CHEMICAL REACTIVITY CONSIDERATIONS IN THE METABOLISM OF *N*-HETEROAROMATICS

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SUMMARY

Enzymic carbon hydroxylations in *N*-heteroaromatics may involve either an electrophilic activated oxygen species (oxene), or a nucleophile (hydroxyl ion, OH^-). A consideration of the chemical reactivities of ring systems often allows tentative predictions of likely reaction products with appropriate enzyme systems. e.g. microsomal cytochrome P-450, or cytosolic molybdenum hydroxylases. Pyrroles and related π-excessive *N*-heteroaromatics are substrates for electrophilic enzymic ring hydroxylations mediated by cytochrome P-450, but the acidic ring nitrogen in pyrroles is not normally a site for metabolic attack. Pyridines and related π-deficient *N*-heteroaromatics are substrates for nucleophilic enzymic ring hydroxylations mediated by molybdenum hydroxylases. The nucleophilic nitrogen in such rings can also be a site for metabolism, affording N-oxides or quaternary N-conjugates as metabolites.

I. CHEMICAL-BIOCHEMICAL CORRELATIONS

There are two practical sources of interest in the biotransformation of *N*-heteroaromatics. Firstly, numerous aromatic nitrogen heterocycles (e.g. purines, pyrimidines) have now been demonstrated to play an important role in cellular biochemistry, and secondly an ever increasing number of such compounds are used as drugs, pesticides, dyes and as industrial and agricultural chemicals. *N*-Heteroaromatics are a special group of xenobiotics in so far as metabolism is concerned; this is because the heteroatom not only acts as an alternate site for metabolic attack, but also influences considerably the chemical and presumably biochemical reactivity at other sites in the ring. Clearly it would be of interest to relate structural and chemical properties of specific ring systems to their biotransformation, particularly since the mechanistic aspects of many drug metabolising enzymes are now reasonably well understood.

It is fair to say that current textbooks and monographs on drug metabolism do not give adequate treatment to this chemical-biochemical correlation. This is partly because the recurring theme,

on scanning the literature, is a poverty of sound experimental evidence to support such correlations. Authors of original papers on *N*-heteroaromatics often only give prominence to qualitative and quantitative aspects of metabolism without any attempts at identifying enzyme systems or mechanisms of individual biotransformations. Conceptualisations from a vast collection of such compound specific data, particularly where the compounds are all very highly complex substituted derivatives, are fraught with danger. This is almost a case of complexity preceding simplicity, since in most cases nothing is known about the metabolism of simple heterocycles. It would seem desirable to establish general principles with such model *N*-heteroaromatics, since it may be possible to employ this information prospectively with more complex drug molecules.

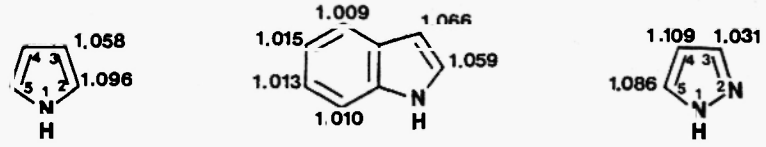
It is the author's aim to provide an introduction to the metabolism of aromatic nitrogen heterocycles along rational lines, and he hopes this short review provides a framework to which each reader can attach new information as it comes his way. Almost all the chemical characteristics of nitrogen heteroaromatic systems are to be found in the behaviour of pyridines and pyrroles. Consequently, in the interest of brevity, only these ring systems have been chosen to exemplify how chemical reactivity considerations provide explanations of events observed *in vitro* and *in vivo* metabolism. More detailed compilations of metabolic data on *N*-heteroaromatics are available in earlier reviews /1,2/.

II. METABOLISM OF PYRROLES AND OTHER π-EXCESSIVE *N*-HETEROAROMATICS

2.1. Reactions at ring carbons

The nitrogen atom in pyrrole (see Figure 1) adds its lone pair of electrons to the four electrons furnished by the two double-bonds, to make up the sextet required for aromaticity. Nitrogen's extra pair of electrons, which is responsible for the usual basicity of amines, is therefore involved in the π-cloud, and not available for reaction with acids, making pyrroles extremely weak bases. However, the contribution of electrons from the nitrogen atom of pyrrole to the

π-cloud results in all the ring carbons having high electron densities /3/. Compared to benzene, pyrrole is a π-excessive aromatic compound and is readily substituted by electrophilic reagents, but is resistant to nucelophilic reagents. Cytochrome P-450 catalysed oxygenations involve generation of a reactive electrophilic active oxygen species from molecular oxygen /4/; this active oxygen would be expected to attack positions of high electron density. As far as can be ascertained, pyrrole metabolism appears not to have been studied, but all ring carbons should be amenable to such enzymic aromatic ring hydroxylations. Indeed, its benzologue derivative (indole, see Figure 1) predictably undergoes oxidation at C-3, the position of highest electron density, affording 3-hydroxyindole as the major *in vitro* metabolite with hepatic microsomes /5/. Similar chemical reactive considerations apply to pyrazole (Figure 1), which affords 4-hydroxypyrazole as a major *in vivo* metabolite in the rat /6/. Whereas pyrrolic compounds are substrates for electrophilic enzymic oxidations via cytochrome P-450, they are not normally substrates for the molybdenum hydroxylases, where a nucleophilic attack by OH^- is involved (see section III on pyridine metabolism).



