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The inhibition of tyrosinase by pyridinones

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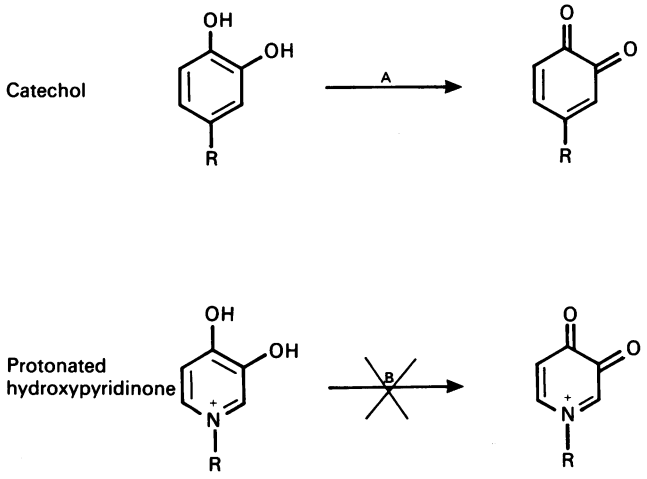
3-Hydroxypyridine-4-ones have potential as orally active chelators of iron(III) and therefore may find application in the treatment of thalassaemia. An undesirable feature of these molecules is that they inhibit tyrosinase. We have established that alkyl substitution at position 2 in the aromatic ring minimizes interaction with tyrosinase and does so without appreciably influencing the affinity for iron(III).

INTRODUCTION

Tyrosinase is a copper-containing mono-oxygenase (EC 1.14.18.1) that catalyses the *ortho*-hydroxylation of monophenols and the oxidation of *o*-diphenols to *o*-quinones (Mason, 1965). The enzyme is involved in the conversion of tyrosine into dopa (3,4-dihydroxyphenylalanine) and in the synthesis of melanin (Lerch, 1981). Tyrosinase interacts with a range of copper-chelating compounds, including aminophenols (Toussaint & Lerch, 1987), aromatic carboxylic acids (Wilcox *et al.*, 1985) and arylamines (Toussaint & Lerch, 1987). Tyrosinase is also inhibited by L-mimosine (Ia), a naturally occurring pyridinone (Hashiguchi & Takahashi, 1977), and as this compound is a powerful chelator of copper(II) (Stünzi *et al.*, 1979, 1980) it probably also binds to the copper atoms situated at the active site of tyrosinase. However, in contrast with catechols, the corresponding quinone (Scheme 1) is unstable, and therefore when mimosine binds to the active-site copper ion it is not transformed into a quinone and consequently remains bound.

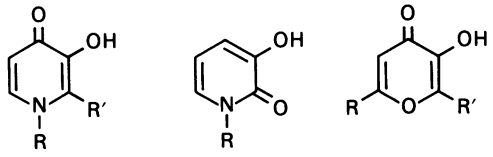
Mimosine is a 3-hydroxypyridin-4-one derivative and



Scheme 1. Tyrosinase-catalysed catechol oxidations

Catechols are converted into *o*-quinones by tyrosinase (route A), but in contrast hydroxypyridinones are not converted into quinone-containing structures (route B).

Table 1. Structures of the chelating inhibitory molecules: (I) 3-hydroxypyridine-4-ones, (II) 3-hydroxypyridine-2-ones and (III) 3-hydroxypyran-4-ones



(I) (II) (III)

