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Inhibition of Naringinase (L-Rhamnosidase) by Piperidine Analogues of L-Rhamnose: Scaffolds for Libraries Incorporating Trihydroxypipecolic Acids

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> Abstract: L-Deoxyrhamnojirimycin 1 does not inhibit naringinase significantly but 5-*epi*-L-deoxyrhamnojirimycin 2 is a potent inhibitor. Conversely, α -C-glycosides of 1 are good inhibitors of L-rhamnosidase whereas those of 2 are not. Intermediate azabicyclic lactones are likely to be of use for the incorporation of a number of trihydroxypipecolic acids into peptide libraries. Copyright © 1996 Elsevier Science Ltd

The preceding paper¹ reports the synthesis and evaluation of a number of potent inhibitors of naringinase which are azafuranose analogues of L-rhamnose. Although aza-D-mannofuranose analogues give rise to many potent inhibitors of D-mannosidases, mannopyranose analogues such as deoxymannojirimycin and 6-*epi*castanospermine are usually only weak mannosidase inhibitors.² L-Deoxyrhamnojirimycin (LRJ) 1 was first synthesised from D-gulonolactone and reported to have no significant inhibition of naringinase.³ Later, 1 was prepared by a sequence involving an aldolase reaction and stated to be a good inhibitor of naringinase.⁴ Wong⁵ repeated both the chemical and biochemical syntheses of 1 and found the materials to be identical by NMR and other spectroscopic data, but confirmed the earlier results that the sample from the chemical synthesis was a very poor inhibitor but that from the enzymic route was quite a good inhibitor of L-rhamnosidase; Wong suggested that the activity might be due to traces of an impurity in 1 which, on the basis of the synthesis, he proposed could be 5-*epi*-LRJ **2**.



This paper reports the unambiguous synthesis of 5-epi-LRJ 2, a related lactam 7 and tetrazole 9 from L-rhamnose and compares them as naringinase inhibitors with LRJ 1 and the corresponding lactam 6 and tetrazole 8, prepared from D-gulonolactone by an identical route to that used for the enantiomer of 8 derived from L-gulonolactone.⁶ Wong's suggestion that 5-epi-LRJ 2 might be a good inhibitor of naringinase is shown to be correct. Homologues of azasugars, such as the natural product homonojirimycin,⁷ are also inhibitors of glycosidases.⁸ This paper reports the synthesis, and effects on naringinase, of α - 3 and β - 4 homo-L-rhamnojirimycin, and of homo-5-epi-L-rhamnojirimycin 5. Remarkably 3 is a powerful inhibitor of L-rhamnosidase whereas 5 has only a marginal inhibitory effect. Easily available bicyclic lactone intermediates in these syntheses provide intermediates that are convenient for the incorporation of the pipecolic acids into combinatorial libraries.



Scheme 1 (i) Tf₂O, pyridine; NaN₃, DMF (ii) H₂, 10% Pd/C, MeOH (iii) Me₂S:BH₃, THF (iv) H₃O⁺ (v) NH₃, MeOH (vi) (CF₃CO)₂O, pyridine (vii) heat

For the synthesis of 5-*epi*-LRJ **2** [Scheme 1], the lactone 10^9 was esterified with triflic anhydride in pyridine and the triflate then treated with sodium azide in DMF to afford the azide, **11**, m.p. 78-81 °C, $[\alpha]_D^{21}$ -118.8 (*c*, 1.33)¹⁰ in 67% yield. Hydrogenation of the azidolactone **11** in methanol with 10% palladium on carbon caused reduction to the azide and spontaneous isomerisation to the lactam **12**, m.p. 151-152°C, $[\alpha]_D^{21}$ +39.0 (*c*, 0.52), 74% yield. Reduction of the lactam **12** with borane:dimethylsulphide in THF followed by acid hydrolysis with hydrochloric acid gave 2^{11} in 71% yield. Acid hydrolysis of **12** gave the lactam **7**, m.p. 192 - 193°C, $[\alpha]_D^{25}$ +66.9 (*c*, 0.86 in H₂O) in 89% yield. Reaction of the azidolactone **11** with ammonia in methanol gave the open chain amide **13**, m.p. 78-81°C, $[\alpha]_D^{23}$ +13.1 (*c*, 1.13 in acetone), 99% yield. Dehydration of **13** with trifluoroacetic anhydride in pyridine gave the nitrile **14** which on heating in toluene formed the protected tetrazole **15**, foam, $[\alpha]_D^{24}$ -23.4 (*c*, 0.84), 71% yield. The ketal was removed from **15** by acid hydrolysis to afford the unprotected tetrazole **9**, oil, $[\alpha]_D^{23}$ +24.3 (*c*, 0.6 in acetone) in 79% yield.