pyrrole indole pyrazole

Fig. 1: Some typical π-excessive *N*-heteroaromatics (* numbers refer to calculated electron densities)

2.2. Reactions at ring nitrogen

The ring nitrogen in pyrrole, since its lone pair is utilised in making up the sextet for aromaticity, is not available for bond formation in metabolic reactions. There is no convincing data to support *N*-hydroxylation at pyrrolic nitrogens. Indeed, conjugations

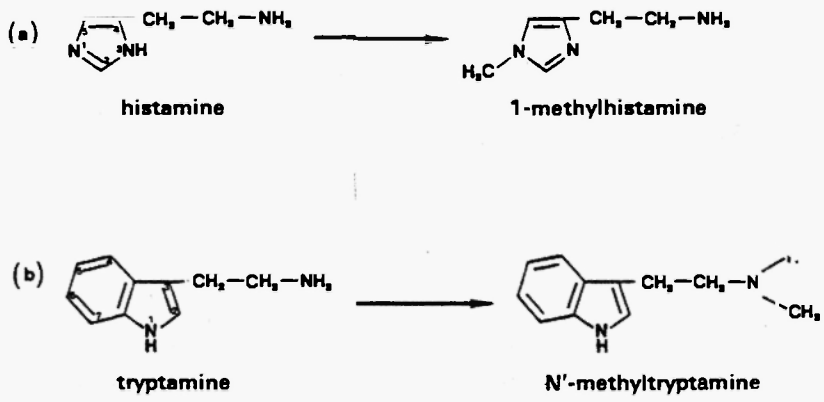


Fig. 2: N-Methylation reactions in histamine (a) and tryptamine (b)

(e.g. *N*-methylations) which require a nucleophilic atom do not occur at the acidic pyrrolic nitrogens. There are no examples of *N*-methylations, *N*-glucuronidation, or any other *N*-conjugation reactions in pyrrole, indole, pyrazole and related π-excessive heteroaromatics. For example in the catabolism of histamine (Figure 2a), the site of *N*-methylation is the tertiary double-bonded N^1 nitrogen atom (note that the final product, 1-methylhistamine, is not quaternary because of intramolecular rearrangement). Interestingly, the most basic nitrogen in histamine, i.e. the side-chain ethylamino nitrogen, is not *N*-methylated by the very specific histamine N-methyltransferase. However, *N*-methylation in tryptamine (Figure 2b) occurs at the *N'*-side chain nitrogen, and not the N^1-indolic nitrogen by fairly non-specific arylamine *N*-methyltransferase /7/. These observations are in accord with the generally accepted view that the mechanism of methyl group transfer in the formation of *N*-methylated metabolites operates via a classic S_N^2 substitution reaction, with a direct displacement of the (+)S-methyl group of the sulphonium compound S-adenosyl-L-methionine by nitrogen with an available lone pair of electrons. Detailed systematic investigations on the chemical mechanisms of various types of *N*-conjugations have not to date been carried out, and therefore the conclusions about the role of nucleophilicity must at present be tentative.

III. METABOLISM OF PYRIDINES AND OTHER π-DEFICIENT *N*-HETEROAROMATICS

3.1. Reactions at ring carbons

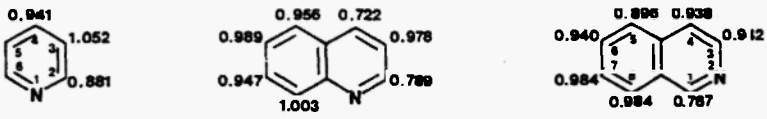


Fig. 3: Some typical π-deficient *N*-heteroaromatics (* numbers refer to calculated electron densities).

The nitrogen atom in pyridine (Figure 3), like each of the ring carbons, is bonded to other members of the ring by the use of sp^2 orbitals, and provides one electron for the aromatic π-cloud. Whereas the third sp^2 orbital of carbons are utilised in forming a bond with hydrogen, the third sp^2 orbital of nitrogen contains an available lone pair of electrons. This ring nitrogen attracts electrons from the π-cloud; pyridine therefore resembles nitrobenzene in being a typical π-deficient aromatic compound, electron densities at C-2 and C-4 being lower than at C-3 /3/. In its benzologue, quinoline (Figure 3), positions of high electron density are C-3, C-6, and C-8. Whereas the nitrogen in pyrrole activates the ring towards electrophilic substitution reactions, the nitrogen in pyridine activates the ring towards nucleophilic reactions, which take place at C-2 or C-4. Pyridine is resistant to electrophilic substitutions, and nitrations, sulphonations and halogenations only occur at C-3 reluctantly, the position of highest electron density.

