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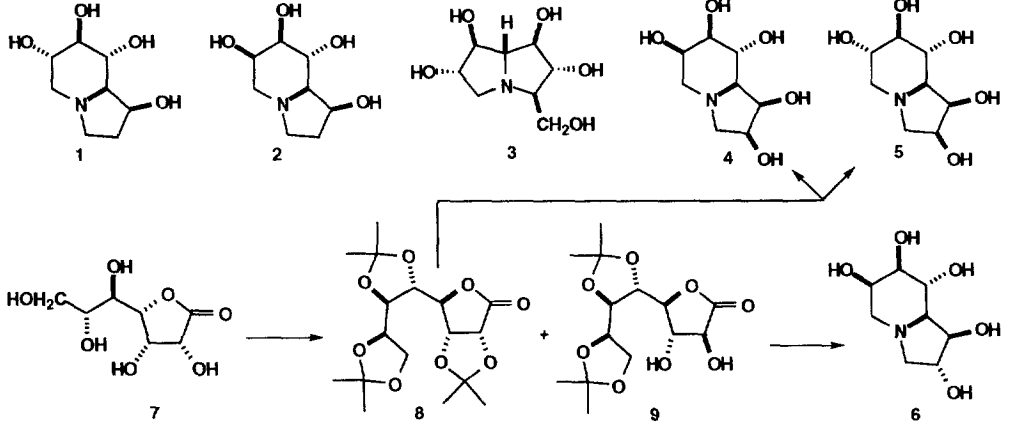
2-HydroxyCastanospermines (Dihydroxy-L-swainsonines) from Octonolactones: Inhibition of Naringinase (L-Rhamnosidase)

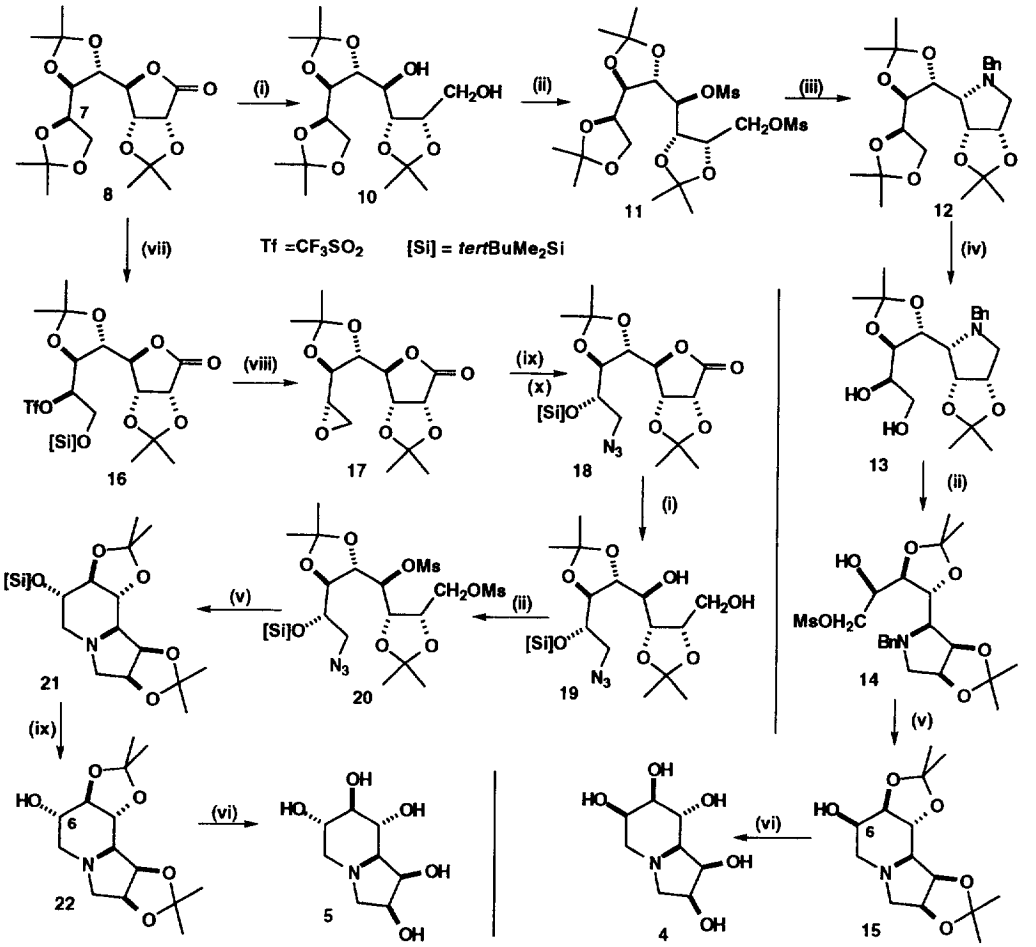
Andrew A. Bell,[a] Lea Pickering,[a] Alison A. Watson,[b] Robert J. Nash,[b] Rhodri C. Griffiths,[c] M. George Jones[c] and George W. J. Fleet[a]*

