Reasearch in Pharmaceutical Sciences 1 (2006) 40-48
Received: Aug 2005
Accepted: Feb 2006

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences and Health Services

Original Article

Synthesis, analysis and determination of partition coefficients of N-arylhydroxypyridinone derivatives as iron chelators

L. Saghaie[1]*, M. Mirmohammad Sadeghi[2] and A. Nikazma[1]

[1]*Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R.Iran.*
[2]*Department of Chemistry, Faculty of Sciences, Isfahan University, Isfahan, I.R.Iran.*

Abstract

Iron overload is a serious clinical condition which can be largely prevented by the use of iron-specific chelating agents. In this study, the synthesis and determination of partition coefficients (K_{part}) of a range of N-alkyl hydroxypyridinones, as orally active iron chelators, are described. Synthesis of N-aryl hydroxypyridinones was achieved via a single step synthetic pathway. In this method, maltol (2-methyl-3-hydroxypyra-4-one) was reacted with an excess of suitable aryl primary amines under reflux condition in dilute hydrochloric acid at pH about 5. The progress of reactions was monitored by TLC. The reaction mixture was adjusted to pH 7 using sodium hydroxide and the product was collected by filtration. Purification was achieved by re-crystallization from hot methanol. In this work, 1-phenyl-2-methyl-3-hydroxypyridin-4-one, 1- (3-chlorophenyl) -2-methyl-3-hydroxypyridin-4-one, 1- (3-hydroxyphenyl) -2-methyl-3-hydroxypyridin-4-one, 1- (3-carboxyphenyl) -2-methyl-3-hydroxy-pyridin-4-one and 1-(4-carboxyphenyl)-2-methyl-3-hydroxypyridin-4-one were synthesized. Identification and structural elucidation of compounds were achieved by ^{1}HNMR, IR, elemental analysis, mass spectra and through physical constants. K_{part} values of the compounds were also determined in an aqueous/octanol system using an automated continuous flow method (a filter probe method).

١

Key words: Hydroxypyridinones, Iron chelator, Iron overload, Partition coefficient

INTRODUCTION

There are a number of inherited diseases which are associated with the gradual accumulation of iron, from them β-thalassaemia and thalassaemia intermedia being particularly well characterized (1). In some regions of the world, the genes are relatively common; in South East Asia, for instance, approximately 100000 children are born each year suffering from thalassaemia. In adults, the normal total body iron ranges between 4g and 5g, whereas some thalassaemic patients may accumulate 50-70 g of iron over a 10-year period. Iron, by virtue of its facile redox chemistry, is toxic when present in excess (2). Desferrioxamine (DFO, Figure 1) a natural siderophore, has been used for the treatment of iron overload for over 30 years (3), and currently it is the only clinically useful drug available for this purpose. However, DFO suffers from one disadvantage i.e. it is inactive when administered orally, and only causes sufficient iron excretion to keep pace with the transfusion regime when given either subcutaneously or intravenously over 12-18h several times per week. For this reason, many patients find it difficult to comply with the treatment, and some even stop taking the drug all together, subsequently developing the complications of iron overload. There is, therefore, no

***Corresponding author: Dr. L. Saghaie
Tel. 0098 311 7922644, Fax. 0098 311 6680011
E-mail: lotfollahs@yahoo.com

doubt that an orally active chelating agent is needed to treat patients on lifelong transfusion programmers. The development of an oral iron chelator might also allow the extension of the therapeutic use of red-cell transfusions in sickle-cell anaemia (3).

3-Hydroxypyridin-4-ones (HPOs) are currently one of the main candidates for the development of orally active iron chelators which can be alternatives to DFO (4). This class of ligands is highly selective for iron (III) and has relatively low complex formation constant for copper, zinc, calcium and magnesium (Table 1). The speciation plot for 3-hydroxypyridine-4-one with iron demonstrates that over the physiological pH range 5.0-9.9, the dominant species is the uncharged 3:1 complex (5). To date, the bidentate hydroxypyridine-4-ones, such as 1,2-dimethyl-3-hydroxy-pyridin-4-one (Deferiprone or L1, Figures 2 and 4, marketed by Apotex Inc. Toronto, Canada, as Ferripox^{TM}) has been extensively studied in thalassaemia patients (6, 7). Unfortunately, the dose required to keep a previously well-chelated patient in negative iron balance with Ferripox^{TM} is relatively high, in the region of 75 to 100 mg/kg/day (8). Side effects have also been observed in some patients receiving L1 (9, 10). One of the major reasons for the limited efficacy of L1 in clinical use is that it undergoes extensive phase II metabolism in the liver as shown in figure 2 (11). The 3-hydroxyl functionality, which is crucial for scavenging iron, is a prime target for glucuronidation. Urinary recovery studies conducted on L1 in both rats and human have shown that respectively >44% and >85% of the administered dose is recovered in the urine as the non-chelating 3-O-glucuronide conjugate (11). Despite these limitations, the ability of these compounds to relieve iron overload in human has made it clear that this class of the chelator has considerable potential as orally active iron chelators (12, 13). In addition to the potential treatment of iron overload in thalassaemic patients,

