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L-(+)-Swainsonine and Other Pyrrolidine Inhibitors of Naringinase: Through an Enzymic Looking Glass from D-Mannosidase to L-Rhamnosidase?

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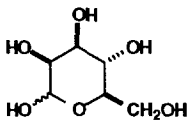
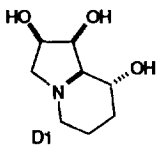
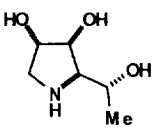
Abstract: The synthesis and inhibitory properties towards naringinase (L-rhamnosidase) of L-(+)-swainsonine and of a number of more highly oxygenated analogues, and of some monocyclic equivalents, are reported. L-(+)-swainsonine and 1,4,6-trideoxy-1,4-imino-L-mannitol are powerful and specific inhibitors of naringinase.


D-mannopyranose


D1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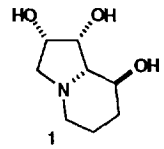
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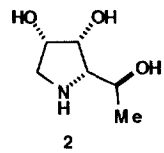
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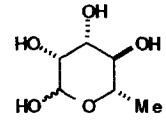
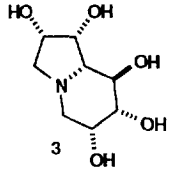

L-rhamnopyranose

Recent papers by Dennis[1] and others[2] have underlined the potential of the natural product swainsonine **D1**[3] as an anticancer agent, probably due to its inhibitory effect on α-D-mannopyranosidases of N-linked glycoprotein processing.[4] In general, the most potent inhibitors of D-mannopyranosidases are azafuranose mimics of which **D1** is the most powerful and also has the requisite physico-chemical properties to get into, and stay inside, cells. Studies have shown that monocyclic equivalents of swainsonine such as **D2**[5] are very potent inhibitors of D-mannosidases. The preceding paper[6] showed that pentahydroxylated indolizidines with a *cis*-diol unit in the pyrrolidine **3** and **4** were weak inhibitors of naringinase but that **5** with a *trans*-diol has no effect on L-rhamnosidase. Perhaps the structural features of D-mannosidases which are responsible for inhibition by **D1** and **D2** are mirrored by the properties that cause inhibition of L-rhamnosidases, so that the enantiomers **1** and **2** might prove to be good inhibitors of naringinase. This paper reports the synthesis and preliminary evaluation of such compounds. It may be that the relative inhibition of L-rhamnosidase by analogues of L-swainsonine at a higher oxidation level will provide a guide to the structure-activity relationship of enantiomeric compounds as D-mannosidase inhibitors. There are no prior reports of the synthesis or properties of such hydroxy- and dihydroxyswainsonine derivatives.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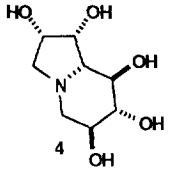
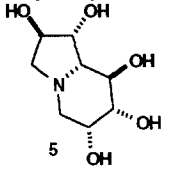
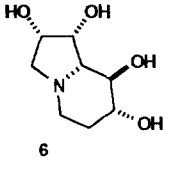
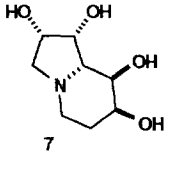
IC_{50} 530 μM


4
IC_{50} 610 μM


5
NI at 700 μM


6
IC_{50} 50 μM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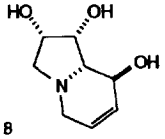
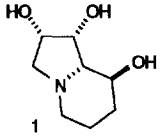
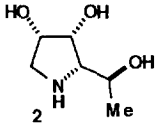
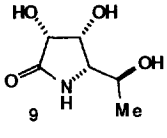
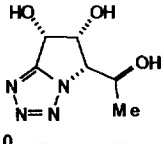
IC_{50} 265 μM