[a]Dyson Perrins Laboratory, Oxford Centre for Molecular Sciences, South Parks Road, Oxford OX1 3QY UK
[b]Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Cardiganshire SY23 3EB UK
[c]Institute of Biological Sciences, University of Wales, Aberystwyth, Cardiganshire SY23 3DD UK

Abstract: Short syntheses of 2S-2-hydroxycastanospermine, and 2R- and 2S-2-hydroxy-6-*epi*castanospermine - in which there are 6 adjacent chiral centres and 8 contiguous carbon atoms containing functional groups from eight carbon sugar lactones - depend on efficient cyclisations to give piperidines with *trans*-acetonides as protecting groups. Inhibition of naringinase (L-rhamnosidase) by 2S-2-hydroxy-6-*epi*castanospermine and 2S-2-hydroxycastanospermine may be due to a structural resemblance to the unnatural L-(+)-swainsonine.

Various potential chemotherapeutic applications of castanospermine **1** including possible approaches to the treatment of cancer and AIDS have caused an extensive investigation into the synthesis of the natural product and of structural variations thereof.[1] 6-*epi*Castanospermine **2** is also a naturally occurring alkaloid[2] and other less oxygenated indolizidines, such as swainsonine and lentiginosine occur naturally. The most highly oxygenated bicyclic sugar mimic that has so far been isolated is casuarine **3**,[3] a glucoside of which has also been identified.[4] More highly oxygenated derivatives of castanospermine with an extra hydroxyl group at C-2 also occur as natural products, although they occur in very small quantities and are difficult to isolate in a pure form; it may be that these new alkaloids have the same stereochemistry at five of their six chiral centres as **1** and **2**, leaving only the additional hydroxyl substituent at C-2 with any stereochemical ambiguity. Short and unambiguous syntheses of such materials would provide samples for biological evaluation and for establishing the presence of such compounds in plants. The synthesis of even more highly oxygenated analogues of castanospermine would appear to increase the difficulty of the synthesis. However, eight carbon sugar lactones **8** and **9** with only isopropylidene and/or silyl ether protecting groups are readily available in large amounts from the cheap heptonolactone **7**.[5] This paper reports the synthesis of the very highly oxygenated castanospermine analogues **4**, **5** and **6** by connecting C-1, C-4 and C-8 of the lactones **8** and **9** with nitrogen. Each of the targets has six contiguous stereogenic centres with functional groups attached to all of the eight carbon atoms. The inhibition of naringinase (L-rhamnosidase) by **4** and **5** - but not **6** - is also reported.





Scheme 1 (i) $LiBH_4$, THF (ii) $MeSO_2Cl$, pyridine, DMAP (iii) $PhCH_2NH_2$ (iv) TsOH, MeOH (v) H_2, Pd black, EtOH; NaOAc (vi) $CF_3COOH:H_2O$, 1:1 (vii) ref.5; then Tf_2O, pyridine (viii) nBu_4NF, THF (ix) NaN_3, DMF (x) *tert*$BuMe_2SiOTf$, pyridine