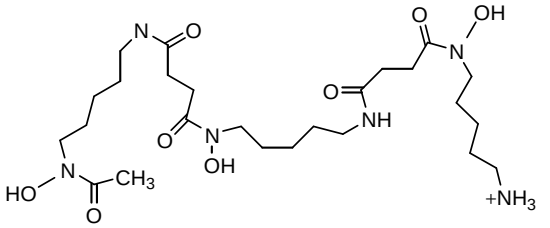


Figure 1. Structure of desferrioxamine (DFO)

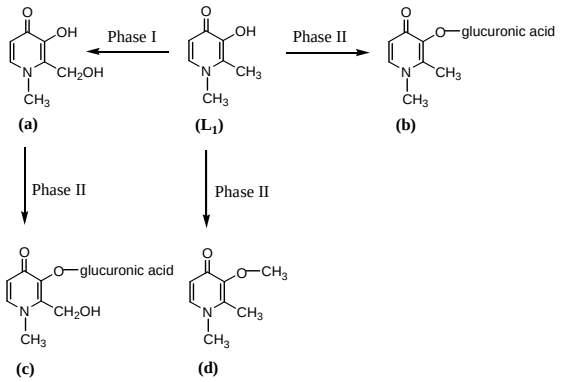


Figure 2. Metabolism of compound L_1 in man/rat; (b) is major metabolite in both species.

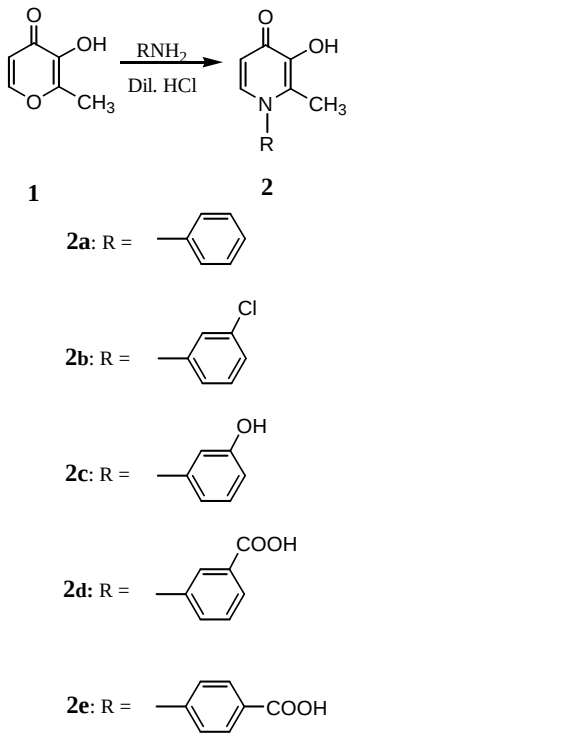


Figure 3. Synthesis of N-aryl-3-hydroxypyridin-4-ones *via* the single step synthetic pathway

hydroxypyridin-4-ones may well find other clinical applications centered on iron removal. The hydroxypyridones are being investigated for the treatment of malaria (14) and aluminium removal, especially aluminium mobilization in renal dialysis patients (15**,** 16).

In order to investigate further ligands which able to scavenge iron effectively at low concentrations, it was decided to synthesize other derivatives of this type of compounds namely N-aryl-3-hydroxy-pyridin-4-one derivatives (5a-e). In this study, synthesis of these compounds and their partition coefficients (K_{part}) were discussed.

The compounds' partition coefficients (K_{part}) were determined in an aqueous/ octanol system using an automated continuous flow method (a filter probe method) (17).

MATERIALS AND METHODS

All chemicals used in this project were obtained from Aldrich (Gillingham, UK). Melting points were determined using an Electrothermal IA 9100 Digital melting point. IR spectra were recorded on a Perkin-Elmer 1420. Proton NMR spectra were determined by EM-390 (80 MHz). Mass spectra were taken using a Vacuum Generaters 16F (35eV). Elemental analyses (Leco CHNCl-932) were performed by micro analytical laboratories at University of Manchester (Manchester, UK).

