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Inhibition of α-L-fucosidase by derivatives of deoxyfuconojirimycin and deoxymannojirimycin

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Deoxyfuconojirimycin (1,5-dideoxy-1,5-imino-L-fucitol) is a potent, specific and competitive inhibitor (K_i 1×10^{-8} M) of human liver α-L-fucosidase (EC 3.2.1.51). Six structural analogues of this compound were synthesized and tested for their ability to inhibit α-L-fucosidase and other human liver glycosidases. It is concluded that the minimum structural requirement for inhibition of α-L-fucosidase is the correct configuration of the hydroxy groups at the piperidine ring carbon atoms 2, 3 and 4. Different substituents in either configuration at carbon atom 1 (i.e. 1α- and β-homofuconojirimycins) and at carbon atom 5 may alter the potency but do not destroy the inhibition of α-L-fucosidase. The pH-dependency of the inhibition by these amino sugars suggests very strongly that inhibition results from the formation of an ion-pair between the protonated inhibitor and a carboxylate group in the active site of the enzyme. Deoxymannojirimycin (1,5-dideoxy-1,5-imino-D-mannitol) is also a more potent inhibitor of α-L-fucosidase than of α-D-mannosidase. This can be explained by viewing deoxymannojirimycin as β-L-homofuconojirimycin lacking the 5-methyl group. Conversely, β-L-homo analogues of fuconojirimycin can also be regarded as derivatives of deoxymannojirimycin. This has permitted deductions to be made about the structural requirements of inhibitors of α- and β-D-mannosidases.

INTRODUCTION

Many synthetic and naturally occurring polyhydroxylated indolizidines, piperidines and pyrrolidines have been shown to be specific and potent inhibitors of glycosidases (Elbein, 1987; Fellows & Fleet, 1988). The isolation or design and synthesis of novel glycosidase inhibitors is very important in view of their potential usefulness as anti-viral agents (Tyms *et al.*, 1987; Gruters *et al.*, 1987; Fleet *et al.*, 1988*a*) and as anti-neoplastic agents (Humphries *et al.*, 1986; Dennis, 1986). The basis of these applications is probably inhibition of the glycosidases involved in processing the glycans attached to glycoproteins. This type of inhibitor can also be used to induce a deficiency of a lysosomal glycosidase in animals or cells in culture (Cenci di Bello *et al.*, 1983). This situation mimics the lysosomal storage disease that results from the genetic deficiency of the enzyme, but in a reversible manner that permits the lysosomal turnover of glycoproteins to be studied *in situ*. This may also be relevant to the biological effects of these inhibitors. Deoxyfuconojirimycin [1,5-dideoxy-1,5-imino-L-fucitol, DFJ; (I) in Fig. 1] is a potent inhibitor of mammalian α-L-fucosidase (Fleet *et al.*, 1985). Although DFJ does not have any anti-(human immunodeficiency virus) activity, its *N*-methyl derivative [(II) in Fig. 1] does (Fleet *et al.*, 1988*a*), presumably through inhibition of human α-L-fucosidase. Human liver lysosomal α-L-fucosidase exists in multiple forms separable on the basis of charge or size even when purified to homogeneity (Thorpe & Robinson, 1978). These forms have slightly different pH optima towards the synthetic substrate 4-methylumbelliferyl α-L-fucoside, accounting for the relatively broad pH–activity profile (Alhadeff, 1981). All these forms are absent in the lysosomal storage disease fucosidosis, indicating that they arise by post-translational events. The enzyme has a broad but ill-defined specificity, catalysing the hydrolysis of α-(1→2)-, α-(1→3)-, α-(1→4)- and α-(1→6)-fucosidic linkages in a variety of natural and synthetic substrates (Chien & Dawson, 1980). To investigate the structural features necessary for the inhibition of human α-L-fucosidase, a series of analogues of DFJ was synthesized. As α-homonojirimycin is a good inhibitor of α-D-glucosidase (Kite *et al.*, 1988), an attempt was made to synthesize α-L-homofuconojirimycin. However, this resulted in the preparation of 6-epi-α-L-homofuconojirimycin [2,6,7-trideoxy-2,6-imino-D-*glycero*-D-*gluco*-heptitol, 6-epi-α-HFJ; (VII) in Fig. 1], which was nevertheless a potent inhibitor of α-L-fucosidase. Deoxymannojirimycin [1,5-dideoxy-1,5-imino-D-mannitol, DMJ; (III) in Fig. 1], the mannose analogue of DFJ, is a more potent inhibitor of α-L-fucosidase than of α-D-mannosidase (Evans *et al.*, 1985). To understand the basis of this specificity, β-L-

