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METABOLIC STUDIES WITH MODEL CYTOCHROME p-450 SYSTEMS

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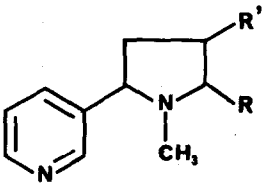
Abstract: The biomimetic potential of metalloporphyrin catalysts has been studied using nicotine as the substrate. Results indicate the formation of products identical to those obtained from in vivo metabolism.

Cytochrome p-450 model systems have attracted much attention in recent years in an attempt to mimic the oxidative metabolism of the natural p-450 process and thus study the mechanistic processes involved. This attention has largely centered on mechanistic studies of aromatic and aliphatic hydroxylation, alkene epoxidation and the nature of the active oxidant capable of performing these oxidative processes[1].

Considering the nature of the research carried out in these laboratories, it was felt appropriate to study the possible effect of model porphyrin systems on the metabolism of substrates as a guide to the in vivo cytochrome p-450 metabolic process. For this study, Fe(III) and Mn(III) TppCl were used as the catalytic model and Nicotine (1) was selected as the substrate due to it being an N-containing heterocycle with an aromatic and aliphatic ring and also because a wealth of data exist on its in vivo metabolism;of which the major metabolites are Cotinine (2) and 3'-hydroxycotinine (3)[2]. Some earlier model studies on nicotine oxidation have been done however these have concentrated on photo and electrochemical oxidizing methods[3].

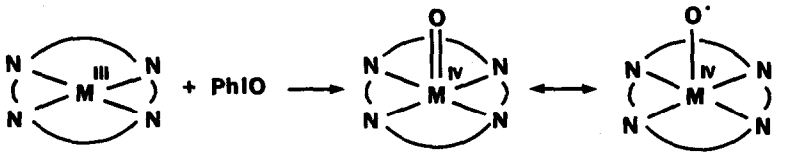


1:R=R'=H
2:R= =O R'=H
3:R= =O R'=OH

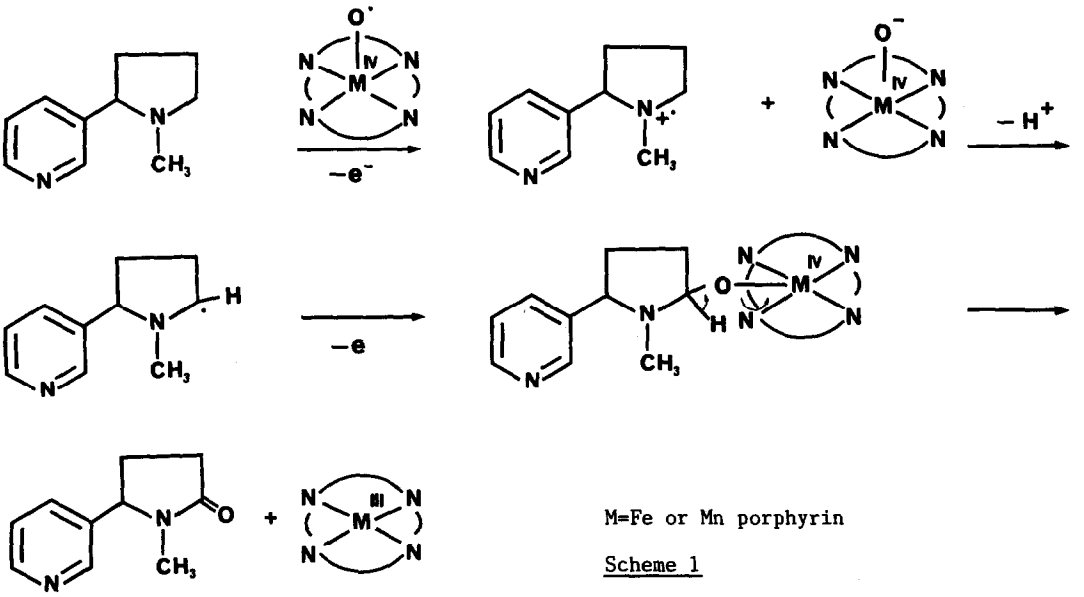
Oxidation studies were carried out in CH_2Cl_2 and benzene using iodosobenzene as oxidant[4]. In the case of Fe(III) TppCl, a molar ratio of oxidant to catalyst greater than 10-fold gave traces or no product. This may be attributed to the irreversible oxidation of the iron system leading to the destruction of the catalyst. Also a ratio of less than 5-fold gave no detectable reaction product. Surprisingly, only one product was obtained from these reactions; this was shown to be cotinine (2) by mass analysis in the FAB measurement mode giving an ion at m/z 177 (M+H) and m/z 161 which are characteristic of cotinine mass

