Tetrahedron Letters,Vol.26,No.11,pp 1465-1468,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

LACK OF GLYCOSIDASE INHIBITION BY, AND ISOLATION FROM XANTHOCERCIS ZAMBESIACA (LEGUMINOSAE) OF, 4-O-(β-D-GLUCOPYRANOSYL)-FAGOMINE [1,2,5-TRIDEOXY-4-O-(β-D-GLUCOPYRANOSYL)-1,5-IMINO-D-ARABINO-HEXITOL], A NOVEL GLUCOSIDE OF A POLYHYDROXYLATED PIPERIDINE ALKALOID

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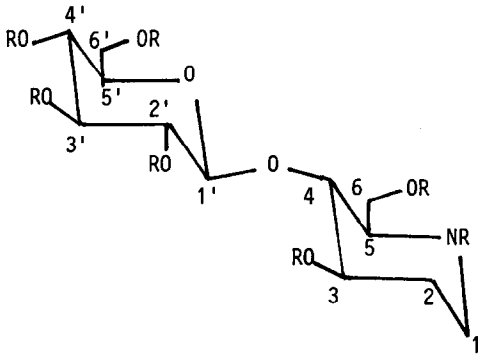
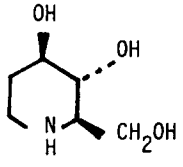
A COSY spectrum has been used to determine the position of inter-residue linkage in 4-O-(β-D-glucopyranosyl) fagomine (1) an example of a new class of glycosides of polyhydroxylated piperidine alkaloids isolated from seed of the legume Xanthocercis zambesiaca. (Bak.) Dunn. Unlike a number of polyhydroxylated piperidines, neither the glucoside (1) nor free fagomine [1,2,5-trideoxy-1,5-imino-D-arabinohexitol] (3) showed any inhibitory activity towards glycosidase enzymes from a variety of sources.

Both naturally occurring and synthetic polyhydroxylated derivatives of piperidine, pyrrolidine and indolizidine are of interest as specific inhibitors of glycosidase enzymes in a variety of organisms;[1] the structural similarity between the protonated form of the heterocyclic base and the cation derived from the corresponding glycoside during hydrolysis may account for the competitive inhibition of enzymic activity.[2] Several such compounds,such as deoxymannojirimycin (5) swainsonine (6) and castanospermine (8), occur as the free base in plants where they may play a possible defensive role by inhibiting digestive glycosidases in potential herbivores such as insects.[3] No glycosides of these polyhydroxylated alkaloids have been reported previously. This paper describes the isolation and characterisation of a novel glucoside (1) of fagomine (3); fagomine [1,2,5-trideoxy-1,5-imino-D-arabino-hexitol] has been detected as a free base in seed of buckwheat (Fagopyrum esculentum Moench)[4] but so far has not been reported to occur elsewhere.

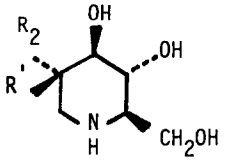
The glucoside (1) was first detected by the colour reactions characteristic of this group of polyhydroxylated alkaloids (pale yellow with ninhydrin, Dragendorff negative) in seed of both species of the legume genus Xanthocercis Baill. and was subsequently isolated[5] from X. zambesiaca as white crystals, m.p. 232-233°C (dec), $[\alpha]_D^{20}$ - 3.1° (c 1.2 in H_2O), MH^+ 310 (NH_3DCI). Treatment of (1) with acetic anhydride in pyridine gave a hepta-acetate (2), $[\alpha]_D^{20}$ -38.0° (c, 0.7 in $CHCl_3$), MH^+ 604 (NH_3DCI); the i.r. spectrum of (2) showed the presence of an amide carbonyl (1640 cm^{-1}) as well as ester groups (1750 cm^{-1}). Dilute acid hydrolysis[6] of (1) gave free fagomine, m.p. 180-184°C, $[\alpha]_D^{20}$+ 24.7° (c, 0.4 in H_2O) [lit.[4] m.p. 186-188°C, $[\alpha]_D^{11}$ +23° (c, 1.0 in H_2O)] with the details of the ^1H NMR identical to those previously reported.[4] The absolute configuration of fagomine [1,2,5-trideoxy-1,5-imino-D-arabino-hexitol] was demonstrated by comparison with a sample synthesised by an enantiospecific route from D-glucose.[7]