Abbreviations used: DFJ, deoxyfuconojirimycin (1,5-dideoxy-1,5-imino-L-fucitol); DMJ, deoxymannojirimycin (1,5-dideoxy-1,5-imino-D-mannitol); HFJ, L-homofuconojirimycin (or methyldeoxymannojirimycin).

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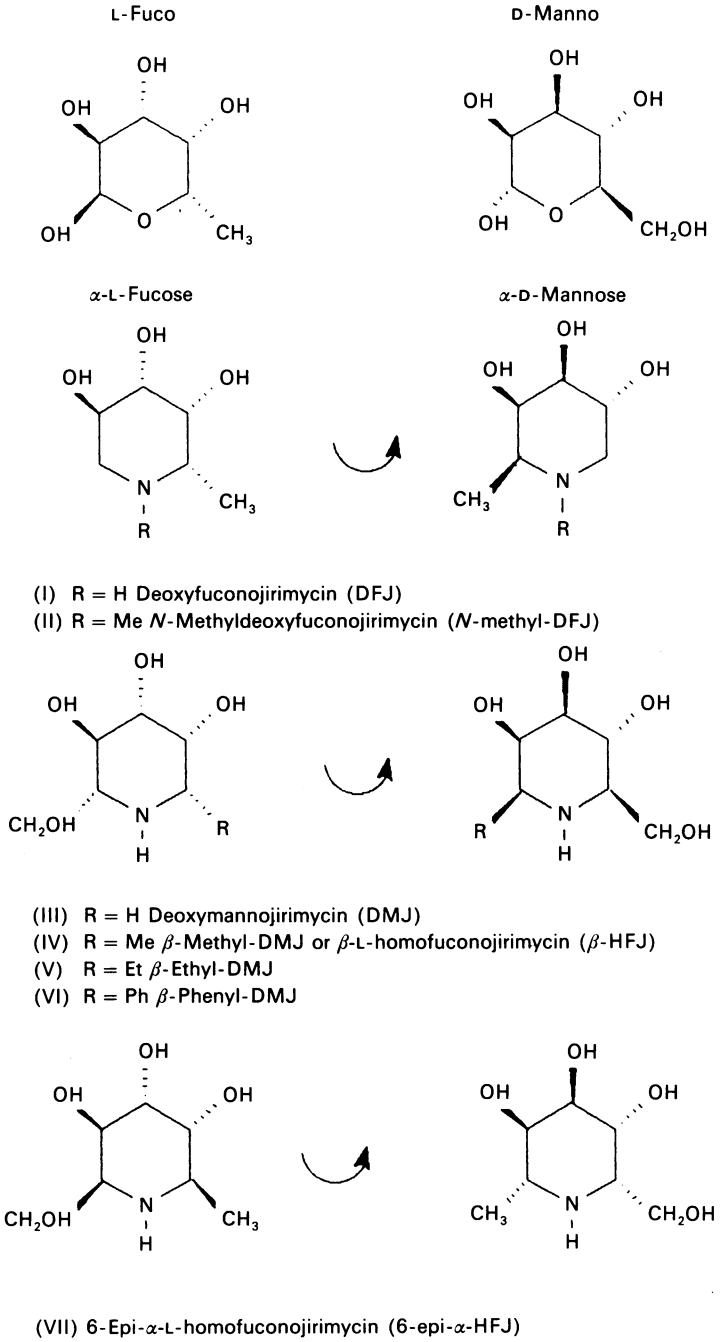

Fig. 1. Structures of inhibitors

homofuconojirimycin [β-HFJ; (IV) in Fig. 1], which can be viewed as β-methyl-DMJ, β-ethyl-DMJ [(V) in Fig. 1] and β-phenyl-DMJ [(VI) in Fig. 1] were also synthesized and tested as inhibitors of α-L-fucosidase and α-D-mannosidase.