(Ia)	$R = CH_2CH(NH_2)CO_2H$,	$R' = H$	(IIa)	$R = H$	
(Ib)	$R = CH_2CH_2NH_2$,	$R' = H$	(IIb)	$R = CH_3$	
(Ic)	$R = CH_2CH_3$,	$R' = H$	(IIIa)	$R = H$,	$R' = CH_3$
(Id)	$R = CH_3$,	$R' = CH_3$	(IIIb)	$R = CH_2OH$,	$R' = H$
(Ie)	$R = CH_2CH_3$,	$R' = CH_3$			
(If)	$R = CH_2CH_2CH_3$,	$R' = CH_3$			
(Ig)	$R = CH_2CH_2CO_2H$,	$R' = CH_3$			
(Ih)	$R = CH_2CH_2OH$,	$R' = CH_3$			
(Ii)	$R = CH_2CH_2NH_2$	$R' = CH_3$			
(Ij)	$R = CH_2CH_2OCH_3$,	$R' = CH_3$			
(Ik)	$R = CH_3$	$R' = CH_2CH_3$			

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Table 2. Inhibition of tyrosinase by iron chelators

Kinetic constants were obtained from Dixon (1953) plots. Assays were carried out with 3 ml of air-saturated L-dopa as substrate (4 mM) dissolved in 0.1 M-potassium phosphate buffer, pH 6.0. The rate of product formation was monitored spectrophotometrically at 30 °C at 475 nm (Lerch, 1987).

Substrate	K_i (mM)	Substrate	K_i (mM)
3-Hydroxypyridin-4-ones		3-Hydroxypyridin-2-ones	
(Ia)	0.045	(IIa)	0.022
(Ib)	0.014	(IIb)	1.5
(Ic)	0.004		
(Id)	5.6		
(Ie)	2.2	3-Hydroxypyran-4-ones	
(If)	2.6		
(Ig)	7.6	(IIIa)	17.0
(Ih)	3.2	(IIIb)	0.008
(Ii)	0.8		
(Ij)	5.6		
(Ik)	6.2		

is therefore closely related to a group of pyridinones that has been developed as orally active iron chelators (Hider *et al.*, 1982; Gyparaki *et al.*, 1987; Porter *et al.*, 1988). High daily doses of pyridinone will be required for the treatment of patients suffering from iron overload, and consequently it is important to establish whether the selected ion chelators inhibit tyrosinase. Three classes of molecule have been tested, namely 3-hydroxypyridin-4-ones (I), 3-hydroxypyridin-2-ones (II) and 3-hydroxypyran-4-ones (III) (Table 1), with *Neurospora crassa* tyrosinase (Lerch, 1987).

EXPERIMENTAL AND RESULTS

All the pyridine-4-ones tested were found to inhibit tyrosinase, but with a wide range of K_i values (Table 2). Significantly the ligands lacking an alkyl substituent on the aromatic ring [(Ia), (Ib) and (Ic)] are highly potent inhibitors. In contrast, those ligands possessing a methyl or ethyl substituent *ortho* to the chelating oxygen atoms [(Id)–(Ik)] were much weaker inhibitors (at least 100-fold decrease in K_i value). Similar findings are obtained with the pyridine-2-ones, where the presence of the methyl function has a dramatic influence on the K_i value (Table 2). Again pyranones show the same trend, maltol (IIIa) being a weak inhibitor, whereas kojic acid (IIIb) is quite potent (Table 2). Maltol possesses a methyl function *ortho* with respect to the chelating oxygen atoms whereas kojic acid does not.

The presence of *o*-alkyl functions does not influence the affinity constant for the interaction between pyridinones and either iron(III) or copper(II) (P. D. Taylor & R. C. Hider, unpublished work). Therefore the presence of these groups must create steric congestion, whereby the ligand is unable to approach the copper atom at the active site of tyrosinase in a bidentate fashion. A similar effect of steric hindrance has been observed previously for different *ortho*-substituted benzoic acids (Wilcox *et al.*, 1985).

DISCUSSION

This simple observation is of critical importance for clinical chelator design. The maximum blood concentration after a normal oral dose is unlikely to exceed 100 μM, a concentration that is at least 20 times lower than the K_i value for alkyl-substituted pyridinones. We consider it unlikely that the pyridinones (Id), (Ie), (Ij) and (Ik) will generate toxic side effects by virtue of tyrosinase inhibition.

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