Synthesis of 1-phenyl-2-methyl-3-hydroxy-pyridin-4-one (2a).

Maltol 1 (6.31 g, 0.05 mole) was added to a solution of aniline (5.00 ml, 0.05 mole) in 98 ml water, 2 ml HCl 37% and 10 ml ethanol. The mixture was refluxed for 60 h. Then the reaction mixture was adjusted to pH 7 using 2 N sodium hydroxide. The precipitated product was collected by filtration and re-crystallized from methanol to afford a white crystalline solid, 1.24 g (22%).

Synthesis of 1- (3-chlorophenyl) -2-methyl-3-hydroxypyridin-4-one (2b).

Maltol 1 (1.26 g, 0.051 mole) and 3-chloroaniline (1.40 g, 0.01 mole) were reacted as described for 2a to give 2b as a white crystalline solid, 0.27 g (11%).

Synthesis of 1- (3-hydroxyphenyl) -2-methyl-3-hydroxypyridin-4-one (2c).

Maltol 1 (6.31 g, 0.05 mole) and 3-aminophenol (6.0 g, 0.05 mole) were reacted as described for 2a to afford 2c as white solid crystals, 1.61 g (14.5%).

Synthesis of 1- (3-carboxyphenyl) -2-methyl-3-hydroxypyridin-4-one (2d).

Maltol 1 (6.31 g, 0.05 mole) and 3-amino-benzoic acid (7.54 g, 0.05 mole) were reacted as described for 2a to give 2d as a white crystalline solid, 5.34 g (43%).

Synthesis of 1- (4-carboxyphenyl) -2-methyl-3-hydroxypyridin-4-one (2e).

Maltol 1 (6.31 g, 0.05 mole) and 4-amino-benzoic acid (7.54 g, 0.05 mole) were reacted as described for 2a to obtain 2c as white solid crystals, 2.49 g (20%).

Determination of partition coefficients by the use of filter probe method.

Partition coefficients (K_{part}) of the ligands used in present study were determined using the automated continuous flow method (filter probe method) as previously described (17). The system was comprised of an IBM compatible PC running the "TOPCAT" program (18) which controlled both Metrohm 665 Dosimat autoburette and a Pye-Unicam Lambda 5 UV/Vis spectro-photometer, as well as performing all calculations of partition coefficients. All K_{part} values were performed using AnalaR grade reagents under nitrogen atmosphere in a sealed titration vessel (250 ml) at a laboratory constant temperature (25 ± 0.5°C). The two phases used, were MOPS [3-(N-morpholino) propane sulphonic

acid] buffer (50 mM, pH 7.4, prepared by the use of distilled water) and n-octanol, each of which was pre-equilibrated with the other phase before use due to the limited solubility of water in n-octanol (2.3 M) (19). The buffer (100 μL) was circulated through a spec-trophotometric flow-cell, which was returned to the mixing chamber with the aid of a peristaltic pump at a flow rate of 1 ml/min.

The filter probe consisted of a polytetrafluoroethylene (PTFE) plunger associated with a gel-filtration column. The aqueous phase was separated from two-phase system (n-octanol/MOPS) by means of a hydrophilic cellulose filter (5-diameter, 589/3 blauband filter paper, Schleicher and Schuell) mounted in the gel-filtration column adjuster SR 25/50, Pharmacia). A known volume (normally 20-100 ml) of MOPS buffer (saturated with n-octanol) was taken in the flat-based glass-mixing chamber. A base-line absorption value of the solution was used as a reference absorbance. A 10^{-4} M solution of the ligand was prepared in the aqueous phase (typically 40 ml) to give an absorbance between 1.5-2.0 at the preselected wavelength (~ 280 nm). Upon commencement of the computer program, absorbance measurements were automatically recorded at preselected time intervals, usually 1 second. When the absorbance readings were stabilized, as determined by the computer from equilibrium conditions selected by the operator (typically a constant absorbance is where the absorbance changes by less than 0.002 units over a minimum of 10 minutes), a suitable volume of n-octanol was added to the aqueous phase from the automatic dispenser. Absorbance readings were subsequently recorded until the system reached to the equilibrium again, at which point a further aliquot of n-octanol was added. This cycle was repeated for at least five additions of n-octanol. At each stage of the addition n-octanol, the corresponding partition coefficient were calculated using the following equation:

$$K_{part} = \frac{A_0 - A_1}{A_1} \times \frac{V_w}{V_0} \qquad \text{(Eq. 1)}$$