MATERIALS AND METHODS

Tissue

Post-mortem human liver, which had been stored at −20°C until required, was homogenized in deionized water (50%, w/v) in a Potter–Elvehjem homogenizer and then centrifuged at 37000 *g* for 30 min in an MSE 18 centrifuge. The resultant supernatant was used as the source of human glycosidases. A freeze-dried extract of liver from *Charonia lampas* was obtained from Seikagaku Kogyo Co. (Tokyo, Japan) for the isolation of α-L-fucosidase by the procedure of Iijima & Egami (1971).

Enzyme assays

The glycosidase activities in an extract of human liver were assayed by using the appropriate fluorigenic 4-umbelliferyl glycoside substrate (Koch–Light, Haverhill, Suffolk, U.K.) at a concentration of 0.5 mM at the optimal pH for each enzyme (Burditt *et al.*, 1980). The specificity of the inhibitors was determined by assaying the glycosidases in the presence of and absence of a 1 mM solution of each compound. The nature of the inhibition

of human α-L-fucosidase, the value of K_i determined by using the Dixon graphical procedure and the effect of pH on the inhibition were investigated as described previously (Al Daher *et al.*, 1989). Bovine epididymal and *Charonia lampas* α-L-fucosidases were assayed with *p*-nitrophenyl α-L-fucopyranoside as substrate in McIlvaine (1921) phosphate/citrate buffer, pH 6.0, and 0.05 M-sodium acetate buffer, pH 4.5, containing 0.15 M-NaCl, respectively. The Lineweaver–Burk graphical procedure and secondary plots of the slope against inhibitor concentration were used to determine the nature of inhibition and the values of K_i for these activities.

Inhibitors

To facilitate understanding of the structural relationships among the compounds tested, they have been named as derivatives of the parent compounds DFJ and DMJ. DFJ (I) was prepared from either D-glucose (Fleet *et al.*, 1985, 1988*b*) or D-lyxonolactone (Fleet *et al.*, 1989*a*). *N*-Methyl-DMJ (II) was synthesized from DFJ as described previously (Fleet *et al.*, 1989*a*). DMJ (III) was synthesized from L-gulonolactone (Fleet *et al.*, 1989*b*). β-HFJ (IV) [m.p. 97–98 °C; $[\alpha]_D^{20}$ −21.5° (*c*. 1.07 in water)] was prepared from L-gulonolactone (Fleet *et al.*, 1989*c*). β-HFJ can also be considered as a derivative of DMJ, namely β-methyl-DMJ. Therefore compounds (V) and (VI), which were synthesized in a similar manner to compound (IV), have been called β-ethyl-DMJ and β-phenyl-DMJ respectively. 6-Epi-α-HFJ (VII) [m.p. 61–64 °C; $[\alpha]_D^{20}$ +40.3° (*c*. 0.90 in water)] was prepared from D-mannose (Fleet *et al.*, 1989*c*).

RESULTS

Inhibition of α-L-fucosidase by DFJ and derivatives

DFJ. DFJ (I), the parent compound for the derivatives described in this study, has been shown previously to be a potent competitive inhibitor of mammalian α-L-fucosidases (Fleet *et al.*, 1985). We show here that it inhibits human liver lysosomal α-L-fucosidase in a competitive manner with a K_i of 1×10^{-8} M at pH 5.5. This effect was highly specific, because none of the other 11 glycosidases assayed was inhibited by more than 15% at its optimal pH value by 1 mM-DFJ. At 0.1 μM DFJ inhibited α-L-fucosidase exclusively. The inhibition of α-L-fucosidase by DFJ was pH-dependent (Fig. 2) and appeared to depend on the ionization of a group with a pK between pH 3.5 and 4.0. The pK_a of the ring imino group of DFJ was found to be 8.4 by titration. Thus the inhibitor would be positively charged over the pH range in which the inhibition varies. It is concluded that the ionizing group is on the enzyme and is probably a carboxy group.

