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GLYCOSIDASE INHIBITION BY PLANT ALKALOIDS WHICH ARE STRUCTURAL ANALOGUES OF MONOSACCHARIDES

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Key Word Index—Glycosidase; alkaloids; 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine; deoxynojirimycin; 1,5-dideoxy-1,5-imino-D-mannitol.

Abstract—The inhibitory activities of three plant alkaloids, deoxynojirimycin, 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine and 1,5 dideoxy-1,5-imino-D-mannitol towards glycosidases from several sources have been compared. These are structural analogues of D-glucose, D-fructose and D-mannose respectively. The occurrence of 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine in *Lonchocarpus sericeus* seed is confirmed and has been shown to be responsible for the glucosidase inhibition wrongly attributed to 1,5-dideoxy-1,5-imino-D-mannitol in a previous report.

INTRODUCTION

Several examples of alkaloids which structurally resemble sugars are now known in nature [1, 2]. Three such analogues of monosaccharides in which the ring oxygen is replaced by nitrogen, have been found in plants: 1,5 dideoxy-1,5-imino-D-glucitol (1) (deoxynojirimycin) in *Morus* (Moraceae), a glucose analogue [3]; 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (2) in *Derris elliptica* (Wall.) Benth. (Leguminosae), a fructose analogue [4]; and 1,5-dideoxy-1,5-imino-D-mannitol (3) in *Lonchocarpus sericeus* H.B.K. (a legume closely related to *Derris*), a mannose analogue [5].

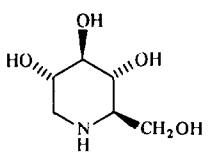
RESULTS AND DISCUSSION

In a previous report [6] we stated that 3 is a potent competitive inhibitor of α- and β-glucosidase and insect derived trehalase. This was incorrect. Seed of *L. sericeus* has now been shown to contain both 2 and 3 in a ratio of approximately 3:1. When the original isolation of 3 was repeated, minor changes in the solvent composition (see Experimental) used in the final crystallization led to the crystallization of 2 rather than 3, and it was 2 not 3 which was responsible for the glucosidase inhibition reported. Confirmation of the structure of 2 from *L. sericeus* has now been obtained by comparison with an authentic sample of 2 unambiguously synthesized [7].

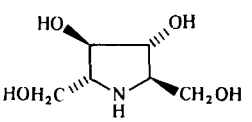
Both 1 and 3 are known to be inhibitors of glucosidase and mannosidase respectively [8–10]. We have now compared the activity of authentic 2 and 3 (isolated from seed of *L. sericeus*) and 1 (isolated from leaves of *Morus nigra*) against several glycosidases from various sources. The results are shown in Table 1.

The fructose analogue 2 proved somewhat surprisingly a more effective inhibitor of α- and β-glucosidases than 1, and was also the only one which inhibited invertase. Predictably 3 inhibited α-mannosidase and not glucosidases but its more potent inhibition of α-fucosidase was unexpected and noteworthy. The insect trehalase inhibition previously attributed to 3 [6] (50% inhibition at 5.5×10^{-5} M) was due to 2. Unfortunately the action of 1 and 3 on this enzyme could not be investigated since the preparation was no longer available, but 1 has been reported to inhibit fungal trehalase [8].

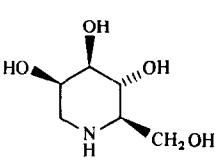
Sugar analogues, both natural and synthetic, are currently of interest as specific inhibitors of carbohydrases in a variety of organisms [11, 12]. In particular 1 and 2 inhibit α- and β-glucosidases, and 3 an α-mannosidase of glycoprotein processing [13–15]. It is likely that their



1 deoxynojirimycin; [1,5 - dideoxy - 1,5 - imino - D - glucitol]



2 2R,5R - dihydroxymethyl - 3R,4R - dihydroxypyrrolidine



3 deoxymannojirimycin [1,5 - dideoxy - 1,5 - imino - D - mannitol]

Table 1. Concentration of inhibitor required to produce 50% inhibition of enzyme activity under the stated conditions.

Enzyme	Inhibitor		
	1,5-Dideoxy-1,5-imino-D-mannitol (3)	2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine (2)	1-Deoxynojirimycin (1)
α-Glucosidase (yeast)	NI	3.3×10^{-6} M	1.9×10^{-4} M
β-Glucosidase (emulsin)	NI	7.8×10^{-6} M	8.1×10^{-5} M
α-Mannosidase (Jack Bean)	1.5×10^{-4} M	NI	NI
α-Galactosidase (*Asp. niger*)	NI	NI	NI
β-Galactosidase (*Asp. niger*)	NI	NI	NI
β-Glucuronidase (*Helix pomatia*)	NI	NI	NI
Invertase (yeast)	NI	5.25×10^{-5} M	NI
α-Fucosidase (bovine epididymis)	2.2×10^{-5} M	NI	NI
β-Xylosidase (*Asp. niger*)	NI	2.5×10^{-4} M	4.0×10^{-4} M

*NI, up to 1×10^{-3} M, no inhibition.

presence contributes to the chemical defence of those plants in which they occur. Larvae of the bruchid *Callosobruchus maculatus*, a major pest of grain legumes, cannot survive levels greater than 0.03% w/w of **2** in the diet, which has been shown to inhibit the gut α- and β-glucosidases [16]. Locusts are deterred from feeding by **2** [17]. The previously observed inhibition by **2** of insect trehalase [6] is particularly noteworthy, since specific trehalase inhibitors could theoretically be useful insecticides, trehalose not having been found in mammals.