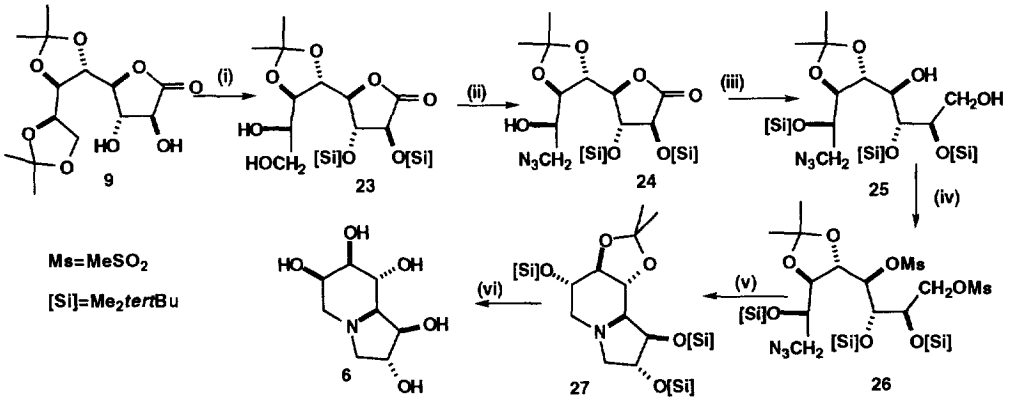
Two different strategies were used for the construction of **4** and **5** by joining C-1 C-4 and C-8 of the triacetonide **8** by nitrogen [Scheme 1]. In the case of 2S-hydroxy-6-*epi*castanospermine **4**, nitrogen was first introduced at C-1 allowing the initial development of a pyrrolidine ring by subsequent attack by the nitrogen nucleophile at C-4 and subsequent elaboration to close the second piperidine ring by attack at C-8. For the epimer **5**, nitrogen was first introduced at C-8, so that the initial piperidine ring formation is followed by a final closure to the bicyclic system. Thus these approaches to castanospermines may also produce monocyclic piperidine and pyrrolidine amino sugar analogues.[6]

For **4**, reduction of the triacetonide **8** with lithium borohydride in THF gave the diol **10**, oil, $[\alpha]_D^{23}$+30.3 (*c*, 0.58)[7] which was esterified by an excess of mesyl chloride in pyridine in the presence of DMAP to give the dimesylate **11**, oil, $[\alpha]_D^{23}$-24.5 (*c*, 0.86) [91% overall yield]. Treatment of **11** with benzylamine at 110°C for 2 days induced displacement of the primary mesylate and subsequent ring closure to give the pyrrolidine **12**, oil, $[\alpha]_D^{22}$+83.8 (*c*, 0.77), in 93% yield. Removal of the terminal isopropylidene group in 12 with *p*-toluenesulfonic acid in methanol afforded the diol **13**, oil, $[\alpha]_D^{23}$+39.2 (*c*, 1.31) [68% yield] which underwent a highly regioselective mesylation to give the primary mesylate **14**, oil, $[\alpha]_D^{23}$+33.7 (*c*, 0.51), in 91% yield. Hydrogenation of **14** in ethanol in the presence of palladium black followed by treatment with sodium acetate caused hydrogenolysis of the benzyl group and subsequent cyclisation to the bicyclic diacetonide **15**, oil, $[\alpha]_D^{24}$+6.6 (*c*, 0.95), in 62% yield. Reaction of **15** with aqueous trifluoroacetic

acid removed both isopropylidene groups to give the target pentahydroxyindolizidine **4**[8] in 80% yield [26% overall yield from **8**].

