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A 2-METHYLENEOXETANE ANALOG OF ORLISTAT DEMONSTRATING INHIBITION OF PORCINE PANCREATIC LIPASE

Lisa M. Dollinger and Amy R. Howell*

Department of Chemistry, The University of Connecticut
Storrs, CT 06269-4060

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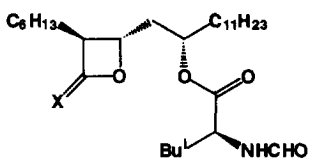
Abstract: The 2-methyleneoxetane analog **2** of orlistat (OLS, **1**) has been synthesized and tested against porcine pancreatic lipase (PPL). Despite the loss of the carbonyl group, a potential site for hydrogen bonding interaction with the enzyme and the key element in the acylation by OLS, **2** has activity comparable to **1**.

We recently described a method for the preparation of 2-methyleneoxetanes **3**[1] from the corresponding β-lactones **4** using the Petasis reagent (**5**)[2] and have embarked on a study of their reactivity under a variety of conditions. One reaction we have investigated is acid-catalyzed hydrolysis to give the corresponding β-hydroxyketones **6**. This successful ring opening of **3** mimics the mechanism via which β-lactones act as inhibitors of a number of different protease and lipase enzymes. Thus, in the case of β-lactones, inhibition occurs via acylation of an essential nucleophilic residue on the enzyme, resulting in cleavage of the O1-C2 bond. For the 2-methyleneoxetanes, alternate reaction pathways can be envisaged involving attack by the nucleophile at C2 or C4 (equation 1) facilitated by the presence of an appropriately located proton donor within the catalytic site. It seems likely that conformational flexibility within the active site would permit attack at either position. Given the obvious structural similarity between 2-methyleneoxetanes and β-lactones and some apparent confluence in their reactivity, we have initiated a program to determine the biological activity of **3**. Our initial target was methyleneoxetane **2**, an analog of orlistat (formerly known as tetrahydrolipstatin; OLS, **1**), which is an inhibitor of pancreatic lipase and shows anti-obesity and hypolipidemic activity.[3] We now wish to present our initial results detailing the successful methylenation of OLS and the high biological activity of the corresponding methyleneoxetane **2**.



1 X = O orlistat
2 X = CH_2

Our earlier study[1] demonstrated that β-lactones were particularly receptive to methylenation, reacting preferentially in the presence of other functional groups such as ketones and alkenes. However, concern for our ability to execute methylenation in the presence of the ester and formamide groups of **1** lead us to initially investigate the benzyl ether **7**. Treatment of this with **5** at 75 °C for 2 h gave **8** in 50% yield. Encouraged by this result and with the intention of avoiding, if possible, the use of protecting groups, we then investigated the tolerance of **5** for a free hydroxyl group. To our great satisfaction, **9** was also successfully transformed to the corresponding methyleneoxetane **10** (69%). The synthesis of **2** from **10** was completed as described in the literature.[4] Even more significantly, **1** was converted directly to **2** in an unoptimised 20% yield.[5]

Analog **2** was compared directly with **1** in an assay versus porcine pancreatic lipase (PPL), using tributyrin as the substrate.[6] Comparative IC_{50}'s were 0.4 μg/mL (**1**) and 1.7 μg/mL (**2**). Preliminary kinetic studies suggest

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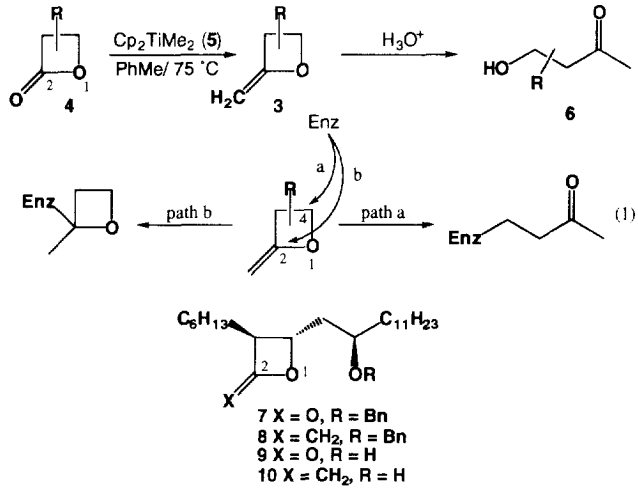
irreversible inhibition, but a definitive statement concerning the mechanism of action must await more detailed studies.



In conclusion, this report describes the preparation and biological activity of the novel OLS analog **2**. The activity is especially significant considering that the carbonyl group of OLS is integral to both interaction and reaction with pancreatic lipase and confirms that further study of 2-methyleneoxetanes is warranted. We are currently pursuing the methylenation of other biologically active β-lactones and also evaluating some of our simple methyleneoxetanes[1] against other hydrolytic enzymes.

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References and Notes

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5. This reaction was done with 1 equiv of **5** (normally 1.5 - 2.0 equiv are used). There were no other major products evident from a proton NMR spectrum of the crude reaction mixture. Nevertheless, the reaction was not as clean as we normally observe. Compound **2** was isolated as described in ref 1.
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