(1) R = H (2) R = COMe

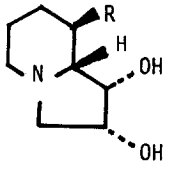


(3)



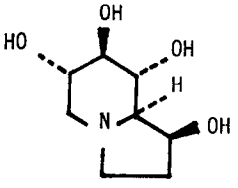
(4) R^1 = H, R^2 = OH

(5) R^1 = OH, R^2 = H



(6) R = OH

(7) R = H



(8)

The details of the structure of (1) were determined by proton-proton shift correlated 2D NMR spectroscopy;[8] with the aid of the COSY spectrum which depicted the correlation of all the protons in (1), the coupling constant and chemical shift data[9] were then obtained from the 1D spectrum. The trans-axial orientations of the protons at positions 3,4,5,1', 2', 3', 4' and 5' are all clearly indicated by the large vicinal J values. The position of the linkage of fagomine to the glucosyl residue in (1) was determined from a n.O.e. experiment and from a COSY spectrum with emphasis in long-range couplings. A 15% n.O.e. enhancement of the H-4 signal was observed on irradiation of H-1'; no enhancement of H-3 or H-6 was observed under these conditions.[10] The COSY spectrum of (1) obtained under conditions calculated to emphasise long-range couplings shows, inter alia, a new cross-peak which arises from the weak inter-residue coupling between H-1' and H-4 (Fig); no long-range coupling is observed between H-1' and H-3 or H-6. The present study provides an example of using COSY spectra to determine the position of interresidue linkage, a powerful technique for structural elucidation of oligosaccharides and related molecules.[11]

Preliminary studies on the effects of both free fagomine (3) and the fagomine glucoside (1) on the hydrolytic activity of α- and β-glucosidases(yeast and apricot emulsin respectively), α-mannosidase (Canavalia ensiformis), α- and β-galactosidases (Aspergillus niger), β-glucuronidase (Helix pomatia), α-fucosidase (bovine epididymis) and β-xylosidase (Aspergillus niger) on their respective o- or p-nitrophenyl glycopyranosides failed to show inhibition by either compound up to 10^{-3}M. This is in marked contrast to the effects of

