# ENZYMATIC RESOLUTION OF NEW ANTI-INFLAMMATORY DRUG ETODOLAC

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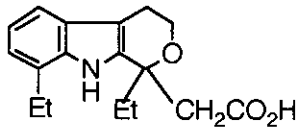
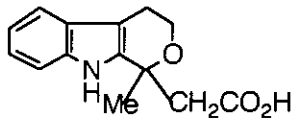
**Abstract** - Optically active etodolac (**1**) was easily prepared by lipase-catalyzed kinetic resolution. The unnecessary enantiomer as a by-product of the resolution could be racemized and was converted to a repeated substrate for the enzymatic reaction.

Etodolac (**1**,1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid) is a newly synthesized effective nonsteroidal anti-inflammatory drug with analgesic and antipyretic activity.[1] It has already been reported that virtually all of the effects of etodolac *in vivo* on prostaglandin synthetase inhibition and on reduce of adjuvant-induced arthritis, were due to the *(S)*-(+)-enantiomer (**1**).[2]

Now we wish to describe the efficient preparation of optically active etodolac (**1**) and its simplified analog (**2**) by utilizing a lipase-catalyzed resolution and subsequent racemization of an unnecessary enantiomer.

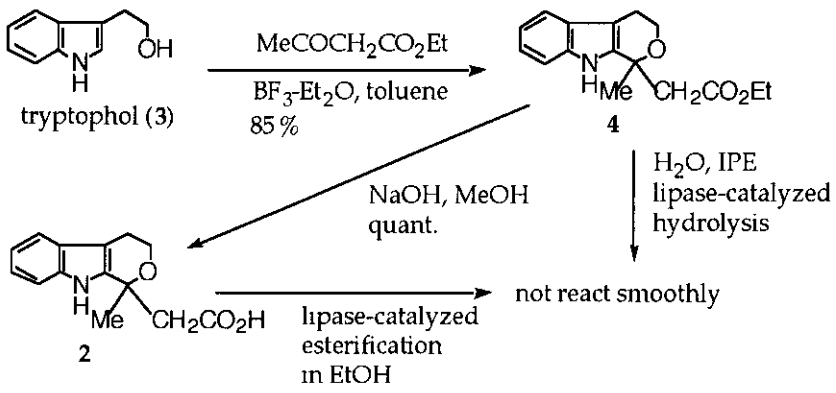



Etodolac (**1**)



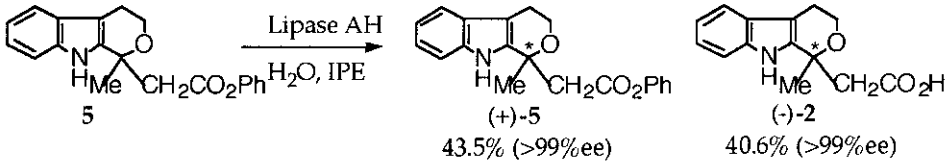

**2**

First, we have synthesized the racemic ethyl ester (**4**) from commercially available tryptophol (**3**) and ethyl acetoacetate. The product (**4**) was then submitted for lipase-catalyzed hydrolysis in water-saturated diisopropyl ether (IPE) with several enzymes. However, there was no enzyme which could smoothly hydrolyze **4** (Scheme 1). Furthermore, esterification of free carboxylic acid (**2**) by the same enzymes in ethanol did not occurr.




Scheme 1

Next, the phenyl ester (**5**) was tested as a more reactive ester under the same conditions. As expected, the phenyl ester (**5**) was effectively hydrolyzed with complete enantioselectivity by using Lipase AH *(Pseudomonas cepacia).* Similarly, the etodolac phenyl ester (**6**) was synthesized by reported procedure[3] and submitted to the enzymatic hydrolysis. To our disappointment, hydrolysis did not proceed, and more activated *m*-nitrophenyl and *p*-nitrophenyl esters of etodolac did not react neither (Figure 1).



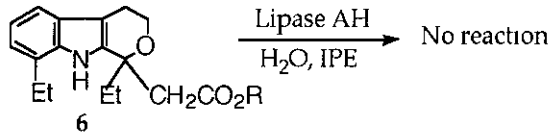
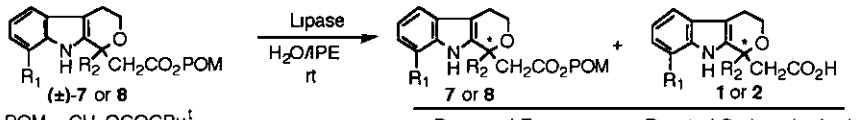