Where

A_0 = Initial absorbance

A_1 = Absorbance at equilibrium after addition of n-octanol

V_w = Volume of MOPS buffer

V_0 = Total volume of n-octanol added to glass vessel

Finally, a mean partition coefficient value and standard deviation were calculated

RESULTS

In this work compounds 1-phenyl-2-methyl-3-hydroxypyridin-4-one, 1- (3-chlorophenyl) -2-methyl-3-hydroxypyridin-4-one, 1- (3-hydroxyphenyl) -2-methyl-3-hydroxypyridin-4-one, 1- (3-carboxyphenyl) -2-methyl-3-hydroxypyridin-4-one and 1-(4-carboxyphenyl)-2-methyl-3-hydroxypyridin-4-one were synthesized via a single step synthetic pathway. Preparation of these ligands are illustrated in Figure 3. Maltol 1 was reacted with different primary aryl amines in diluted HCl to give the corresponding 3-hydroxypyridin-4-ones 2a -e in 11-43% yields.

Identification and structural elucidation of compounds were achieved by ^{1}HNMR, IR, elemental analysis, mass spectra and through physical constants:

Compound 2a- ^{1}H NMR (DMSO-d_6): δ 2.0 (s, 3H, 2-CH_3), 5.7 (bs, H, 3-OH), 6.2 (d, 1H, 5-H (pyridinone ring), 7.3 -7.7 (m, 6H, phenyl ring & 6-H (pyridinone ring)).

MS (EI): m/z = 201 (M^+), 200 (M-H), 184 (M-OH), 124 (M-C_6H_5).

IR (KBr): 3200 (OH), 1630 (C=0), 1580 (C=C), 1300 (C-N) cm^{-1}

Anal. Calcd for $C_{12}H_{11}NO_2$: C, 71.63; H, 5.51; N, 6.69%. Found: C, 71.78; H, 5.45; N, 6.88 %. mp 221-222 ^oC.

Compound 2b-^{1}H NMR (DMSO-d_6): δ 2.0 (s, 3H, 2-CH_3), 4.0 (bs, 1H, 3-OH), 6.2 (d, 1H, 5-H (pyridinone ring)), 7.3 -7.7 (m, 5H, phenyl ring & 6-H (pyridinone ring)).

MS (EI): m/z = 236 (M^+), 235 (M-H), 200 (M-Cl), 168 (M-Cl, OH, CH_3), 78 (M-OH, CH3, C_6H_4Cl).
IR (KBr): 3100 (OH), 1630 (C=0), 1580 (C=C), 1300 (C-N) cm^{-1}
Anal. Calcd for $C_{12}H_{10}NO_2Cl$: C, 61.16; H, 4.28; N, 5.95; Cl,15.04%. Found: C, 61.36; H, 4.35; N, 5.87 Cl, 14.95%. mp 184-185 ^oC.
Compound 2c-^{1}H NMR (DMSO-d_6): δ 2.0 (s, 3H, 2-CH_3), 4.5 (bs, H, 3-OH), 6.3 (d, 1H, 5-H (pyridinone ring)), 7.4 –8.0 (m, 5H, phenyl ring & 6-H (pyridinone ring)).
MS (EI): m/z = 217 (M^+), 216 (M-H), 200 (M-OH).
IR (KBr): 3100 (OH), 1630 (C=0), 1600 (C=C), 1300 (C-N) cm^{-1}
Anal. Calcd for $C_{12}H_{11}NO_3$: C, 66.35; H,5.10; N,6.45%. Found: C, 66.50; H, 5.04; N, 6.37 %. mp 268-269 ^oC
Compound 2d-^{1}H NMR (DMSO-d_6): δ 2.0 (s, 3H, 2-CH_3), 5.8 (bs, H, 3-OH), 6.3 (d, 1H, 5-H (pyridinone ring)), 7.6 –8.3 (m, 5H, phenyl ring & 6-H (pyridinone ring)).
MS (EI): m/z = 245 (M-HCl), 244 (M-H, HCl), 200 (M-COOH, HCl).
IR (KBr): 3100 (OH), 1690 (C=0), 1580 (C=C), 1300 (C-N) cm^{-1}
Anal. Calcd for $C_{13}H_{11}NO_4$. HCl: C, 55.43; H, 4.29; N, 4.97; Cl, 12.58%. Found: C, 55.31; H, 4.23; N, 4.91 Cl, 12.69%. mp 264-265 ^oC.
Compound 2e-^{1}H NMR (DMSO-d_6): δ 2.0 (s, 3H, 2-CH_3), 4.5 (bs, H, 3-OH), 6.3 (d, 1H, 5-H (pyridinone ring)), 7.6 (d, 3H, 6-H (pyridinone ring) & 2'-H and 6'-H (phenyl ring)), 7.6 (d, 2H, 3'-H and 5'-H (phenyl ring)),
MS (EI): m/z = 245 (M-HCl), 244 (M-H, HCl), 200 (M-COOH, HCl).
IR (KBr): 3100 (OH), 1630 (C=0), 1580 (C=C), 1300 (C-N) cm^{-1}
Anal. Calcd for $C_{13}H_{11}NO_4$. HCl: C, 55.43; H, 4.29; N, 4.97; Cl, 12.58%. Found: C, 55.30; H, 3.33; N, 5.05 Cl, 12.51%. mp 310-311 ^oC.
The K_{part} values of the compounds were also determined in an aqueous/octanol system using an automated continuous flow method (a filter probe method) (17). Table 2 shows the K_{part} values of bidentate ligands, 2a-e, and the partition coefficients of their corresponding Fe-complexes (K_{part} Fe-complex).
The ligands covered a range of K_{part} values of 0.43-10.20. The charged molecules at pH 7.4 (2d and 2e) show low K_{part} values. Among the neutral ligands reported in this study, the compound 2a possesses the highest partition coefficients (K_{part} = 10.20, Table 2).