analysis. Comparison with an authentic sample using both M.S. and t.l.c showed the product to be cotinine.

When using iodosoarenes as the oxidant in these porphyrin model systems, the view is that the active oxidant is a metalloporphyrin oxyl radical[5] i.e.



From this, it appears reasonable to suggest that the oxidation of nicotine to cotinine may proceed via a one-electron transfer from the pyrrolidine to the active oxidant followed by proton loss and the formation of an intermediate complex which is eliminated to give the ketone (Scheme 1).



Scheme 1

When using Mn(III)TppCl as the catalyst with iodosobenzene, the yield of cotinine was substantially increased (Table 1); Manganese is well known for its ability to bind reversibly with oxygen which may be an explanation for the observed increase in yield. However, as with the Fe(III) system, only one reaction product was found. Use of molecular

oxygen requires a reducing species to produce the singlet oxygen state; the use of zinc in the presence of oxygen gave low yields with both Fe and Mn systems but ascorbate gave no product with these systems.

Tablel Yields of oxidation products from model oxidation systems

Fe(III)	Yield(%)	2	3	4	Mn(III)	Yield(%)	2	3	4
PhIO		14	4	ND	PhIO		22	7	3
Zn+O		7	ND	ND	Zn+O		8	ND	ND
Zn		-	ND	ND	Zn		-	ND	ND
O		-	ND	ND	O		-	ND	ND
Ascorbate+O +Ptr		-	ND	ND	Ascorbate+O + Ptr		-	ND	ND
Zn+O +AcOH		-	ND	ND	Zn+O +AcOH		4	ND	ND

Ptr:t-BuNH4Br;omission of catalyst in each case gave no product.
ND:Not Detected. Unchanged starting material was recovered in low yield reactions.

Having only obtained a single product from the systems used, it was appropriate to use cotinine itself as the substrate in the hope of obtaining further oxidation products. Table 1 indicates the oxidation systems used for cotinine and gives the yields of products obtained. As can be seen, the Fe model only oxidized cotinine to 3-OH cotinine while using PhIO, no other system giving any product. The Mn model also only oxidized with PhIO but in this case, one further product was obtained which has been shown to be (4)[6]; this has been isolated as a urinary metabolite from the Rhesus monkey[7] and analysis by mass and n.m.r.[8] has indicated that it is tautomeric[9] with the keto-amide which is in accord with data obtained in this study.

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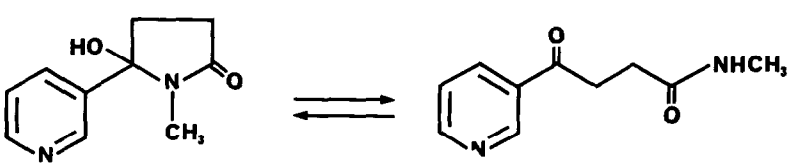

In our hands Mn has proved, in each case, to be the more efficient model catalyst with respect to yield; each material isolated has proven to be identical to in vivo metabolites. Comparative studies are currently in progress using isolated hepatocyte systems; p-450 model oxidation of other substrates is also being studied.

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4. General procedure: To the substrate (0.6mmol) and MTppCl (0.006mmol) in dry CH_2Cl_2 (3ml) was added iodosobenzene (0.06mmol) and the mixture stirred until the oxidant had been consumed. Evaporation of the solvent and purification by prep. t.l.c. gave the product.
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