At first sight, it would appear to be easy to invert the stereogenic centre bearing the free hydroxyl group in **15** to give **22** and thus gain access to the castanospermine analogue **5**. However, all attempts to achieve this transformation - either by displacement of a leaving group at C-6 of the indolizidine or by oxidation of the alcohol to a ketone and subsequent reduction - were unsuccessful. Accordingly, a sequence that involved inversion of the C-7 stereogenic centre of the lactone **8** prior to cyclisation was devised. Mild acid hydrolysis of the triacetonide **8** with aqueous acetic acid followed by selective silylation of the primary alcohol and esterification of the remaining alcohol with triflic anhydride gave the silyltriflate **16**, m.p. 80-81°C, $[\alpha]_D^{24}$-12.2 (*c*, 0.72). Treatment of **16** with tetrabutylammonium fluoride in THF caused removal of the silyl protecting and spontaneous formation of the epoxide **17**, m.p. 130-131°C, $[\alpha]_D^{24}$-22.8 (*c*, 0.64), in 99% yield. Reaction of **17** with sodium azide in DMF induced ring opening with introduction of the azide functionality at C-8, and the free hydroxyl group at C-7 was reacted with *tert*-butyldimethylsilyl triflate in pyridine to give the fully protected azidolactone **18**, m.p. 101-103°C, $[\alpha]_D^{24}$-31.8 (*c*, 0.84) in 44% overall yield. Reduction of the lactone **18** with lithium borohydride in THF gave the diol **19** $[\alpha]_D^{24}$+6.8 (*c*, 1.4) [96% yield], mesylation of which afforded the azidodimesylate **20** $[\alpha]_D^{24}$-18.8 (*c*, 1.2), in 85% yield. Hydrogenation of the azidomesylate **20** in ethanol in the presence of palladium black effected reduction of the azide to the corresponding amine which, in the presence of sodium acetate, in refluxing ethanol cyclised to give the fully protected castanospermine **21**, $[\alpha]_D^{23}$+36.6 (*c*, 0.82), in 36% yield. The silyl ethyl protecting group in **21** was removed by tetrabutylammonium fluoride in THF to give the diacetonide **22**, $[\alpha]_D^{23}$+45.8 (*c*, 0.36) in quantitative yield. The ketals were removed from **22** by acid hydrolysis to give 2S-2-hydroxycastanospermine **5** in 81% yield.[9] The ^{1}H NMR spectra of the diacetonides **15** and **22** and of the final targets **4** and **5** provided strong supporting evidence of the stereochemistry at C-6 in the two epimers. Thus in the ^{1}H NMR spectra of both **5** and **22** the proton at C-7 has 2 large *trans*-diaxial coupling constants, but the corresponding proton in **4** and **15** has one large and one small coupling constant.

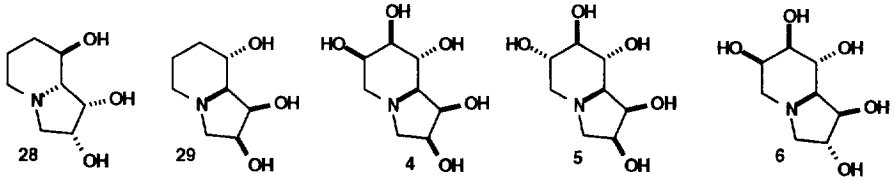
For the synthesis of 2R-2-hydroxy-6-*epi*castanospermine **6**, the diacetonide **9** was converted into the disilyl-monoacetonide **23** as previously described. Regioselective esterification of **23** with tosyl chloride in pyridine gave the primary tosylate which with sodium azide in DMF afforded the azide **24**, m.p. 98-100 °C. $[\alpha]_D^{23}$+25.9 (*c*=2.22) in an overall yield of 63%. Silylation of the remaining free hydroxyl group in **24**, followed by reduction of the lactone with lithium borohydride in THF gave the diol **25**, oil, $[\alpha]_D^{23}$ +18.5 (*c*, 1.39) [60% yield] which was converted to the dimesylate **26**, $[\alpha]_D^{23}$ +53.2 (*c*, 0.48) [68% yield]. Hydrogenation of the azide **26** in ethanol in the presence of palladium black, followed by sodium acetate, gave the protected bicycle **27**, $[\alpha]_D^{23}$ -35.5 (*c*, 0.67), in 64% yield. All the protecting groups were removed from **27** by treatment with aqueous trifluoroacetic acid to give the target indolizidine **6**[10] in 47% yield.



Scheme 2 (i) Ref. 5 (ii) TsCl, pyridine; then NaN_3, DMF (iii) *tert*$BuMe_2SiOTf$; then $LiBH_4$, THF (iv) $MeSO_2Cl$, pyridine, DMAP(v) H_2, Pd black, EtOH; then NaOAc (vi) $CF_3COOH:H_2O$, 1:1

The syntheses of **4**, **5** and **6** rely on relatively high yield cyclisations to form piperidines with *trans*-acetonides; the overall yield of the hydroxycastanospermines is better than any of the corresponding attempts

to make either castanospermine itself or diastereomers thereof by similar approaches. The success of the cyclisation may be that, even if epoxides are formed, the nature of the ketal protection precludes the formation of pyrrolidines by 5-*exo*-tet processes and only allows the formation of piperidines by competing 7-*endo*-tet cyclisations.