Effect of *N*-methylation on inhibition: *N*-methyl-DFJ. Methylation of the piperidine ring nitrogen atom to form *N*-methyl-DFJ (II) had very little effect on the competitive inhibition of α-L-fucosidase (K_i 5×10^{-8} M). However, it did broaden the specificity of inhibition of glycosidases. *N*-Acetyl-β-D-hexosaminidase and β-D-galactosidase were inhibited by 59% and 22% respectively by 1 mM-*N*-methyl-DFJ. The inhibition of α-L-fucosidase by *N*-methyl-DFJ had a pH-dependence identical with that of the unmethylated compound (Fig. 2). Methylation was found to lower the pK_a of the ring imino group to 7.8. The observation that this did not alter the pH-dependence of inhibition reinforces the suggestion that the degree of inhibition depends on the state of ionization of a group on the enzyme.

Substitution at C-1: β-HFJ. The addition of a hydroxymethyl group to ring carbon atom 1 to create a β-HFJ (IV) did not diminish the inhibition of α-L-fucosidase (K_i 1×10^{-8} M). It also created weak inhibitory activity towards β-D-galactosidase, which was inhibited by 27% by 1 mM-β-HFK. The inhibition of α-L-

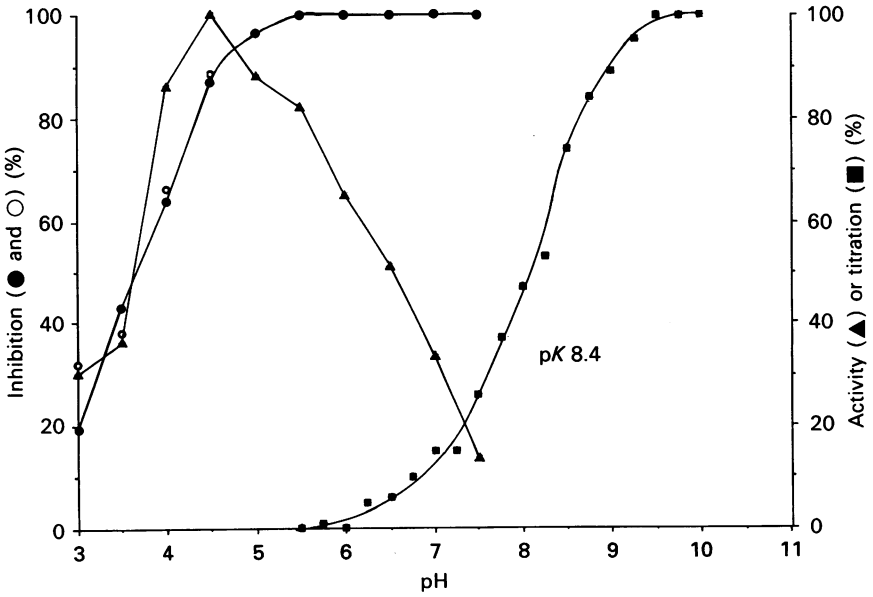

Fig. 2. Effect of pH on activity of α-L-fucosidase and its inhibition by DFJ and *N*-methyl-DFJ

The activity of human liver α-L-fucosidase was measured in the absence (▲) and in the presence of DFJ (●) or *N*-methyl-DFJ (○). Inhibition was measured as percentage of uninhibited reaction. ■, Titration curve for DFJ.