EXPERIMENTAL

Isolation and characterization of **1**. Finely ground leaves of *Morus nigra* (2 kg) collected in RBG Kew was extracted with 70% aq. MeOH (4.5 l.). The extract was applied to an Amberlite IR-120 column (3 × 35 cm, NH_4^+ form) previously equilibrated in 70% MeOH, and **1** was eluted with 300 ml 1 M pyridine. On evaporating to dryness, the residue was dissolved in 15 ml H_2O and applied to an Amberlite CG-50 column (1.5 × 40 cm, NH^+ form). Slow elution with H_2O enabled **1** to be separated from brown pigment. Fractions of pure **1** were applied to an Amberlite CG-400 column (1 × 10 cm, OH^- form) and **1** passed through unbound. Removal of solvent and recrystallization from MeOH containing a small proportion of EtOH and Me_2CO yielded 2.1 g of crystalline free base. Comparison of **1** with authentic deoxynojirimycin (see Acknowledgements) by NMR analysis showed that they were identical.

Isolation and characterization of **2** *and* **3**. Finely ground seed 100 g of *Lonchocarpus sericeus* H.B. & K. (collected by W. O. Boateng in Ghana, Accession N° KOTH/BD/3/81) was defatted with Me_2CO (500 ml) and extracted with 70% aq. MeOH (3 × 400 ml). Pooled filtered extracts were applied to an Amberlite CG-120 column (1.5 × 40 cm, NH_4^+ form) previously equilibrated in 70% aq. MeOH. After washing with H_2O (700 ml), **2** and **3** were eluted with 1 M aq. pyridine (500 ml). On evaporating to dryness the residue dissolved in H_2O (15 ml) was applied to an Amberlite CG-400 column (1 × 10 cm, OH^- form). Both **2** and **3** were removed by washing with H_2O. Removal of the solvent and recrystallization from hot EtOH yielded 960 mg of **2** free base as a crystalline solid, mp 116–118°, $[\alpha]_D^{20}$ + 54.3° (c 1.2; H_2O). The NMR parameters of this compound were identical to those previously described [4], in particular the characteristically simple ^{13}C NMR. The absolute configuration of **2** was confirmed as 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine by comparison with an authentic sample unambiguously synthesized from D-glucose [7].

The mother liquor containing **2** and **3** was treated as previously described [5]. Adjustment of the pH to 5.0, evaporation to dryness, dissolution in MeOH and addition of a few drops of EtOH and Me_2CO gave 70 mg of a crystalline monohydrochloride of **3**. Spectral characterizations were identical to those of a synthetic sample of **3** (see Acknowledgements).

Enzyme assays. Compounds **1**, **2** and **3** were incorporated into assay buffers where appropriate to give a final concentration range of 10^{-8} to 10^{-3} M. Sources and assay techniques for α- and β- glucosidase, α-mannosidase α- and β- galactosidase and β-glucuronidase were as described in ref. [6]. Other enzymes were assayed as follows: β-xylosidase (Sigma X-5375, *Aspergillus niger*). 400 μl 50 mM trisodium citrate, pH 5.0; 200 μl 2 mM *o*-nitrophenyl-β-D-xylopyranoside, 200 μl enzyme (4.5 μg/ml). Incubated for 10 min at 25°. Added 600 μl 0.25 M NaOH. Read at 410 nm. α-fucosidase (Sigma F-7753, bovine epididymis). 200 μl 50 mM trisodium citrate, pH 6.5; 200 μl 1 mM *p*-nitrophenyl-α-L-fucopyranoside, 200 μl enzyme (5 μg/ml). Incubated for 10 min at 25°. Added 400 μl 0.25 M NaOH. Read at 410 nm. Invertase (Sigma I-4504, bakers yeast). Assay involved an incubation and an assay mixture. Incubation mixture; 500 μl 0.1 M acetate buffer, pH 4.6; 250 μl sucrose (100 mg/ml); 50 μl enzyme (10 μg/ml). Incubated for 10 min at 25°. Added 400 μl

0.3 M Tris buffer. Assay mixture; 1.66 ml 0.1 M triethanolamine buffer, pH 7.6; 100 μl 0.2 M $MgCl_2$; 100 μl ATP (10 mg/ml); 100 μl NADP (10 mg/ml); 10 μl hexokinase (10 mg/ml); 10 μl phosphoglucose isomerase (10 mg/ml); 20 μl incubation solution; 10 μl glucose-6-phosphate dehydrogenase (5 mg/ml). Incubated for 5 min at 25°. Read at 360 nm.

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