Specific inhibition of human β-D-glucuronidase and α-L-iduronidase by a trihydroxy pipecolic acid of plant origin

Isabelle Cenci di Bello, Peter Dorling[+], Linda Fellows* and Bryan Winchester[†]

MRC Human Genetic Diseases Research Group, Department of Biochemistry, Queen Elizabeth College (University of London), Campden Hill Road, London W8 7AH, England, [+]School of Veterinary Studies, Murdoch University, Perth, Western Australia 6150, Australia and **Jodrell Laboratory, Royal Botanic Gardens, Kew TW9 3DS, England*

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The glucuronic acid analogue of 1-deoxynojirimycin, 2(*S*)-carboxy-3(*R*), 4(*R*), 5(*S*)-trihydroxypiperidine, recently isolated from seeds of *Baphia racemosa*, is a novel specific inhibitor of human liver β-D-glucuronidase and α-L-iduronidase. No other glycosidases are inhibited. The inhibition of β-D-glucuronidase is competitive, with a K_i of 8×10^{-5} M and is pH-dependent. This inhibitor may be useful to induce a mucopolysaccharidosis or to investigate the function of microsomal β-D-glucoronidase.

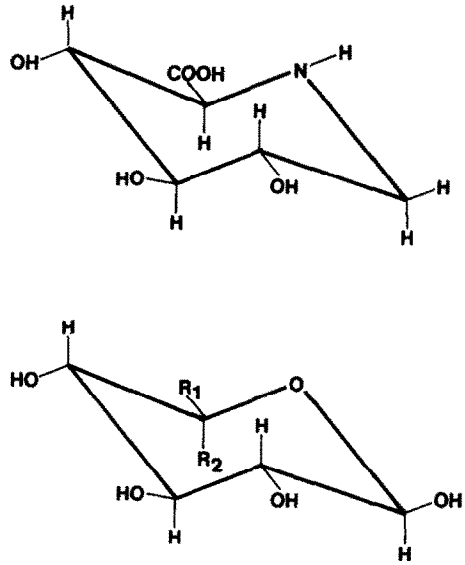
β-D-Glucuronidase *α-L-Iduronidase* *Novel glycosidase inhibitor* *Trihydroxypipecolic acid*

1. INTRODUCTION

Naturally occurring inhibitors of glycosidases have been found to induce lysosomal storage phenomena [1] and to alter the processing of N-linked oligosaccharides of glycoproteins [2]. The bacterial piperidine nojirimycin and its derivatives, 1-deoxynojirimycin and *N*-methyl-1-deoxynojirimycin [3–5] and the plant indolizidine alkaloid, castanospermine [6] all alter the processing of N-linked glycans by inhibiting α-glucosidase I. α-D-mannosidase I and α-D-mannosidase II in the processing pathway are inhibited by the synthetic mannose analogue, 1-deoxymannojirimycin [7] and the plant indolizidine alkaloid, swainsonine, respectively [8,9]. Swainsonine also induces the accumulation of partially hydrolysed and abnormally processed oligosaccharides by completely inhibiting lysosomal α-D-mannosidase [10,11].

2(*S*)-Carboxy-3(*R*),4(*R*),5(*S*)-trihydroxypiperidine (BR1), the glucuronic acid analogue of 1-deoxynojirimycin and 1-deoxymannojirimycin, has

BR I



	R_1	R_2
α – L – iduronic acid	H	COOH
β – D – glucuronic acid	COOH	H

Fig.1. The structure of 2(*S*)-carboxy-3(*R*), 4(*R*), 5(*S*)-trihydroxypiperidine (BR1), α-L-iduronic acid and β-D-glucuronic acid.

[†] To whom correspondence should be addressed

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been isolated from seeds of *Baphia racemosa* [12]. We show that BR1 inhibits human liver β-D-glucuronidase in vitro in a pH-dependent and competitive manner. The only other liver glycosidase inhibited is α-L-iduronidase.

2. MATERIALS AND METHODS

2.1. *Tissue*

Post-mortem human liver, which had been stored at −20°C until required was homogenized (50%, w/v) in a Potter-Elvehjem homogenizer and then centrifuged at 37000 × *g* for 30 min in an MSE 18 centrifuge. The resultant supernatant was used as the source of enzymic activity.