Table 1. Summary of inhibition of α-L-fucosidase and other glycosidases

Compound	pK_a	Inhibition of α-L-fucosidase K_i (M)	Other specificities (% inhibition at 1 mM)
(I) DFJ	8.4	1×10^{-8}	
(II) *N*-Methyl-DFJ	7.8	5×10^{-8}	*N*-Acetyl-β-D-hexosaminidase (59%)
(III) DMJ	7.2	5×10^{-8}	α-D-Mannosidase (64%)
(IV) β-HFJ	7.3	1×10^{-8}	β-D-Galactosidase (27%)
(V) β-Ethyl-DMJ	7.8	7×10^{-8}	*N*-Acetyl-β-D-hexosaminidase (36%)
(VI) β-Phenyl-DMJ	6.3	1×10^{-6}	*N*-Acetyl-β-D-hexosaminidase (50%)
(VII) 6-Epi-α-HFJ	7.7	5×10^{-6}	β-D-Glucuronidase (24%)

Table 2. Comparative inhibition of α-L-fucosidases by compounds (IV), (V) and (VI)

Compound	K_i (M) Bovine epididymis enzyme	K_i (M) *Charonia lampas* enzyme
(I)	–	1.4×10^{-7}
(II)	–	1.1×10^{-6}
(IV)	2×10^{-8}	1.4×10^{-7}
(V)	2×10^{-6}	5.8×10^{-7}
(VI)	5.5×10^{-6}	2×10^{-3}

fucosidase by this compound (pK_a 7.25) showed the characteristic pH-dependence.

Different substitutions at C-5: β-ethyl-DMJ and β-phenyl-DMJ. As compound (IV) retained the essential features for the inhibition of α-L-fucosidase, the effect of changing the 5-methyl group on this compound to ethyl [β-ethyl-DMJ (V)] and phenyl [β-phenyl-DMJ (VI)] was investigated. Neither substitution abolished inhibition of α-L-fucosidase (Table 1), but the bulky phenyl group lowered K_i to 10^{-6} M. Both compound (V) and compound (VI) also inhibited *N*-acetyl-β-D-hexosaminidase, by 37% and 50% respectively, at a concentration of 1 mM. Compound (V) showed the characteristic pH-dependence of inhibition of α-L-fucosidase, consistent with a measured pK_a of 7.75. Compound (VI) showed an increase in inhibition with pH at lower pH values, but at higher pH values the inhibition decreased. This may reflect ionization of the ring imino group, the pK_a of which was measured to be 6.3.

To determine whether a non-mammalian form of α-L-fucosidase responded in a similar way to changes of substituent at C-5, the effects of compounds (IV), (V) and (VI) on the α-L-fucosidase activities from the marine gastropod *Charonia lampas* and bovine epididymis were compared (Table 2). The compounds were generally more powerful inhibitors of the mammalian enzyme. Substitution of an ethyl for a methyl group slightly decreased the inhibition of both activities, but substitution of a phenyl group essentially abolished inhibitory activity towards the invertebrate enzyme selectively. *N*-Methylation lowered the potency of DFJ towards the non-mammalian enzyme, whereas addition of the β-1-homo group had no effect, as for the human enzyme.

Lack of substituent at C-5: DMJ. Compounds (IV), (V) and (VI) can be viewed as β-1-substituted derivatives of DMJ (III) (see Fig. 1). It has been reported previously that DMJ can inhibit α-L-fucosidase (Evans *et al.*, 1985), suggesting that the presence of the 5-methyl group in an inhibitor is not essential for inhibition of α-L-fucosidase. The K_i for inhibition of human α-L-fucosidase by DMJ is 5×10^{-6} M, compared with a value of 1×10^{-8} M for β-HFJ (β-methyl-DMJ), which differs from DMJ only in the presence of the 5-methyl group. This suggests that the absence of this group increases K_i by approx. 500-fold.

Configuration at C-1 and C-5: 6-epi-α-HFJ. Inversion of the configuration at ring carbon atoms 1 and 5 yielded 6-epi-α-HFJ (VII). This decreased the potency of the competitive inhibition of α-L-fucosidase (K_i 5×10^{-6} M). It showed the characteristic effect of pH on inhibition, consistent with its value of pK_a of 7.7 for the ring imino group ionization.