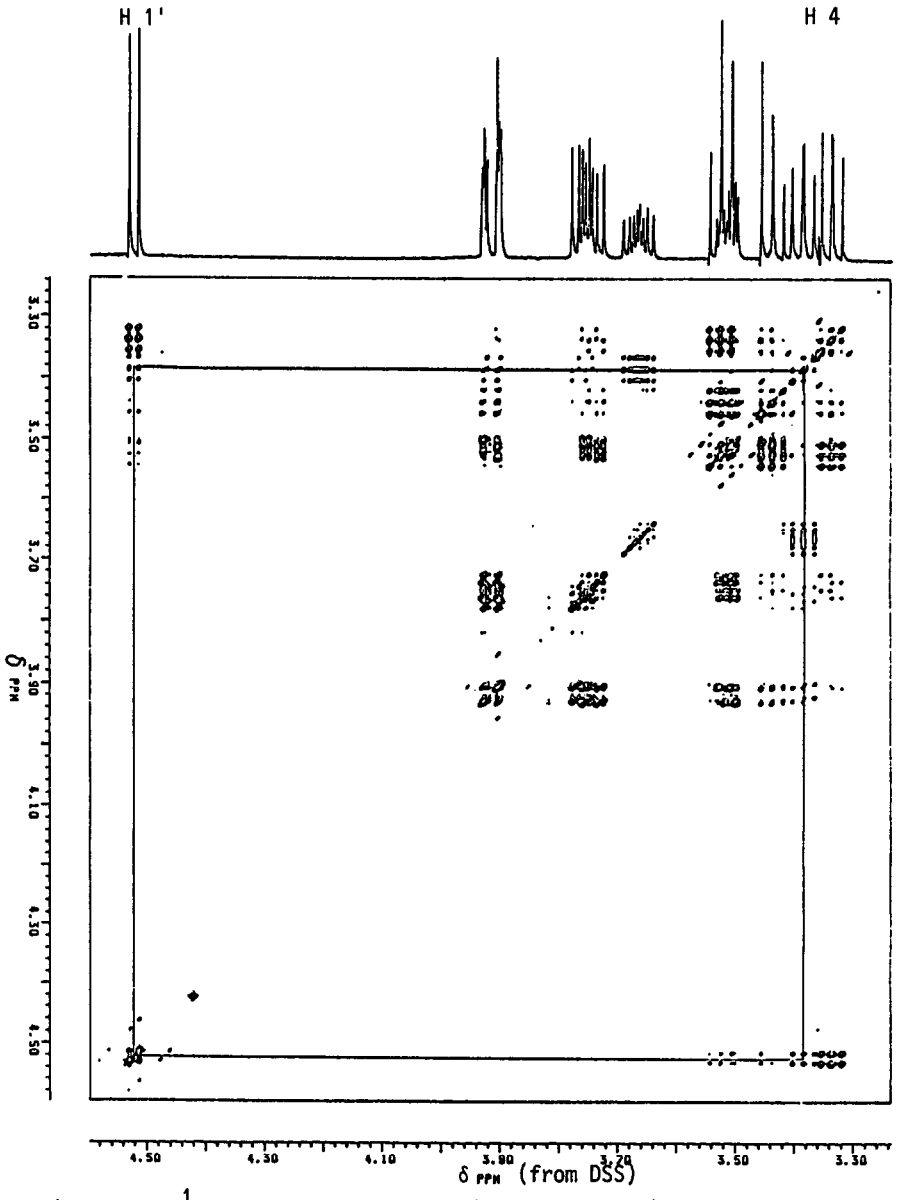


Fig. 1. Combined ^{1}H NMR and COSY spectra (500 MHz) of (1) with emphasis of correlations due to long range coupling (90^{o} - t_1 - Δ - 45^{o} - Δ sequence, fixed delay Δ = 0.25 s, absolute value mode contour display with 0.66 Hz/pt resolution in both dimensions). Highfield protons are not shown.

deoxymannojirimycin[13] (5) and deoxynojirimycin (4),[14] both of which are known to be glycosidase inhibitors. This is in support of evidence that, for a number of glycosidases, interaction of the protein with the hydroxyl at position 2 of the glycoside is important in catalysis.[15] It has recently been reported that although swainsonine (6) is a powerful and specific mannosidase inhibitor, the indolizidinediol (7) lacking the hydroxyl group on the piperidine ring is only a weak inhibitor of α-mannosidase.[16,17]

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5 Isolation. Finely ground mature seed of Xanthcercis zambesiaca (Bak.)Dunn (600 g) was defatted (hexane) and extracted with 70% aqueous MeOH. The extract was applied to an Amberlite IR-120 column (NH_4^+ form) and (1) was eluted with aqueous pyridine. On evaporating to dryness the residue, dissolved in water, was applied successively to Amberlite CG-50 (NH_4^+ form) and Amberlite CG-400 (OH^- form) ion exchange columns, (1) being removed from each by washing with water. Removal of solvent and recrystallisation from MeOH/EtOH/Me_2CO gave the glucoside (1) (4.5 g).

6 The glucoside (1) in 2M HCl (10 ml) was heated at 100° for 2 h. The hydrolysate was applied to a column of Amberlite CG-120 (H^+ form), and the column washed with water. Fagomine was eluted with 2M NH_4OH.

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9 500 MHz spectrum of (1) in D_2O δ (From DSS) 1.45, ddt, $H2_a$; 2.05, dddd, $H2_e$; 2.60, dt, $H1_a$; 2.66, ddd, H5; 3.02, ddd, $H1_e$; 3.33, dd, H2'; 3.38, dd, H4; 3.44, dd, H4'; 3.51, ddd, H5'; 3.53, t, H3'; 3.67, ddd, H3; 3.74, dd, H6'; 3.76, dd, H6; 3.91, dd, H6; 3.92, dd, H6'; 4.52, d H1' Coupling constants: $J_{1a,1e}$13.0; $J_{1a,2a}$13.0; $J_{1a,2e}$2.7; $J_{1e,2a}$ 4.5; $J_{1e,2e}$2.3; $J_{2a,2e}$13.0; $J_{2a,3}$11.6; $J_{2e,3}$5.0; $J_{3,4}$8.8; $J_{4,5}$9.6, $J_{5,6}$5.6 ; $J_{5,6}$3.0; $J_{6,6}$11.5 ; $J_{1',2'}$7.9; $J_{2',3'}$9.3;$J_{3',4'}$9.3;$J_{4',5'}$9.8; $J_{5',6'}$5.8;$J_{5',6'}$2.2; $J_{6',6'}$12.2 Hz.

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17 An SERC postdoctoral fellowship (to TKMS) and a CASE award(to AHH) are acknowledged.
(Received in UK 2 January 1985)