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ASCORBIC ACID-BASED INHIBITORS OF α-AMYLASES

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Abstract: A series of ascorbic acid and isoascorbic acid derivatives has been evaluated as inhibitors of malt, bacterial, fungal, pancreatic and salivary α-amylases using a simple and quick assay procedure. The results demonstrate that the enediol moiety of ascorbic acid is essential for α-amylase inhibition. Acylation of the primary and secondary alcohols, and the absolute configuration of the secondary alcohol, do not affect the potency of inhibition.

Starch is an important source of energy for most living organisms and is utilised in a series of degradation reactions catalysed by a variety of amylolytic enzymes of varying specificity. These include α-amylases, which have attracted considerable attention in recent years.[1] Amylase inhibition is known to induce carbohydrate tolerance, satiety and weight loss, and it also prolongs gastric emptying. α-Amylase inhibitors, therefore, have possible therapeutic potential in the treatment of obesity and non-insulin-dependent diabetes mellitus.[2-4] They are also of technological importance in the food industry when excessive α-amylase activity causes problems during food processing.[5-7]

α-Amylase inhibitors generally fall into one of two categories: naturally occurring proteins, found in many plants, which are thought to act as a defense mechanism, e.g. against insect predators; and substrate analogues, particularly pseudooligosaccharides.[8] Proteinaceous α-amylase inhibitors have been studied extensively and are now characterised in molecular detail providing further targets for synthetic mimics.[9,10]

Our attention was drawn to an obscure report[11] suggesting that ascorbic acid is an inhibitor of α-amylase that appears to have been overlooked in subsequent literature. In this paper, we report the evaluation of a series of readily available ascorbic acid derivatives as inhibitors of α-amylases using a simple and rapid screening procedure based on the Ceralpha method from Megazyme Australia.[12,13] This provides a rapid and convenient method for quantifying α-amylase activity spectrophotometrically, based on liberation of *p*-nitrophenol from a modified oligosaccharide substrate. The assay is absolutely specific for α-amylase and is linear, allowing adaptation of the method to provide detailed kinetic

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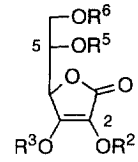
information. The current study was undertaken to provide initial structure-activity information on this simple class of α-amylase inhibitor.

Table 1. Inhibition of malt α-amylase

no.	substituent				inhibition[a]
	R^6	R^5	R^3	R^2	
1a	H	H	H	H	95%
1b	H	H	H	H	96%
	(C5 epimer)				
1c	COMe	COMe	H	H	89%
1d	$CO(CH_2)_{14}Me$	H	H	H	90%
1e	H	H	Me	Me	4%
1f	H	H	Me	H	9%
1g	COMe	COMe	Me	Me	0%
1h	COMe	H	Me	Me	3%
1i	COEt	H	Me	Me	7%
1j	$CO(CH_2)_2Me$	H	Me	Me	10%
1k	H	H	H	H	4%
	(2,3-dihydro)				



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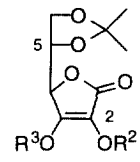

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no.	substituent		inhibition[a]
	R^3	R^2	
2a	H	H	90%
2b	H	H	93%
	(C5 epimer)		
2e	Me	Me	2%
2f	Me	H	29%
2l	COMe	Me	9%

[a] Malt α-amylase was used for the initial screening process since it is inexpensive, readily available and stable at 4 °C. All inhibition values refer to a 5 mM solution of inhibitor and the values are expressed as a % relative to a control without inhibitor.

Two series of ascorbic acid derivatives were assayed for their ability to inhibit α-amylases, one without acetal protection (compounds **1**, Table 1) and the other with an acetal at C5 and C6 (compounds **2**, Table 1). All the compounds were readily prepared from either ascorbic acid or isoascorbic acid using literature-based methods. Ascorbic acid **1a** was conveniently converted into the acetal **2a** on reaction with acetone and acetyl chloride.[14] Compound **2a** was a key intermediate to **2e**, **2f**, **2l** and indirectly, to **1e-1j**.[15] Ascorbic acid was also readily converted into **1c**,[16] **1d**[17] and **1k**[18] and isoascorbic acid **1b** gave **2b**.[19]

All of the most potent inhibitors (compounds **1a-1d**, **2a** and **2b**, Table 1) of malt α-amylase possess a hydrogen at R^2 and R^3. A range of substituents at R^5 and R^6 would appear to be tolerated, including H, acetal, COMe and an extended carbon chain at R^6 (compound **1d**).[20] The configuration at C5 would appear to be unimportant, with the isoascorbic acid examples **1b** and **2b** displaying similar

potency to the corresponding epimers, **1a** and **2a**. The introduction of methyl groups at both R^2 and R^3 results in a marked decrease in potency. This is also true when a single methyl group is introduced at R^3 (c.f. compounds **1f/1a** and **2f/2a**). Reduction of the C2-C3 double bond of **1a**, to give the 2,3-dihydro-derivative **1k**, also resulted in a marked decrease in inhibition. These combined results suggest that the enediol moiety of ascorbic acid is essential for α-amylase inhibition. Finally, a similar pattern of inhibition was displayed against α-amylases from malt, bacterial, fungal, pancreatic and salivary α-amylases (see Tables 1 and 2). Kinetic studies are currently in progress to establish the mode of inhibition of α-amylases by ascorbic acid and its derivatives.

Table 2. Inhibition of α-amylases from other sources.

no.	α-amylase[a]			
	bacterial	fungal	pancreatic	salivary
1b	99%	98%	100%	100%
1c	92%	91%	99%	
1d	84%	70%	88%	69%
2a	98%	94%	99%	88%
2b	93%	91%	99%	98%
2f	8%	17%		0%

[a] All inhibition values refer to a 5 mM solution of inhibitor and the values are expressed as a % relative to a control without inhibitor. All enzymes were purchased from Sigma Chemical Company.

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