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AN ALTERNATIVE PROPOSAL FOR THE MODE OF INHIBITION OF GLYCOSIDASE ACTIVITY BY POLYHYDROXYLATED PIPERIDINES, PYRROLIDINES AND INDOLIZIDINES: IMPLICATIONS FOR THE MECHANISM OF ACTION OF SOME GLYCOSIDASES

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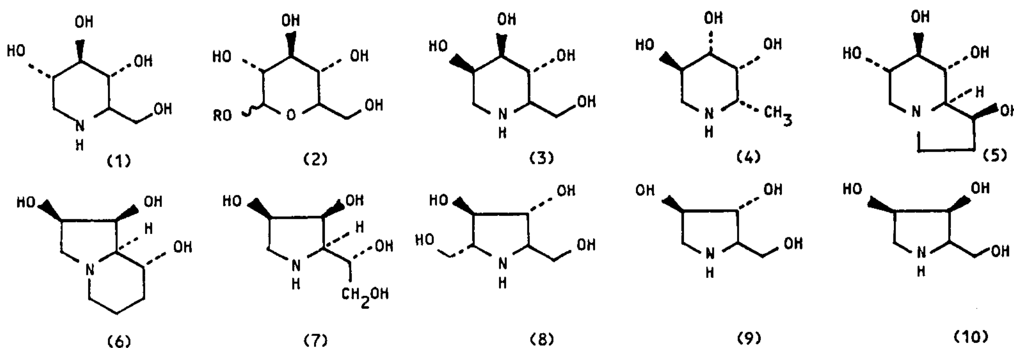
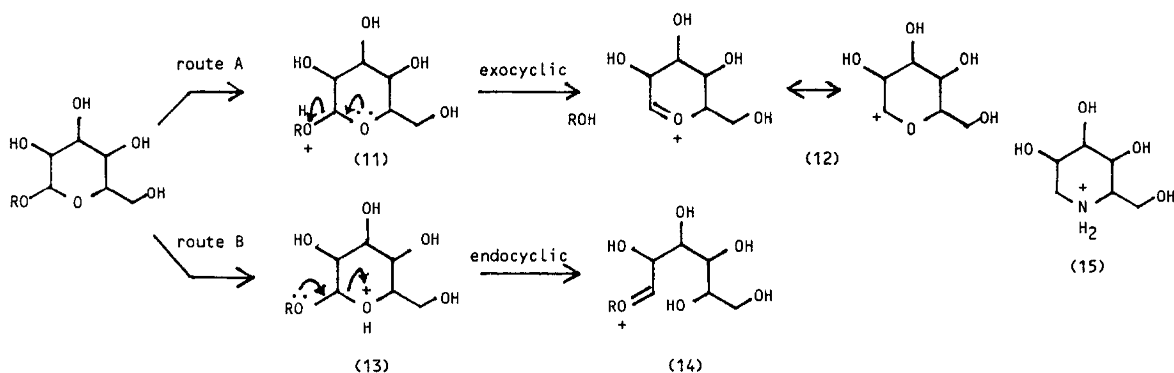
It is proposed that polyhydroxylated alkaloids inhibit glycosidase activity by a mechanism which suggests that the enzyme-catalysed hydrolysis of some glycosides may proceed by cleavage of the endocyclic (ring) carbon-oxygen bond, rather than the exocyclic (anomeric) carbon-oxygen bond.

Polyhydroxylated piperidines and pyrrolidines have recently been shown to be a powerful set of inhibitors of glycosidase activity.[1] Thus deoxynojirimycin (1), related to glucosides (2) by substitution of the pyranose oxygen with the amino function and deoxygenation of the anomeric position, inhibits the activity of several glucosidases;[2] deoxymannojirimycin (3), related to mannopyranosides in the same way as (1) is related to (2), inhibits several mannosidases.[3] 1,5-Dideoxy-1,5-imino-L-fucitol (4), the corresponding analogue of L-fucosides, is an exceptionally potent and specific inhibitor of several α-L-fucosidases; for example, 50% of the bovine epididymis α-L-fucosidase catalysed hydrolysis of p-nitrophenyl α-L-fucopyranoside [$K_M \sim 10^{-3}$M] is inhibited at 2.5×10^{-8}M of (4) [K_I 4.8×10^{-9}M].[4] Also the indolizidine alkaloid, castanospermine (5), in which the piperidine ring is structurally related to glucopyranosides (2), inhibits various glucosidases.[5] In contrast, the indolizidine alkaloid swainsonine, in which the pyrrolidine