DISCUSSION

All the derivatives of DFJ were good competitive inhibitors of human liver α-L-fucosidase and were more effective than L-fuconolactone (Wiederschain & Rosenfeld, 1969) and L-fuconic-δ-lactam (Fleet *et al.*, 1988*b*). *N*-Methylation of DFJ to give compound (II) only slightly decreased the inhibition, suggesting that the active site of the enzyme can tolerate addition of an aliphatic group to this part of the substrate/inhibitor. A similar deduction has been drawn by us from studies on the effect of *N*-alkylation of 1,4-dideoxy-1,4-imino-L-allitol, a moderate inhibitor of α-L-fucosidase (Al Daher *et al.*, 1989). Interestingly, both *N*-methyl-DFJ and *N*-methyl-1,4-dideoxy-1,4-imino-L-allitol are moderate inhibitors of *N*-acetyl-β-D-hexosaminidase. Compounds (IV), (V) and (VI), which have an alkyl or aryl substituent adjacent to the ring nitrogen atom, also inhibit *N*-acetyl-β-D-hexosaminidase moderately.

The presence of a group on the 'anomeric' ring carbon atom 1 was not necessary for inhibition of α-L-fucosidase, e.g. DFJ. Furthermore, substitution of a β-linked anomeric hydroxymethyl group, as in compound (III) (β-HFJ), did not alter the value of K_i. In contrast, compound (VII), which has an α-linked anomeric hydroxymethyl group but in which the configuration of the 5-methyl group is inverted, was the weakest inhibitor.

This suggests that the correct configuration at C-1 cannot compensate for the incorrect configuration of the methyl group.

The effect of changing the C-5 substituent can be seen by comparing the inhibition due to compounds (IV), (V) and (VI). Although changing the methyl group to an ethyl or phenyl group progressively decreases the potency, it does not abolish inhibition of α-L-fucosidase. Thus the active site of the enzyme can tolerate an increase in the size and hydrophobicity of the C-5 substituent on an inhibitor, but not a change in configuration. This also suggests that there might be a hydrophobic region in the active site close to where this part of the inhibitor/substrate binds.

In contrast, the lack of inhibition of *Charonia lampas* L-fucosidase by compound (VI) indicates that the active site of the invertebrate enzyme may be different from that of the mammalian enzyme. The invertebrate enzyme has a broad specificity, hydrolysing α-(1→2)-, α-(1→3)-, α-(1→4)- and α-(1→6)-fucosidic linkages to a variety of glycan structures (Nishigaki *et al.*, 1974). The precise specificity of the human enzyme has not been defined.

The common structural feature to all these inhibitors of human α-L-fucosidase is the configuration of the hydroxy groups at C-2, C-3 and C-4. It is tempting to speculate that this is the minimum structural feature on a piperidine or pyranose ring necessary for the inhibition of or recognition by mammalian α-L-fucosidase. Support for this hypothesis comes from the observation that both DFJ and DMJ, which have the correct substituents at C-2, C-3 and C-4 but lack substitutions at C-1 and C-5 respectively, are potent inhibitors of α-L-fucosidase. However, the nature and orientation of substituents at C-1 and C-5 can moderate the inhibition. Our results suggest that interaction between the enzyme and the C-5 substituent of an inhibitor or substrate is more important than interaction with the C-1 region. The latter is probably important in determining the specificity of catalysis. The precise configuration of the anomeric group in an inhibitor may not be important for other mammalian glycosidases. Nojirimycin (1,5-dideoxy-1,5-imino-D-glucitol) specifically inhibits α-D-glucosidase but not β-D-glucosidase, whereas α-homonojirimycin (2,6-dideoxy-2,6-imino-L-*glycero*-D-*gulo*-heptitol) inhibits both α-D-glucosidase and β-D-glucosidase, albeit the former more strongly (Kite *et al.*, 1988).