Figure 1

Thereupon, the pivaloyloxymethyl (POM) ester (**7**), which was developed by us previously as a useful ester in lipase-catalyzed hydrolysis,[4] was chosen. The hydrolysis of **7** with several lipases was examined and the reaction proceeded easily with the moderate enantioselectivity, although the chiral center was remote from the reacting ester site. The etodolac POM ester (**8**) was also tested in the same manner and the results were listed in Table 1. Among lipases tested, five enzymes could catalyze the hydrolysis of both POM esters [Lipase AH (Amano: *Pseudomonas cepacia* ), Lipase OF (Meito: *Candida cylindracea* ), Lipase QL (Meito: *Alcaligenes sp* .) IM(Lipozyme IM, Novo: *Mucor miehei* ), CHE (Amano: Cholesterol esterase) ].

Table1 Lipase-catalyzed Resolution of Racemic POM Ester




| | Entry | $R_1$ | $R_2$ | Enzyme | Time | Recoverd Ester Yield(%) | Recoverd Ester % ee | Reacted Carboxylic Acid Yield(%) | Reacted Carboxylic Acid % ee |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | H | Me | AH | 30h | 41 | 13 1(+) | 51 | 5 3(-) |
| | 2 | H | Me | OF | 3d | 32 | 35 9(+) | 62 | 19.4(-) |
| 7 | 3 | H | Me | QL | 5d | 41 | 89 0(-) | 56 | 58 4(+) |
| | 4 | H | Me | IM | 8h | 49 | 37 7(+) | 51 | 30 5(-) |
| | 5 | H | Me | CHE | 8h | 54 | 35 9(+) | 42 | 22 0(-) |
| | 6 | Et | Et | AH | 11d | 86 | 4 7(R) | 3 | 42 8(S) |
| | 7 | Et | Et | OF | 4d | 46 | 19.4(R) | 39 | 21 0(S) |
| 8 | 8 | Et | Et | QL | 8d | 33 | 84 1(S) | 58 | 45 6(R) |
| | 9 | Et | Et | IM | 5d | 35 | 7 6(R) | 53 | 10 3(S) |
| | 10 | Et | Et | CHE | 7d | 78 | 6 2(R) | 7 | 41 5(S) |

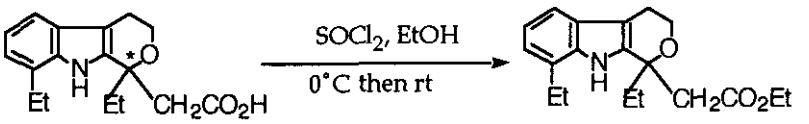
The reactions were carried out with a substrate (50 mg) and lipase (50mg), in IPE saturated with water at room temperature Yields were isolated ones Optical purerities were determined by HPLC analyses with Daicel Chiralcel AS, (Hexane-IPA=10-1) Absolute configurations of the product of entries 1~5 were not determined

There was resemblance of stereoselectivities between hydrolyses of **7** and etodolac ester (**8**) with listed five enzymes. Lipase QL showed the best selectivity in the both reactions. Absolute configuration of the carboxylic acid (**1**, etodolac) thus obtained (45.6% ee, entry 8)[5] was determined by comparison of the sign of specific rotation with that of specimen.[2]

The reaction mixture was filtered to remove the enzyme, and then treated with 0.5M $Na_2CO_3$ to separate the each products. From the organic phase, the optically active unreacted POM ester was obtained and the aqueous phase gave the resulting carboxylic acid. It was facile to separate the each chiral products without using chromatography.

Moreover, the undesired enantiomer was examined to be racemized. Etodolac ethyl ester (**9**) was prepared from the optically active carboxylic acid (**1**, 45.6% ee) by treatment with $SOCl_2$ in EtOH. After purification, we observed the racemization of **9** thus obtained, by HPLC analysis and measurement of specific rotation (Scheme 2). In the racemization step, it seemed to proceed the cleavage of the pyran ring C-O bond in acidic condition.

Optically active etodolac (**1**) was easily synthesized by lipase-catalyzed kinetic resolution using the POM ester methodology.[4]




optically active **1** racemic **9**

Scheme **2**

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5. *(S)*-**1** [84.1%ee] $^1$H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7 Hz), 1.32 (3H, t, J=7 Hz), 1.93-2.20 (2H, m), 2.71-2.92 (4H, m), 3.05 (2H, s), 3.98-4.18 (2H, m), 7.01 (1H, d, J=7 Hz), 7.07 (1H, t, J=7 Hz), 7.35 (1H, d, J=7 Hz), 8.65 (1H, br). $[\alpha]_D^{20}$ -20.3 (c=1.5, EtOH).

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