8
28% at 830 μM


1
IC_{50} 0.3 μM
K_i 0.45 μM


2
IC_{50} 4.0 μM
K_i 1.0 μM

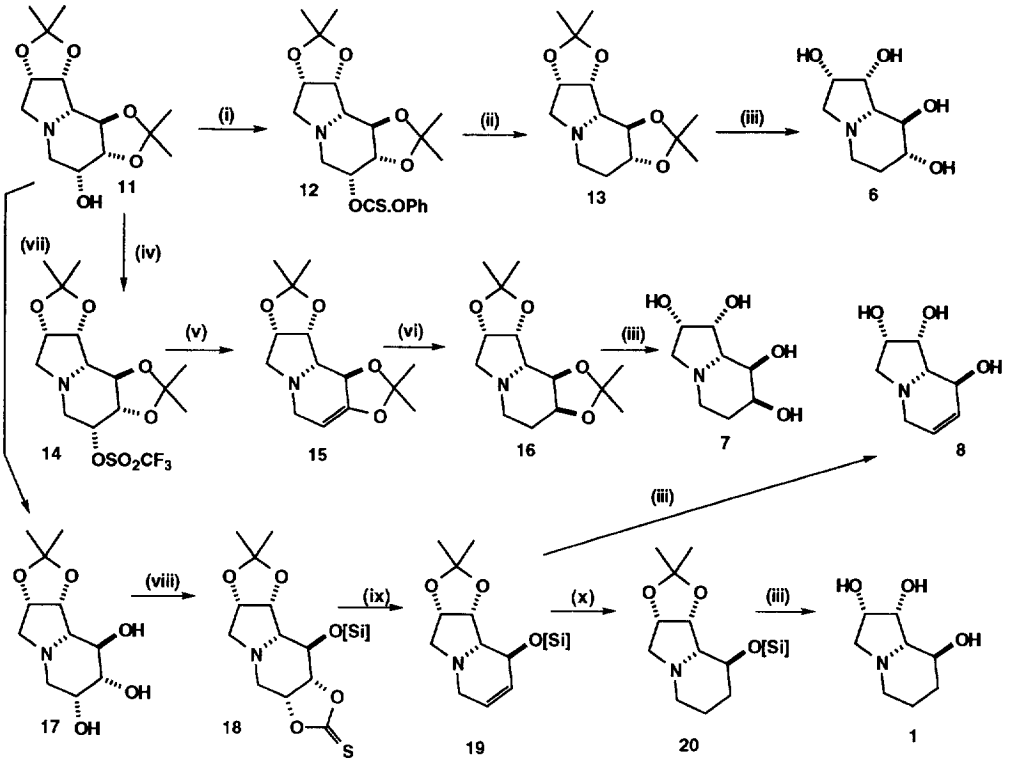

9
NI at 890 μM


10
IC_{50} 70 μM
K_i 56 μM

IC_{50} indicates concentration of compound needed to cause 50% inhibition of naringinase

NI means no inhibition at stated concentration

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

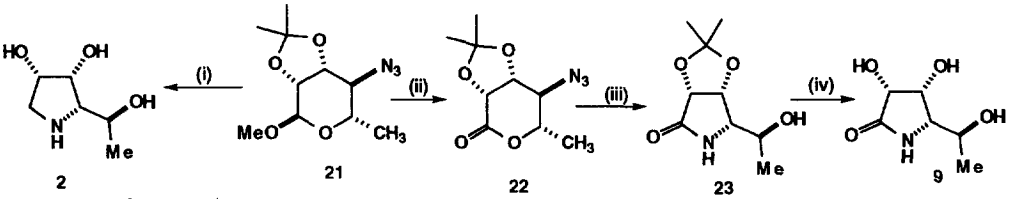


Scheme 1: (i) PhO.CS.Cl, DMAP, MeCN (ii) Bu_3SnH, AIBN, toluene (iii) $CF_3COOD:D_2O$, 1:1 (iv) Tf_2O, pyridine (v) DBU, THF (vi) H_2, Pd black, MeOH (vii) $CH_3COOH:H_2O$, 80:20 (viii) $(Im)_2CS$, toluene; then *tert*$BuMe_2SiOTf$, pyridine CH_2Cl_2 (ix) $(EtO)_3P$, heat (x) H_2, Pd black, EtOAc