The pH-dependence of inhibition of α-L-fucosidase by all these compounds suggests that the mechanism of action of these inhibitors depends on the ionization of a group on the enzyme with a pK_a between 3.5 and 4.0. A carboxy group with a pK_a value of 3.7–3.8 has been implicated in the catalytic mechanism (White *et al.*, 1985) and active-site inactivation (White *et al.*, 1986) of human liver α-L-fucosidase. It is postulated that this active-site carboxylate anion binds to the positively charged inhibitor to form an ion-pair. This could mimic the transition-state stabilization of a fucosyl oxocarbonium ion by an active-site carboxylate group, which has been suggested as a possible mechanism action for human liver α-L-fucosidase (White *et al.*, 1987). DFJ and analogues completely inhibit all the soluble α-L-fucosidase activity in human liver, indicating that all the post-translationally generated multiple forms of the enzyme are susceptible to inhibition. A preliminary report has suggested that rat liver organelles, particularly plasma membrane, contain membrane-associated α-L-fucosidase activity that is not completely inhibited by DFJ (Nuck *et al.*, 1987). It will be interesting to see if human cells contain corresponding activities and if they are inhibited differentially by the compounds described in the present paper.

Just as DMJ can be considered as a derivative of DFJ, so can the β-L-homo analogues of DFJ, compounds (IV), (V) and (VI), be viewed as analogues of DMJ (Fig. 1). They can thus provide information about the structural requirements for inhibition of α- and β-D-mannosidases. DFJ and derivatives only inhibit α- or β-D-mannosidase by less than 15%, despite the configuration of the hydroxy groups at C-2, C-3 and C-4 being the same as in DMJ. This suggests that greater complementarity between inhibitor and active site is required for inhibition of mannosidases than for α-L-fucosidase. DMJ inhibits α- but not β-D-mannosidase, showing that α-D-mannosidase does not require the α-anomeric linkage. However, compounds (IV), (V) and (VI), which only differ from DMJ by the presence of an anomeric β-substituent, do not inhibit α-D-mannosidase. Thus the enzyme does not tolerate an incorrect anomeric linkage, and this may be the basis of its specificity.

These compounds (IV), (V) and (VI) also fail to inhibit β-D-mannosidase, although all the ring substituents are in the correct configuration. This shows that the structural requirements of an inhibitor of β-D-mannosidase are more rigorous than those for α-D-mannosidase. This conclusion agrees with our previous deductions based on the selective inhibition of α-D-mannosidase but not β-D-mannosidase by Swainsonine and analogues (Cenci di Bello *et al.*, 1989). Perhaps β-D-mannosidase requires a β-linked anomeric oxygen atom for interaction between enzyme and inhibitor/substrate as well as the correct substituents at all the other ring positions. However, the possibility that substitution of nitrogen for oxygen in the ring may prevent binding cannot be excluded.

Comparison of the specificity of inhibition of closely related amino sugars can provide much indirect information about the active sites of glycosidases and suggest structures that might be more effective or specific inhibitors.

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REFERENCES

Al Daher, S., Fleet, G., Namgoong, S. K. & Winchester, B. (1989) Biochem. J. **258**, 613–615

Alhadeff, J. A. (1981) in Lysosomes and Lysosomal Storage Diseases (Callahan, J. W. & Lowden, J. A., eds.), pp. 299–314, Raven Press, New York

Burditt, L. J., Chotai, K., Hirani, S., Nugent, P. G. & Winchester, B. (1980) Biochem. J. **189**, 467–473

Cenci di Bello, I., Dorling, P. & Winchester, B. (1983) Biochem. J. **215**, 693–696

Cenci di Bello, I., Fleet, G., Namgoong, S. K., Tadano, K.-I. & Winchester, B. (1989) Biochem. J. **259**, 855–861