ring is the analogue of a mannofuranoside, is a potent inhibitor of several enzyme catalysed mannosidase[6] hydrolyses of p-nitrophenyl mannopyranosides. Also, the synthetic pyrrolidine (7) is a powerful inhibitor of several mannosidases as is swainsonine,[7,8] showing that the azafuranose analogue of mannose is the important feature in this inhibition of enzyme catalysed hydrolysis of mannopyranoside substrates. It is not easy to predict which glycosidases will be inhibited by hydroxylated pyrrolidines; thus, the azafructofuranose analogue (8)[9] and 1,4-dideoxy-1,4-imino-D-arabinitol (9) are both potent inhibitors of some glucosidases, whereas the iminolyxitol (10) is a powerful inhibitor of galactosidase activity.[10]

In studies on the inhibition of glycosidase activity by this group of compounds, it has been found generally that the nature of the inhibition is competitive and reversible. It is also the case that, where the effect of changing the pH on the glycosidase inhibition by these compounds has been studied, it has invariably been found that increasing the pH up to pH7 leads to a higher percentage of inhibition of enzymic activity;[11] this has been interpreted as showing that the ionization state of the nitrogen is important, and that the unprotonated amines are the effective inhibitors of the hydrolysis. The present view[12] of the mechanism of the inhibition of enzyme catalysed hydrolysis of glycosides by this group of compounds is that the hydrolysis proceeds by the equivalent of acid catalysed cleavage (11) of the exocyclic (anomeric) carbon-oxygen bond (route A) giving a pyranose cation (12), and that the protonated form of the amine inhibitor (15) acts by mimicing this cation (12). Since several of these amines are potent inhibitors of highly specific enzyme catalysed processes, this explanation seems open to criticism. First, the stereochemical arrangements in (12) and (15) are very different; the cation (12) contains two adjacent three coordinate atoms, whereas in the protonated amines (15) the corresponding atoms are both tetrahedral. Secondly the chemical similarity between the equivalent of a protonated carbonyl group (12), with the positive charge delocalised on both carbon and oxygen and the protonated amine (12), in which the positive charge on nitrogen is delocalised over those atoms to which it is bonded,[13] is somewhat tentative. Because of these difficulties, I would therefore like to propose an alternative and apparently unrecognized explanation. That is, were the hydrolysis to take place by protonation of the ring oxygen, (13) simultaneous to or followed by cleavage of the endocyclic (ring) carbon-oxygen bond, to (14) then this group of amines would provide excellent transition state analogues for



enzymic inhibition of the ring opening reaction by the formation of the protonated amines (15) (route B). In the case of cleavage of the exocyclic carbon-oxygen bond (route A), stereoelectronic factors make it quite clear that α-glycosides must hydrolyse via their ground state chair conformation whereas β-glycosides must first assume a boat conformation in order to fulfil the stereoelectronic requirement.[14] In contrast, cleavage of the endocyclic ring carbon-oxygen bond (route B) may take place in a chair conformation for both the α- and β-anomers, since in both anomers an anomeric oxygen lone pair may be suitably oriented to assist in the fragmentation of the ring C-O bond. There is no evidence that the hydrolysis of the glycosides catalysed by any of the enzymes inhibited by these heterocyclic amines generally proceeds by fragmentation of one acetyl bond rather than the other. It may be that whether exocyclic or endocyclic carbon-oxygen fragmentation occurs - or indeed whether there is competition between the two pathways - may depend on the enzyme and/or the substrate.

In designing inhibitors for particular glycosidases, consideration should be given to features affecting endocyclic carbon-oxygen bond fragmentations. It is also apparent that if this class of inhibitor is indeed inhibiting initial ring fragmentation of the glycoside, then other enzyme catalysed reactions of sugar substrates in which the opening of a pyranose or furanose ring is involved may also be inhibited by these compounds; we are currently pursuing this line of investigation.[15]

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