Tetrahedron Letters,Vol.26,No.26,pp 3127-3130,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

POTENT COMPETITIVE INHIBITION OF α-GALACTOSIDASE AND α-GLUCOSIDASE ACTIVITY BY 1,4-DIDEOXY-1,4-IMINOPENTITOLS: SYNTHESES OF 1,4-DIDEOXY-1,4-IMINO-D-LYXITOL AND OF BOTH ENANTIOMERS OF 1,4-DIDEOXY-1,4-IMINOARABINITOL

G.W.J. Fleet,[a] S.J. Nicholas,[a] P.W. Smith,[a] S.V. Evans,[b] L.E. Fellows[b] and R.J. Nash[b]

[a] Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford, UK.

[b] Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey. UK.

The syntheses of 1,4-dideoxy-1,4-imino-D-lyxitol (3), 1,4-dideoxy-1,4-imino-D-arabinitol (4) and 1,4-dideoxy-1,4-imino-L-arabinitol (5) are reported; (3) is a potent competitive inhibitor of α-galactosidase (green coffee beans) and (4) a competitive inhibitor of α-glucosidase (Brewer's yeast) suggesting that iminopentitols have considerable potential as glycosidase inhibitors. (4) was found to be identical to an alkaloid recently isolated from Angylocalyx boutiqueanus.

There is considerable interest in the chemistry and biological activity of polyhydroxylated piperidines, many of which are glycosidase inhibitors by virtue of their structural resemblance to pyranose sugars.[1] In contrast, only two reports of glycosidase inhibition by simple hydroxylated pyrrolidines have appeared; 2R,5R-dihydroxymethyl-3R,4R-dihydroxypyrrolidine (1), isolated from certain legumes[2,3] and recently synthesised from glucose,[4] inhibits a viral glycoprotein processing glucosidase I,[5] yeast α-glucosidase and invertase, almond α-glucosidase, insect trehalase and fungal β-xylosidase.[3] Also, 1,4-dideoxy-1,4-imino-D-mannitol (2) is an inhibitor of a plant α-mannosidase[6] and of a glycoprotein processing mannosidase.[7] This paper describes the synthesis from D-mannose of 1,4-dideoxy-1,4-imino-D-lyxitol (3), and from

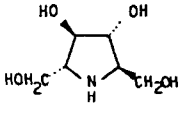
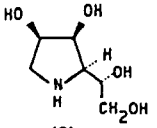
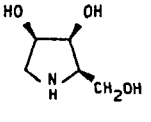
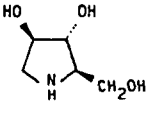
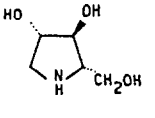
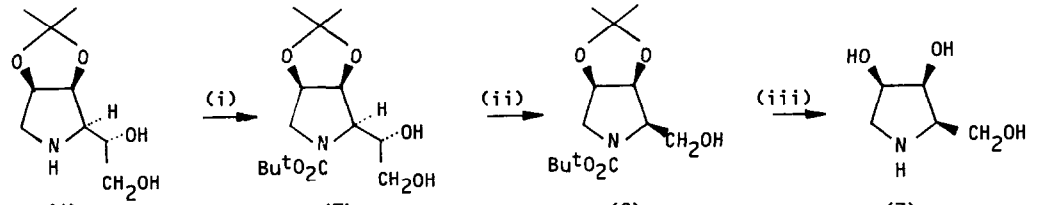
Inhibitor / Enzyme	 (1)	 (2)	 (3)	 (4)	 (5)
α-Glucosidase (yeast)	3.3×10^{-6}	5.0×10^{-4}	NI	1.8×10^{-7}	1.0×10^{-5}
β-Glucosidase (emulsin)	7.8×10^{-6}	4.5×10^{-4}	3.5×10^{-4}	2.0×10^{-4}	NI
α-Mannosidase (Jack Bean)	NI	5.0×10^{-7}	1.4×10^{-5}	1.0×10^{-4}	NI
α-Galactosidase (green coffee beans)	NI	4.0×10^{-4}	2.0×10^{-7}	NI	NI
β-Galactosidase Asp. niger	NI	1.6×10^{-4}	1.4×10^{-4}	NI	NI
α-L-Fucosidase (bovine epididymis)	NI	NI	NI	NI	NI
β-Xylosidase Asp. niger	2.5×10^{-4}	NI	NI	NI	NI

Table. Inhibition of glycosidase activity by hydroxylated pyrrolidines (1) - (5). Concentration [M] of inhibitor required to reduce enzyme-catalysed hydrolysis of corresponding nitrophenyl glycopyranoside by 50% under standard assay conditions.[8] (NI indicates no inhibition up to 3.3×10^{-4}M).

