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3R,4R-DIHYDROXY-L-PROLINE: A POTENT AND SPECIFIC β-D-GLUCURONIDASE INHIBITOR

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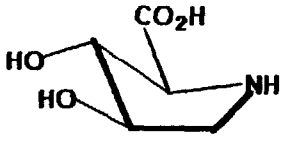
Summary: The title compound, a naturally-occurring amino acid found in virotoxins, competitively inhibits bovine β-D-glucuronidase but does not affect other glycosidases.

Naturally-occurring iminosugars such as deoxynojirimycin[1] and deoxymannonojirimycin[2] can induce lysozomal storage phenomena and disrupt glycoprotein biosynthesis by inhibiting glycosidases which process cell membrane glycolipids and modify N-linked oligosaccharides. These inhibitors (and related indolizidine alkaloids) contain hydroxylated piperidine rings which are homochiral with D-gluco and D-mannopyranose structures, and they bind competitively at the enzymatic site of pyranosyl cation formation. Even hydroxylated pyrrolidines, which bear a closer resemblance to furanose sugars, have recently been reported to inhibit the hydrolysis of glycopyranosides.[4] As part of our research in this area, we recognized that the all-*trans*-dihydroxy-L-proline **1**, an amino acid found in toxic peptides of Amanita virosa mushrooms,[5] was structurally and stereochemically analogous to D-glucuronic acid **2**, an important component of such mucopolysaccharides as heparin, hyaluronic acid and chondroitin. We now disclose that (-)**1** is a potent competitive inhibitor of β-D-glucuronidase, Its activity is comparable to that of (+)**3**, a naturally-occurring trihydroxypipecolic acid of plant origin.[6]



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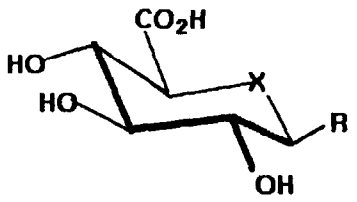

2 X= O, R= OH
3 X= NH, R= H

Proline 1 strongly inhibited the hydrolysis of p-nitrophenyl-β-D-glucuronide by bovine β-D-glucuronidase, causing 50% inhibition of activity at 1 x 10^{-4} M.[7] Besides the standard Lineweaver-Burk analysis, a plot of 1/V vs [I] at different inhibitor concentrations revealed that 1 was a competitive inhibitor (K_i = 9 x 10^{-5}M) of enzymic activity (K_M = 1.4 x 10^{-3}M). All other glycosidases tested (almond β-glucosidase, jackbean α-mannosidase, bovine β-galactosidase, green coffee α-galactosidase, and β-N-acetylhexosaminidase) were unaffected at 10^{-3}M. Pipecolate (+)3, which we have recently synthesized,[8] demonstrated comparable activity towards human liver β-D-glucuronidase (50% inhibition of the competitive type at 0.3 x 10^{-4}M; K_i = 8 x 10^{-5}M at pH 5.0).[6] Besides shedding light on pyrrolidine/piperidine structure-activity relationships, rationally designed sugar-specific glucuronidase inhibitors like 1 may prove useful in studying the clinical effects of mucopolysaccharidosis.

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7. All enzymes and p-nitrophenylglycopyranosides were purchased from Sigma. Assays were conducted at pH 5.00 (50 mM HOAc-NaOAC buffer) using a substrate concentration of 0.5 mM. Mixtures (200 μL) were incubated at 37°C for 15 min, quenched with pH 10.4 glycine buffer and absorbances read at 400nm.
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