

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. © 1998 Elsevier Science Ltd. All rights reserved.

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}

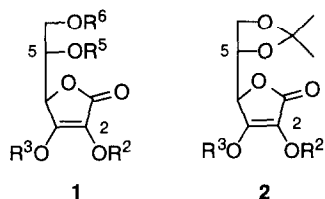
Our attention was drawn to an obscure report¹¹ suggesting that ascorbic acid is an inhibitor of α -amylase that appears to have been overlooked in subsequent literature. In this paper, we report the evaluation of a series of readily available ascorbic acid derivatives as inhibitors of α -amylases using 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

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) ₂ Me	H	Me	Me	10%
1k	H	H	H	H	4%
	(2,3-dihydro)				

no.	substituent		inhibition ^a
	R ³	R ²	
2a	H	H	90%
2b	H	H	93%
	(C5 epimer)		
2c	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 **2c**, **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 R² and R³. A range of substituents at R⁵ and R⁶ would appear to be tolerated, including H, acetal, COMe and an extended carbon chain at R⁶ (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 R² and R³ results in a marked decrease in potency. This is also true when a single methyl group is introduced at R³ (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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20. The acyl substituents have be shown to be stable under the conditions of the assay.