D-xylose of 1,4-dideoxy-1,4-imino-D-arabinitol (4) and of the L enantiomer (5). The inhibitory action of each compound on a range of commercially available glycosidases was studied.[8] The iminolyxitol (3) proved a highly potent competitive inhibitor of α-galactosidase (ex green coffee beans) and a moderate inhibitor of α-mannosidase (Jack Bean), while the D-iminoarabinitol (4) was found to be a strong competitive inhibitor of yeast α-glucosidase; its enantiomer (5) also inhibited α-glucosidase, but to a lesser extent. Further, the identity of (4) with a recently isolated[9] alkaloid from Angylocalyx boutiqueanus was established. These results confirm the potential of 1,4-dideoxy-1,4-iminopentitols as glycosidase inhibitors (Table).

1,4-Dideoxy-1,4-imino-D-lyxitol (3) is related to the iminomannitol (2) by a one carbon degradation of the side chain. The acetonide (6), prepared from benzyl α-D-mannopyranoside as previously described,[6] was treated with tert-butoxycarbonyl anhydride to give the



(6) (7) (8) (3)

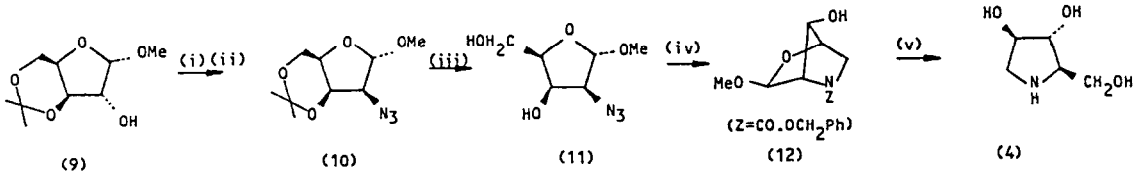
(i) $(Bu^tOCO)_2O$,pyridine, room temp. (ii) $NaIO_4$ in EtOH, then add $NaBH_4$ (iii) CF_3COOH/H_2O (4:1). room temp; then purify by ion exchange chromatography.

SCHEME 1

carbamate (7),[10] $[\alpha]_D^{20}$ -44.5° (c,0.16 in $CHCl_3$) in 47% yield. Periodate oxidation of the diol (7), followed by borohydride reduction of the resulting aldehyde led to the formation of (8), $[\alpha]_D^{20}$ -42.2° (c, 0.23 in $CHCl_3$) (78% yield) (Scheme 1). Both the isopropylidene and t-BOC protecting groups were removed by hydrolysis with aqueous trifluoroacetic acid to give, after ion exchange chromatography, the free base (3) (71 % yield) $[\alpha]_D^{20}$ -15.8° (c, 0.14 in H_2O) which formed a crystalline hydrochloride,[11] m.p. 157-159°, $[\alpha]_D^{20}$ +18.8° (c, 0.16 in H_2O).

The iminomannitol (2) was previously found[6] to be a powerful competitive inhibitor of Jack Bean α-mannosidase (K_m 2.0 x 10^{-3}M) with 50% inhibition at 5.0 x 10^{-7}M and K_I 7.6 x 10^{-7}M; in contrast the iminolyxitol (3) only moderately inhibits α-mannosidase activity (50% inhibition at 1.4 x 10^{-5}M). However, (3) was found to be a very potent competitive inhibitor of α-galactosidase (ex green coffee beans) (K_m 8.3 x 10^{-4}M) with 50% inhibition at 2.0 x 10^{-7}M and K_I 1.0 x 10^{-7}M. Only weak inhibition of β-galactosidase (Aspergillus niger) by (3), or of α- and β-galactosidase by (2), was observed (see table). This is the first report of inhibition of galactosidase activity by either a polyhydroxylated piperidine or pyrrolidine.