The hydroxycastanospermines **4**, **5** and **6** were tested as inhibitors of a number of glycosidases. Both **4** (IC_{50} 530 μM) and **5** (IC_{50} 610 μM) [11] in which there is a *cis*-diol unit in the pyrrolidine moiety are moderate inhibitors of naringinase (L-rhamnosidase) whereas no such inhibition was found for **6** with a corresponding *trans*-diol unit; neither castanospermine **1** nor 6-*epi*castanospermine **2** caused any inhibition of naringinase. Thus, D-(-)-Swainsonine **28** is a natural product which is a very powerful inhibitor of mannopyranosidases; **4** and **5** have a structural resemblance as dihydroxy derivatives of L-(+)-swainsonine **29**[12] and this feature may be responsible for the L-rhamnosidase inhibition. Additionally, although castanospermine is a very powerful inhibitor of intestinal sucrase, the 2-hydroxy analogue **5** is only a very weak inhibitor of the rabbit gut disaccharidases. It thus appears that the pyrrolidine azafuranose mimic predominates over the piperidine azapyranose mimic and so **4** and **5** may be better described as dihydroxy-L-swainsonines. In summary, this paper presents the first use of an octonolactone in the synthesis of a non-carbohydrate target in which all the chiral centres and functionalities are present in the products, and relies on the highly efficient cyclisation . The following paper demonstrates the value of such materials for the synthesis of L-swainsonine **29** and various more highly oxygenated analogues thereof, and thus easy access to highly efficient pyrrolidine inhibitors of L-rhamnosidase.[13]

REFERENCES

1. Bridges, C. G., Ahmed, S. P., Kang, M. S., Nash, R. J., Porter, E. A., Tyms, A. S., *Glycobiology*, 1995, **5**, 249; Picasso, S., Chen, Y., Vogel, P., *Carbohydr. Lett.*, 1994, **1**, 1; Furneaux, R. H., Gainsford, G. J., Mason, J. M., Tyler, P. C., *Tetrahedron*, 1995, **51**, 12611; Goti, A., Cardona, F., Brandi, A., Picasso, S., Vogel, P., *Tetrahedron: Asymm.*, 1996, **7**, 1659; Asano, N., Kizu, H., Oseki, Tomioka, E., Matsui, K., Okamoto, M., Baba, M., *J. Med. Chem.*, 1995, **38**, 2349.

2. Molyneux, R. J., Roitman, J., Dunnheim, G., Szumilo, T., Elbein, A.D., *Arch. Biochem. Biophys.*, 1986, **251**, 450.

3. Nash, R. J., Thomas, P. I., Waigh, R. D., Fleet, G. W. J., Wormald, M. R., Lilley, P. M. D., Watkin, D. J., *Tetrahedron Lett.*, 1994, **35**, 7849.

4. Wormald, M. R., Nash, R. J., Watson, A. A., Bhadoria, B. K., Langford, R., Sims, M., Fleet, G. W. J., *Carbohydr. Lett.*, 1996, **2**, in press.

5. Bell, A. A., Nash, R. J., Fleet, G. W. J., *Tetrahedron: Asymm.*, 1996, **7**, 595.

6. Berger, A., Dax, K., Gradnig, G., Grassberger, V, Stutz, A. E., Ungerank, M., Legler, G., Bause, E., *Bioorg. Med. Chem. Lett.*, 1992, **2**, 27; Baudat, A., Picasso, A., Vogel, P., *Carbohydr. Res.*, 1996, **281**, 277.

7. Unless otherwise stated, all specific rotations were measured in chloroform.

8. 2S-2-hydroxy-6-epicastanospermine **4**: hygroscopic white solid $[\alpha]_D^{23}$+13.1 (*c*, 0.88, H_2O); $\delta_H(D_2O)$: 2.04 (1H, dd, $J_{8a,1}$ 3.8 Hz, $J_{8a,8}$ 9.7 Hz, H-8a), 2.26 (1H, dd, $J_{5,6}$ 1.5 Hz, $J_{5,5'}$ 12.4 Hz, H-5), 2.54 (1H, dd, $J_{3,2}$ 7.9 Hz, $J_{3,3'}$ 10.7 Hz, H-3), 2.84 (1H, dd, $J_{3',2}$ 2.8 Hz, $J_{3',3}$ 10.7 Hz, H-3'), 3.00 (1H, dd, $J_{5',6}$ 2.9 Hz, $J_{5',5}$ 12.4 Hz, H-5'), 3.45 (1H, dd, $J_{7,6}$ 3.6 Hz, $J_{7,8}$ 9.6 Hz, H-7), 3.85 (1H, dd, $J_{8,8a}$ 9.6 Hz, $J_{8,7}$ 9.6 Hz, H-8), 3.93 (1H, m, H-6), 4.18 (1H, dd, $J_{1,8a}$ 3.8 Hz, $J_{1,2}$ 5.9 Hz, H-1), 4.34 (1H, ddd, $J_{2,3'}$ 2.8 Hz, $J_{2,1}$ 5.9 Hz, $J_{2,3}$ 7.9 Hz. H-2); $\delta_C(D_2O)$: 63.8 (t, C-5), 65.1 (t, C-3), 67.4 (d, C-8), 68.0 (d, C-6), 68.3 (2 x d, C-1, C-2), 68.4 (d, C-8a), 69.9 (d, C-7); *m/z* (NH_3, CI): 206 ($M+H^+$, 100%).