Scheme 2 (i) Tf₂O, pyridine, CH₂Cl₂ (ii) H₂, Pd black, NaOAc, EtOAc (iii) LiBH4, THF, then H₃O⁺ (iv) LiBHEt₃, THF; then HCl, MeOH (v) PCC, CH₂Cl₂, molecular sieve (vi) (EtO)₃P, THF, reflux (vii) NaCNBH₃, MeCOOH (viii) NaOAc, MeOH

The readily available azidolactone 16^{12} is a common starting material for all of the C-azaglycosides 3, 4 and 5 [Scheme 2]. The synthesis of 5 requires formation of a bond between nitrogen and C-6 of the lactone with inversion of configuration. Esterification of the free alcohol in 16 with triflic anhydride and pyridine in dichloromethane gave the triflate 17 which on hydrogenation in ethyl acetate in the presence of palladium black and sodium acetate gave the corresponding amine 18 which spontaneously cyclised to the lactone, 19, m.p. 98-100°C, $[\alpha]_D^{21}$ +23.2 (c 1.0), in an overall yield of 61%.¹³ Reduction of the aminolactone 19 with lithium borohydride in THF, followed by treatment with acidic ion exchange resin, gave the deprotected α -homo-epi-LRJ 5¹⁴ in 92% yield.

In order to retain the configuration at C-6 of the lactone 16 during the formation of the piperidine ring, the alcohol 16 was oxidised with pyridinium chlorochromate in dichloromethane in the presence of molecular sieve to afford the ketone 20, m.p. 123-4°C, $[\alpha]_D^{21}$ -8.2 (c 0.92) in 82% yield. Treatment of the azidoketone 20 with triethyl phosphite at reflux in THF induced an intramolecular aza-Wittig reaction to form the bicyclic imine 21. 183-4°C. $[\alpha]_{0}^{21}$ -175.8 (c 1.06), in 61% yield, Reduction of the imine with sodium cyanoborohydride in acetic acid resulted in hydride delivery from the least hindered side of the iminium ion to give the bicyclic aminolactone 22, m.p. 118-9°C; $[\alpha]_D^{21}$ +32.8 (c 1.34), 83% yield; the structure of 22 was firmly established by X-ray crystallographic analysis, showing that the ring had been formed with overall retention of configuration.¹⁵ Treatment of 22 with superhydride in THF followed by treatment with hydrogen chloride in methanol gave α -homoLRJ 3¹⁶ in 80% yield. Ring opening of the lactone 22 by sodium acetate in methanol gave a mixture of 23, m.p. $128-9^{\circ}$ C, $[\alpha]_{D}^{24}$ -23.7 (c. 0.60) together with 24 m.p. 175-6°C. $\left[\alpha\right]_{0}^{23}$ +44.9 (c 0.81); the proportion of products depends on the length of time of the reaction. It is clear that the initially formed 23 is less stable than 24 in which both the ester and methyl groups are equatorial: furthermore, 23 can be isomerised to 24 under the reaction conditions without appreciable elimination taking place. Superhydride reduction of 24, followed by work up with hydrogen chloride in methanol, gave BhomoLRJ 4¹⁷ in 63% yield. Similar treatment of 23 afforded 3 in 71% yield. Both the bicyclic lactones 19 and 22 undergo rapid and efficient ring opening reactions with amines and should provide access to libraries containing trihydroxypipecolic acid.18



 Table 1: Inhibition of naringinase (L-rhamnosidase) [from Penicillium decumbens] activity by piperidine analogues of rhamnose in the hydrolysis of p-nitrophenyl- α -L-rhamnopyranoside