2.2. *Inhibitor (BR1)*

2(*S*)-Carboxy-3(*R*),4(*R*),5(*S*)-trihydroxypiperidine (BR1) was isolated from *B. racemosa* [12].

2.3. *Glycosidase assays*

Glycosidases were assayed with the appropriate fluorigenic (4-methylumbelliferyl) substrate (Koch-Light, Haverhill, Suffolk) in the McIlvaine phosphate-citrate buffer system as described previously [13]. α-L-iduronidase was assayed at pH 3.5 using 0.5 mM 4-methylumbelliferyl α-L-iduronide (Koch-Light) in 0.067 M sodium formate buffer. This substrate is known to be contaminated with 4-methylumbelliferyl β-D-glucuronide [14]. As α-L-iduronidase has negligible activity at pH 2.8 [14] it was possible to measure the amount of contaminating β-D-glucuronide by comparing the rates of hydrolysis by the liver extract at pH 2.8 of 0.5 mM 4-methylumbelliferyl α-L-iduronide with a series of known concentrations of 4-methylumbelliferyl-β-D-glucuronide. The concentration of contaminating β-D-glucuronide was found to be 0.067 mM (approx. 14%). To assay the true α-L-iduronidase activity at pH 3.5 the rate of hydrolysis at pH 3.5 of a control containing 0.067 mM 4-methylumbelliferyl β-D-glucuronide was subtracted from the apparent α-L-iduronidase activity. The effect of BR1 on glycosidase activities was investigated by including 10^{-5}–10^{-3} M BR1 in the assay mixtures. The nature of the inhibition of β-D-glucuronidase was determined by Lineweaver-Burk and Dixon graphical procedures, using a computer program to get the lines of best fit. One unit of activity is a rate of 1 μmol of substrate hydrolysed per min.

2.4. *Measurement of pK*

The p*K* values of ionizing groups were measured by manual titration of a 50 mM solution of BR1 with NaOH over the pH range 2–10 using a Kent Electronics 7060 pH meter.

3. RESULTS AND DISCUSSION

The only human liver glycosidases inhibited by BR1 were β-D-glucuronidase and α-L-iduronidase (table 1). Concentrations of BR1 of 2.6×10^{-4} M and 3.5×10^{-5} M produced 50% inhibition of β-D-glucuronidase and α-L-iduronidase respectively under the standard assay conditions. BR1 is a competitive inhibitor of β-D-glucuronidase with a K_i value of 8×10^{-5} M at pH 4.75 (fig.2). The K_m value for the substrate at this pH is 3.7 ± 0.5 mM (mean ± SD, $n = 5$). Rigorous kinetic studies could not be carried out on the effect of BR1 on α-L-iduronidase because of the contamination of the substrate. However, limited studies indicated that the mode of inhibition was the same as for β-D-glucuronidase.

Table 1

Inhibition of human liver glycosidases by BR1

Glycosidase	% Inhibition[a]	
	BR1	
	10^{-5} M	10^{-3} M
β-D-Glucuronidase (pH 4.75)	5	78
α-L-Iduronidase (pH 3.5)	20	96
α-D-Glucosidase	0	7
β-D-Glucosidase (pH 5.5)	0	2
α-D-Galactosidase	0	0
β-D-Galactosidase	0	0
α-D-Mannosidase	0	3
β-D-Mannosidase	0	0
β-D-*N*-Acetylglucosaminidase	0	0
α-L-Fucosidase	0	0

[a] Expressed as the fraction of activity of control lost in presence of inhibitor. All assays at pH 4.0 with 0.5 mM substrate unless otherwise indicated

The effect of pH on the inhibition of β-D-glucuronidase by BR1 was investigated (fig.3). It can be seen that the inhibition is pH dependent, increasing with pH over the range pH 3.5–5.0. To ascertain whether this pH dependence was a reflection of an ionization on the inhibitor, the titration curve of BR1 was measured. Groups with pK values of 6.5 and approx. 2.5 were observed. It was assumed that these corresponded to the ionization of the ring N-atom and carboxyl group respectively. This suggests that BR1 with an uncharged N-atom and an overall negative charge like the substrate is a more potent inhibitor. Conversely, the inhibition of α-D-mannosidase by swainsonine is greater at pH values below the pK (7.4) of the indolizidine ring nitrogen. It has been suggested that protonated swainsonine is a powerful competitive inhibitor (K_i 8 × 10^{-8} M) because of its configurational and conformational similarity to the mannosyl cation intermediate in the hydrolysis of mannosides [15]. BR1 may inhibit β-D-glucuronidase by competing with the substrate rather than by being a transition state analogue. The greater value of K_i for BR1 inhibition may reflect a difference in mechanism of inhibition of glycosidases by piperidine and indolizidine alkaloids.