1,4-Dideoxy-1,4-iminoarabinitols (4) and (5) may both be synthesised from D-xylose by forming the pyrrolidine ring between either C-2 and C-5, or C-1 and C-4, of xylose. Thus the D enantiomer (4) requires the introduction of a nitrogen function with inversion



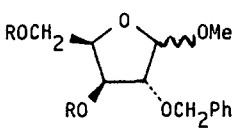
(9) (10) (11) (12) (4)

(i) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2 (ii) NaN_3, DMF, 100°, 12 h (iii) Dowex 50W-8x resin, MeOH room temp., 4h (iv) p-$Me.C_6H_4SO_2Cl$, pyridine, 0°; H_2, Pd black, EtOH with NaOAc, 50°; then $PhCH_2OCOCl$, ether, water containing $NaHCO_3$ (v) CF_3COOH/H_2O (4:1); then $NaBH_4$, EtOH; then H_2, Pd Black, MeCOOH.

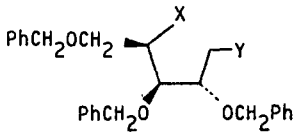
SCHEME 2

of configuration at C-2, followed by intramolecular cyclisation by nitrogen onto C-5 (Scheme 2). The acetonide of methyl α-D-xylofuranoside (9), readily available from D-xylose directly,[12] is esterified with triflic anhydride and the resulting triflate treated with sodium azide to give the azide (10), $[\alpha]_D^{20}$ +128° (c, 0.96 in $CHCl_3$) in 76% yield. Methanolysis of the acetonide under mild conditions gave methyl 2-azido-2-deoxy-D-ribofuranoside (11), $[\alpha]_D^{20}$ +132° (c, 0.38 in $CHCl_3$) (83% yield). Selective tosylation of the primary hydroxyl group in (11), followed by palladium catalysed hydrogenation of the azide, gave the corresponding amine which cyclised on heating with sodium acetate in ethanol to form a bicyclic amine isolated as the carbamate (12) [36% yield from (11)]. Hydrolysis of (12) by aqueous trifluoroacetic acid, followed by borohydride reduction of the resulting aldehyde, hydrogenolytic removal of the carbamate protecting group and purification by ion exchange chromatography, gave the free base (4), $[\alpha]_D^{20}$ +7.8° (c, 0.46 in H_2O) which was crystallised as the hydrochloride m.p. 113-115° $[\alpha]_D^{20}$ +37.9° (c, 0.53 in H_2O) in 65% yield from (12).

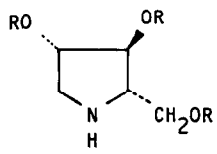
1,4-Dideoxy-1,4-imino-L-arabinitol (5) was synthesised from the xylitol (15), in which only hydroxyl groups from C-1 and C-4 of D-xylose are left unprotected. The xylofuranoside (9)



(13) R=H (14) R=CH_2Ph



(15) X=Y=OH (16) X=Y=OSO_2Me (17) X=OSO_2Me; Y=N_3



(18) R=CH_2Ph (5) R=H

was benzylated and the isopropylidene protecting group removed (Dowex 50W-8x resin, H^+ form in methanol) to give a mixture of the anomeric furanosides (13) which was treated with benzyl bromide, sodium hydride and tetrabutyl ammonium iodide in tetrahydrofuran to form (14) in 45% yield from (9). Hydrolysis of (14) by trifluoroacetic acid in water, followed by reduction of the resulting lactol with sodium borohydride in ethanol (room temp, 1 h) gave 2,3,5-tri-O-benzyl-D-xylitol (15) (87% yield), $[\alpha]_D^{20}$ -11.3° (c, 0.9 in $CHCl_3$). Esterification of (15) with mesyl chloride (2.5 equiv) in pyridine formed the dimesylate (16), $[\alpha]_D^{20}$ +16.0° (c, 1.3 in $CHCl_3$) [90% yield]. Nucleophilic displacement of the primary mesylate in (16) by sodium azide in dimethyl formamide gave the azidomesylate (17)

in 66% yield, $[\alpha]_D^{20}$ +13.8° (c, 0.5 in $CHCl_3$) $M+NH_4^+$ 543 (NH_3 DCI), with an azide absorption (2100 cm^{-1}) and a low field proton (δ 4.88) on the carbon bearing the mesylate in ^1H NMR. Reduction of the azide function (palladium black, H_2, in ethanol) gave the protected pyrrolidine (18) as the only isolated compound; further hydrogenation of (18) (palladium black in acetic acid), followed by ion exchange chromatography, gave the free base (5) which was crystallised as the hydrochloride, m.p. 107-111° $[\alpha]_D^{20}$ -34.6 (c, 0.37 in H_2O) [48% yield from (17)].