The readily available diacetonide **11** is a highly divergent intermediate for the synthesis of L-swainsonine itself **1** and the more highly oxygenated analogues [Scheme 1]. Thus reaction of the free hydroxyl group in **11** with phenyl chlorothioformate in acetonitrile in the presence of DMAP gave the thionocarbonate **12**, oil, $[\alpha]_D^{23}$ -29.1 (*c*, 0.75) [68% yield] which underwent the Barton deoxygenation on treatment with tributyltin hydride to afford the deoxygenated *trans*-diacetonide **13**, oil, $[\alpha]_D^{25}$+55.5 (*c*, 0.8),[7] in 59% yield. Removal of the ketal protecting groups in **13** by aqueous trifluoroacetic acid gave the *trans*-hydroxyswainsonine **6**[8] in 81% yield; the deprotections of **13** and the other acetonides were performed in D_2O so that the progress of the reaction could easily be monitored by ^1H NMR. For the epimer **7**, **11** was first esterified with triflic anhydride in pyridine to give the triflate **14**, m.p. 92-94°C, $[\alpha]_D^{24}$-6.8 (*c*, 0.30) [61% yield] which with diazabicyclo[5.4.0]-undec-7-ene (DBU) in THF afforded the enol ether **15**, $[\alpha]_D^{24}$-43.1 (*c*, 0.20), [100% yield]. Hydrogenation of **15** in methanol in the presence of palladium black gave the *cis*-diacetonide **16**, $[\alpha]_D^{25}$+143.5 (*c*, 0.9) [81% yield] which on acid hydrolysis afforded *cis*-hydroxy compound **7**[9] [79% yield].

For the synthesis of L-swainsonine itself **1**, **11** was first hydrolysed in aqueous acetic acid to afford the monoacetonide **17**, m.p. 138 - 139°C, $[\alpha]_D^{22}$ +26.6 (*c*, 0.98 in MeOH) [85% yield] and sequentially reacted with 1,1'-thiocarbonyldiimidazole in toluene, followed by *tert*-butyldimethylsilyl triflate in dichloromethane in the presence of pyridine, to give the fully protected cyclic thionocarbonate **18**; m.p. 156 - 158°C, $[\alpha]_D^{21}$ -4.7(*c*, 1.29 in EtOAc), in 72% yield. Treatment of **18** with triethylphosphite at reflux induced a Corey-Winter fragmentation to **19**, oil, $[\alpha]_D^{21}$ +88.6 (*c*, 0.71 in EtOAc) in 76% yield. Acid hydrolysis of **19** gave dehydro-L-swainsonine **8**[10] in 80% yield. Alternatively, hydrogenation of the double bond in **19** in

ethyl acetate in the presence of palladium black afford the saturated silyl ether 20, oil, $[\alpha]_D^{24}$ +68.2 (*c*, 0.76) [89% yield] which was completely deprotected to give crystalline L-(+)-swainsonine **1** m.p. 143-144°C, $[\alpha]_D^{21}$ +84.3 (c, 1.02 in H_2O); [lit[11] m.p. 143-145°C; $[\alpha]_D^{24}$ +83.3 (c, 0.5 in MeOH); lit. for enantiomer **D1**, m.p. 143-145°C; $[\alpha]_D^{23}$ -87.2 (c, 2.1 in MeOH)] in 74% yield.



2 21 22 23 9

Scheme 2: (i) H_3O^+; then H_2, Pd black, EtOH (ii) ref. 14 (iii) H_2, Pd black, EtOAc (iv) CF_3CO_2H:H_2O, 1:1

Some monocyclic pyrrolidine analogues of L-rhamnose have been shown to be inhibitors of naringinase[12] and as the 6-deoxy-D-rhamnofuranose pyrrolidine analogue **D2** is a powerful inhibitor of D-mannopyanosidases,[13] it was decided to prepare the L-rhamnose analogues **2** and **9**. The known azide **21** had been used previously for the synthesis of the L-rhamnofuranotetrazole **10** and is a suitable divergent intermediate for these targets [Scheme 2].[14] Hydrolysis of the isopropylidene and anomeric methyl protecting groups in **21** gave the corresponding lactol which, on hydrogenation in ethanol in the presence of palladium black, afforded the azafuranose **2**, 70% yield, isolated as the hydrochloride, m.p. 183-185°C, $[\alpha]_D^{24}$ +25.9 (c, 0.39 in MeOH) {lit.[15] for the enantiomer **D2**, m.p. 184-185°C, $[\alpha]_D^{27}$ -21.5 (*c*, 1.0 in MeOH)}. For the five-ring lactam **9**, **21** was first converted to the azido-lactone **22**[14] which on hydrogenation in ethyl acetate in the presence of palladium black gave the protected lactam **23**, m.p. 170-173 °C, $[\alpha]_D^{22}$ -11.4 (*c*, 0.72 in CH_3OH), in quantitative yield. Hydrolysis of the acetonide in **23** with aqueous trifluoroacetic acid afforded the target L-rhamnonolactam **9**[16] in 83% yield.