Chien, S.-F. & Dawson, G. (1980) Biochim. Biophys. Acta **614**, 476–488

Dennis, J. W. (1986) Cancer Res. **46**, 5131–5136

Elbein, A. D. (1987) Annu. Rev. Biochem. **56**, 497–534

Evans, S. V., Fellows, L. E., Shing, T. K. M. & Fleet, G. W. J. (1985) Phytochemistry **24**, 1953–1955
Fellows, L. E. & Fleet, G. W. J. (1988) in Alkaloidal Glycosidase Inhibitors from Plants (Wagman, G. H. & Cooper, R., eds.), pp. 540–560, Elsevier, Amsterdam
Fleet, G. W. J., Shaw, A. N., Evans, S. V. & Fellows, L. E. (1985) J. Chem. Soc. Chem. Commun., 841–842
Fleet, G. W. J., Karpas, A., Dwek, R. A., Fellows, L. E., Tyms, A. S., Petursson, S., Namgoong, S. K., Ramsden, N. G., Smith, P. W., Son, J. C., Wilson, F., Witty, D. R., Jacob, G. & Rademacher, T. W. (1988*a*) FEBS Lett. **237**, 128–132
Fleet, G. W. J., Ramsden, N. G., Dwek, R. A., Rademacher, T. W., Fellows, L. E., Nash, R. J., Green, D. St. C. & Winchester, B. (1988*b*) J. Chem. Soc. Chem. Commun. 483–484
Fleet, G. W. J., Petursson, S., Campbell, R. A., Mueller, R. A., Behling, J. R., Babiak, K. A., Ng, J. S. & Scaros, M. G. (1989*a*) J. Chem. Soc. Perkin Trans. 1 665–666
Fleet, G. W. J., Ramsden, N. G. & Witty, D. R. (1989*b*) Tetrahedron **45**, 319–326
Fleet, G. W. J., Namgoong, S. K., Barker, C., Baines, S. & Winchester, B. (1989*c*) Tetrahedron Lett. **30**, 4439–4442
Gruters, R. A., Neefjes, J. J., Tersmette, M., De Goede, R. E. Y., Tulp, A., Huisman, H. G., Miedma, F. & Ploegh, H. L. (1987) Nature (London) **330**, 74–77
Humphries, M J., Matsumoto, K., White, S. L., Molyneux, R. J. & Olden, K. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 1752–1756
Iijima, Y. & Egami, F. (1971) J. Biochem. (Tokyo) **70**, 75–78
Kite, G. C., Fellows, L. E., Fleet, G. W. J., Liu, P. S., Scofield, A. M. & Smith, N. G. (1988) Tetrahedron Lett. **29**, 6486–6489
McIlvaine, H. (1921) J. Biol. Chem. **49**, 183–186
Nishigaki, M., Muramatsu, T., Kobata, A. & Maeyama, K. (1974) J. Biochem. (Tokyo) **75**, 509–517
Nuck, R., Sauvageot, D., Paulsen, H., Tauber, R. & Reutter, W. (1987) Proc. Int. Symp. Glycoconjugates 9th abstr. E37
Thorpe, R. & Robinson, D. (1978) Clin. Chim. Acta **86**, 21–30
Tyms, A. S., Berrie, E. M., Ryder, T. A., Nash, R. J., Hegarty, M. P., Taylor, D. L., Mobberley, M. A., Davis, J. M., Bell, E. A., Jeffries, D. J., Taylor-Robinson, D. & Fellows, L. E. (1987) Lancet **ii**, 1025–1026
White, W. J., Schray, K. J. & Alhadeff, J. A. (1985) Biochim. Biophys. Acta **829**, 3030–310
White, W. J., Schray, K. J., Legler, G. & Alhadeff, J. A. (1986) Biochim. Biophys. Acta **873**, 198–203
White, W. J., Schray, K. J., Legler, G. & Alhadeff, J. A. (1987) Biochim. Biophys. Acta **912**, 132–138
Wiederschain, G. Y. & Rosenfeld, E. L. (1969) Bull. Soc. Chim. Biol. **51**, 1075

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