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# Lipase Mediated Diastereoselective Hydrolysis of Steroidal 3-(O-Carboxymethyl) Oxime Methyl Esters

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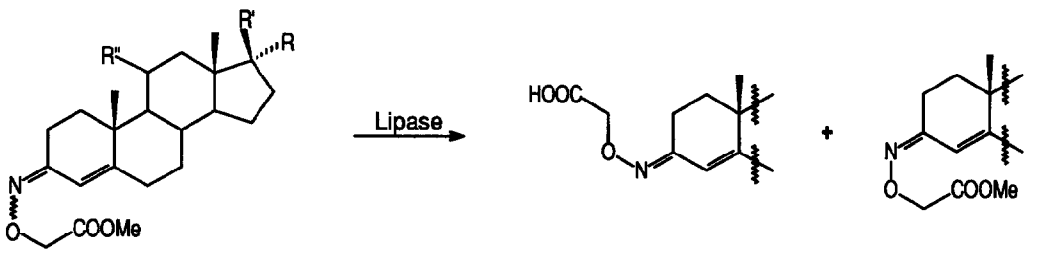
**Abstract:** A series of geometric isomeric mixtures of steroidal 3-(O-carboxymethyl) oxime methyl esters **1-4** were hydrolyzed with high diastereoselectivity for the *anti* isomer with crude lipase from *Candida cylindracea*.

We are currently undertaking structural studies of several acid/base sensitive steroids and other naturally occurring macromolecules which required a gentle hydrolysis method that was diastereoselective. Enzyme-mediated reactions are known for their stereoselectivity and are generally carried out under very mild conditions.[1] Lipase is well documented as a "broad spectrum" enzyme with high efficiency for hydrophobic substrates.[1] This work describes the use of lipase (from *Candida cylindracea*, Aldrich, type VIII) in the diastereoselective hydrolysis of 3-(O-carboxymethyl oxime) methyl esters 17-α-OH-progesterone (**1**), progesterone (**2**), testosterone (**3**), and cortisol (**4**).




**R, R', R"**; **1.** OH, C(O)Me, H **2.** H, C(O)Me, H **3.** H, OH, H **4.** OH, $C(O)CH_2OH$, OH

In a 1.5 mL polypropylene micro centrifuge tube was place 1 mg ester (≈2.4 μmol), as a 1:1 isomeric mixture, 980 μL lipase solution (7.5 mg/mL lipase in 100 mM sodium phosphate, pH 7.2), and 20 μL of Triton X-100.[2] The heterogeneous mixture was incubated for 48 hours (**4** was incubated for 24 hours) at 25 °C with agitation by rotation. The mixture was then acidified with 100 μL of 2 N HCl and extracted with 1 mL ethyl acetate. The organic layer was collected, concentrated by reduced pressure, and the resulting colorless residue was dissolved in 200 μL of methanol. The methanol solution was immediately evaluated by reverse phase HPLC on a 3 μ $C_{18}$ 6.2 × 150 mm column, eluting at 1 mL/min with water/methanol (see

Table 1) containing 0.1% vol/vol trifluoroacetic acid. Elution peaks corresponding to steroidal 3-carboxymethyl oximes and unreacted methyl esters were integrated (the relative percentage of the *syn* and *anti* product acids and unreacted starting esters are recorded in the Table 1). Retention times and elution order for the esters and free acids were determined using synthetically prepared materials.[3-5] The yield of the reaction is a summation of the *syn* and *anti* hydrolysis products determined by HPLC from the relative peak ratios of acids to unreacted esters. We did not observe any degradation of starting esters or product acids.

In summary lipase from *Candida cylindracea* proved to be effective in carrying out hydrolysis of methyl esters of steroidal 3-carboxymethyl oximes **1** - **4** in a mild manner. The enzyme exhibited preference for the *anti* isomer. The faster rate and greater selectivity observed for **4** is probably due to cortisol's better solubility in the reaction media. Currently, we are adapting these methods for the hydrolysis of esters of highly acid/base sensitive naturally occurring macromolecules.

**Table 1**

| Substrate* | % Product Acid | | % Yield | % Unreacted Ester | | Elution Buffer |
|---|---|---|---|---|---|---|
| | *syn* | *anti* | of Acids | *syn* | *anti* | MeOH:$H_2O$ |
| **1** | 14 | 86 | 53 | 89 | 11 | 70:30 |
| **2** | 20 | 80 | 56 | 80 | 20 | 75:25 |
| **3** | 20 | 80 | 55 | 81 | 19 | 70:30 |
| **4** | 6 | 94 | 52 | 98 | 2 | gradient** |

*1:1 isomeric mixture **Initial: 60:40, 35 min: 30:70

**References**

1. T. Itoh, E. Ohira, Y. Takagi, S. Nishiyama, and K. Hakamura, *Bull. Chem. Soc. Jpn.* 1991, **64**, 624-627, and the references therein.

2. Hydrolysis was not observed when Triton X-100 was omitted. It is presumed the detergent provided a hydrophilic interface required by lipase to facilitate measurable catalytic activity.
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3. A. H. Janoski, F. C. Shulman, and G. E. Wright, *Steroids* 1974, **23**, 49-64.

4. M. T. Shipchandler, J. R. Fino, L. D. Klein, and C. L. Kirkemo, *Anal. Biochem.* 1987, **162**, 89-101.

5. The synthesis and separation of the methyl esters and acids will be described in a future article. all synthetic products gave correct analytical data ($^1H$, $^{13}C$ NMR, MS, HPLC, and elemental analysis).