The results of studies on the inhibition of naringinase (L-rhamnosidase) from *Penicillium decumbens* by the piperidine analogues are summarised in Table 1; all inhibition of naringinase by compounds 1 - 9 was competitive.¹⁹ LRJ 1 showed no inhibition at 750 μ M whereas 5-*epi*-LRJ 2 was a strong inhibitor of naringinase with K_i 1.0 μ M. This would be entirely consistent with Wong's proposal that small amounts of 2 may also be formed in the enzymic route to 1 and this would account for the observed inhibition of naringinase by such samples. 2 was also a mild inhibitor of almond emulsin β -glucosidase [60% at 970 μ M]. Some surprising results were obtained for the homologues. Thus, although 1 gave no inhibition of the L-rhamnosidase, α -homoLRJ 3 is a potent inhibitor [K_i 5.3 μ M]. β -HomoLRJ 4 is a much weaker inhibitor but

is a powerful inhibitor of coffee bean α -galactosidase with IC50 4 μ M; this will be discussed elsewhere. In contrast to the potent inhibition shown by 2, homo-epi-LRJ 5 only has weak inhibitory effects on Lrhamnosidase. Both 3 and 5 at 800 μ M showed weak inhibition of green coffee bean α -galactosidase. E. coli β -galactosidase and Jack Bean α -mannosidase. No inhibition of naringinase was found by either of the two lactams 6 and 7 or by the *epi*-tetrazole 9. Only very weak inhibition of naringinase was observed for the pyranose tetrazole 8 [25% inhibition at 770 µM] in marked contrast to the furano-tetrazole analogue³ which has K. 56 μ M; 8 is also a weak inhibitor of almond emulsin β -glucosidase [44% at 770 μ M].

In summary, Wong's proposal that an impurity, probably 2, in the enzymic synthesis of 1 could cause the difference in the properties of apparently the same material from two different sources looks correct. Some niperidine analogues of L-rhamnose are very good inhibitors of naringinase, so that both pyranose and furanose analogues of rhamnose are recognised by the enzyme. This is in contrast to most D-mannosidases where azafuranose mimics are good inhibitors, but azapyranose analogues usually are much weaker. Naringinase is a convenient enzyme to study in regard to epitopes of L-rhamnose, and this work may provide clues for finding inhibitors of enzymes which are involved in the incorporation of rhamnose into mycobacterial cell walls²⁰ in novel approaches to the treatment of tuberculosis.^{21,22}

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9. Dominguez, J. N., Owen, L. N., Carbonyar, Res., 1979, 78, 101. 10. Unless otherwise stated, all specific rotations were measured in chloroform. 11. Data for 5-epi-LRJ \mathbf{Z} [α] D^{24} +6.3 (c, 0.96 in H₂O); $\delta_{\rm H}$ (D₂O, 500MHz, pH 8) 0.94 (3H, d, H-6, J 6.9Hz), 2.59 (1H, dd, H-1, J 10.3Hz, J 12.8Hz), 2.71 (1H, dd, H-1', J 4.7Hz, J 12.9Hz), 2.91 (1H, m, H-5), 3.61 (1H, dd, H-4, J 2.0Hz, J 4.4Hz), 3.77 (1H, dd, H-2, J 3.4Hz, J 4.4Hz, J 10.0Hz), 3.81 (1H, m, H-3); $\delta_{\rm C}$ (D₂O, 50MHz) 15.7 (q, C-6), 44.8 (t, C-1), 49.3 (d, C-5), 66.6, 71.3, 72.9 (d x 3, C-2, C-3, C-4).