DISCUSSION

The general methodology (methodology of Harris) (20) which has been adopted for the synthesis of N-alkyl-3-hydroxypyridin-4-ones, is summarized in Figure 4. The commercially available maltol 1 was benzylated to give 3. Reaction of 3 with alkylamines (or ammonia), gives the benzylated pyridinones 4, which is subsequently subjected to catalytic hydrogenation to remove the protecting group, yielding the corresponding bidentate N-alkyl-3-hydroxypyridin-4-ones 5 .

Table 1- Logarithms of overall stability constants for DFO and HPOs with selected metal ions.

Metal ion	DFO	HPOs
Fe (III)	31	37
Cu (II)	14	17
Zn (II)	11	12.5
Mg (II)	4	7
Ca (II)	2.3	4.5

Table 2. The partition coefficient values (K_{part}) of ligands (**5a-e**) and their corresponding iron (III) complexes between n-octanol and MOPS buffer at pH 7.4. Number of determinations = 6.

Ligand	K_{part} of Ligand	K_{part} of Fe-Complex
5a	10.2 ± 0.20	53.85
5b	5.6 ± 0.10	12.32
5c	3.5 ± 0.06	3.88
5d	0.52 ± 0.02	0.036
5e	0.43 ± 0.01	0.022

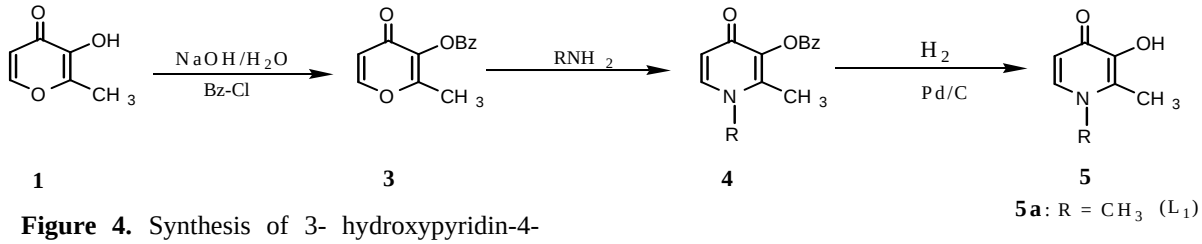
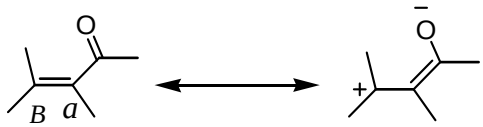


Figure 4. Synthesis of 3- hydroxypyridin-4-ones by utilizing of the methodology of Harris and co-worker.

Figure 5. Mesomerism of an α, β-unsaturated carbonyl moiety.