9. 2S-2-hydroxycastanospermine **5**: hygroscopic white solid $[\alpha]_D^{23}$+66.5 (*c*, 1.33, H_2O); $\delta_H(D_2O)$: 1.91 (1H, dd, $J_{5,5'}$ 10.7 Hz, $J_{5,6}$ 10.7 Hz, H-5), 2.01 (1H, dd, $J_{8a,1}$ 3.5 Hz, $J_{8a,8}$ 9.8 Hz, H-8a), 2.54 (1H, dd, $J_{3,2}$ 8.1 Hz, $J_{3,3'}$ 10.9 Hz, H-3), 2.71 (1H, dd, $J_{3',2}$ 2.6 Hz, $J_{3',3}$ 11.0 Hz, H-3'), 2.95 (1H, dd, $J_{5',6}$ 5.2 Hz, $J_{5',5}$ 10.8 Hz, H-5'), 3.12 (1H, dd, $J_{7,6}$ 9.2 Hz, $J_{7,8}$ 9.2 Hz, H-7), 3.42 (1H, ddd, $J_{6,5'}$ 5.3 Hz, $J_{6,5}$ 9.9 Hz, $J_{6,7}$ 9.9 H-6), 3.47 (1H, dd, $J_{8,7}$ 9.5 Hz, $J_{8,8a}$ 9.5 Hz, H-8), 4.06 (1H, dd, $J_{1,8a}$ 3.8 Hz, $J_{1,2}$ 5.8 Hz, H-1), 4.24 (1H, m, H-2); $\delta_C(D_2O)$: 55.8 (t, C-5), 60.0 (t, C-3), 69.3, 69.8. 70.3, 70.4, 70.9, 79.3 (6 x d, C-1, C-2, C-6, C-7, C-8, C-8a); *m/z* (APCI): 206 ($M+H^+$, 100%)

10. 2R-2-hydroxy-6-epicastanospermine **6**: hygroscopic white solid $[\alpha]_D^{23}$ -25.5 (*c*=0.81,H_2O). $\delta_H(D_2O)$: 1.98(1H, dd, $J_{3',2}$5.6, $J_{3',3}$10.2, H-3'), 2.12(1H, dd, $J_{8a,1}$4.2, $J_{8a,8}$9.7, H-8a), 2.24(1H, app d, H-5'), 2.96(1H, dd, $J_{5,6}$2.8, $J_{5,5'}$12.5, H-5), 3.36(1H, dd, $J_{3,2}$7.0, $J_{3,3'}$10.3, H-3), 3.42(1H, dd, $J_{7,6}$3.5, $J_{7,8}$9.5, H-7), 3.72(1H, app t, H-8), 3.86(1H, app q, H-6), 3.94(1H, dd, $J_{1,8a}$4.4, H-1), 4.07(1H, app t, H-2); $\delta_C(D_2O)$: 55.90(t, C-5), 60.67(t, C-3), 67.51, 69.61, 70.30, 75.89, 77.93, 78.13(6 x d, C-2, C-3, C-4, C-5, C-6, C-7); MS APCI (+ve) *m/z*= 206(M+H)$^+$100%.

11. IC_{50} is the concentration of inhibitor required to cause 50% inhibition of the activity of naringinase *(Penicillium decumbens)* in the hydrolysis of *p*-nitrophenyl-α-L-rhamnopyranoside (K_m 1.1 mM), for further details of the assays, see following paper.

12. Oishi, T., Iwakuma, T., Hirama, M., Ito, S., *Synlett*, **1995**, 404; no enzyme inhibition studies for L-swainsonine were reported in this paper.

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