The results of studies on the inhibition of naringinase (L-rhamnosidase) from *Penicillium decumbens* for compounds **1** - **10** are summarised in Table 1; all of the inhibition observed was competitive.[17] 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 (green coffee bean,), β-galactosidase (*E.coli*, rabbit gut, rabbit liver), α-mannosidase (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 stated in the following text. The dihydroxy-L-swainsonines **3** and **4** were weak inhibitors of naringinase with IC_{50} of 530 μM and 610 μM, respectively, whereas the epimer **5** with a *trans*-relationship of the diol in the pyrrolidine moiety caused no inhibition of naringinase at 700 μM [**3** caused 34% inhibition of *E. coli* β-galactosidase and 23% inhibition of Jack Bean α-mannosidase]. Removal of the hydroxyl groups at C-6 in **3** or **4** to give **6** resulted in a ten-fold increase in the naringinase inhibition to IC_{50} 50 μM. However, the epimeric hydroxy-L-swainsonine **7** is a significantly weaker inhibitor [IC_{50} 264 μM]. Dehydro-L-swainsonine **8** was a very weak L-rhamnosidase inhibitor [IC_{50} 830 μM] and also was a very weak inhibitor of almond β-glucosidase [42% at 830 μM].

L-Swainsonine **1** is a very potent inhibitor of naringinase with K_i of 0.45 μM; it is also highly specific - the only other inhibition found was that of Jack Bean α-mannosidase with K_i of 2500 μM. In contrast, D-Swainsonine **D1** shows no inhibition of the L-rhamnosidase at all. The monocyclic L-rhamnitol **2** is also a good inhibitor with K_i of 1.0 μM, and again is specific with the only other observed inhibition being 49% inhibition of *E. coli* β-galactosidase at 1 mM. The lactam **9** shows no inhibition of any glycosidases at all, while in contrast the neutral tetrazole **10** inhibits naringinase with K_i of 56 μM. There is no inhibition of D-mannosidases by D-tetrazole analogues of D-mannose[18] and this may indicate a significant difference in the degree of protonation[19] required for the inhibitor in the D- and L- series.

In summary, this paper reports the first syntheses of mono- and di-hydroxylated swainsonine derivatives in either the D-(-) or L-(+)- series. The value of very highly functionalised higher sugars, such as

the octonolactones, in giving easy access to such materials in enantiomerically pure form is firmly established by this paper. The low inhibition given by L-(+)-swainsonine **1** against D-mannopyranosidase shows the value of enantiospecific syntheses for establishing such matters in regard to biological activity; D-(-)-swainsonine **D1** has no effect on naringinase. There are clearly considerable similarities between the mirror-image relationships of azafuranose compounds which inhibit D-mannosidase and L-rhamnosidase activities. Even these preliminary studies show there may be significant differences between the structural specificities of the enzymes, as is the case with the differential inhibition by the non-basic D- and L-tetrazoles. These studies may have major implications in the studies of enzymes which deal with enantiomeric sugars, as in the case of D-galactose *versus* L-fucose and D-mannose *versus* L-rhamnose. The following paper shows that azapyranose analogues of L-rhamnose can provide potent inhibitors of L-rhamnosidase, whereas azapyranose analogues of D-mannose are usually weak inhibitors of D-mannosidase; enzymic mirrors may not always be transparently exact. Enzymes that process L-rhamnose are not confined to hydrolases and this work may have implications in regard to the study of mycobacterial cell wall biosynthesis and approaches to the treatment of tuberculosis.[20]