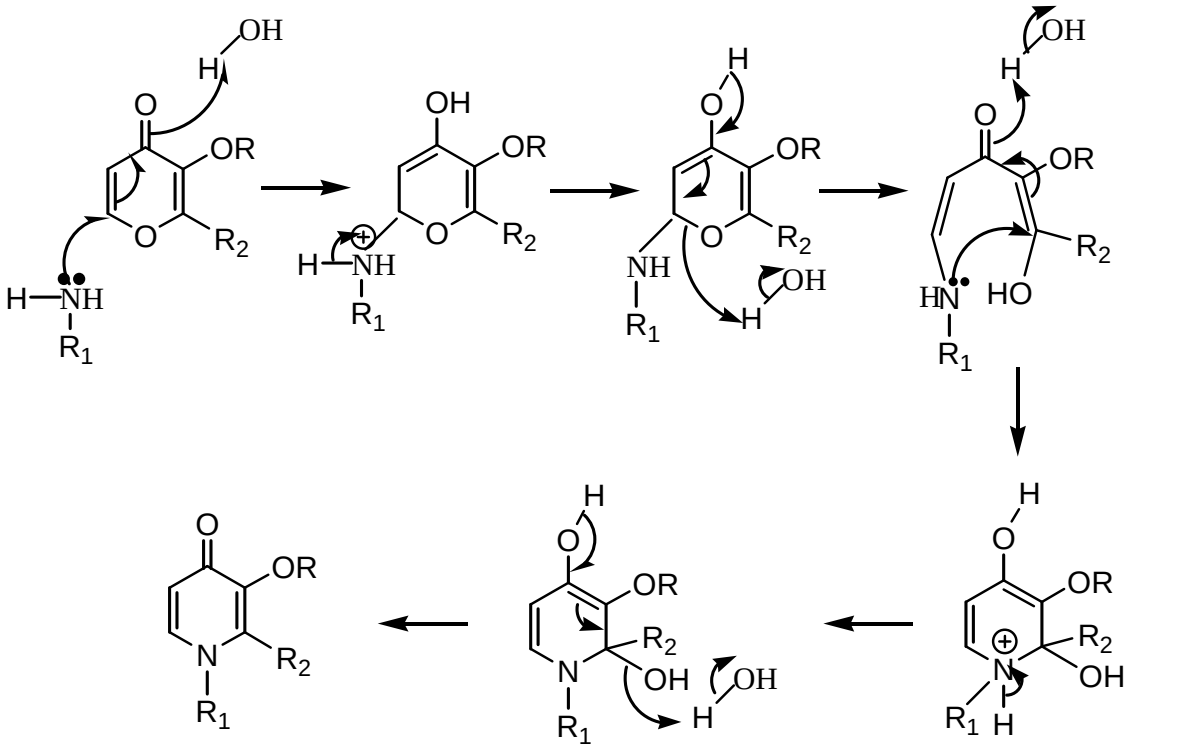


Figure 6. Formation of a N-substituted-3- hydroxypyridin-4-one from reaction of corresponding 4(1*H*)-pyranone with primary amine by double Michael-type addition.

Mechanism of pyridinone formation**.** Mesomerisation of α,β-unsaturated carbonyl compound causes the β-carbon to be electron deficient and therefore prone to nucleophilic attack (Figure 5). When the nucleophile is a carbanion, the reaction is termed Michael addition whereas for any other species, it is known as Michael-type addition. In fact a 3-hydroxypyran-4-one (maltol) (1) consists of two α, β-unsaturated carbonyl components and as such is prone to these additions at both the 2- and 6- positions. When the nucleophile is a primary amine double attack at both

α,β-unsaturated functions of the 3-hydroxypyran-4-one leads to formation of 3-hydroxypyridin-4-one with the loss of a water molecule (Figure 6) (21).

Protection of the 3-hydroxy substituent. For bulky amines, it is necessary to protect the hydroxyl function of the pyran-4-one because the hydroxyl function interferes with the reaction. Under the basic conditions employed in the amination reaction it is likely that the unprotected hydroxyl group could undergo a Michael-type reaction with intermediates formed during the amination step (Figure 7). Further condensation products lead to significant consumption of starting material and therefore influence the overall yield. In general the bulkier the amine, the lower the yield (21). The protection of the 3-hydroxyl group of HPOs avoids this difficulty and therefore leads to the desired product in acceptable yield. Conversion of 1 with small primary alkyl amines to the corresponding pyridinones can be achieved without protection of the 3-hydroxyl group (22). However, this one step reaction is limited to primary mines of short length (with yields of less than 40%), longer or branched-chain amines give yields of less than 10% (23). This investigation, prompted us to attempt a direct one-step preparation. Reaction of maltol 1 (instead of benzyl maltol) with aniline (the primary aromatic amine) in water and reflux condition gave no product (only oily polymeric compounds were obtained). Reaction of 1 with aniline in dilute hydrochloric acid (98 ml water and 2 ml conc. HCl) then was attempted (Figure 3). A reaction period of sixty hours at reflux resulted in 1-phenyl-2-methyl-3-hydroxy-pyridin-4-one (Figure 3, 2a). Fortunately, the product was not soluble in the methanol and was separated from side products and starting materials relatively easily. Further purification was achieved by recrystallization from hot methanol (in 22% yield). The reaction of 1 in dilute hydrochloric acid then was extended to 3-