Recently, a 1,4-dideoxy-1,4-iminopentitol of unknown stereochemistry has been isolated from the legume Angylocalyx boutiqueanus; the spectral data reported[9] for this new alkaloid [^1H and ^{13}C NMR, MS] are in agreement with the data obtained for the synthetic compounds (4) and (5). Also, the NMR and mass spectra of the hydrochlorides of (4) and (5) are identical to each other. The natural product[9] and 1,4-dideoxy-1,4-imino-D-arabinitol (4) showed identical inhibitory activity towards the range of glycosidases tested (see Table), in particular the potent competitive inhibition of yeast α-glucosidase (K_m 3 x 10^{-4}M) with 50% inhibition of enzymic activity at 1.8 x 10^{-7}M (K_I 1.0 x 10^{-7}M). The L enantiomer (5) was, in contrast, only a moderate inhibitor of α-glucosidase, with 50% inhibition at 1.0 x 10^{-5}M.

In summary, this work demonstrates that 1,4-dideoxy-1,4-iminopentitols have potential as glycosidase inhibitors. It is worth noting that, although the free bases (3), (4) and (5) are hygroscopic oils, the corresponding hydrochlorides are crystalline and relatively easily handled.

A SERC award (to PWS) and NERC award (to RJN) are gratefully acknowledged.

References

1. R.C. Bernotas and B. Ganem, Tetrahedron Lett., 1985, 26, 1123; Y. Kodama, T. Tsuruoka, T. Niwa and S. Inouye, J.Antibiotics, 1985, 38, 116; S.V. Evans, A.R. Hayman, L.E. Fellows, T.K.M. Shing, A.E. Derome and G.W.J. Fleet, Tetrahedron Lett., 1985, 26, 1465 and references therein.

2. A. Welter, G. Dardenne, M. Marlier and J. Casimir, Phytochemistry, 1976, 25, 747.

3. S.V. Evans, L.E. Fellows, T.K.M. Shing and G.W.J. Fleet, Phytochemistry, in press.

4. G.W.J. Fleet and P.W. Smith, Tetrahedron Lett., 1985, 26, 1469.

5. A.D. Elbein, M. Mitchell, B.A. Sanford, L.E. Fellows and S.V. Evans, J.Biol.Chem., 1984, 259, 12409.

6. G.W.J. Fleet, P.W. Smith, S.V. Evans, and L.E. Fellows, J.Chem.Soc., Chem.Commun., 1984, 1240.

7. G. Palmartczyk, M. Mitchell, P.W. Smith, A.D. Elbein, and G.W.J. Fleet, submitted for publication.

8. The enzymes and the nitrophenyl glycopyranoside substrates were purchased from Sigma. The details of the individual enzyme assays are given in reference 3.

9. R.J. Nash, J.M. Williams and E.A. Bell, Phytochemistry, 1985, in press.

10. All new compounds have satisfactory analytical and/or spectral data.

11. NMR data on the hydrochloride of iminolyxitol (3) in D_2O; ^{13}C NMR:δ70.70 (d,CHOH), 70.55 (d,CHOH), 63.22 (d,CHN), 58.38 (t,CH_2OH), 47.87 (t,CH_2N). ^1H NMR:δ3.03 (dd, H-1, $J_{1,1'}$=12.2 Hz,$J_{1,2}$=7.3 Hz), 3.36 (dd, H-1', $J_{1',2}$ =7.3 Hz), 3.56 (m, H-4), 3.72 (dd, H-5, $J_{5,5'}$=12.1 Hz,$J_{4,5}$=8.4 Hz), 3.81 (dd, H-5', $J_{4,5'}$=5.0 Hz), 4.17 t, H-3, $J_{3,4}$=4.1 Hz), 4.31 (dt, H-2,$J_{2,3}$=4.1 Hz).

12. B.R. Baker, R.E. Schaub, and J.H. Williams, J.Amer.Chem.Soc., 1955, 77,7.

(Received in UK 10 April 1985)