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A NEW STRONG INHIBITOR OF BETA-MANNOSIDASE

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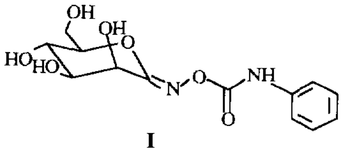
Abstract: N-phenyl-carbamate of D-mannonohydroxymolactone (**I**) was synthesized from mannose and was shown to be the best competitive inhibitor of beta-mannosidase so far reported (K_i = 25 nM).

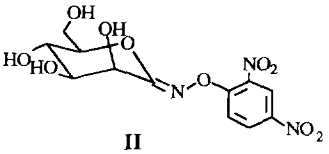
Natural as well as synthetic inhibitors of α-mannosidases have been the focus of much interest, since some of them (such as swainsonine) have been shown to have potential therapeutic applications as anticancer drugs[1]. On the other hand, only a few inhibitors of β-mannosidases have been described. Moreover, most of the numerous known inhibitors of α-mannosidases have poor, if any, action on β-mannosidases.

β-mannosidases are involved in number of biological processes like degradation of glycoproteins, germination of seeds and sporangia[2]. It is one of the numerous glycosidases acting on hemicelluloses present in wood degrading organisms[3] (fungi, termites, bacteria). In all these cases, inhibitors acting at very low concentration would be of interest in theoretical studies as well as in practical applications.

The syntheses of several derivatives of glyconolactone-oximes which are transition-state analogue inhibitors of glucosidases[4,5] and N-acetylglucosaminidase[6] have been published. We report here the synthesis of two strong inhibitors of β-mannosidase belonging to this class of products.



I



II

Compounds **I** and **II** were synthesized in a moderate yield of 50 %, following the general method of Vasella, *via* the known mannonohydroximolactone peracetate[4,7] and as previously reported for derivatives of several glyconolactone-oximes[4,5]. **I** and **II** were tested for their inhibitory activities on α-mannosidase from Jack beans and β-mannosidase from snails, using p-nitrophenylmannopyranosides as substrates (K_M = 1.2 and 0.57 mM respectively). Both compounds **I** and **II** were found to be competitive inhibitors of the two enzymes with comparable, modest activities on α-mannosidase (K_i 80 and 50 μM respectively). **II** was found to be a good inhibitor of β-mannosidase (K_i = 10 μM). Compound **I** strongly inhibited β-mannosidase, with K_i = 25 nM (Fig. 1). Thus, compound **I** is the best inhibitor of β-mannosidase so far reported.

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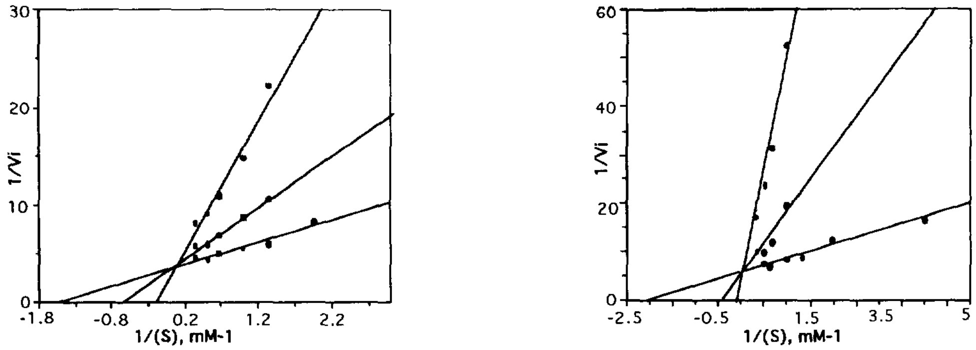

Fig. 1: Lineweaver-Burk plots of inhibition of β–mannosidase by **I** (left) and **II** (right). Conditions: p-nitrophenyl-β–mannoside as substrate, acetate buffer pH 4.5, 34°C; reaction stopped after 2-4 min by addition of sodium borate, then lecture of absorbance at 410 nm. Concentrations of inhibitor: 40 and 200 nM of **I** (left); 40 and 100 μM of **II** (right).

The *gluco*-analogue of **I** was previously reported to be a good inhibitor of β-glucosidase, and a modest inhibitor of α–glucosidase (K_i = 2.5 and 75 μM respectively)[4,5]. It is a better inhibitor of β-glucosidase than gluconolactone (K_i = 30 μM). Mannonolactone itself is a good inhibitor of β–mannosidase (K_i = 17 μM). Thus, the behavior of **I** on β–mannosidase is quite parallel to that previously reported for the *gluco*-analogue on β-glucosidase. As demonstrated for this last compound, glyconohydroximolactones are transition-state analogues[4]. In addition, like other inhibitors bearing an heteroatom on the "anomeric" position, they are thought to act by hydrogen bounding between this heteroatom and the catalytic carboxylic acid of the active site[8]. The efficiency of the inhibitor varies with the strength of this hydrogen bond, and, consequently, with the basicity of the heteroatom (for example, gluconohydroxymolactam is a better inhibitor of β–glucosidase than gluconohydroximolactone: K_i = 2.9 μM, versus 100 μM[9]). **II**, although a good inhibitor, is less performant than **I**: One can expect the nitrogen atom in **II** to be less basic than in **I**; The aromatic ring in **II** is closer to the nitrogen than in **I**, and in that part of the molecule, there is less flexibility in **II** than in **I**. This could prevent the formation of a strong hydrogen bond, since this has been demonstrated to be in the plane of the sugar ring[8].

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