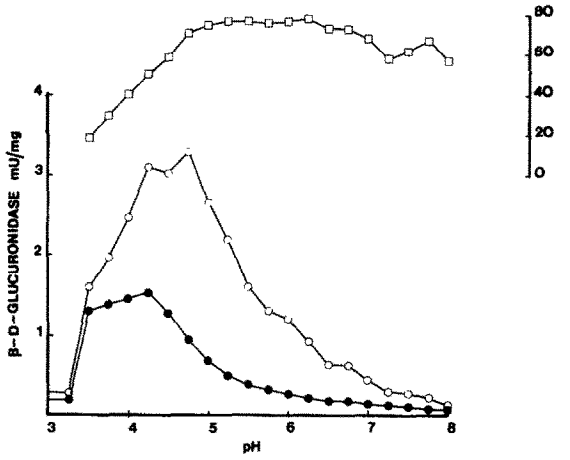

Fig.3. pH dependence of inhibition of β-D-glucuronidase by BR1. Human liver β-D-glucuronidase was assayed at various pH values, alone (○) and in the presence of 0.25 mM BR1 (●). The percentage inhibition by 0.25 mM BR1 is shown at each pH value (□).

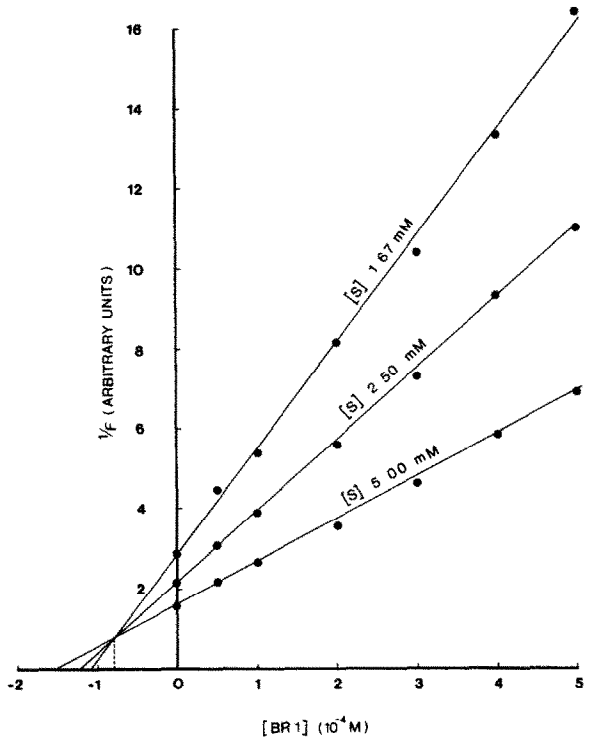

Fig.2. Inhibition of human liver β-D-glucuronidase by BR1 at pH 4.75. Different concentrations of BR1 (1.0–5.0 × 10^{-4} M) were included in assay mixtures containing 1.67, 2.5 and 5 mM substrate. The nature of the inhibition and the value of K_i were determined graphically by the Dixon procedure.

BR1 is not as potent an inhibitor of β-D-glucuronidase as D-glucaro-δ-lactam, which is prepared from nojirimycin by oxidation, or D-glucaro-1,4-lactone [17]. These latter compounds have K_i values of 4 × 10^{-8} and 4.6 × 10^{-7} M, respectively, for the inhibition of the hydrolysis of p-nitrophenyl β-D-glucuronide by bovine liver β-glucuronidase. The conformation of the lactone and stable lactam probably resemble the half-chair conformation of the transition state.

It is interesting that BR1 inhibits the α-L-iduronide and the β-D-glucuronide, which only differ in their configuration at C_5. The β-D-glucoside, which has the same configuration as the β-D-glucuronide for the ring substituents, is not inhibited by BR1, indicating the importance of the charged carboxyl in the binding or recognition mechanism.

If fed to laboratory animals in large quantities

or administered to cells in culture, BR1 may prove to be a useful compound to induce a mucopolysaccharidosis and throw some light on the function of microsomal β-D-glucuronidase.

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