REFERENCES

1. Goss, P. E., Baker, M. A., Carver, J. P., Dennis, J. W., *Clin. Cancer Res.*, 1995, **1**, 935.
2. Das, P. C., Roberts, J. D., White, S. L., Olden, K., *Oncology Res.*, 1995, **7**, 425; Taeda, Y., Nose, M., Hiraizumi, S., Ohuchi, N., *Breast Cancer Res. Treatment*, 1996, **38**, 313.
3. Colegate, S.M., Dorling, P.R., Huxtable, C.R., *Aust. J. Chem.*, 1979, **32**, 2257; Schneider, M.J., Ungemach, F.S., Broquist, H.P., Harris T.M., *Tetrahedron*, 1983, **39**, 29.
4. Winchester, B., Fleet, G. W. J., *Glycobiology*, 1992, **2**, 199; Asano, N., Oseki, K., Kizu, H., Matsui, K., *J. Med. Chem.*, 1994, **37**, 3706.
5. Winchester, B., Al-Daher, S., Carpenter, N. M., Cenci di Bello, I., Choi, S. S., Fairbanks, A. J., Fleet, G. W. J., *Biochem. J.*, 1993, **290**, 743.
6. preceding paper
7. Unless otherwise stated, all specific rotations were measured in chloroform.
8. Data for *trans*-hydroxyswainsonine **6**: $[\alpha]_D^{25}$+14.0 (*c*, 0.1, H_2O); $\delta_H(D_2O)$: 1.47 (1H, dddd, *J* 4.5 Hz, *J* 12.4 Hz *J* 12.4 Hz, *J* 12.4 Hz, H-6), 1.89 (1H, ddd, *J* 2.4 Hz, $J_{6',7}$ 4.9 Hz *J* 10.8 Hz, H-6'), 2.02 (1H, m, H-8a), 2.07 (1H, dd, *J* 11.9 Hz *J* 11.9 Hz, H-5), 2.53 (1H, dd, *J* 8.4 Hz, *J* 10.7 Hz, H-3), 2.81 (1H, d, *J* 11.1 Hz, H-3'), 2.88 (1H, d, *J* 11.5 Hz, H-5'), 3.43 (1H, ddd, $J_{7,6'}$ 5.1 Hz, $J_{7,8}$ 9.0 Hz, *J* 11.2 Hz, H-7), 3.52 (1H, dd, $J_{8,8a}$ 9.3 Hz, $J_{8,7}$ 9.3 Hz, H-8), 4.16 (1H, dd, *J* 3.8 Hz, *J* 5.7 Hz, H-1), 4.32 (1H, m, H-2); $\delta_C(D_2O)$: 31.6 (C-6), 49.7 (C-5), 60.1 (C-3), 69.8, 70.2, 71.2, 71.3, 73.8 (C-2, C-1, C-7, C-8, C-8a).
9. Data for *cis*-hydroxyswainsonine **7**: $[\alpha]_D^{25}$+22.0 (*c*, 0.05, H_2O); $\delta_H(D_2O)$: 1.66 (1H, dddd, $J_{6,5}$ 2.6 Hz, *J* 4.8 Hz *J* 13.9 Hz, *J* 13.9 Hz, H-6), 1.75 (1H, ddd, $J_{6',5'}$ 1.9 Hz, *J* 5.4 Hz, *J* 14.8 Hz, H-6'), 2.22 (1H, ddd, $J_{5,6}$ 3.0 Hz, *J* 12.0 Hz *J* 12.0 Hz, H-5), 2.33 (1H, dd, *J* 3.1 Hz, $J_{8a,8}$ 10.2 Hz, H-8a), 2.55 (1H, dd, $J_{3,2}$ 8.2 Hz, $J_{3,3'}$ 11.0 Hz, H-3), 2.68 (1H, ddd, $J_{5',6'}$ 1.6 Hz, *J* 4.5 Hz, *J* 11.5 Hz, H-5'), 2.82 (1H, dd, $J_{3',2}$ 2.5 Hz, $J_{3',3}$ 11.2 Hz, H-3'), 3.73 (1H, dd, $J_{8,7}$ 3.1 Hz, $J_{8,8a}$ 10.3 Hz, H-8), 3.99 (1H, dd, $J_{7,8}$ 3.0 Hz, *J* 6.0 Hz, H-7), 4.13 (1H, dd, *J* 3.6 Hz, $J_{1,2}$ 5.9 Hz, H-1), 4.29 (1H, ddd, $J_{2,3'}$ 2.7 Hz, $J_{2,1}$ 6.0 Hz, $J_{2,3}$ 8.0 Hz. H-2); $\delta_C(D_2O)$: 30.3 (t, C-6), 46.1 (t, C-5), 60.6 (t, C-3), 66.0 (d, C-8a), 67.9 (d, C-7), 68.4 (d, C-8), 69.4 (d, C-2), 70.0 (d, C-1).