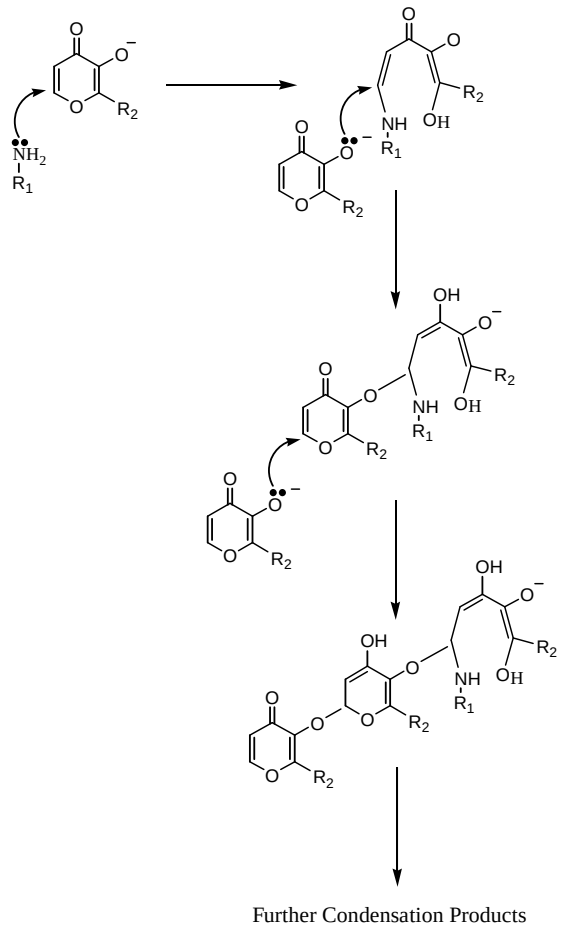
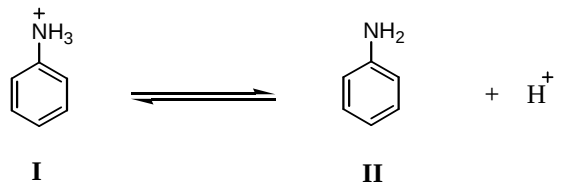


Figure 7. A possible condensation product in the synthesis of bidentate pyridin-4-ones.



I **II**

Figure 8. The equilibrium between protonated (**I**) and un-protonated (**II**) forms of aniline.

chloroaniline, 3-aminophenol, 3-amino-benzoic acid and 4-aminobenzoic acid to give the N- aryl-3-hydroxypyridin-4-ones 2b, 2c, 2d and 2e respectively, and as expected, the yields were poor (Figure 3).

Under the acidic conditions employed in the amination reaction, it is unlikely that the unprotected hydroxyl function could undergo a Michael-type reaction with intermediates formed during the amination step. It should be noted that, in acidic

condition, aryl amines, unlike alkyl amines, are not completely protonated (Form I) and a small fraction of amine is as an un-protonated (Form II) specious (Figure 8). The following equilibrium between two forms of I and II may occur under the reaction conditions employed in the syntheses described (Figure 3).

The nitrogen atom of this specious (Form II) would be sufficiently nucleophilic to explain attack at $C_{(6)}$ [or $C_{(2)}$] of the maltol 1. Although, the yields for synthesis of N-aryl-3-hydroxypyridin-4-ones *via* the single step synthetic pathway are lower than the "Harris method" this method is much easier.

The K_{part} values of ligands: In addition to determination of the K_{part} values of bidentate ligands (by using the filter probe method) (17), the partition coefficients of their corresponding Fe-complexes (K_{part} Fe-complex) can be also calculated from equation 2 (21) (Table 2).

$\log K_{part}$ Fe-complex = -2.46 $\log K_{part}$ Ligand- 0.75 (Eq. 2).

As expected, the introduction of an hydrophilic functional group such as hydroxyl, halide and carboxylic groups at the 3 or 4-position of N-substituted phenyl group resulted in an decrease in the K_{part} value of ligands in comparison to the un-substituted counterpart (compound 2a). This trend also holds for their iron (III) complexes. For those compounds which have K_{part} values greater than 3 (Figure 3, 2a and 2b), iron (III) complexes are more hydrophobic than their corresponding free ligands.

