefore be separated from the reaction mixture by simple filtration; reactions with water-insoluble compounds are very well possible, and there are indications that enzyme selectivity is improved when working in organic solvents (ref. 8). On the other hand, reaction rates are generally lower than in water (ref. 9). When working with lipases under these conditions, water is not available as a nucleophile, so a transesterification with an alcohol or a carboxylic acid has to be done. We chose to transesterify 3 with ethanol or *n*-butanol using CCL in a dry organic solvent. The enzyme was not active in acetonitrile but reacted quite fast in octane, in complete accordance with the fact that most biocatalysts work best in hydrophobic solvents (ref. 10). Just as in the aqueous system, the reaction rate strongly decreased when about half of the substrate molecules had been transesterified, which indicated that the reaction is stereoselective (see Fig. 2 for the reaction with *n*-butanol; ethanol gave almost the same graph).

The transesterification reaction with butanol is relatively slow ; 100 mg of enzyme per ml had to be used in order to complete it in about five hours. This means that for large-scale reactions a lot of enzyme is needed, which would make the process rather uneconomical. To check the reusability of the enzyme, it was filtered off from the reaction mixture, washed with octane and added to a fresh solution of substrates. As can be seen from Fig. 2, the course of the reaction with the reused enzyme is exactly superimposable on that in

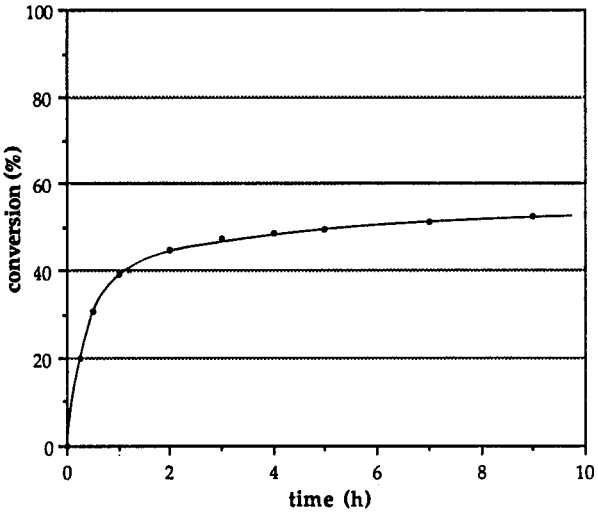


Fig. 1. Time course for the hydrolysis of 3 in water by the lipase from *Candida cylindracea*. Conditions: 3.0 mmol 3 and 1000 mg enzyme were brought in about 30 ml bidistilled water at 37° C; the liberated acid was titrated with 199 mM KOH using a pH-stat.

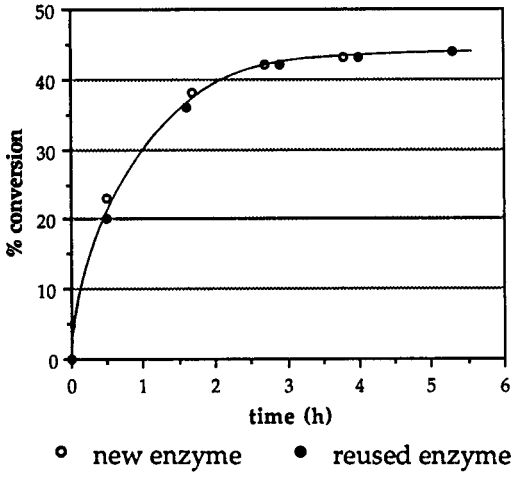


Fig. 2. Time course for the transesterification of 3 with *n*-butanol by the lipase from *Candida cylindracea* in octane. Conditions: 100 mM 3, 500 mM *n*-butanol, 100 mg/ml enzyme in dry octane; reactions were performed in 1 ml vials in an incubator /shaker set at 350 rpm and 45° C.
o: reaction with fresh enzyme
•: reaction with enzyme from previous reaction (reused enzyme)