π-Deficient *N*-heteroaromatics as a general rule appear to be poor substrates for cytochrome P-450 mediated aromatic ring hydroxylations, and when such reactions do occur, they are predictably at positions of high electron densities /1,2/. In benzologue derivatives such as quinoline, the electrophilic enzymic attack is predominantly at C-6 and C-8 in the benzene ring, although 3-hydroxyquinoline is also formed as a minor metabolite. π-

Deficient *N*-heteroaromatics are generally better substrates for the molybdenum-containing enzymes, such as xanthine oxidase and aldehyde oxidase /8,15/. These enzymes catalyse the oxidation of electron-deficient carbons, the reactions usually represented as in Equation 1.

$$E_{oxd} + RH + OH^- \rightarrow E_{red} + R\text{-}OH + 2e^- + H^+$$

$$E_{red} + O_2 \rightarrow E_{oxd} + H_2O_2$$

– Equation 1

The oxygen introduced into the substrate is derived from water, the reaction involving a nucleophilic attack by OH^-. The enzyme undergoes a cycle of oxidation/reduction, the ultimate physiological electron acceptor may be oxygen, although a wide variety of other compounds can be utilised in this capacity. Pyridine does not appear to react with "molybdenum-hydroxylases" *in vitro* /9/, but interestingly affords 4-pyridone as a major metabolite *in vivo* in several animal species /10/. Substituted pyridines (e.g. 3-acetylpyridine, metyrapone) also undergo nucleophilic enzymic α-C-oxidation /11-14/. The charged nitrogen in *N*-methylpyridinium and *N*-methylquinolinium compounds activates the α-carbon even further to nucleophilic attack, and such compounds are good substrates for the molybdenum enzymes /12,15/. Quinoline and isoquinoline predictably undergo such α-C-oxygenations *in vitro* at positions of low electron densities (C-2 and C-1 respectively, see Figure 3), and furthermore appropriate substitutions can increase reaction rates with the enzymes through electronic or lipophilicity factors. Such chemical reactivity considerations have been applied to several other *N*-heteroaromatics in an effort to rationalise the observed sites of metabolic reactions /1,2,8/.

3.2. Reactions at ring nitrogen

As explained above, the nitrogen of pyridine and related azaheterocycles is considerably different from the nitrogen of pyrrole. The lone pair of electrons on the pyridyl nitrogen can participate in dative or semi-polar bond formation to afford *N*-oxides as metabolites. Such metabolic reactions readily occur in

pyridines, diazenes, and other fused analogues, very often as major pathways of metabolism /13/. This is in contrast to the rarity of metabolic *N*-oxygenations at pyrrolic nitrogens. The nucleophilic nitrogens of pyridines can also participate in conjugation reactions, e.g. *N*-methylation, *N*-glucuronidations, to afford water soluble quaternary metabolites. The significance of such "biological quaternisation reactions" /13/ has only now been recognised. Whereas in most cases the highly water soluble quaternary metabolites are readily eliminated as harmless urinary excretion products, in some instances such products may be of toxicological significance if they are generated and trapped at specific sites (e.g. the CNS).

IV. CONCLUDING REMARKS

It is hoped that this short review outlines how chemical reactivity considerations may be useful in drug metabolism. These reactivity parameters are particularly useful in *in vitro* experimnents, when often the experiment is designed to achieve a specific objective by excluding one enzyme system altogether by use of an appropriate cell fraction. *In vivo* the drug "takes the line of least resistance", and the proportion of different metabolites produced will depend on a variety of factors. It must be recognised that substituents often have a profound influence on lipophilicity and the "steric shape" of the molecule, which in turn determines access to enzyme active sites. Considerations of all these parameters are ultimately needed in any predictions. It is clear that *N*-heteroaromatics are metabolically no more or less stable than aromatic carbocycles. In this respect it is interesting to quote from Albert's excellent book on Heterocyclic Chemistry /3/. "The current use of heterocyclic nuclei as a basis for drugs and for pesticides is connected with the usually high resistance of heterocyclic substrates to biological degradation, examples of which stability are provided by the persistence of the nitrogenous vitamins in the human body, and also of the purine and pyrimidine bases of deoxyribonucleic acid". The metabolic stability of endogenous *N*-heteroaromatics has a different biochemical basis, but exogenous *N*-heterocycles, in most instances, undergo extensive

biotransformation, as studies carried out in the last two decades have demonstrated.

VIII. REFERENCES

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