However, this trend did not hold for charged chelators which possess K_{part} values less than 3 (Figure 3, 2d and 2e). In this case the iron (III) complexes were found to be more hydrophilic than their corresponding free ligands. The K_{part} values of both complex and ligand were close for only one ligand, namely 2c ($K_{part} \approx 3.5$).

ACKNOWLEDGEMENT

The authors wish to thank Prof. Hider, Department of Pharmacy, King's College London for providing necessary facilities in connection with determination of K_{part} values of iron chelating agents.

REFERENCES

1. Weatherall DJ and Clegg JB. The thalassaemia syndromes. 3rd ed. Oxford: Blackwell scientific publications; 1981.
2. Halliwell B and Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J. 1986;219:1-14.
3. Hershko C, Konijn AM and Link G. Iron chelators for thalassaemia. Br J Haematol. 1998;101:399-406.
4. Tilbrook GS and Hider RC. Iron chelators for clinical use. In: Sigel A, Sigel H, editors. Metal Ions in Biological Systems. Vol. 35: Iron transport and storage in micro organisms, plants and animals. New York: Marcel Dekker; 1998.p. 691-697.
5. Dobbin PC, Hider RC. Iron chelation therapy. Chem in Britain. 1990; 565-568.
6. Hoffbrand AV. Deferiprone therapy for trans-fusional iron overload. Best Pract Res Clin Haematol. 2005;18:299-317.
7. Taher A, Aoun E, Sharara AI, Mourad F, Gharzuddine E, Koussa S,et al. Five-year trial of deferiprone chelation therapy in thalassaemia major patients. Acta Haematol. 2004;112:179-183.
8. Balfour JAB, Foster RH. Deferiprone-a review of its clinical potential in iron overload in beta-thalassaemia major and other transfusion-dependent diseases. Drugs 1999;58:553-578.
9. Liu ZD, Hider RC. The development of iron chelators for clinical use. Med Res Rev. 2003; 22:26-64.
10. Hider RC, Liu ZD, Khodr HH. Iron chelating agents in medicine. Curr Med Chem. 2004; 10:1051-1064.
11. Singh S, Epemolu Ro, Hider RC. Urinary metabolic profiles in man and rat of 1,2-dimethyl and 1,2-diethyl pyridinones. Drug Metab Dispos. 1992;20:25-30.
12. Tricta F, Spino M. Iron chelation with oral deferiprone in patients with thalassaemia. New Engl J Med. 1998;339:1710-1715.

13. Taher M, Saghaie L, Abrahimi M. Investigation of intestinal absorption of pyridinones in rat. Ir Pharm Res. 2004;4:201-207.
14. Hider RC, Liu ZD. The treatment of malaria with iron chelators. J Pharm Pharmacol. 1997; 49:59-64.
15. Blanusa M, Prester L, Varnai VM, Paylovic D, Kostial K, Jones MM, Singh PK. Chelation of aluminium by combining DFO and L1 in rats. Toxicology. 2000;147:151-156.
16. Kotoghiorghes GJ, Barr J, Baillord RA. Studies of aluminium mobilization in renal dialysis patients using the oral chelator 1,2-dimethyl-3-hydroxy-pyrid-4-one. Arzneimittelforschung. 1994;44:522-526.
17. Saghaie L, Hider RC and Mostafavi S. Comparison automated continuous flow method with shake-flask method in determining of partition coefficients of bidentate hydroxy-pyridinone ligands. Daru. 2003;11:38-48.
18. Hall AD. TOPCAT Program for determination of partition coefficients. Department of Pharmacy, King's College London, University of London, London, UK. 1990.
19. Khodr H. A modified version of TOPCAT program (KDHK94 program) for determination of partition coefficients, Department of Pharmacy, King's College London, University of London, London, UK. 1994.
20. Wolfenden R, Radzicka A. On the probability of finding a water molecule in a nonpolar cavity. Science. 1994;265:936-937.
21. Harris RLN. Potential wood growth inhibitors. Improved synthesis of mimosine and related 4(1H)-pyridinones. Aust J Chem. 1976;29:1329 -1334.
22. Saghaie L. Design of orally active iron (III) chelators for clinical use [Ph.D. dissertation]. London: King's College University; 1996.
23. Imafuku K, Takahashi and Matsumurg H. Constituent effects on 6-substituted 3-hydroxyl-1-methyl-4-pyridinones, Bull Chem Soc Japan. 1979;S2:111-113.