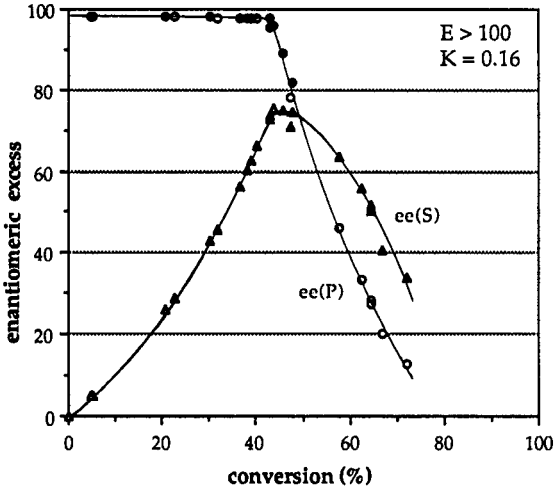


Fig. 3. Course of the enantiomeric excess during the transesterification of 3 with ethanol by the lipase from *Candida cylindracea* in octane. Conditions: 100 mM 3, 500 mM ethanol, 100 mg /ml enzyme in dry octane; reactions were performed in 1 ml vials in an incubator/ shaker set at 350 rpm and 45° C. E.e. values were obtained by injection on a chiral GC-column (SP-Cyclodextrin-B-236-M-19).
Δ: remaining substrate (3); ▲: duplicate reaction
o: enzymatic product (ethyl ester of 3);
•: duplicate reaction

which fresh enzyme was used. This means that there is essentially no enzyme inactivation during the transesterification, so the large amount of enzyme needed for the first reaction can be reused afterwards without any difficulty.

The enantiomeric excess of the enzymatic reaction product and the remaining substrate during the transesterification is shown in Fig. 3 for the case of ethanol; almost the same curves were obtained for *n*-butanol. As can be seen in this Figure the enantiomeric excess of the enzymatic product is at least 98% up to 45% conversion. The e.e. of the remaining substrate reaches about 75% but this can of course be improved by reincubation of this material (ref. 11). The curves match very nicely with the theory for reversible enzymatic pseudo-first order reactions as formulated by Chen et al. (ref. 11), although in our case the reactions are most probably second order because the ratio of ethanol/3 is only 5. It can be calculated from this theory that the equilibrium constant (K) is 0.16 (0.20 for *n*-butanol, with a *n*-butanol/3 ratio of 2.5) and the enantiomeric ratio (E) is more than 100 (also found for *n*-butanol).

These results were encouraging enough to proceed with an enzymatic resolution on a larger scale. For this, 12.8 g of 3 (200 mM) were incubated with 500 mM of *n*-butanol and 40 g of enzyme in octane up to a total volume of 400 ml in two well-capped erlenmeyer flasks. The flasks were shaken at 300 rpm/45° in a shaker/incubator and after 2.5 h (43% conversion) the enzyme was filtered off. Most of the octane was removed by evaporation and the resulting mixture of methyl and *n*-butyl esters was separated by preparative gas chromatography. This latter technique is very useful for this purpose: the methyl- and butyl esters were excellently resolved without racemization and the method is quite fast (± 2 g per injection, 45 min per chromatogram). Thus, this whole procedure from the enzymatic reaction to the GC-separation was done in one working day. Yields were close to 100% and the e.e. values were 98% for the butyl ester and 70% for the remaining methyl ester.

In this way it is easy to obtain substantial amounts of "enantiomerically pure" 2-methoxy-3-carbomethoxytetrahydrofuran (3). Of course, the products are not pure enantiomers since they still contain cis- and trans stereo-isomers which have to be separated later on. Unfortunately, the absolute configuration of the fast-reacting stereoisomer is still unknown. Data on the stereospecificity of CCL-catalyzed resolution of chiral carboxylic acid derivatives are relatively scarce and not applicable to our substrate. It is therefore necessary to convert the enzymatic product into a crystalline derivative and determine its absolute configuration by X-ray crystallography. This work is being carried out at the moment.

CONCLUSIONS

- The four stereo-isomers of 2-methoxy-3-carbomethoxytetrahydrofuran (3) can be separated into two enantiomeric pairs by the lipase of *Candida cylindracea* (CCL) with good selectivity. Yield of 7 g of pure material can easily be obtained in one run.
- Because of the higher solubility of the substrate in water, it is easier to work in an organic solvent. This remarkable paradox shows the great potential of enzymatic reactions in non-aqueous systems.
- Transesterification of 3 with ethanol or *n*-butanol proceeds without noticeable inactivation of the enzyme.
- The reactions of CCL with 3 in water and in octane have comparable enantioselectivities.

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