10. Data for dehydro-L-swainsonine **8**: m.p. 132 - 135°C; $[\alpha]_D^{23}$ +63.4 (c, 1.0 in H_2O); δ_H (D_2O, 500MHz) 2.24 (1H, dd, H-8a, *J* 3.7Hz, *J* 8.6Hz), 2.63 (1H, dd, H-3, *J* 8.1Hz, *J* 11.0Hz), 2.61 (1H, br d, H-5, *J* 16.4Hz), 2.86 (1H, dd, H-3', *J* 3.5Hz, *J* 11.1 Hz), 3.22 (1H, br d, H-5', *J* 16.4Hz), 4.21 (1H, dd, H-1, *J* 4.1Hz, *J* 5.7 Hz), 4.32 (1H, ddd, H-2, *J* 3.5Hz, *J* 5.6Hz, *J* 8.1Hz), 4.44 (1H, ddd, H-8, *J* 1.9 Hz, *J* 3.6Hz, *J* 8.8Hz) 5.63 (1H, dd, H-6, *J* 1.1Hz, *J* 10.3Hz) 5.72 (1H, br d, H-7, *J* 10.1Hz); δ_C (D_2O, 50MHz) 52.3,59.8 (t x 2, C-3,C-5), 64.9,69.2,69.9, 70.3 (d x 4, C-1, C-2, C-8, C-8a), 127.5,129.9 (d x 2, C-6, C-7).
11. Oishi, T., Iwakuma, T., Hirama, M., Ito, S., *Synlett*, **1995**, 404.
12. Provencher, L., Steensma, D. H., Wong, C.-H., *Biorg. Med. Chem.*, 1994, **2**, 1179.
13. Elis, M. J., Rule, C. J., Wurzberg, Ganem, B., *Tetrahedron Lett.*, 1985, **26**, 5397.
14. Davis, B., Brandstetter, T. W., Smith, C., Hackett, L., Winchester, B. G., Fleet, G. W. J., *Tetrahedron Lett.*, 1995, **36**, 7507.
15. Carpenter, N., Fleet, G. W. J., di Bello, I. C., Winchester, B., Fellows, L. E., Nash, R. J., *Tetrahedron Lett.*, 1989, **30**, 7261.
16. Data for L-rhamnonolactam **9**: ; m.p. 185-188°C $[\alpha]_D^{24}$ +14.8 (c, 1.1 in MeOH); ν_{max} (film) 3307 (OH,NH), 1698 (C=O) cm^{-1}; δ_H (D_2O, 500MHz) 1.20 (3H, d, H-6, $J_{5,6}$ 6.4Hz), 3.36 (1H, dd, H-4, $J_{3,4}$ 3.6Hz, $J_{4,5}$ 8.2Hz), 3.90 (1H, dq, H-5, $J_{4,5}$ 8.2Hz, $J_{5,6}$ 6.4Hz), 4.31 (1H, d, H-2, $J_{2,3}$ 4.9Hz), 4.42 (1H, dd, H-3, $J_{2,3}$ 4.9Hz, $J_{3,4}$ 3.6Hz); δ_C (CD_3OD) 19.0 (q, C-6), 60.4,65.1,69.7,71.7 (d x 4, C-2, C-3, C-4, C-5), 177.5 (s, C-1); *m/z* (NH_3, DCI): 179 ($M+NH_4^+$,40), 162 ($M+H^+$,100%).
17. 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). Details of the other enzyme assays will be given in a full paper.
18. Brandstetter, T. W., Davis, B., Hyett, D., Smith, C., Hackett, L., Winchester, B. G., Fleet, G. W. J., *Tetrahedron Lett.*, 1995, **36**, 7511.
19. Jeong, J. H., Murray, B. W., Takayama, S., Wong, C. H., *J. Am. Chem. Soc.*, 1996, **118**, 4227.

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