ada, H-2, J 3, H12, J 4, H12, J 10, DH2), 3, S1 (TH, III, H-3); 6C (D2O, 50MH2) 13.7 (q, C-6), 44.8 (t, C-1), 49.3 (d, C-3), 66.6, 11.3, 72.9 (d x 3, C-2, C-3, C-4). 12. Estevez, J. C., Smith, M. D., Wormald, M. R., Besra, G. S., Brennan, P. J., Nash, R. J., Fleet, G. W. J., *Tetrahedron Asymm.*, 1996, 7, 391. 13. The structure of lactone 19 was firmly established by X-ray crystallographic analysis of a cyclohexylidene derivative. 14. Data for homo-epi-LRJ 5 [α]D²⁻³-38.1 (c, 0.9 in H₂O, pH 8); v_{max} (thin film)/cm⁻¹: 3401 (br OH, NH); δH (500 MHz; D2O, pH 9): 1.11 (3H, d, J₆ 7 6.8, H-7), 2.93 (1H, dt, J 40, J 10.7, H-2), 3.20 (1H, q, J 6.8, H-6), 3.71-3.75 (3H, m), 3.77 (1H, dd, J 3.1, J 10.7), 3.98 (1H, t, J 3.5); δC (50 MHz; D2O, pH 9): 15.9 (q, C-7), 61.0 (t, C-1), 49.3, 55.9 (2d, C-2, C-6), 65.4, 71.4, 72.5 (3d, C-3, C-4, C-5); *m/z* (DCI; NH3): 178 (MH⁺, 100%). 15. Details of the X-ray structure of **22** will be provided in the full paper. 16. Data for α-homoLRJ 3 oil(α]D²⁻⁵ +15.0 (c, 0.9 in H₂O, pH 8), δH (500MHz; D2O, pH 8) 1.05 (3H, d, J₆, 7 6.3, H-7), 2.59 (1H, dd, J₅, 6.9, J, J₄, 3.3, H-4), 3.56 (1H, dd, J₁, 1' 17, J', 2, 7.1, H-1), 3.62 (1H, dd, J₅, 6.9.4, J₄, 5.9.5, H-5), 3.51 (1H, dd, J₄, 5.9.5, J_3, 4.3.3, H-4), 3.56 (1H, dd, J₁, 1' 17, J', 2, 7.1, H-1), 3.62 (1H, dd, J₅, 6.9.4, J₄, 5.9.5, H-5), 3.51 (1H, dd, J₆, 7 6.3, H-3); bC (50 MHz; D2O, pH 10): 17.2 (q, C-7), 59.3 (t, C-1), 51.2, 59.7 (2d, C-2, C-6), 69.3, 71.8, 73.6 (3d, C-3, C-4, C-5); *m/z* (Electrospray) 178 (MH⁺, 100%). 17. Data for β-homoLRJ 4 oil, [α]D²⁻⁴ +12.1 (c 0.95 in H₂O, pH 8); δH (500MHz; D2O, pH 8) 1.07 (3H, d, J₆, 7 6.4, H-7), 2.45 (1H, dd, J₆, 5.9, J₄, 4.3, 2.9, H-3), 3.49 (1H, dd, J₁, 2.6.9, J₁, 2.6.7, J₂, 3.1.3, H-2), 3.21 (1H, dd, J₅, 6.9.6, J₆, 7.6.4, H-7), 2.45 (1H, dd, J₅, 6.9.6, J₆, 7.6.4, H-7), 2.45 (1H, dd, J₆, 5.7, 6.4, C-5); *m/z* (Electrospray) 178 (MH⁺, 100%). 17. Data for β-homoLRJ 4 oil, [α]D²⁻⁴ +12.1 (c 0.95 in H₂O, pH 8); δH (5

15.4 (3d, C-3, C-4, C-3); m/2 (APC1) 176 (M-H⁻¹, 100%). 18. The details of ring opening of the lactones with amines will be presented in a full paper. 19. Naringinase (Sigma) (0.25 µg/ml) was assayed against 5mM p-nitrophenyl- α -L-rhamnopyranoside (Sigma) at pH 4.0 (K_m 1.1 mM). The compounds were also assayed for potential inhibition of α -glucosidase (Brewers yeast, rabbit gut, β-glucosidase (almond emulsin, rabbit gut, rabbit liver), α -galactosidase (Jack Bean), β-N-acetylglucosaminidase (Jack Bean, bovine), xylanase (*Trichoderma viride*), pectinase (*Aspergillus niger*), and rabbit gut sucrase, maltase, trehalase and lactase; there was no significant inhibition of any of these enzymes other than where stude in the text. Details of the argumen will be given in a full argument.

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